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1P AGONIST AND ANTAGONIST EFFECTS OF 5-HT_{1A} RECEPTOR LIGANDS ON EXCITATORY SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS *IN VIVO*

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The relative agonist and antagonist properties of a series of 5-HT_{1A} receptor ligands administered systemically was assessed electrophysiologically in the hippocampus of alert rats. The ability to reduce excitatory synaptic transmission was used as a measure of 5-HT_{1A} receptor mediated responsiveness (O'Connor et al, 1990).

A pair of stimulating and recording wire electrodes were implanted in the stratum radiatum of the CA1 region of the dorsal hippocampus of pentobarbitone (40 mg/kg, i.p.) anaesthetized male Wistar rats (200-250 g). Animals were allowed at least 1 week to recover before electrically evoked population excitatory post-synaptic potentials (EPSP) were recorded whilst lightly restrained in a hammock. Changes in the amplitude of the EPSP (60% maximum) were recorded after i.p. injection of the drugs.

8-OH-DPAT (8-hydrox-2-(di-n-propylamino)tetralin, 25-75 µg/kg), gepirone (0.5-10 mg/kg) and its metabolite 1-(2-pyrimidinyl)-piperazine (1-PP, 0.25-1 mg/kg) produced dose-dependent transient reductions in the amplitude of the EPSP with maximum decreases of 73 ± 4% (n = 4), 38 ± 3% (n = 4) and 32 ± 2% (n = 6) respectively.

Whereas MDL 73005EF (2 mg/kg, Moser et al, 1990) had no effect on its own it significantly blocked the reduction of the EPSP amplitude produced by either 50 µg/kg 8-OH-DPAT (from 57 ± 6% to 15 ± 2%, n = 4, $P < 0.01$, mean ± s.e. mean % decrease, Student's t test), 3 mg/kg gepirone (from 27 ± 2% to 5 ± 1% decrease, n = 4, $P < 0.01$) and 1-PP (from 28 ± 0.1% to 9 ± 2% decrease, n = 4, $P < 0.01$).

Pretreatment with gepirone (3 mg/kg) or 1-PP (1 mg/kg) prevented a further significant reduction in the EPSP amplitude by 50 µg/kg 8-OH-DPAT (26 ± 3% and 20 ± 3% decrease for gepirone + water and gepirone + 8-OH-DPAT respectively; 29 ± 1% and 27 ± 2% decrease for 1-PP + water and 1-PP+8-OH-DPAT respectively, n = 4 per group).

These findings suggest that with regard to 5-HT_{1A} receptor modulation of excitatory synaptic transmission in the hippocampus of the alert rat 8-OH-DPAT behaves as an agonist, gepirone and 1-PP as partial agonists and MDL 73005EF as an antagonist when given systemically.

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2P (-)-PENBUTOLOL ANTAGONIZES 8-OH-DPAT-INDUCED INHIBITION OF RAT 5-HYDROXYTRYPTAMINERGIC DORSAL RAPHÉ NEURONS

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A number of compounds, such as BMY 7378, NAN-190, and RK-153, which display antagonist actions in "postsynaptic" models of serotonin type 1A (5-HT_{1A}) receptor function, appear to act predominantly in an agonist fashion at "presynaptic" 5-HT_{1A} receptors located on cell bodies and dendrites of serotonergic dorsal raphe (DR) neurons (Hjorth & Sharp, 1990; Sharp *et al.*, 1990; VanderMaelen & Braselton, 1991). The β-adrenoceptor and 5-HT_{1A} ligand (-)-penbutolol [(-)-P] has displayed antagonist actions in biochemical and behavioral studies of 5-HT_{1A} function. A recent microdialysis study by Hjorth *et al.*, (1991) suggests that (-)-P does not exert 5-HT_{1A} agonist actions on serotonergic DR neurons, and can block the inhibitory effects of 8-OH-DPAT. The present study examined this question directly, using extracellular single unit recording and microiontophoretic electrophysiological techniques in chloral hydrate anesthetized adult male Sprague-Dawley rats. Intravenous doses of (-)-P, ranging from 0.001 to 2.0 mg/kg produced weak and variable inhibitory effects, with most cells partially inhibited, and a few completely inhibited. Overall, impulse flow was preserved, consistent with the actions of a very weak 5-HT_{1A} partial agonist. Surprisingly, pretreatment of rats for 10 min with 0.5 mg/kg, i.v. of (-)-P had absolutely no antagonist-like effect on the dose-response curve for inhibition of DR neuronal firing induced by 8-OH-DPAT. However, when a protocol similar to that used by Hjorth *et al.* (1991) was utilized, with (-)-P administered s.c. 60 min or more before 8-OH-DPAT, an 11-fold shift to the right of the 8-OH-DPAT dose-response curve was observed (ED_{50} values = 2.13 and 24.8 µg/kg, i.v.). This high dose of (-)-P by itself produced partial inhibition of firing for most cells tested, but in general, impulse flow was still preserved. Microiontophoretic administration of 8-OH-DPAT produced inhibition of firing of serotonergic DR neurons. This response showed little net change over time following s.c. saline injection. However, the inhibitory response to microiontophoretic 8-OH-DPAT was reduced an average of 85.5% by (-)-P when the same protocol described above was used (60 min or more after 8.0 mg/kg, s.c. (-)-P). These results are consistent with the notion that (-)-P is a very weak 5-HT_{1A} partial agonist that can effectively antagonize "presynaptic" 5-HT_{1A} receptors located on serotonergic DR neurons, without shutting down impulse flow in these cells. Why the lower i.v. dose with the shorter pre-treatment time failed to produce any detectable antagonism remains to be explored. (-)-Penbutolol was graciously provided by J. Chamberlain and S. Maayani.

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3P THE DIFFERENTIAL PHARMACOLOGY OF THE (-) AND (+) STEREOISOMERS OF WAY 100339 AT 5-HT$_{1A}$ AND α_2-ADRENOCEPTORS IN THE RAT

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WAY 100339 (7-(1-azetidinyl)-5,6,7,8-tetrahydroquinoline) was synthesised as part of a programme designed to identify 5-HT$_{1A}$ receptor ligands. Here we report the pharmacological profile of WAY 100339 and its stereoisomers, WAY 100507 (-) and WAY 100508 (+). Standard radioligand binding assays on WAY 100339, WAY 100508 and WAY 100507 demonstrated affinities at 5-HT$_{1A}$ receptors with IC$_{50}$ s of 136, 60 and 2994 nM and at α_2 adrenoceptors of 219, 25 and 458 nM respectively. WAY 100339 produced a typical 5-HT$_{1A}$ behavioural syndrome with an ED$_{50}$ (95% confidence limits) = 0.77 (0.53-1.2) mg/kg i.v, similar to that produced by the selective 5-HT$_{1A}$ receptor agonist flesinoxan (ED$_{50}$ = 1.5 (1.1-2.0) mg/kg i.v.). WAY 100507 (10 mg/kg, i.v.) did not produce syndrome. WAY 100508 (0.3-10 mg/kg, i.v.) produced sedation and ataxia at all doses. Co-administration of WAY 100508 and WAY 100507 (1.5 mg/kg, i.v.) produced the 5-HT$_{1A}$ syndrome. Also, when WAY 100508 was co-administered with either of the two α_2 antagonists, Wy 26392 (Paciorek *et al*, 1984) or idazoxan (both at 0.5 mg/kg, i.v.), the 5-HT$_{1A}$ syndrome was evoked. The sedative potentials of WAY 100339 (0.3-10mg/kg), WAY 100508 (0.3-3 mg/kg), WAY 100507 (1-100 mg/kg) and the α_2-adrenoceptor agonist clonidine (0.03-1 mg/kg) were assessed using groups of 6 to 8 rats tested 60 min after oral dosing in automated open fields. All the compounds tested produced dose related decreases in locomotor activity. The doses of each compound required to inhibit locomotor activity to 50 % of control levels were 4.3, 0.53, >100 and 0.14 mg/kg p.o. respectively. In pentobarbitone (70 mg/kg, i.p.) anaesthetised rats, cumulative administration of WAY 100339 (0.01-1 mg/kg, i.v.), WAY 100508 (0.003-1 mg/kg, i.v.) and clonidine (0.3-30 µg/kg, i.v.) produced dose related falls in MABP (mean arterial blood pressure). These responses and the effects of antagonists on them are tabulated below. WAY 100507 alone (0.01-10 mg/kg) did not signifcantly reduce MABP.

Agonist	Control ED$_{20}$ (µg/kg) (n)	95% Confidence limits	Antagonist	Dose (mg/kg)	New ED$_{20}$ (µg/kg) (n=4)	95% Confidence limits	Dose ratio
WAY 100339	42.4 (6)	15.2-117.8	Wy 26392	1.0	331	124-883**	7.8
Clonidine	1.9 (4)	0.7-4.8	Wy 26392	1.0	40.4	20.1-78.7**	21.3
WAY 100508	13.3 (4)	2.8-61.7	WAY 100507	10	372	127-1084***	28.0
Clonidine	1.7 (5)	0.5-5.9	WAY 100507	10	clonidine response abolished		>>18

** P<0.01, *** P<0.001 compared with control ED$_{20}$ (defined as the dose producing a 20% fall in MABP), unpaired Student's t-test.

In conclusion, the pharmacology of WAY 100339 is similar to that of flesinoxan: producing a fall in blood pressure, 5-HT$_{1A}$ syndrome and little sedation. The effects of WAY 100508 are more like those of clonidine i.e. a fall in blood pressure with marked sedation; whilst the activity of WAY 100507 resembles that of Wy 26392. These results suggest that WAY 100508 acts as an agonist at both α_2 adrenoceptors and 5-HT$_{1A}$ receptors, whereas WAY 100507 acts as an α_2 antagonist. In the racemic mixture (WAY 100339) therefore, the α_2 agonist effects of WAY 100508 are masked by WAY 100507 allowing the 5-HT$_{1A}$ agonist activity of WAY 100508 to predominate. These experiments illustrate interesting differential pharmacological properties of the two enantiomeric forms of WAY 100339 in the rat.

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4P THE EFFECTS OF THE 5-HT$_{1A}$ LIGAND MDL 73005EF ON THE SPATIAL LEARNING IMPAIRMENT PRODUCED BY EITHER GEPIRONE OR ATROPINE

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The 5-HT1A ligands buspirone (Rowan et al, 1990), gepirone (Rowan & Barrett, 1990) and 8-OH DPAT (Carli & Samanin, 1992) have been reported to impair the spatial learning of rats in the Morris water maze. The finding (Barrett & Rowan, 1990) that a sub-threshold dose of gepirone greatly enhanced deficits produced by atropine is indicative of a strong interaction between cholinergic and serotonergic systems in spatial learning. The present study examined the effect a 5-HT1A ligand with relatively low intrinsic activity, MDL 73005EF (MDL, Moser et al 1990), on the performance deficits produced by either gepirone or atropine. Male Wistar rats (250-300 g) were trained to escape onto a platform submerged in a pool of opaque water (1 m in diameter) over three days (12 trials). On the fourth day the platform was removed and a 2 min recall (probe) trial was carried out and videotaped. The tape was later analysed using a computer tracking system. MDL (5 mg kg^{-1}) or water (1 ml kg^{-1}) was administered i.p. 15 min prior to an injection of either water (1 ml kg^{-1}) or gepirone(7 mg kg^{-1}) or atropine (20 mg kg^{-1}) which was given i.p. 30 min before the first trial on each day.

Table 1 **Water maze performance**

	ESCAPE LATENCY (s)			PROBE TRIAL (DAY 4)	
	DAY 1	DAY 2	DAY 3	No. of target annulus crossings	Swimming speed(cm/s)
water + water	63.8 ± 13.5	31.6 ± 4.8	19.2 ± 6.2	6.4 ± 1.7	15.3 ± 1.2
water + MDL	60.9 ± 6.8	36.0 ± 8.1	20.3 ± 2.0	4.9 ± 0.7	9.6 ± 0.8*
water + gepirone	66.9 ± 9.4	43.2 ± 15.7	34.6 ± 9.9	5.4 ± 1.0	15.5 ± 0.4
MDL + gepirone	108.4 ± 6.9	75.7 ± 5.7*	60.0 ± 7.4*	3.5 ± 0.9*	6.8 ± 0.7*
water + atropine	84.2 ± 15.7	72.1 ± 9.0	36.5 ± 9.8	4.7 ± 1.0	13.3 ± 1.0
MDL + atropine	110.4 ± 7.4	112.2 ± 3.1*	81.0 ± 9.4*	2.7 ± 0.9	15.4 ± 1.3

Values are the mean ± s.e.mean. * P<0.05 ANOVA or t-test compared to reference group, n=8 per group, n=16 for water + MDL group

Although MDL induced a decrease in swimming speed it did not impair the acquisition or recall of the task at the dose tested (Table 1). It did however appear to increase the deficits in performance produced by either gepirone or atropine.These results are consistent with the view that 5-HT1A ligands impair spatial learning in the rat and that anticholinergic effects may be enhanced by such agents.

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5P ANTAGONISM OF PRESYNAPTIC AND POSTSYNAPTIC 5-HT$_{1A}$ RECEPTORS *IN VIVO* BY THE SELECTIVE 5-HT$_{1A}$ RECEPTOR ANTAGONIST, WAY100135

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WAY 100135 [N-tert-butyl 3- 4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide dihydrochloride] is a selective, silent 5-HT$_{1A}$ receptor antagonist (Fletcher et al., 1991). We have examined the effects of WAY 100135 on two in vivo responses evoked by the selective 5-HT$_{1A}$ receptor agonist 8-hydroxy-N,N-dipropylamino-tetralin (8-OH-DPAT), i.e. attenuation of endogenous 5-HT release measured by microdialysis, and the behavioural syndrome (forepaw treading, flat posture, hyperlocomotion). These effects are mediated by presynaptic (somatodendritic) and postsynaptic 5-HT$_{1A}$ receptors, respectively.

24 hours after implantation of guide cannulae into the ventral hippocampus of anaesthetised male Wistar rats (280-320g), dialysis probes were inserted for the measurement of extracellular 5-HT (Brazell et al. 1985). Probes were perfused with artificial cerebrospinal fluid pH 7 (1.0 ul/min) and 20 min samples were collected following a 2.5 h stabilisation period. Three control samples were taken followed by subcutaneous administration of vehicle (0.3 % methyl cellulose) or drug; 8-OH-DPAT (100 ug/kg s.c.) or saline (1 ml/kg s.c.) was administered 30 min later and samples collected for a further 3.5 h. Induction of the '8-OH-DPAT syndrome' in male Sprague-Dawley rats (320-400g) was used to assess post-synaptic 5-HT$_{1A}$ agonist activity in vivo. The syndrome was scored quantally (unequivocal response or non-response) 0-5 min after intravenous administration of the test compound. ED$_{50}$ values (with 95% confidence limits) were determined using a sequential up/down method ($n \geq 10$ per treatment) and calculated by modified probit analysis (Kimball et al., 1957). For 5-HT$_{1A}$ receptor antagonist evaluation, test compound or vehicle was administered subcutaneously 30 min prior to ED$_{50}$ determination for 8-OH-DPAT.

WAY 100135 (10 mg/kg s.c.) had no significant effect on hippocampal extracellular levels of 5-HT. In contrast, the 5-HT$_{1A}$ receptor partial agonists BMY 7378 (5 mg s.c.) and buspirone (1 mg/kg s.c.) and the potent agonist 8-OH-DPAT significantly ($P < 0.05$) decreased hippocampal 5-HT levels to 37.6 ± 6.2, 39.9 ± 15.0, and 19.2 ± 9.9 % of pre-injection control values. Pre-treatment with WAY 100135 (10 mg/kg s.c.) completely blocked the 8-OH-DPAT-induced decrease in extracellular levels of 5-HT ($P < 0.05$). 8-OH-DPAT and buspirone induced the '8-OH-DPAT syndrome' with ED$_{50}$ values of 0.04 (0.03-0.05) and 0.34 (0.27-0.42) mg/kg i.v. respectively. In contrast, WAY100135 and BMY7378 ($\leq$ 10 mg/kg i.v.) failed to induce any components of the '8-OH-DPAT syndrome'. Pretreatment with WAY100135 (1-3 mg/kg s.c.) and BMY7378 (0.1-3 mk/kg s.c.) dose-dependently antagonised all components of the syndrome induced by 8-OH-DPAT.

These data demonstrate that WAY100135 is a selective 5-HT$_{1A}$ receptor antagonist at both presynaptic and postsynaptic 5-HT$_{1A}$ receptors. Unlike BMY7378 and buspirone, WAY100135 did not attenuate 5-HT release; indicating that it is a 5-HT1A receptor antagonist devoid of agonist properties.

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6P PHARMACOLOGICAL CHARACTERIZATION AND LOCALIZATION OF 5-HT$_{1B}$ AND 5-HT$_{1D}$-LIKE RECOGNITION SITES IN RAT BRAIN

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Quantitative autoradiography was applied to determine the pharmacology and distribution of the binding sites labelled with [^{125}I]GTI (serotonin-O-carboxy-methyl-glycyl-[^{125}I]tyrosinamide) in rat brain. Displacement studies with the 5-HT$_{1B}$ selective compound CP 93129 (5-hydroxy-3(4-1,2,5,6-tetrahydropyridyl)-4-azaindole) and (-)pindolol resulted in biphasic competition curves showing 60-80 % high affinity and 20-40 % low affinity sites in the globus pallidus and the substantia nigra. In contrast, PAPP (1-[2-(4-aminophenyl)]piperazine), which has some 5-HT$_{1D}$ versus 5-HT$_{1B}$ selectivity and sumatriptan produced biphasic curves with an inversed ratio (i.e. a minor component of high affinity sites, Table 1).

Table 1. Affinity values (pK$_D$, -log mol/l ± SEM) of different drugs for 5-HT$_{1B}$ and 5-HT$_{1D}$-like binding sites in the rat substantia nigra and percentage of specific binding (SB) when biphasic curves were found (mono = monophasic displacement).

DRUG	5-HT$_{1B}$	% SB	5-HT$_{1D}$-like	% SB
5-CT	9.02 ± 0.14	mono	9.02 ± 0.14	mono
(-)PINDOLOL	8.52 ± 0.14	82 %	5.96 ± 0.13	18%
CP 93129	7.92 ± 0.05	77 %	5.76 ± 0.11	23 %
SUMATRIPTAN	6.69 ± 0.16	49 %	7.83 ± 0.14	51 %
YOHIMBINE	6.24 ± 0.08	ND	7.97* ± 0.41	ND
PAPP	6.07 ± 0.15	38 %	7.80 ± 0.24	62 %
RAUWOLSCINE	5.11 ± 0.15	ND	7.15* ± 0.06	ND

ND = not determined. * Affinity values obtained in the presence of 100 nM CP 93129

A mapping of the 5-HT$_{1D}$-like sites was made using [^{125}I]GTI in the presence of 100 nM CP 93129 to block 5-HT$_{1B}$ receptors. 5-HT$_{1D}$-like sites were found in substantia nigra, globus pallidus, caudate-putamen, subthalamic nucleus, medial terminal nucleus of the accessory optic tract and nucleus of the optic tract. Thus, [^{125}I]GTI labels mainly 5-HT$_{1B}$ sites in rat brain, and a minor population of sites with a 5-HT$_{1D}$-like binding profile. Recent cloning work suggests the coexistence of two members of the 5-HT$_{1B/1D}$ receptor family in rat and man (termed 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$, see Hartig et al., 1992). The majority of the sites labelled with [^{125}I]GTI in rat brain represents the 5-HT$_{1B}$ variant (the rat analogue of 5-HT$_{1D\beta}$). It remains to be seen whether the minor component of [^{125}I]GTI binding equates with the putative 5-HT$_{1D\alpha}$ receptor.

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7P 5-HT AGONIST REGULATION OF 5-HT$_{1C}$ RECEPTORS

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5-Hydroxytryptamine$_{1C}$ (5-HT$_{1C}$) and 5-HT$_2$ receptors share considerable structural homology but subserve different CNS functional roles. 5-HT$_2$ receptors are readily down-regulated by both agonists and antagonists (Leysen *et al.*,1989) but similar regulation of 5-HT$_{1C}$ receptors has yet to be studied. To examine 5-HT$_{1C}$ receptor regulation, the behavioural response and 5-HT$_{1C}$ receptor protein-like immunoreactivity (5-HT$_{1C}$-LI) were measured in rat brain and spinal cord following repeated treatment with the 5-HT$_2$/5-HT$_{1C}$ agonist 1-[2,5-dimethoxy-4-methylphenyl]-2-aminopropane (DOM) and the 5-HT$_{1C}$/5-HT$_{1B}$ agonist 1-(3-chlorophenyl)piperazine (m-CPP).

Adult male Wistar rats (300-335g) received twice daily injections of either DOM (2.5 mg kg^{-1} i.p), m-CPP (5 mg kg^{-1} i.p), or saline (0.154M 0.1ml kg^{-1} i.p., n=6 each) for 5 days. Following the first and alternate injections, the number of wet-dog shakes, back muscle contractions, rears (DOM and m-CPP) and 90^o turns and yawns (m-CPP) were measured separately but continously for 30min in behavioural chamber. After (30min) the last injection, rats were decapitated and 5-HT$_{1C}$-LI assayed using a polyclonal antiserum raised against the rat 5-HT$_{1C}$ receptor protein (Sharma *et al.*,1992). Results are presented as mean±s.e.mean and ANOVA followed by Dunnett t-test (behaviour) and Student's t-test (5-HT$_{1C}$-LI) used for statistical analysis.

Repeated DOM injection progressively reduced back muscle contractions ($P<0.05$, by 67% from 43±7 in 30 mins initially) and wet-dog shakes (by 52% from 19±5, not significant). DOM treatment also significantly elevated 5-HT$_{1C}$-LI in the dorsal spinal cord (being 3450±241 in DOM compared with 2297±193 fmols mg^{-1} protein in controls; $P<0.01$) and reduced levels in the hypothalamus (836±126 compared with1335±157 fmols mg^{-1} protein; $P<0.05$) and ventral spinal cord (2708±337 compared with 4164±867 fmols mg^{-1} protein, not significance). In a novel environment, on the first injection, m-CPP attenuated both turns (from 25±4 in 30min to 63±12 with saline) and rears (18±2 compared with 25±6), this reduction was maintained to the ninth injection (turns $P<0.01$, 10±2 and 39±7, rears $P<0.001$, 3±1 and 11±1). With repeated m-CPP, yawns increased significantly ($P<0.05$,from 5±1 to 13±1) whereas no such behaviour was observed in saline-treated rats and 5-HT$_{1C}$-LI remained unaltered in any brain or cord region.

The DOM-induced behavioural tolerance is consistent with the previously reported rapid agonist-induced downregulation of 5-HT$_2$ receptors (Leysen *et al.*,1989). In contrast, no downregulation of either the hypolocomotor effect of m-CPP (reported to be 5-HT$_{1C}$ mediated, Kennett & Curzon, 1988) or 5-HT$_{1C}$-LI receptor protein levels was observed in the current study despite repeated agonist administration.

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8P INTRA-PERIAQUEDUCTAL GREY ADMINISTRATION OF mCPP POTENTIATES A CHEMICALLY-INDUCED DEFENCE RESPONSE

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Specific stimulation of cell bodies using glutamate analogues such as D, L-Homocysteic acid (DLH) has revealed the dorsal periaqueductal grey (PAG) as the site of integration of the defence response (Hilton and Redfern 1986). The role played by serotonin in modulating this response has been studied in terms of the effect of agonists and antagonists on the electrical aversive stimulation threshold. Jenk et al. (1989) suggested that activation of 5-HT$_{1A}$ receptors was proaversive while activation of 5-HT$_{1C}$ receptors was antiaversive. More recently dorsal PAG administration of the 5-HT$_{1A}$ agonists 5-carboxamidotryptamine (5-CT) and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT), attenuated the DLH induced defence response (Beckett et al. 1992). The present study has examined the effect of direct administration of the 5HT$_{1C}$ agonist 1-(3-chlorophenyl)piperazine (mCPP) into the PAG on DLH induced defence. In addition the effect of systemic treatment, prior to mCPP and DLH, with the 5-HT antagonist mianserin has also been investigated.

Male Lister hooded rats (200-250g) anaesthetised with a halothane N_2O/O_2 mixture had stainless steel guide cannulae, implanted into the dorsal PAG (AP -7mm, ML +0.2mm, DV -5.1mm relative to bregma). Seven days post surgery the animals were placed in a circular arena and DLH (5nmols in 250nl) was injected 10 minutes after saline (250nl) or mCPP (18.5nmols in 250nl). Subsequent behaviour was recorded on video tape for 5 minutes Defence behaviours were quantified in terms of response duration, number of jumps and arena revolutions. Twenty four hours later the animals were given mianserin (2mg/kg i. p.) 10 minutes prior to mCPP (20 minutes before DLH). Injection sites were verified by histological examination.

DLH produced the characteristic overt pattern of defence behaviour following saline pretreatment; mean response duration 32±2s, jumps 4±1, revolutions 10±1 (n=8), whereas pretreatment with mCPP significantly potentiated the response; duration 57±3s; $p< .005$, jumps 9±2; $p< .05$, revolutions 19±1; $p< .005$ (n=8). Systemic administration of mianserin partially reversed the proaversive effect of mCPP; duration 46±4s; $p< .05$, jumps 5±1, revolutions 16±2; $p< .05$ (n=8).

These data suggest that activation of 5-HT$_{1C}$ receptors within the dorsal PAG potentiates the DLH induced defence response, indicating a proaversive role for 5-HT$_{1C}$ receptors located in this area.

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9P EFFECT OF 21 DAYS FLUOXETINE ON 5-HT AUTORECEPTOR FUNCTION IN RAT DORSAL RAPHÉ AND SUPRACHIASMATIC NUCLEUS *IN VITRO*

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Moret & Briley (1990) showed that 5-HT uptake inhibitors given for three weeks in rats cause a down regulation of the axon terminal 5-HT autoreceptor. Here we report changes in the sensitivity of 5-HT autoreceptors in rat brain slices containing the dorsal raphe (DRN) or suprachiasmatic nucleus (SCN) after 21 days treatment with fluoxetine or water. Electrically stimulated 5-HT overflow ([5-HT]ex) was monitored using fast cyclic voltammetry (FCV);(O'Connor and Kruk, 1991). Rats were given either water or fluoxetine ($5mgkg^{-1}day^{-1}$ i.p.) for 21 days. 24h after the last injection, slices containing either the DRN or SCN were dissected and superfused with oxygenated ACSF at 32°C. [5-HT]ex was monitored with a carbon fibre electrode placed centrally in the DRN or SCN. Local electrical stimulation using 5 pulses at 100Hz (0.1ms; 20V) was applied every 5min with a bipolar electrode. [5-HT]ex could be measured for more than 5h;(10-15nM 5-HT, DRN; 8-12nM 5-HT, SCN).

[5-HT]ex was significantly enhanced in both brain regions after three weeks treatment with fluoxetine (201% of control in DRN; 217% of control in SCN; n=6). 5-HT uptake was not affected; the half time of uptake was 2.3±0.4s (water controls) 1.9±0.3 s (fluoxetine 21 days) in the DRN and 3.2±0.5s (water) 3.3±0.4s (fluoxetine) in the SCN; $P>0.01$; n=6 in each experiment). The 5-HT1 ligand RU24969 (10-9M to 10-6M) produced a concentration dependent decrease in [5-HT]ex in the SCN of water treated rats with a maximum inhibition of 73.0±8.8%; n=6. In fluoxetine treated rats the concentration response curve was significantly shifted to the right when compared to controls ($P<0.001$; n=6) with a maximum inhibition of 49.7±7.6%. In control rats, the 5HT1a agonist 8-OH-DPAT (10-9M to 10-6M) caused a concentration dependent decrease in [5-HT]ex in the DRN with a maximum inhibition of 45.0±9.6%. In fluoxetine treated rats, the concentration response curve to 8-OH-DPAT was shifted to the left with a maximum inhibition of 70.3±4.7%.

Our results confirm that chronic treatment with a 5-HT reuptake blocker causes 5-HT axon terminal autoreceptor down regulation (in the SCN). An interesting finding is that there may be sensitisation of 5-HT somatodendritic autoreceptors in the DRN.
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10P EFFECTS OF PAROXETINE ON 5-HT_2 RECEPTOR-MEDIATED PHOSPHOINOSITIDE HYDROLYSIS AND 5-HT_2 RECEPTOR BINDING IN GUINEA-PIG BRAIN

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Both biochemical and pharmacological studies indicate that the specific 5-HT uptake inhibitors and the tricyclic antidepressants down-regulate 5-HT_2 receptor number and function in rodent brain after chronic administration (Johnson, 1991). In this study the effects of acute and chronic paroxetine and amitriptyline administration on the 5-HT-stimulated phosphoinositide (PI) hydrolysis and 5-HT receptor binding were determined in guinea-pig cerebral cortex. The guinea-pig was used in this study rather than the rat as its 5-HT autoreceptor is of the 5-HT_{1D} subytpe, similar to that of the human.

5-HT-stimulated PI hydrolysis was examined in cross-chopped guinea-pig cortical slices from both treated and untreated animals, using a prelabelling assay with [^{3}H]myo-inositol and assaying [^{3}H]inositol phosphates in the presence of lithium as previously described by Kendall and Nahorski (1985). 5-HT_2 receptor binding was evaluated using [^{3}H]ketanserin (Leysen *et al.*, 1982).

5-HT stimulated [^{3}H]inositol phosphates accumulation in a dose-related but biphasic manner. The high affinity portion of the dose-response had an EC_{50} of 2.8±0.3μM (n=12) and reached a maximum at 100μM 5-HT. 5-Carboxamidotryptamine (5-CT), 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI) and 4-bromo-2,5-dimethoxyphenylisopropylamine (DOB) elicited similar biphasic PI responses to 5-HT with EC_{50} values of 22±1.8μM (n=4), 0.8±0.07μM (n=3) and 0.7±0.07μM (n=3) respectively. Quipazine and 1-m-chlorophenylpiperazine (mCPP) were ineffective as agonists. The 5-HT-stimulated PI response was antagonized in a competitive manner by ritanserin (Ki=0.2±0.08nM), methiothepin (Ki=1.4±0.8nM) and mianserin (Ki=22±1.8nM). Ondansetron (10μM) was ineffective as an antagonist. Only the high affinity portion of the agonist dose-response curve was sensitive to antagonists. These findings are largely consistent with the response to 5-HT being mediated by 5-HT_2 receptors.

Acute paroxetine treatment (10mg/kg p.o, for 24h) had no significant effect on 5-HT-stimulated PI hydrolysis but chronic paroxetine treatment (10mg/kg p.o., for 21 days) increased the 5-HT response significantly ($p<0.01$, unpaired Student's t-test, n=5). Chronic administration of amitriptyline (10mg/kg p.o., for 21 days) produced no change compared with control. There was no change in the total number of 5-HT_2 receptors as measured by [^{3}H]ketanserin binding.

These results are in marked contrast to the decreases seen in 5-HT-stimulated PI hydrolysis following treatments with certain antidepressants in rats. Whether this represents a specific effect of antidepressant treatment in guinea-pig brain or is related to a species difference is unclear at the present time.

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11P ACTIVATION OF 5-HT$_2$ RECEPTORS ENHANCES DEPOLARIZATION OF NEOCORTICAL NEURONS BY N-METHYL-D-ASPARTATE

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Serotonin (5-HT) enhances NMDA induced depolarization of both rat and mouse cortical neurons (Nedergaard et al., 1987; Mally et al., 1991). However, the 5-HT receptor responsible has not previously been clearly identified. In the present report, the 5-HT receptor subtype mediating the enhancement was assessed using the "grease-gap" recording technique.

Methods for cortical wedge preparation from rat motor cortex and recording were similar to those of Harrison and Simmonds (1985). ACSF had the following composition (mM): NaCl 126, KCl 3.5, $CaCl_2$ 2, $MgCl_2$ 1.3, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11. ACSF was gassed with 95% O_2/5% CO_2 and had a pH of 7.4. Responses were calculated as % Response = ((Treatment-Control)/Control) X 100.

The amplitude of the depolarization induced by 50 μM NMDA (2 min) was enhanced during co-administration of 5-HT. Testing only one 5-HT concentration on a wedge (to prevent tachyphylaxis), yielded a bell shaped dose response curve (93, 238 and 30% enhancement respectively at 10, 30 and 100μM). Enhancement was not observed when quisqualate and kainate were substituted for NMDA. TTX significantly reduced the amplitude of the NMDA depolarization, however the 5-HT enhancement persisted (70 and 172% at 10 and 30 μM respectively). Methysergide, ritanserin and spiperone (0.1 to 10 nM) dose dependently reduced the 5-HT enhancement. ICS 205-930 (10 nM), a 5-HT$_3$ antagonist, was ineffective. DOI, a 5-HT$_{1C\text{-}2}$ agonist, enhanced the NMDA depolarization (135 and 179% at 2 and 5 μM, respectively), whereas TFMPP (5μM), a 5-HT$_{1B\text{-}1C}$ agonist had no effect and 8-OH-DPAT (10 μM), a 5-HT$_{1A}$ agonist, reduced the NMDA response (-36%). Carbachol and phenylephrine (10μM) mimicked the action of 5-HT and were antagonized by scopolamine and prazosin respectively. However, neither antagonist significantly reduced the 5-HT enhancement.

The results with agonists and antagonists suggest that 5-HT enhances the NMDA depolarization via activation of 5-HT$_2$ receptors. This is in keeping with the high density of 5-HT$_2$ receptors in layer V$_a$ (Pazos et al., 1985) and the fact that layer V pyramidal neurons likely make a substantial contribution to the recorded potential. The TTX, scopolamine and prazosin data are consistent with a direct effect of 5-HT on pyramidal (projection) neurons, although the possibility of an indirect effect cannot be eliminated.

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12P COCAINE ANTAGONIZES 5-HT ACTIVATION OF 5-HT$_3$ CHANNELS IN NEURONES FROM RAT NODOSE GANGLION

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Serotonin can activate a ligand-gated ion channel designated the 5-HT$_3$ receptor or channel, and 5-HT$_3$ type binding sites have been reported in mammalian CNS (Costall *et al*, 1990). Several recent studies have suggested that 5-HT$_3$ receptors may play a role in the action of drugs of abuse, and there are reports suggesting that behavioral effects related to cocaine administration are altered by 5-HT$_3$ antagonists (Costall *et al*, 1990). To elucidate the interaction between cocaine and 5-HT$_3$ receptors, we have used the whole-cell patch-clamp technique to study the effect of cocaine on serotonin activation of 5-HT$_3$ channels in neurones freshly dissociated from rat nodose ganglion (Ikeda *et al*, 1986). Using a fast-flow pipette perfusion system, application of serotonin to these neurones activated an inward current with fast activation and deactivation kinetics. With serotonin concentrations greater than 1 μM, inward current activated to a peak and then desensitized. The average concentration-response curve for peak serotonin-activated current was between 0.1 and 100 μM with an EC_{50} = 4.55 μM and a Hill coefficient of 1.33. Increasing concentrations of cocaine produced increasing reduction in peak serotonin-activated current amplitude for a given concentration of serotonin. With 1 μM serotonin, the cocaine IC_{50} was 0.99 μM and the Hill coefficient 1.15. With increasing concentrations of serotonin, higher concentrations of cocaine were required to reduce peak current amplitude. Thus, for example, with 10 μM serotonin, the cocaine IC_{50} was 4.9 μM and the Hill coefficient 1.26. In neurones particularly sensitive to serotonin, the current activated by 250 nM serotonin could be reduced in amplitude by 30% or more by cocaine concentrations as low as 100 nM. Schild plots of increasing serotonin and cocaine concentrations indicated a pA_2 value of 5.4 and a K_B of 3.8 μM The results suggest that cocaine competitively antagonizes serotonin activation of neuronal 5-HT$_3$ channels. In addition, this effect is in a concentration range that occurs in the plasma of individuals during cocaine abuse.

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13P EFFECTS OF KAINIC ACID MICROINJECTIONS INTO THE CAT MEDULLARY LATERAL TEGMENTAL FIELD ON THE SYMPATHOLYTIC ACTION OF 8-OH-DPAT

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The lateral tegmental field (LTF) has been implicated as a putative source of the generation of basal sympathetic activity. Sympathetically related neurons have recently been identified in this area. A sub-population of these, the sympathoexcitatory (LTF-SE) neurons, have been shown to project to and provide an excitatory input to the rostral ventrolateral medulla (Barman and Gebber, 1987). We have demonstrated that these LTF-SE neurons are inhibited by i.v. 8-OH-DPAT and iontophoretic 8-OH-DPAT and 5-HT. On the other hand, i.v. 8-OH-DPAT increased the firing rate of sympathoinhibitory neurons in the LTF (LTF-SI). LTF-SI units were insensitive to iontophoretic 8-OH-DPAT but were excited by iontophoretic 5-HT (Clement and McCall, 1992). The present experiments were designed to investigate the effects of kainic acid microinjections into the LTF on the sympatholytic effects of 8-OH-DPAT. Kainic acid has been reported to destroy cell bodies while leaving fibers of passage intact (see McGeer and McGeer, 1982).

Cats were given a pre-operative dose of ketamine HCl (11 mg/kg) and anesthetized by an intravenous injection of diallybarbiturate sodium (60 mg/kg), urethane (240 mg/kg), and monoethylurea (240 mg/kg). Animals were immobilized with gallamine triethiodide (4 mg/kg, i.v.) and artificially respired. Mean arterial pressure (MAP), heart rate (HR), and cardiac nerve activity (CNA) were recorded simultaneously. The animals were placed in a David Kopf Instruments stereotaxic apparatus and spinal investigation unit, and obex was used as a landmark for microinjections. Bilateral microinjections of 1.0 - 2.5 μM (50 - 100 nl) kainic acid were performed using a 1 μL Hamilton syringe connected to a glass micropipette (tip diameter 50 microns). Animals were given incremental doses of 8-OH-DPAT (10 - 300 μg/kg) or clonidine (5 - 30 μg/kg) one hour after microinjections.

Bilateral microinjections of kainic acid resulted in slight (6% ± 5.5 %) increases in MAP, negligible alterations in HR, and a 20% ± 16% increase in CNA. The maximum dose of 8-OH-DPAT caused a 19% decrease in MAP, an 8% decrease in HR, and a 26% increase in CNA (n = 14). Cardiac nerve activity could subsequently be inhibited by administration of clonidine. These values differed significantly from control animals in which 8-OH-DPAT reduced CNA and MAP 95% and 40% respectively. Histological examination of microinjection sites revealed the extent of diffusion of kainic acid to range from 300 to 500 microns from the point of injection, with diffusion being slightly greater along the dorso-ventral axis.

These results indicate that the sympathetically related neurons in the LTF play an important role in the sympatholytic action of 8-OH-DPAT, and suggest disparate central sites for the action of 8-OH-DPAT and clonidine.

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14P REGIONAL HAEMODYNAMIC EFFECTS OF 5-HT i.c.v. IN CONSCIOUS LONG-EVANS AND BRATTLEBORO RATS

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In anaesthetized rats, the pressor effect of 5-HT i.c.v. is mediated by vasopressin release and sympathoexcitation due to activation of $5\text{-HT}_{2/1C}$ and 5-HT_{1A} receptors respectively (Anderson et al., 1992). In the present experiments the regional haemodynamic effects of i.c.v. 5-HT were examined in conscious Long-Evans and Brattleboro (i.e. vasopressin deficient) rats, chronically instrumented with a right lateral ventricular cannula, pulsed Doppler flow probes and an intra-arterial catheter. Surgery was carried out under sodium methohexitone anaesthesia (60 mg kg^{-1} i.p.) in 3 stages with at least 7 days between each stage (Gardiner et al., 1988). Each animal received an i.c.v. dose (5μl over 20s) of saline and of 5-HT (4, 40, 120 nmol kg^{-1}).

Table: Changes (mean ± s.e.mean) 2 min after i.c.v. 5-HT. * $P<0.05$ ** $P<0.01$ (ANOVA) compared with saline.

	Long-Evans (n=8-11)			Brattleboro (n=8)
Dose (nmol kg^{-1})	4	40	120	120
MAP (mmHg)	6 ± 1**	13 ± 1**	15 ± 2**	-4 ± 2
HR (bpm)	30 ± 16*	-47 ± 10**	-53 ± 9**	19 ± 19
Renal flow (%)	1 ± 2	4 ± 2	5 ± 2	2 ± 6
Mesenteric flow (%)	-8 ± 3	-19 ± 3**	-30 ± 5**	-21 ± 3**
Hindquarters flow (%)	14 ± 7*	23 ± 5**	36 ± 8**	54 ± 4**
Renal conductance (%)	-5 ± 3	-8 ± 2*	-9 ± 2*	5 ± 6
Mesenteric conductance (%)	-13 ± 3**	-28 ± 3**	-38 ± 5**	-18 ± 4**
Hindquarters conductance (%)	7 ± 7	9 ± 4	19 ± 7	59 ± 5**

The pressor and mesenteric vasoconstrictor effects of 5-HT i.c.v. in Long-Evans rats are consistent with vasopressin release. This is supported by the absence of a pressor effect in Brattleboro rats. However, Brattleboro rats did show a mesenteric vasoconstrictor response to 5-HT i.c.v. consistent with sympathoexcitation; the absence of a pressor effect was, presumably, due to the concurrent marked hindquarters vasodilator response.

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15P FURTHER INVESTIGATION OF THE ROLE OF CENTRAL 5-HT_{1A} RECEPTORS IN THE REFLEX VAGAL BRADYCARDIA ELICITED BY i.v. PHENYLBIGUANIDE IN ANAESTHETIZED RATS

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The putative 5-HT_{1A} antagonists, spiperone, methiothepin, pindolol and buspirone, applied centrally, have been shown to attenuate the reflex vagal bradycardia elicited by i.v. phenylbiguanide (PBG) in anaesthetized rats (Bogle et al., 1990). Experiments were carried out to determine whether the putative 5-HT_{1A} antagonists 8-MeO-CLEPAT (Fozard et al., 1987), MDL 73005EF, MDL 72832, BMY 7378, NAN 190 and spiroxatrine (see Hoyer, 1991), given centrally (i.c.), have a similar effect.

Blood pressure (BP) and heart rate (HR) were measured from the femoral artery in spontaneously breathing Sprague-Dawley rats (250-350g) anaesthetized with α-chloralose (70 mg kg^{-1}). Atenolol (1 mg kg^{-1}) was given into the jugular vein and bradycardias were induced with i.v. PBG (10μg kg^{-1}) before and after administration (i.c. ; 10μl over 20s) of the test drug (see Bogle et al.,1990). Results are mean ± s.e.mean.

Table 1 Changes in Heart Rate (beats min^{-1}) evoked by PBG. * $P < 0.05$ Student's unpaired t-test

Test Drug	n	Control	5 min	20min	35min
MDL 73005EF 200μg kg^{-1}	5	157±18	115±26	156±22	149±15
MDL 72832 200μg kg^{-1}	5	152±9	138±11	147±5	144±9
BMY 7378 200μg kg^{-1}	5	100±18	98±14	99±18	120±26
Saline Control	5	112±16	112±11	115±15	112±15
NAN 190 100μg kg^{-1}	5	140±33	146±35	130±35	151±27
Tween 20 1% (control NAN 190)	5	102±22	117±20	90±23	122±8
Spiroxatrine 100μg kg^{-1}	5	120±20	**63±18***	**91±36***	103±38
0.01N HCl (control spiroxatrine)	5	123±25	123±38	140±36	143±38
8-MeO-CLEPAT 200μg kg^{-1}	5	120±13	77±5	**60±6***	**83±7***
Saline pH 3-4 (control CLEPAT)	5	115±10	102±17	114±13	105±10

The failure of MDL 73005EF, MDL 72832, BMY 7378 and NAN 190 to attenuate this reflex may reflect their ability to have an agonist action on the "pre-synaptic" 5-HT_{1A} receptor.

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16P EFFECT OF INTRATHECAL CALCITONIN GENE-RELATED PEPTIDES ON THYROTROPHIN-RELEASING HORMONE AND 5-HT AGONIST-INDUCED MOTOR BEHAVIOURS

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Calcitonin gene-related peptide (CGRP), which co-exists with acetylcholine in motoneurones, may enhance acetylcholine-induced contractions and nicotinic receptor number at the neuromuscular junction (Uchida *et al.*, 1991). CGRP may also be released from motoneurone colaterals and/or dendrites within the ventral spinal cord where it could interact with adjacent neurones, such as bulbospinal raphe terminals containing 5-hydroxytryptamine (5-HT) and thyrotrophin-releasing hormone (TRH). The present study examines the behavioural effect of intrathecal (i.t.) pretreatment with rat $\alpha CGRP_{1-37}$ and the antagonist human $CGRP_{8-37}$ on 5-HT agonist- and TRH analogue-induced motor behaviours in adult rats

Indwelling i.t. cannula (extending to the thoraco-lumbar cord) were implanted in rats under sodium methohexitone anaesthesia (60 $mgkg^{-1}$ i.p.). Following 7d recovery, the number of back muscle contractions and wet-dog shakes were counted separately for 30 min and tail elevation scored (on a scale of 0 to 3 for 20s every min) for 20 min following i.t. injection of the non-selective 5-HT agonist 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT, 15µg) or the TRH analogue (3,3'-dimethyl TRH, RX 77368, 50ng) both given alone and after i.t. treatment with either rat $\alpha CGRP_{1-37}$ or $CGRP_{8-37}$ (both at 0.01, 0.1 and 1µg, n=7 and 6, respectively). At the end of the behavioural study the distribution of radioactivity in the cannula and brain and spinal cord was examined 36 min after i.t. injection of 4.2KBq [^{125}I]tyr_0CGRP_{28-37} with rat $\alpha CGRP_{1-37}$ (1µg, and 6 mins after 50ng RX 77368 i.t. as in the behavioural protocol, n=6).

Neither CGRP peptide (1µg i.t.) elicited any notable motor behaviour when given alone. Pretreatment i.t. with $CGRP_{1-37}$ (0.01µg, 46±27 in 30 min, $P<0.05$; 0.1, 59±25, NS and 1, 49±25 $P<0.05$ Dunnett t-test) significantly reduced (ANOVA, $F=3.97$, $P=0.0059$) RX 77368-induced wet-dog shakes (113±20) without altering the time course of the behaviour, while only the highest dose of $CGRP_{8-37}$ attenuated wet-dog shakes. The marked tail elevation produced by RX 77368 (16 from a possible score of 60) was also significantly attenuated (Kruskal-Wallis, $H=18.68$, $P=0.001$) by $CGRP_{1-37}$ (scores being 20, NS; 3, $P<0.05$ and 1, $P<0.05$ with 0.01, 0.1 and 1µg respectively) but was not reduced by $CGRP_{8-37}$. Both $CGRP_{1-37}$ (69±13 in 30 min) and $CGRP_{8-37}$ (110±22) tended to elevate the back muscle contractions elicited by 5-MeODMT (being 52±13 and 75±13, respectively with 5-HT agonist alone) but this did not reach significance. At the peak of the TRH peptide-induced behavioural response (36 min after CGRP pretreatment) the majority of the radioactive CGRP recovered, 78±4%, was within the spinal cord with only 3±1% in the brainstem and little in any more rostral brain area, suggesting that the behavioural interaction between these two neuropeptides may occur in these CNS regions.

Neither CGRP peptide altered 5-HT agonist-induced spinal motor behaviours such as back muscle contractions (Fone *et al.*, 1991) but $CGRP_{1-37}$ potently attenuated TRH analogue-induced bulbospinal motor behaviours and the antagonist $CGRP_{8-37}$ appears to possess partial agonist activity in these behavioural paradigms.

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17P ATP IS ABLE TO INCREASE $[Ca^{2+}]_i$ IN N1E-115 CELLS, POSSIBLY VIA ACTIVATION OF AN ATYPICAL P_2 RECEPTOR

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ATP has been linked with increases in $[Ca^{2+}]_i$ in a number of cell lines (Dubyak, 1986; Hallam and Pearson, 1986). In this abstract we report its effects in the neuroblastoma clone N1E-115 via a receptor which differs from the classically-described P_{2X}- or P_{2Y}-purinergic receptors.

Addition of ATP (100 μM) to populations of fura-2-loaded N1E-115 cells produced a transient increase in $[Ca^{2+}]_i$ of 243 ± 17 nM from a resting value of 138 ± 7 nM. A similarly transient increase in $[Ca^{2+}]_i$ was seen following addition of UTP but adenosine was without effect. The response was concentration-dependent with an EC_{50} value of 21 ± 3 μM. Folowing addition of higher concentrations of the purine nucleotide (1 mM) the transient nature of the response was lost and $[Ca^{2+}]_i$ remained significantly above the pre-stimulus value. However this secondary plateau was not seen when calcium was omitted from the extracellular medium. Similarly, addition of the calcium channel blocker Ni^{2+} (1 mM) before or after ATP removed the plateau response.

In a second series of experiments, a number of poorly-hydrolysable analogues of ATP were investigated. 2-Methylthio-ATP and α,β-methylene-ATP were without effect, however, $ATP\gamma S$ produced a concentration-dependent increase in $[Ca^{2+}]_i$ with a similar potency to ATP (EC_{50} 30 ± 17 μM, n=3) but with decreased efficacy.

In conclusion, ATP at sub-millimolar concentrations is able to transiently increase $[Ca^{2+}]_i$ in populations of N1E-115 cells. The lack of effect of adenosine suggests the involvement of a P_2 receptor, however, the results from the analogue studies indicate a receptor distinct from the classically defined P_{2X}- and P_{2Y}-purinergic receptor. This may be similar to the 5'-nucleotide receptor suggested by Brown *et al.* (1991) to exist in human airway epithelial cells.

P.A.I. holds an SERC/CASE award with Boots Pharmaceuticals.

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18P ETHANOL-INDUCED CHANGES IN G-PROTEIN α-SUBUNIT EXPRESSION IN NG108-15 CELLS ARE NOT MEDIATED BY ADENOSINE

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Recent work has implicated a role for adenosine in ethanol-induced heterologous desensitization of adenylate cyclase in NG108-15 cells. It was suggested that chronic ethanol causes adenosine to accumulate extracellularly, and so leading to a reduction in Gsα mRNA and Gsα protein (Nagy *et al.*, 1989). In this study we further investigated the effect of chronic ethanol on G-protein expression in NG108-15 cells. G-protein α-subunit levels were determined by Western blotting using commercially available antibodies (BioMac), and G-protein mRNA by Northern blotting using partial cDNA sequences. Blots were quantified by cutting out bands and radioactive counting. Pretreatment of NG108-15 cells with ethanol (100 mM; 48 h) induced heterologous desensitization of agonist-stimulated cyclic AMP levels. For example, the fold stimulation of cAMP levels over basal produced by 1 μM iloprost was reduced from 3.7 ± 0.2 in control to 2.2 ± 0.2 following ethanol (means ± SEM; $p < 0.01$). This ethanol treatment also reduced membrane levels of Gsα by 27 ± 2%, Giα by 34 ± 2% but increased Goα by 43 ± 3% (mean changes ± SEM, compared to control; $p < 0.05$ in each case). The effects of ethanol on α-subunit expression in each case were not reversed by co-inclusion of adenosine deaminase (1 unit/ml; 48 h) in the incubation medium and could not be mimicked by the adenosine agonist 5'-(N-ethyl)-carboxamidoadenosine (10 μM; 48 h). Ethanol did not reduce levels of Gsα or Giα mRNA. These results suggest that although chronic ethanol induces heterologous desensitization in NG108-15 cells, the effects of ethanol on G-protein α-subunit expression are more complex than previously suggested and are not mediated by adenosine.

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19P CHARACTERISTICS OF ILOPROST-INDUCED LOSS OF Gsα PROTEIN IN NG108-15 CELLS

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Heterologous desensitization of adenylate cyclase activation in NG108-15 cells is known to be mediated by a reduction in membrane levels of the Gsα-subunit protein (Kelly *et al.*, 1990; McKenzie & Milligan, 1990), however the fate of the Gsα-subunit is not known. In this study, we further investigated this phenomenon. G-protein α-subunit levels were determined by Western blotting using a commercially available antibody (BioMac). In NG108-15 cells pretreatment with the prostacyclin agonist iloprost (100 nM; 17h) reduced the subsequent ability of iloprost (1 μM) to increase cyclic AMP levels over basal (fold-stimulation in control cells 3.5 ± 0.6, and in iloprost pretreated cells 1.82 ± 0.1; means ± SEM $p < 0.05$). Iloprost pretreatment reduced membrane levels of Gsα in NG108-15 cells (with 1 μM iloprost for 7h, maximum reduction was to 49.8 ± 1.3% of control) with an EC50 of about 10 nM and a half-time of 1.25 ± 0.03 hours. Inclusion of cycloheximide at 20 μg/ml did not affect iloprost-induced loss of Gsα. However this concentration of cycloheximide completely blocked the increase in Gsα (resensitization) seen following cessation of prolonged iloprost treatment of the cells. Treatment of cells for varying times (1,2,3,4 and 7h) with 1 μM iloprost demonstrated clear time-dependent decreases in Gsα levels in both membrane and crude homogenate (membranes + cytosol) fractions of NG 108-15 cells (e.g. 4h pretreatment reduced membrane levels of Gsα to 49.8 ± 2.7% of control and that in homogenates to 66.6 ± 1.6% of control, taken as 100%). This suggests that Gsα is not simply translocated into the cytoplasm during iloprost treatment. The inclusion of the lysosomotropic agent chloroquine (500 μM) with iloprost (1 μM ; 7h) in the medium partially reversed the loss of membrane Gsα induced by iloprost. Iloprost alone reduced Gsα levels to 51.6 ± 3.9% of control whereas with iloprost and chloroquine together the levels were only reduced to 73.6 ± 1.7% of control (iloprost versus iloprost + chloroquine $p < 0.05$). Chloroquine itself did not increase Gsα levels. Preliminary experiments suggest that the iloprost induced down-regulation of membrane Gsα is temperature dependent; down-regulation was blocked at 4°C and partially blocked at 20°C. These results suggest that the iloprost-induced loss of Gsα in NG108-15 cells involves relatively rapid down-regulation of the protein, possibly involving lysosomal enzymes.

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20P TOXIN SENSITIVITY AND MEMBRANE LOCALISATION OF THE nAChR: THE EFFECT OF A β_2/β_4 HYBRID SUBUNIT

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It is well documented that the α subunits of both peripheral and central nicotinic acetylcholine receptors (nAChRs) contain the binding site for agonists and toxins such as α-BgT and its neuronal counterpart neuronal BgT. However the ß subunit in neuronal receptors also influences both the agonist and toxin sensitivity of the receptor (Leutje 1990, Leutje 1991). If α_3 and β_2 subunits are injected pairwise into *Xenopus* oocytes, the resulting receptor is inhibited by 100nM neuronal BgT whereas the pairwise combination of α_3 β_4 is not. The ß subunit is also thought to play an important role in 43KDa mediated receptor localisation at the neuromuscular junction (Burden 1983), and the nature and type of ß subunit appears to be important (Wheeler unpublished data). In these experiments we have constructed a hybrid ß subunit containing the N-terminus of the β_2 subunit and the C-terminus of the β_4 subunit to investigate subunit differences in toxin sensitivity and receptor localisation. The neuronal subunits β_2 and β_4 exhibit 64% homology overall, with the β_2 species featuring two glycosylation sites within the N-terminal cytoplasmic domain at positions 26 and 145, the β_4 species is unusual in having four glycosylation sites within the N-terminal cytoplasmic domain, two at position 15 and at the conserved site 145 and two additional sites at positions 72 and 117. A hybrid ß subunit consisting of residues 1-80 of the β_2 subunit attached to residues 80-495 of the β_4 subunit was constructed by polymerase chain reaction (PCR) mediated site directed mutagenesis. Messenger RNA was produced by *in vitro* transcription from the plasmid encoded T3 promoter. The resulting mutant contains three N-terminal glycosylation sites rather than the initial four and can be co-injected with muscle α_1, γ and δ subunits plus the 43KDa protein into *Xenopus* oocytes. The resulting receptor is fully functional and exhibits an agonist profile similar to the normal muscle receptors. If these receptors are visualised using confocal laser scanning microscopy (CLSM) they can be seen to form discrete clusters on the oocyte surface analogous to those receptors containing the neuronal species β_4 whose C-terminal it contains. Incubation of the mutant receptors with neuronal BgT produces ≈46% inhibition of peak current (ACh 10μM n=12), which is intermediate to that seen with muscle receptors containing β_2 (≈70% inhibition of peak current to ACh 10μM) and β_4 (≈20% inhibition of peak current to ACh 10μM). These data indicate that the cytoplasmic N-terminal region of the mutant does not influence receptor localisation on the membrane, but does influence the response to neuronal BgT. Decreasing the number of glycosylation sites increases the sensitivity of the receptor to neuronal BgT, thus glycosylation is implicated in the influence in both the agonist and toxin profile of the nAChR.

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21P MORANTEL: AN AGONIST AND A SLOWLY DISSOCIATING BLOCKER OF NICOTINIC-ACETYLCHOLINE CHANNELS FROM *ASCARIS SUUM*?

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The anthelmintic morantel has been shown to activate nicotinic-acetylcholine receptors in muscle cells of the parasitic nematode Ascaris suum (Harrow & Gration, 1985). Using muscle membrane vesicles from Ascaris we have now studied the activation by morantel of these receptors at the single-channel level (for methods refer to Pennington & Martin, 1990). Standard patch-clamp techniques were employed. Morantel (0.006-600μM) was applied via the pipette solution. Single-channel currents were observed throughout this concentration range, although qualitatively the probability of opening (Popen) at 0.006μM was very low relative to the maximum observed at 6μM. The conductance of morantel-activated single-channel currents was estimated from the slope of I/V plots obtained from excised inside-out patches exposed to symmetrical [Cs^+] and non-symmetrical [Cl^-]. The I/V relationship was linear and exhibited a reversal potential close to 0mV, as would be expected for a channel permeable to cations rather than anions. Two conductance states were observed, the larger being approximately 40pS and the smaller approximately 25pS. Increasing the concentration of morantel had no effect on the open-channel conductance. Analysis of the kinetics of the main conductance state was carried out using vesicle-attached patches to avoid run-down. In the presence of 6 and 10μM morantel the single-channel open-times were best fitted by up to 2 exponentials (suggesting at least 2 open-channel states) and the closed-times by up to 4 exponentials (suggesting at least 4 closed states). The mean open-times (approximately 1ms) exhibited a modest increase on membrane hyperpolarisation, while the mean closed-times exhibited no clear voltage-dependence. This was in stark contrast to our findings with 60-600μM morantel in the pipette solution. Here the mean open-times decreased and the mean closed-times increased with membrane hyperpolarisation. There was also a dramatic reduction in the frequency of opening on hyperpolarisation. These effects increased with increasing morantel concentration, giving rise to both a concentration-dependent and voltage-dependent reduction in Popen. For example, in the presence of 60 and 100μM morantel the Popen (mean$\pm$s.e.m.) fell from 0.004$\pm$0.0001 (n=3) and 0.004$\pm$0.0007 (n=4) respectively at +75mV, to 0.001$\pm$0.0005 (n=3) and 0.0002$\pm$0.00008 (n=4) respectively at -75mV. We can explain these results by suggesting that while morantel is an agonist of the nicotinic acetylcholine channels, it also acts as a slowly dissociating cation open-channel-blocker. Interestingly what appeared to be fast flickering open-channel block was also observed with morantel (100 and 600μM), but only at depolarised potentials.

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22P (18-36) NEUROPEPTIDE Y IS A POTENT RAT MAST CELL DEGRANULATOR

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Neuropeptide Y (NPY) is a potent pressor molecule while the C-terminal analogue (18-36) NPY is predominantly depressor (Boublik et al, 1989). High doses of NPY have been shown to reduce blood pressure following the initial pressor response and it has been suggested that this is due to histamine release from mast cells (Grundemar et al, 1990). We have compared the effects of (18-36) NPY with an NPY analogue which has pressor activity, (13-36) NPY (Potter et al, 1989), and investigated the contribution of mast cell amine release to the depressor effect of (18-36) NPY.

Mast cell degranulation was estimated in vitro by measurement of [^{14}C]-5HT release from rat peritoneal mast cells (Stechschulte & Austen, 1974). Effects on blood pressure were measured in pentobarbitone anaesthetised, syrosingopinised (5mgkg^{-1}day^{-1} s.c. for 2 days), bilaterally vagotomised AP rats, weighing appproximately 300g. (18-36) NPY was a potent mast cell degranulator in vitro (EC_{50}, 41nM) and was 24-fold more potent than (13-36) NPY (EC_{50} 970nM). In vivo, (18-36) NPY caused a transient pressor followed by a prolonged depressor response. Cummulative dosing (2.4 to 72nmol) produced a maximal pressor response of 32$\pm$5mmHg (mean$\pm$s.e.m., n=4) and depressor response of 42$\pm$2mmHg. In contrast, (13-36) NPY (2.0 to 60nmol) caused only a pressor response (32$\pm$4mmHg maximum).

Treatment with a combination of tiotidine (5mgkg^{-1} i.v.), mepyramine (0.5mgkg^{-1}) and methysergide (5mgkg^{-1}) reduced but did not abolish the depressor response to (18-36) NPY. Only if rats were additionally pretreated with Dextran T70 (Macrodex, 1ml) to degranulate mast cells and plasma volume was restored by a further Macrodex injection did (18-36) NPY fail to lower blood pressure. Following this treatment, the cummulative dose of (18-36) NPY required to increase systolic pressure by 25mmHg decreased 4-fold, from 48.8$\pm$9.8 to 11.0$\pm$2.9nmol, when compared with controls ($p<0.01$). There was no significant change in the pressor response to (13-36) NPY in rats treated with dextran, tiotidine, mepyramine and methysergide.

These results indicate that (18-36) NPY is a potent rat mast cell degranulator, releasing both histamine and 5HT which mediate the depressor response. The genuine pressor effect of (18-36) NPY is therefore masked because of these mast cell products. The possibility of NPY and its analogues causing mast cell degranulation should be considered when interpreting effects on blood pressure.

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23P CYTOPROTECTION BY OCTREOTIDE AGAINST ETHANOL-INDUCED LESIONS IN THE RAT GASTRIC MUCOSA

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Gastric mucosal protection by somatostatin against ethanol-induced hemorrhagic erosions in the rat has been reported (Szabo & Usadel 1982). We have observed similar effects with octreotide. Thus, oral administration of ethanol (75 %, v/v, 1ml bolus) to fasted rats produced gastric hemorrhagic lesions in the mucosa which could be inhibited by octreotide at doses of 0.01- 10.0 ng/kg given subcutaneously (s.c.). The dose-response relationship was bell shaped with maximum inhibition occurring at the extremely low dose of 0.1 ng/kg at which there was no effect on gastric acid secretion (unpublished results). The low dose needed for cytoprotection contrasts with the dose of octreotide required to inhibit growth hormone (GH) secretion where the ID_{50} was 0.13 µg/kg (0.103 - 0.159, n=18, 68 % conf. limit). The somatostatin analogue, SDZ 217-717 (5-(l)-citrullin-octreotide), neither prevented mucosal lesions at doses up to 10 ng/kg s.c. nor inhibited GH release at doses up to 100 µg/kg (s.c.).

In order to elucidate further the cytoprotective mechanism of octreotide we investigated its effect on ethanol-disturbed microcirculation of the rat mucosa using an in vivo microscopy system. The abdomen of the nembutal anaesthetized rat was opened and the posterior wall of the stomach was fixed on a heated metal plate (37° C). The stomach was opened along the curvature and the mucosa was rinsed with electrolyte solution. The mucosal microcirculation was continuously observed with a video camera attached to a microscope and the experiments were analysed by an image analysing system. Blood flow was assesssed with a semiquantitave scale from 0 to 4: (0=stasis; 4=normal flow with no particle identification possible). Light intensity measurements were performed in rectangles superimposed on regions of capillaries or collecting venules and corrected for light intensity changes of the total image (Kusterer, 1991). After an equilibration time of 15 min octreotide or SDZ 217-717 (0.01, 0.1, and 1.0 ng/kg s.c.) were injected. Twenty min later FITC-dextran (fluoresceine-isothiocyanate dextran, 10% solution, MW 150 000) was given i.v. and 10 min later 1 ml of ethanol (50%) or physiological saline was applied topically for 5 min to the mucosa. The microcirculation was observed for another 60 min. Ethanol induced stasis of the microcirculation and a fall in mucosal blood flow within seconds. Stasis could be observed first in the collecting venules followed by the capillaries. Octreotide prevented the stasis dose dependently and significantly reversed the fall in blood flow. In parallel to the macroscopic lesion experiments these inhibitory effects showed a bell shaped dose-response relationship with maximal effects at 0.1 ng/kg (s.c.). The ethanol-induced permeability changes in the microvessels of the mucosa monitored by extravasation of FITC from the capillaries were also reduced by octreotide. SDZ 217-717 had no cytoprotective effect.

These results suggest that octreotide maintains the cellular integrity of the gastric mucosa by a mechanism other than the inhibition of gastric acid secretion. This effect is a feature of the endocrinologically active somatostatin analogue as shown by the ineffectiveness of 217-717. Although the mechanism of gastric cytoprotection in ng/kg doses is unknown, octreotide appears to inhibit ethanol induced stasis in the microcirculation concomitantly with an inhibition of an extravasation from the capillaries.

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24P RELATIVE CONTRIBUTIONS OF β-ADRENOCEPTOR SUBTYPES TO ISOPRENALINE-INDUCED RELAXATION OF RAT DISTAL COLON

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Atypical β-adrenoceptors (β_3-adrenoceptors) are characterized by their resistance to classical β-adrenoceptor antagonists e.g. propranolol and by their sensitivity to synthetic agonists, such as BRL 37344 (for review see Zaagsma & Nahorski, 1990). Previous studies on rat distal colon using propranolol and BRL 37344 demonstrated the presence of atypical β-adrenoceptors mediating relaxation of the smooth muscle (McLaughlin & MacDonald, 1990). The present studies were carried out to investigate the effects of the β_1- and β_2- selective antagonists, respectively CGP 20712A and ICI 118551, on responses to isoprenaline to determine the contributions of these subtypes to relaxation. In addition, the properties of ICI D7114, a novel putative stimulant of atypical β-adrenoceptors (Holloway et al., 1991), were investigated. Our preliminary experiments with ICI D7114 showed that it lacked agonist activity in rat distal colon and in fact antagonized responses to isoprenaline, a situation reported previously in rat small intestine (Growcott et al., 1992). We therefore studied the antagonism of isoprenaline by ICI D7114 in more detail.

Longitudinal segments of rat colon were suspended in Krebs solution at 37°C for isometric recording. The Krebs solution contained EDTA (23 µM) and prazosin (0.1 µM) and was gassed with 95/5% O_2/CO_2. After an initial equilibration period, reproducible contractions to a submaximal concentration of methacholine (1 µM) were obtained before carrying out a concentration response curve (CRC) to isoprenaline in a non-cumulative manner. Four consecutive CRCs to isoprenaline were carried out in each tissue with a 1h interval between each curve. Antagonists were present in increasing concentrations during the intervals between CRCs. Control tissues received no antagonist to allow estimation of the magnitude of time-dependent changes.

Isoprenaline produced a concentration-dependent inhibition of methacholine-induced contractions. CRCs to isoprenaline were reproducible with no significant time-dependent changes. Propranolol produced no shift of the isoprenaline CRC at 0.01 µM and an approximately 5-fold shift at 0.1 µM. No further shift was observed with 1 µM. CGP 20712A had no effect on the CRC to isoprenaline at 0.1, 1 and 3 µM. ICI 118551 produced little or no shift at 0.1 µM and an approximately 6-fold shift with 1 µM. No further shift was observed with 3 µM. ICI D7114 produced a concentration-dependent rightward shift of the CRC to isoprenaline. Schild analysis gave a slope of 0.99 ± 0.29 (95% C.L., n = 14) and a mean pA_2 value of 7.29 ± 0.08 (n = 14).

These results with propranolol and β_1- and β_2-adrenoceptor antagonists confirm the mainly atypical nature of β-adrenoceptors in rat distal colon. There also appears to be a small contribution from β_2-adrenoceptors to the response to isoprenaline but β_1-adrenoceptors are absent. ICI D7114 has no agonist activity and appears to behave as a reversible competitive antagonist of atypical β-adrenoceptors in this preparation.

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25P THE ROLE OF HISTAMINE IN RESPONSE TO ELECTRICAL FIELD STIMULATION OF THE VAGUS IN ISOLATED STOMACHS FROM MOUSE, GUINEA-PIG AND RAT

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Frequency-effect curves to electrical field stimulation (e.f.s.) of the vagus in isolated mouse stomachs were abolished both by ganglionic and histamine H_2-receptor blockade (Angus & Black, 1982). However, in a mucosal sheet preparation from the rat, the responses were refractory to both (Baird & Main, 1978). The role of histamine in gastric acid secretory responses to e.f.s. has now been investigated in isolated whole stomachs from mouse (Black & Shankley, 1985) and immature guinea-pig and rat (Welsh, 1992).

In all three species, the frequency-response curves (1-30Hz, 0.5ms pulsewidth, 10V) were abolished by TTX (0.1μM for 30min) indicating neuronal origin. In the guinea-pig and mouse assays, hexamethonium (0.1mM) and atropine (20μM) each abolished the e.f.s. responses. However, although the responses to stable ACh M-receptor agonists were relatively refractory to histamine H_2-receptor blockade, e.f.s. responses were completely abolished in these species. This is consistent with selective vagal innervation of the histamine-containing cell of the gastric mucosa.

In contrast, in the rat whole stomach, as in the mucosal sheet, the e.f.s. response curves were partially refractory to hexamethonium (0.1mM) suggesting post-ganglionic, as well as pre-ganglionic, stimulation in this species. Atropine (20μM) also failed to totally inhibit the responses either alone, or in combination with hexamethonium, suggesting an element of non-cholinergic (NC) innervation. The selective histamine H_2-receptor antagonist, tiotidine (0.1mM), was unable, alone, to totally inhibit the e.f.s. responses. However, when tiotidine was present in combination with atropine the responses were abolished. These results suggest that the putative NC transmitter may act by releasing endogenous histamine, a property we have previously assigned to gastrin, which has been shown to be present in vagal nerves from cats, dogs and humans (Uvnas-Wallensten *et al.*, 1977).

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26P INHIBITORY EFFECT OF ADENOSINE ON VASODILATION BY SENSORY-MOTOR NERVES VIA PREJUNCTIONAL A_1 RECEPTOR IN THE RAT MESENTERIC ARTERIAL BED

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The presence of capsaicin-sensitive sensory-motor nerves, releasing calcitonin gene-related peptide (CGRP) and having a role in the control of vascular tone, has been demonstrated in the rat mesenteric arterial bed (Kawasaki et al., 1988). Adenosine is well known as a regulator of vascular tone acting directly on smooth muscle or through prejunctional inhibition of sympathetic perivascular nerve activity (Burnstock and Kennedy, 1986). However there is little information on the role of adenosine as a modulator of sensory-motor nerves in the vasculature.
The present study investigates the effect of adenosine on the vasodilator response to electrical field stimulation (EFS) of perivascular sensory-motor nerves in the rat mesenteric arterial bed.
This preparation was isolated and set up as previously described (Ralevic et al., 1991) and vascular responses were detected as changes in perfusion pressure (mmHg). In the presence of guanethidine (5 µM) to block sympathetic neurotransmission and of methoxamine (30 µM) to raise the tone of the preparation, EFS (8 Hz, 60 V, 0.1 ms for 30 s) elicited relaxation of $34.4\pm3.3\%$ (n=12). Adenosine (0.3-10 µM) concentration-dependently reduced the vasodilator response to EFS without affecting significantly the tone of preparations and reaching an inhibition of $69.1\pm7.6\%$ at 10 µM. The adenosine receptor antagonist 8-phenyltheophylline (1 µM) shifted the concentration-inhibitory effect curve for adenosine to the right, in a parallel manner. Adenosine 10 µM did not affect the vasodilator response to 0.3 µM exogenous CGRP, thus indicating that the inhibitory action of adenosine was mediated by prejunctional receptors.
In order to characterize the adenosine receptor subtype involved in this effect, stable adenosine analogues selective for A_1- and A_2-receptor subtypes were tested on the vasodilator response to EFS. R-phenylisopropyl-adenosine (R-PIA, 0.3-30 µM), 2-chloroadenosine (2-ClAdo, 1-100 µM), N-ethylcarboxamideadenosine (NECA, 3-300 µM) and S-phenylisopropyladenosine (S-PIA, 10-300 µM) mimicked the inhibitory effect of adenosine with a rank order of potency (R-PIA > 2-ClAdo $\geq$ NECA $\geq$ S-PIA) consistent with the involvement of an A_1 receptor. Furthermore the A_1 selective antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 10 nM), used at a concentration that fully antagonizes A_1-receptor mediated adenosine effects displaced the concentration-effect curve of adenosine to the right. These results provide the first evidence for adenosine as a prejunctional modulator of perivascular sensory-motor nerves via the A_1 subclass of P_1-purinoceptors.

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27P PREJUNCTIONAL MODULATION OF SENSORY-MOTOR NERVE-INDUCED VASODILATATION OF THE RAT MESENTERIC ARTERIAL BED BY [Met^5]ENKEPHALIN

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Electrical field stimulation of the preconstricted rat mesenteric arterial bed during sympathetic nerve blockade elicits vasodilatation shown to be due to the action of the neuropeptide calcitonin gene-related peptide (CGRP) released from perivascular sensory-motor nerves (Kawasaki et al., 1988). Few studies have looked at regulatory mechanisms involved in modulating the activity of these nerves. The present study was undertaken to determine the effects of [Met^5]enkephalin (mENK) on vasodilatation due to stimulation of perivascular sensory-motor nerves in the rat mesenteric arterial bed.

Mesenteric arterial beds of male Wistar rats (300-350g) were set up for perfusion at a constant flow rate of 5 $mlmin^{-1}$ as described previously (Ralevic and Burnstock, 1988). Vascular responses were detected as changes in perfusion pressure (mmHg). Electrical field stimulation of the preparation in the presence of guanethidine (5 x 10^{-6} M) to block sympathetic neurotransmission and methoxamine (10^{-5} - 3 x 10^{-5} M) to raise the tone of the preparations (by 54.17 ± 3.09 mmHg, n = 29), elicited vasodilator responses which increased with increasing frequency of stimulation (2 - 12Hz, 0.1ms, supramaximal voltage for 30 s). The δ-opioid receptor agonist mENK (10^{-8} - 10^{-6}M) inhibited vasodilator responses due to electrical stimulation (8Hz) of sensory-motor nerves in a concentration-dependent manner without affecting the tone of the preparation. The maximum effect reached with 10^{-6}M mENK was 45.45 ± 4.74 % inhibition (n = 4) and naloxone (10^{-7}M) completely reversed this inhibitory effect. A sub-maximal concentration of mENK (10^{-7}M) reduced the vasodilator responses to increasing frequencies of electrical field stimulation and this inhibitory effect was more pronounced at the lower frequencies, being 89.28 ± 7.44 (n = 7), 44.21 ± 8.59 (n = 9), 30.51 ± 5.1 (n = 7) and 18.16 ± 7.06 (n = 7) % inhibition at 2, 4, 8 and 12 Hz respectively. mENK had no effect on motor responses produced by exogenously applied CGRP (5 x 10^{-11} moles injected as a bolus of 50µl); 28.92 ± 6.16 and 25.57 ± 5.87 % relaxation (n = 6) in the absence and presence of mENK respectively.

The results of this study showing a prejunctional inhibitory action of mENK on sensory nerve mediated vasodilatation suggest that opioids can be involved in regulating the peripheral activity of sensory nerves in the rat mesenteric arterial bed.

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28P EFFECTS OF TIMOLOL, TERBUTALINE AND FORSKOLIN ON AQUEOUS HUMOUR FORMATION AND CYCLIC AMP CONTENT OF CILIARY PROCESSES IN THE BOVINE PERFUSED EYE

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Much evidence suggests that timolol decreases aqueous humour formation (AHF) by a mechanism other than beta-adrenergic blockade (Leopold & Duzman, 1986). The present work tests the correlation between cyclic AMP levels in the ciliary processes and the effects on AHF induced by drugs in isolated arterially perfused bovine eye.

Bovine eyes collected from a local abattoir, were cannulated and perfused with modified Krebs solution, gassed with O_2 containing CO_2 (5%), via a long posterior ciliary artery within 45 min of slaughter (Wilson, 1988). AHF rate (given as $K_{out}.min^{-1}$) was monitored fluorimetrically by continuous perfusion of the anterior chamber with an aqueous humour substitute containing fluorescein. Drugs or vehicle were applied into the arterial perfusate by bolus injection (3 or 10 µl). For assay of cyclic AMP, perfused eyes were quickly dissected and frozen 6 min after drug injection. The ciliary epithelium was scraped into tricholoroacetic acid and extracted with tri-n-octylamine in freon. Cyclic AMP was assayed by RIA (Du Pont).

Terbutaline, a beta-adrenoceptor agonist, and timolol, a beta-adrenoceptor antagonist, both caused significant reduction of aqueous humour flow and produced no effect on ciliary cyclic AMP. Forskolin, which stimulates cyclic AMP synthesis without interacting with the cell surface receptor, was found to produce a highly significant increase in ciliary cyclic AMP content but had no effect on AHF rate. Direct application of 8-Br cyclic AMP, a cell permeable analogue, also had no effect on AHF rate (Table 1). Thus in bovine eye, there is no correlation between the AHF rate and the ciliary epithelium cyclic AMP content.

Table 1 AHF rates and ciliary cyclic AMP levels in perfused bovine eyes

Treatment	Dose (nmol)	AHF (K_{out}x10^4)	n	P	Cyclic AMP (pmol. mg^{-1} protein)	n	P
Control (Krebs)	-	46 ± 1	11	-	9.8 ± 0.5	20	-
Timolol	10	40 ± 1	14	<0.01	9.8 ± 0.4	10	ns
Terbutaline	30	38 ± 2	7	<0.05	8.7 ± 0.4	9	ns
Control (DMSO)	-	40 ± 2	10	ns	8.4 ± 0.3	10	-
Forskolin	30	39 ± 2	10	ns	12.5 ± 0.8	10	<0.001
8-Br cyclic AMP	100	41 ± 2	7	ns	-		

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29P INCREASING INTRACELLULAR cAMP SUPPRESSES BRADYKININ-INDUCED OEDEMA IN RABBIT SKIN ONLY IN THE ABSENCE OF VASODILATION

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The stimulation of adenylate cyclase by agents such as prostaglandins, neuropeptides, ß-adrenoceptor agonists or phosphodiesterase (PDE) inhibitors can either promote or inhibit the development of inflammatory oedema despite acting via a common second messenger. We tested the hypothesis that this discrepancy may be explained by the presence or absence of vasodilation. That is, those agents which increase intracellular adenosine 3':5'-cyclic adenosine monophosphate (cAMP) in vascular smooth muscle will cause vasodilation and be pro-inflammatory, whereas those acting primarily on the endothelium, such as ß-adrenoceptor agonists, will increase the permeability barrier and be anti-inflammatory.

Four agents which increase intracellular cAMP were tested by local i.d. injection in rabbits anaesthetized with sodium pentobarbitone. We measured the effect of the type IV PDE inhibitor rolipram 10^{-7} moles/site (ROL), iloprost 10^{-9} moles/site (ILO), salbutamol 10^{-9} moles/site (SAL) or PGE_2 10^{-10} moles/site on oedema induced by bradykinin 10^{-10} moles/site (BK). Oedema formation was measured locally in multiple skin sites by the accumulation ^{125}I-labelled albumin which had been injected intravenously and skin blood flow changes were measured with a laser Doppler flow probe (Warren et al., 1992). Both oedema and blood flow were measured 30 min after injection of each test agent.

BK induced oedema (36 ± 3 µl per site) and this was suppressed by SAL (25 ± 4, $P<0.05$), but not by ROL (37 ± 3). Both ILO and PGE_2 potentiated the BK response (113 ± 11 & 90 ± 5 respectively, $P<0.05$ in each case). The combined response to PGE_2 and BK was suppressed by both SAL (64 ± 5, $P<0.05$) and ROL (74 ± 6, $P<0.05$). Data are mean $\pm$s.e.mean µl per site for 6 animals, each experiment repeated x6 in each animal. The vasodilator effect of the four agonists was assessed in a further experiment using the same model but changes in local red cell flux were measured with a laser Doppler flow probe. The % change in basal blood flow was -3 ± 12, 6 ± 4, 16 ± 13, 111 ± 34 & 102 ± 11% with vehicle control, SAL, ROL, PGE_2 & ILO respectively; data are mean $\pm$s.e.mean of 4 rabbits, experiments repeated x4 in each animal in a balanced site pattern.

The correlation coefficient between the effect of the four agonists on BK-induced oedema and their vasodilator effect was 0.96 ($P<0.05$). This suggests that stimulants of adenylate cyclase will suppress inflammatory oedema only if they do not cause vasodilation of the microcirculation. These findings support the hypothesis that vasodilation is pro-inflammatory.

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30P THE NATURE OF THE BRADYKININ RECEPTORS INVOLVED IN PHOSPHATIDYLINOSITOL HYDROLYSIS AND FUNCTIONAL RESPONSES IN THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA CAECI

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In the smooth muscle of the guinea-pig taenia caeci, bradykinin produces a contraction, often preceded by an initial relaxation resulting from the opening of an apamin-sensitive Ca^{2+}-activated K^+-channel (Carter *et al.*, 1986). In the present study we have investigated coupling mechanisms for these responses, and the nature of the receptors involved. The affinity of the bradykinin-receptor antagonist HOE140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK), was determined against bradykinin-mediated mechanical responses in normal Krebs solution. Similar studies were made for HOE140 in antagonising bradykinin-induced contractions in a high-K^+ medium. In this medium, pharmacomechanical-coupling mechanisms not involving changes in membrane potential, are responsible for contraction. The affinity of HOE140 on bradykinin-induced accumulation of [^{3}H]-inositol phosphates, as a measure of phosphatidylinositol (PI) hydrolysis, was also estimated to determine the possible involvement of this Ca^{2+}-mobilising pathway in the response (Hall & Morton, 1991). The affinity of HOE140 in each system was calculated from the shift in the log-concentration response curve for the bradykinin-induced response.

Table 1. Estimates of pK_B for HOE140

Response	Medium	pK_B	s.e.mean; n
Contraction	Krebs	8.4	(0.1; 12)
Relaxation	Krebs	8.5	(0.1; 12)
Contraction	High-K^+	8.4	(0.1; 7)
PI hydrolysis	Krebs	8.4	(0.1; 4)

Table 1 summarises the recognition properties of the receptors involved in terms of the affinity of HOE140 against bradykinin responses in these systems. It is not a BK_1-receptor, since BK_1-selective ligands are inactive (results not shown): however, it has a lower affinity for HOE140 and other BK_2-receptor antagonists than found in a number of BK_2 preparations (for discussion, see Field *et al*, 1992).

In conclusion, it is not possible to deduce the complete mechanistic sequence of receptor-effector coupling from these observations alone. One hypothesis is that some of the Ca^{2+} involved in contraction, and also for the opening of Ca^{2+}-activated K^+-channels leading to relaxation, is sequential to receptor-mediated PI hydrolysis. However, it seems clear that a receptor with the same recognition properties mediates all these facets of the response to bradykinin.

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31P EFFECTS OF THIORPHAN AND CAPTOPRIL, AND BRADYKININ RECEPTOR ANTAGONISTS, IN FERRET ISOLATED TRACHEAL SMOOTH MUSCLE

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Ferret tracheal smooth muscle, unlike that from most other species, contracts in response to bradykinin (BK) *in vitro* (Farmer, 1991). In guinea-pig trachea, BK elicits weak, highly variable, prostaglandin-mediated contractions or relaxations. Thus, ferret trachea is a useful isolated tissue preparation for studying airway kinin receptors. Since very little is known about the mechanisms underlying BK-induced contractions in ferret trachea, we have examined the actions of thiorphan, captopril, and various other pharmacological inhibitors and antagonists.

Male ferrets (1.5-2.5 kg) were killed by pentobarbitone overdose, and transverse strips of trachea were suspended, under 3 g tension, in organ baths containing modified Krebs-Henseleit solution (37°C) gassed with 95% O_2/5% CO_2. Tissues were incubated for $\geq$ 90 min, and washed every 15 min. Concentration-response curves to BK were constructed, after its addition cumulatively, in the absence and presence of other drugs.

BK caused concentration dependent contractions (pD_2 7.55 ± 0.08; Max. 83.3 ± 2.5 % of response to 10 mM methacholine) that were unaffected by tetrodotoxin (1 µM), hexamethonium (10 µM), atropine (1 µM), propranolol (1 µM), pyrilamine (10 µM), ketanserin (10 µM), capsaicin (10 µM), indomethacin (3 µM), or nordihydroguaiaretic acid (10 µM). A cocktail of thiorphan and captopril (both 10 µM) caused marked, sustained contractions in ≈ 70% of preparations. Tissues that did not respond initially to 10 µM captopril+thiorphan, often did to higher concentrations (30-100 µM). This response was unaffected by any of the above named inhibitors except, occasionally, by atropine. In strips, precontracted with captopril+thiorphan, the B_2 receptor antagonists HOE 140 (DArg-[Hyp^3,Thi^5,DTic^7,Oic^8]-BK), NPC 16731 (DArg-[Hyp^3,Thi^5, DTic^7,Tic^8]-BK) and NPC 567 (DArg-[Hyp^3,DPhe^7]-BK) caused concentration-dependent relaxation, with respective -log IC_{50} values of 8.60 ± 0.24, 8.49 ± 0.30, and 6.75 ± 0.39 (n = 7-10). This rank order of antagonist potency was similar to their ability to reverse contractions in response to exogenous BK (0.1 µM). The B_1 receptor antagonist, desArg^9[Leu^8]-BK (10-100 µM), did not inhibit responses to BK, or captopril+thiorphan but, rather, caused further contraction. Moreover, this agent (100 µM) significantly increased sensitivity to exogenous BK (control pD_2, 7.92 ± 0.09 vs 8.75 ± 0.27 in presence of desArg^9[Leu^8]-BK). Interestingly, atropine-sensitive responses to the enzyme inhibitors were unaffected by BK antagonists. In tissues where the inhibitors had little or no effect on tracheal basal tone, sensitivity to exogenous BK was potentiated by captopril (pD_2 8.74 ± 0.14, n = 6), thiorphan (pD_2 8.65 ± 0.20, n = 6), or by a combination of both inhibitors (pD_2 8.93 ± 0.16, n = 6).

BK-induced contractions of ferret trachea are not mediated by endogenous acetylcholine, tachykinins, histamine, serotonin, or arachidonic acid metabolites. The increase in sensitivity to BK by thiorphan and captopril confirms the peptide's degradation by neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE) (Dusser *et al.*, 1988). Moreover, ferret trachea *in vitro* appears capable of releasing kinins, which are 'normally' degraded by NEP and/or ACE. Under conditions of enzyme inhibition, however, the endogenous BK accumulates in concentrations sufficient to elicit tracheal contraction by activating B_2 receptors. Furthermore, the ability of desArg^9-[Leu^8]-BK to increase sensitivity to (endogenous and exogenous) BK may be due to inhibition of BK degradation enzymes other than ACE or NEP.

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32P EFFECTS OF KININ RECEPTOR AGONISTS AND ANTAGONISTS IN FERRET ISOLATED TRACHEAL SMOOTH MUSCLE: EVIDENCE THAT BRADYKININ-INDUCED CONTRACTIONS ARE MEDIATED BY B_2 RECEPTORS

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Ferret trachea is useful in kinin pharmacology as tracheal smooth muscle from several other species, including dogs, cats, rats and man, fails to respond to bradykinin (BK) (Farmer, 1991). Although, ferret trachea is a convenient preparation in which to study airway kinin responses, very little is known concerning the BK receptor subtypes involved. In the present study, we examined effects of BK receptor agonists and antagonists.

Cumulative concentration-response curves to kinin agonists were obtained in transverse strips of trachea prepared from male ferrets (see Farmer *et al.*, this meeting). Several preparations from one animal were used to study different concentrations of each antagonist. Tissues were incubated with antagonists for at least 30 min prior to application of agonists. Apparent antagonist affinities (pK_b values) were determined from agonist EC_{50} ratios in the absence and presence of each concentration of antagonist.

BK-, kallidin- (KD) and [Tyr(Me)^8]-BK-induced contractions had pD_2 values, respectively, of 7.28 ± 0.08 (n = 22), 7.27 ± 0.21 (6) and 7.74 ± 0.10 (6). They were efficacious, with BK, for example, eliciting 89.8 ± 1.4% of the maximum response to methacholine (10 mM). B_2 receptor antagonists, DArg-[Hyp^3,Thi^{5,8},DPhe^7]-BK (NPC 349), DArg-[Hyp^3,DPhe^7]-BK (NPC 567), DArg-[Hyp^3,Thi^5,DTic^7,Tic^8]-BK (NPC 16731) and DArg-[Hyp^3,Thi^5,DTic^7,Oic^8]-BK (HOE 140) caused right shifts in agonist curves, with no effect on maxima. pK_b values decreased as antagonist concentrations increased (Table 1), resulting in Schild slopes of less than unity. Similar results were obtained with NPC 349 and NPC 16731, and also with the antagonists vs. KD. The B_1 antagonist, desArg^9-[Leu^8]-BK (100 µM), *increased* sensitivity to BK (see Farmer *et al.*, this meeting). DArg-[Hyp^3,DHypE(transpropyl)^7,Oic^8]-BK (NPC 17731), a novel B_2 receptor antagonist, (Burch & Kyle, 1992), caused contractions that were inhibited by HOE 140. DesArg^9-BK, a B_1 receptor agonist (1-100 µM), caused weak contractions that were inhibited by atropine (1 µM), but not by desArg^9-[Leu^8]-BK or HOE 140. This may have been a nonspecific action of the B_1 agonist on cholinergic nerves.

Table 1. pK_b values for NPC 567 and HOE 140 in ferret tracheal contractions in response to bradykinin. Data are expressed as mean ± s.e. mean of (n) observations.

NPC 567	1 µM; 6.47 ± 0.16 (3)	3 µM; 6.08 ± 0.15 (8)[1]	10 µM; 5.69 ± 0.07 (9)[2]	30 µM; 5.42 ± 0.10 (9)[2]
HOE 140	10 nM; 8.38 ± 0.11 (5)	30 nM; 8.17 ± 0.11 (5)	100 nM; 7.80 ± 0.14 (5)	300 nM; 7.16 ± 0.27 (3)[3]

P < 0.05 (ANOVA) compared to corresponding value at [1] 30 µM; [2] value at 1 µM; [3] values at 10 & 30 nM.

Kinin-induced contractions of ferret trachea appear to be mediated by B_2 receptors. Whereas NPC 17731 was a partial agonist, the other B_2 antagonists caused right shifts, with no effect on maximum response, in kinin concentration-response curves. The data, however, are inconsistent with classical competitive antagonism. Ferret trachea may contain a heterogeneous population of BK receptors, one of which exhibits lower affinity for BK than B_2 receptors, and which is not blocked by B_2 antagonists. Alternatively, being modified peptides, the antagonists may act as substrate inhibitors of kinin degradation and, thus, have a self limiting effect as B_2 antagonists, due to higher than anticipated agonist concentrations in the biophase. This is supported indirectly by the observation that desArg^9-[Leu^8]-BK enhanced sensitivity to BK.

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33P SUBSTANCE P AND NEUROKININ A IN A RAT STRAIN WITH A GENETIC NEUROTROPHIC DEFECT

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In the GH inbred Wistar rat, there is exaggerated death of sympathetic neurons neonatally, which may be due to deficiency of a neurotrophic factor (Messina & Bell, 1991). There is, as well, excessive sprouting within GH sympathetic ganglia of sensory axon collaterals containing Substance P (SP) (Gurusinghe & Bell, 1989). We have assessed the content of SP and neurokinin A (NKA), co-localized with SP in sensory neurons (Dalsgaard *et al.*, 1985), in a tissue innervated by sensory SP neurons of spinal origin (trachea) and in one with non-spinal SP neurons (gastric fundus) together with the superior cervical ganglion in GH rats and normal Wistar rats at 6 weeks of age.

Tissue content of immunoreactive SP and NKA was measured after homogenization and extraction with acetic acid by specific RIA (kits from Peninsula Labs). Tracheal content of SP in GH rats (3.6±0.5 pmole/g) was double that in normal rats (1.8±0.2 pmole/g; $P<0.05$, $n>4$). By contrast, assay of the fundus showed no difference between the strains (GH, 31.4±4.6 pmole/g; normal, 23.7±4.2 pmole/g; n=4). The ganglia from GH rats contained 60% more SP (30.1±2.6 pmole/g) than those from normals (18.9±1.9 pmole/g; $P<0.05$, $n>4$). For NKA, values in peripheral tissues were comparable to those found for SP. However, no differences between strains was found for trachea (GH, 2.7±0.4 pmole/g; normal, 3.0±0.5 pmole/g) or fundus (GH, 45.5±5.3 pmole/g: normal, 44.0±5.4 pmole/g; n=4 for each). By contrast, the NKA content of ganglia was below detection levels (at least 4-fold less than that of SP) in normal and GH rats.

Our results confirm histochemically demonstrated overgrowth of SP axons in GH sympathetic ganglia (Gurusinghe & Bell, 1989). The increased SP levels in ganglia and in trachea but not in fundus of GH rats suggests SP axon overgrowth is restricted to SP neurons of spinal origin. This is compatible with overgrowth being related specifically to competition for neurotrophins between spinal and sympathetic neurons. The absence of elevated NKA in GH trachea and the greatly different ratios of SP:NKA in trachea and ganglia implies that, although these two tachykinins may be co-localized, their intraneuronal levels vary independently.

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34P THE STIMULANT ACTIONS OF MAMMALIAN TACHYKININS ON RAT UTERUS ARE MEDIATED BY NK_2 RECEPTORS AND ARE ENHANCED BY PEPTIDASE INHIBITORS

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The aim of the present study was to compare the actions of three mammalian tachykinins, neurokinin A (NKA), neurokinin B (NKB) and substance P (SP), on the uterus of the oestrogen-primed rat, and to ascertain the influence of peptidase inhibitors on their effects. The stimulant effects of substance P on the isolated uterus of the oestrogen-primed rat are potentiated by phosphoramidon (Pennefather & Gould, 1991), indicating that endopeptidase 24.11 plays a role in the inactivation of this tachykinin in this tissue.

Uterine horns were taken from rats treated with oestradiol cypionate 20μg/kg s.c. 24 hr beforehand. Log concentration response curves to the three tachykinins were constructed in the absence and combined presence of inhibitors of endopeptidase 24.11 (phosphoramidon 1μM), angiotensin converting enzyme (captopril 10μM) and aminopeptidase (bestatin 10μM). In addition we have examined the ability of the tachykinins to displace specific binding of 50pM [^{125}I]-iodohistidyl-NKA from membranes of whole uterus. These were prepared as previously described (Geraghty *et al.*, 1992) and finally suspended in 500mM Tris (pH = 7.4) with 3mM $MnCl_2$, 0.02% bovine serum albumin, 1μM phosphoramidon and 4μg/ml chymostatin.

In the absence of the peptidase inhibitors neurokinin A was the most potent and neurokinin B the least potent of the three tachykinins. The relative order of potency remained the same in the presence of the peptidase inhibitors, but the potency of each was increased significantly. For example for NKA the pD_2 was 6.9 ± 0.13 in the absence of inhibitors, and 8.52 ± 0.01 in the presence of inhibitors (n=6). The membrane preparations incubated with [^{125}I]-iodohistidyl-NKA showed saturable specific binding. The rank order of potency of the tachykinins in displacing this binding was NKA > SP > NKB, with IC_{50} values of 1.29 ± 0.41 (n=4), 8.98 ± 3.02 (n=4) and 76.20 ± 28.75 (n=5) nM, respectively.

These data indicate that the tachykinin NK_2 receptor may mediate the uterotonic effects of the mammalian tachykinins on the uterus of the oestrogen-primed rat, and that peptidase/s play a role in their inactivation. Further investigations are required to determine the receptor subtype involved.

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35P THE EFFECTS OF ISLET AMYLOID POLYPEPTIDE AND PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE ON GUINEA-PIG TRACHEAL SMOOTH MUSCLE *IN VITRO*

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Mammalian airways contain a complex network of non-adrenergic, non-cholinergic nerves that utilize a number of peptides as neurotransmitters, including vasoactive intestinal polypeptide (VIP), which is a highly potent bronchodilator, and calcitonin gene-related peptide (CGRP), whose precise role in the airways is not known (Barnes, 1988). We have now investigated the effects on guinea-pig isolated trachea (GPT) of VIP and CGRP and two structurally-related peptides, pituitary adenylate cyclase activating polypeptide (PACAP) and islet amyloid polypeptide (IAPP). PACAP is a 38 amino acid residue, which has 60% homology with VIP, whereas IAPP contains 37 amino acids and has 45% homology with CGRP. The GPT used in this study were prepared as strips denuded of epithelium, and suspended in 4ml organ baths containing oxygenated modified Krebs - Henseleit solution at 37°C.

None of the peptides, in concentrations up to 10μM, contracted GPT ($n \geq 3$). In contrast, VIP and PACAP (3nM - 3μM, $n \geq 15$) caused concentration-related relaxations of preparations contracted with a submaximally effective concentration of KCl (40mM). Relaxant responses to PACAP were about 3-times slower (~ 150min) in reaching a maximum than those to VIP. Both peptides, however, caused a maximal relaxation approximately equal to 90% of that to isoprenaline (100μM). The IC_{50} values for VIP and PACAP were 72 (56-93)nM and 224 (142-355)nM respectively. CGRP and IAPP (3nM - 10μM, $n \geq 20$) also caused concentration-related relaxation, but responses were slow to develop (~ 100min) and the curves shallow. Maximal relaxation was not obtained with either compound, concentrations of 10μM only producing 50% of the response to isoprenaline (100μM). Concentration-effect curves on a single tissue could not be repeated with any of the peptides, subsequent responses always being depressed when compared to the initial curve. In experiments which compared across tissues, a combination of phosphoramidon (1μM), leupeptin (5μM), bestatin (100μM), soya bean trypsin inhibitor (1μM) and aprotinin (5μM) ($n \geq 4$) potentiated relaxations to VIP by two-fold, had no effect on those to PACAP or CGRP, and depressed those to IAPP. Subsequent studies revealed that aprotinin was responsible for the depression of the IAPP responses, whereas leupeptin potentiated IAPP responses by approximately 7-fold (n=4). The specific VIP-receptor antagonist, [4-Cl-D-Phe^6, Leu^{17}]-VIP (up to 10μM) did not inhibit responses to VIP or PACAP (n=4). Similarly, the specific CGRP-receptor antagonist, CGRP (8-37) (up to 10μM) had no effect on responses to CGRP or IAPP (n=4).

These findings demonstrate that the novel peptides, PACAP and IAPP, relax GPT in a similar manner to their respective homologues, VIP and CGRP. However, characterisation of the receptors mediating these effects remains to be determined.

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36P CHARACTERISATION OF THE CCK_A AND GASTRIN RECEPTORS COUPLED TO MUSCLE CONTRACTION IN GUINEA-PIG FUNDUS USING RECEPTOR DESENSITISATION

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The contractile effects of CCK-8 and gastrin on gastric fundus smooth muscle are well-recognised. However, there have been contradictory reports about the characterisation of the receptor types involved (Grider & Makhlouf, 1990; Huang *et al.* 1989). We report the use of homologous receptor desensitisation in conjunction with selective receptor antagonists in a further attempt to characterise the receptors involved. Strips (3x10mm) of fundic smooth muscle, separated from the mucosa by blistering, were suspended in 20ml organ baths, containing Krebs-Henseleit with 15.7mM K^+ at 22°C. Responses were measured isotonically (1g load) and single agonist concentration-effect (E/[A]) curves obtained by cumulative dosing.

CCK-8, E/log[A] curves were flat, extending over four log units with maxima consistently 50% greater than that produced by pentagastrin and tetragastrin. In contrast, the E/log[A] curves to both pentagastrin and tetragastrin obtained over 2.5 log units were not significantly different from one another. The selective CCK_A-receptor antagonist, devazepide, caused dose-dependent rightward displacement of the upper region of the CCK-8 E/log[A] curve rendering it biphasic. The lower, devazepide-resistant, region of the CCK-8 E/log[A] curve was displaced by the selective gastrin receptor antagonist, L365,260 (0.3μM). The CCK-8 E/log[A] curves in the presence of L365,260 alone at 0.3μM, the maximum concentration for selective gastrin receptor block, (Lotti and Chang 1989), remained complex, indicating that both CCK_A- and gastrin receptors were still being simultaneously activated to produce contraction. Therefore, tetragastrin (1μM, pre-incubated for 1h and present throughout) was used for the selective desensitisation of the putative gastrin receptors in an attempt to expose the CCK_A-receptors.

Although at higher concentrations tetragastrin behaved as a partial agonist at CCK_A-receptors on the guinea-pig gallbladder assay, 1μM had no effect on the CCK-8 E/log[A] curve on the guinea-pig gallbladder (Bishop *et al.*, 1992). Similarly, the tetragastrin desensitisation was shown not to affect responses to a selective ACh M-receptor agonist, 5-methylfurmethide on both the fundus and gallbladder assays. Following tetragastrin-desensitisation, monotonic CCK-8 E/log[A] curves ($p[A_{50}]=6.9\pm0.1$) were obtained which extended over a reduced range (2.5 log units) and which were were abolished by 0.1μM devazepide. These results are consistent with the existence of CCK_A and gastrin receptors coupled to muscle contraction in the guinea-pig fundus. A similar conclusion was reached by Boyle *et al.* (1992).

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Shim et al (1961) demonstrated that intravenous serotonin increased thoracic duct lymph flow in the dog but were unable to determine whether it was acting on blood vessels to increase lymph production or acting directly on lymphatic vessels to increase their pumping activity. In the present study we have examined the effects of serotonin on the spontaneous contractions of isolated bovine mesenteric lymphatics. The main lymph duct was dissected from the mesenteries of cows within 30 min after slaughter. Rings (2-3 mm in diameter and 8 mm in length) were suspended in Krebs at 37°C and their spontaneous isometric contractions were recorded.

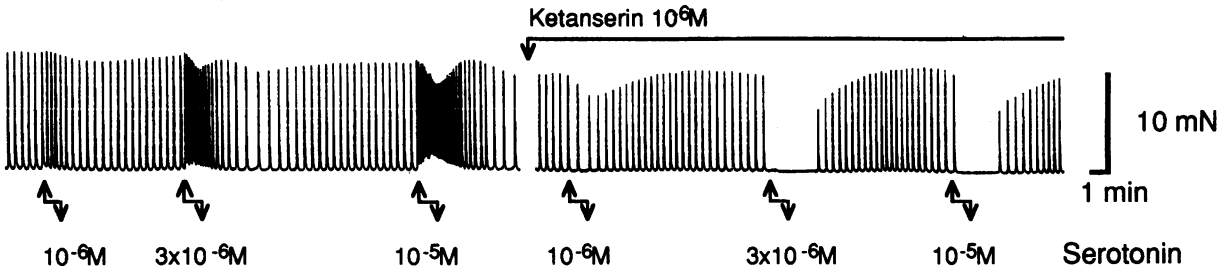

Fig 1 shows the effect of three doses of serotonin on frequency of spontaneous contractions. Under control conditions frequency of contraction increased from a control value of 2 contractions/ min to 4/min at 10^{-6}, 6.5/min at $3x10^{-6}$ an 10/min at 10^{-5}M. In the presence of Ketanserin this excitatory effect of serotonin was converted to an inhibitory one and this could be blocked in turn by propranolol 10^{-6}M. The excitatory effect of serotonin can be blocked also by pirenperone and by methysergide suggesting that $5HT_2$ receptors are being activated. However the observations that noradrenaline, which has a similar excitatory effect on these vessels, can also be blocked by pirenperone though not by ketanserin while serotonin can be blocked by phentolamine and rauwolscine would cast some doubt the above interpretation.

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38P EFFECTS OF GENISTEIN ON EXOCYTOSIS AND PROTEIN PHOSPHORYLATION IN THE EXOCRINE PANCREAS OF THE RAT

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The exocrine pancreas of the rat is a useful model tissue in which to investigate the molecular mechanism of regulated exocytosis. Pancreatic acini permeabilised with the bacterial toxin streptolysin O secrete amylase when provided with Ca^{2+} at concentrations in the micromolar range. This secretory response is enhanced when either GTPγS (Edwardson *et al.*, 1990) or a purified protein tyrosine phosphatase (Jena *et al.*, 1991) is introduced into the cells. These observations point to the involvement of both a GTP-binding protein and a protein phosphorylated on tyrosine in the control of exocytosis in this cell type. We have used the tyrosine kinase inhibitor genistein (Akiyama *et al.*, 1987) to examine these ideas further and to look for a candidate phosphoprotein on the membrane of the zymogen granule.

Pancreatic acini were prepared by collagenase digestion of rat pancreas as described previously (Edwardson *et al.*, 1990). Cells were permeabilised by incubation in a high-potassium buffer containing 0.2 U ml^{-1} streptolysin O. Exocytosis was triggered by addition of 10 μM free Ca^{2+}, and assayed through the release of amylase from the cells. Zymogen granules were isolated as described by Nadin *et al.* (1989).

Genistein inhibited exocytosis in permeabilised acini, to 41±4% (n=13) of control at the highest concentration tested (100 μg ml^{-1}). When isolated zymogen granules were incubated with [γ^{32}P]ATP, two major phosphoproteins, of M_r 45 kDa and 67 kDa, became radiolabelled. The extent of phosphorylation of both proteins was affected by GTPγS (p45 reduced and p67 increased) over the same concentration range (1-100 μM) that enhanced exocytosis in permeabilised acini. Genistein inhibited phosphorylation of p45 (but not p67) over the same concentration range that inhibited exocytosis - the residual phosphorylation at 100 μg ml^{-1} genistein was 52±4% (n=4). A polyclonal antiserum that recognises phosphotyrosine-containing proteins detected two major bands of M_r 45 kDa and 30 kDa on immunoblots of zymogen granule proteins. When granules were isolated from permeabilised acini that had been stimulated with 10 μM Ca^{2+}, the intensity of the 45 kDa band (but not the 30 kDa band) was significantly increased. Pre-treatment of the cells with genistein (200 μg ml^{-1}) caused the virtual disappearance of the 45 kDa band, which was only partially reversed by stimulation of the cells with 10 μM Ca^{2+}.

The properties of the 45 kDa protein on the zymogen granule membrane, and in particular the sensitivity of its phosphorylation state to GTPγS, genistein and Ca^{2+}, suggest that it may be an important component of the exocytotic machinery in pancreatic acinar cells.

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39P EFFECTS OF THE IMMUNOSUPPRESSANTS CYCLOSPORIN A AND FK 506 ON EXOCYTOSIS IN THE RAT EXOCRINE PANCREAS *IN VITRO*

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Cyclosporin A (CsA) and FK 506 are powerful immunosuppressive drugs that are used to prevent rejection of transplanted tissues and organs. Although the major side-effects of these drugs are nephro- and hepatotoxicity, adverse effects on both endocrine and exocrine pancreatic function have also been reported following their use *in vivo* (Lopez-Miranda *et al.*, 1991; Doi *et al.*, 1992). In this study we have examined the effects of CsA and FK 506 on exocytosis in the rat exocrine pancreas *in vitro*.

Acini were prepared by collagenase digestion of rat pancreas, as described by Edwardson *et al.* (1990). Exocytosis in intact acini was triggered by addition of the cholinergic secretagogue bethanechol, and was assayed through the release of amylase. Acini were permeabilised by incubation in a high-potassium buffer containing the bacterial toxin streptolysin O (0.2 U ml^{-1}); in these cells exocytosis was triggered by raising the free Ca^{2+} concentration to 10 μM. Exocytosis is complete after 15 min, the time-point chosen in this study.

Bethanechol stimulated amylase release from intact acini in a dose-dependent manner (EC_{50} 10^{-5}M). A 10-min pre-treatment of the acini with either CsA (1 μM) or FK 506 (100 nM), reduced the amylase release elicited by bethanechol. Cyclosporin did not inhibit basal amylase release, although FK 506 did cause a small inhibition. At a bethanechol concentration of 10^{-4}M, CsA and FK 506 reduced amylase release to 43±8% (n=10) and 45±5% (n=17) of control, respectively; approximate IC_{50} values were 50 nM (CsA) and 3 nM (FK 506). In permeabilised acini, CsA (1 μM) and FK 506 (100 nM) reduced exocytosis to 44±6% (n=14) and 66±6% (n=18) of control, respectively. When pancreatic lobules were prepared from rats that had been treated with the protease inhibitor FOY-305, in order to activate secretion (Rausch *et al.*, 1987), and then further stimulated *in vitro* by the secretagogue caerulein, a pronounced degranulation of the acinar cells was observed; this de-granulation was inhibited by pre-treatment (30 min) of the lobules with FK 506 (1 μM). Hence, the effect of FK 506 on exocytosis has a morphological correlate.

We have shown that both CsA and FK 506 cause a rapid and potent inhibition of exocytosis in pancreatic acinar cells *in vitro*. A similar inhibition of exocytosis has been demonstrated in the mast cell line RBL-2HB (Hultsch *et al.*, 1991), and it has been suggested that the effects of these immunosuppressive drugs on T-cell activation and on other cellular functions, such as secretion, may share a common molecular mechanism. Our results indicate that the effects on secretion may be widespread.

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40P ATRIAL NATRIURETIC PEPTIDE REDUCES THE NEPHROTOXICITY, BUT NOT THE ANTI-TUMOUR ACTIVITY, OF CISPLATIN

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Although cisplatin is an effective anticancer agent its clinical use is complicated by nephrotoxicity, (Hutchinson *et al.*, 1988). Current clinical manoeuvres used to reduce the nephrotoxic effects of cisplatin include hydration with the administration of diuretics or infusion of hypertonic saline, (Hayes, *et al.*, 1977; Ozols *et al.* 1984). While these manoeuvres decrease the frequency of cisplatin associated nephrotoxicity, they require administration of a significant solute load which may be poorly tolerated in patients with poor cardiac function. Atrial natriuretic peptide (ANP) is a natually occuring protein which improves renal function in several models of acute renal failure (Kurnik, *et al.* 1990). We examined the effect of ANP on cisplatin nephrotoxicity and antitumour activity in a conscious rat model.

In study one, where the nephrotoxic effects of cisplatin + ANP were examined, rats received a bolus i.v. dose of ANP (12μg/kg) followed by a bolus of cisplatin (5mg/kg, i.v.) and this was followed by an infusion of ANP of 1μg/kg/min for 45 minutes. Animals were killed 3 days after treatment. In study two, the antineoplastic activity of cisplatin + ANP was examined using the the Walker 256 carcinosarcoma in male Sprague Dawley rats. Treatment with cisplatin + ANP was initiated 5 days after rats were innoculated with the tumour. Treatment consisted of a bolus dose of ANP (12μg/kg), followed by 0.5 mg/kg cisplatin, which is the minimum dose required to eradicate the tumour. Cisplatin treatment was followed by a 45 minute infusion of ANP (1μg/kg/min). Animals were sacraficed 9 days after the initial tumour induction and tumours were removed and weighed.

Co-treatment with ANP resulted in a significant reduction in cisplatin-induced nephrotoxicity when compared to the group receiving cisplatin alone, as indicated by the plasma creatinine (0.76 ± 0.05 vs 1.2 ± 0.12 mg/dl; $p<0.05$) and blood urea nitrogen levels (33.7 ± 2.2 vs 54.9 ± 8.0 mg/dl; $p<0.05$). Renal platinum levels were not significantly altered by ANP co-treatment. Control rats had significantly larger tumours than rats receiving cisplatin treatment (9.5 ± 2.9 vs 1.8 ±0.7g; $p<0.001$). ANP administration alone did not affect tumour growth. When ANP was administered with cisplatin there was a significant reduction in tumour size when compared to the control group (1.4 ± 1.0 vs 9.5 ± 2.9g; $p<0.001$). ANP co-treatment with cisplatin was as effective in reducing tumour size as cisplatin alone.

Our results indicate that ANP infusion reduces the nephrotoxic effects but not the antineoplastic activity of cisplatin. We propose that this peptide hormone may have a role in the treatment and prevention of cisplatin -induced nephrotoxicity and may have advantages over current treatment regimes in certain clinical situations.

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41P TRIMETHYLAMINE N-OXIDE AS A SOURCE OF DIMETHYLAMINE

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Dimethylamine (DMA) is a normal component of the urine, thought to be generated via intermediary metabolism and also liberated from foods, although the exact dietary sources are unsure. Interest has focussed on this compound because it is readily nitrosated to the reactive dimethylnitrosamine which possesses carcinogenic properties. Evidence is provided in this abstract that trimethylamine N-oxide (TMAO) is a major source of DMA. Rats (male Wistar, 250g) given TMAO by several routes show a profound increase in urinary DMA levels only after oral administration, implicating a role for gut microbes. Small increases noticed after parenteral administration suggested an additional tissues mediated reaction. That the route to DMA is not through the intermediate trimethylamine (TMA) is shown by the lack of urinary DMA increase following TMA administration (Table 1). Such a reaction, producing DMA directly from TMAO with the liberation of formaldehyde has been previously shown to occur in both microorganisms (Myers & Zatman, 1971) and fish muscle (Parkin & Hultin, 1981)

Table 1. Increase in 0-24h urinary excretion of amines following TMA & TMAO administration to rats.

compound/route		DMA mg	DMA % dose	TMA mg	TMA % dose	TMAO mg	TMAO % dose
TMA	p.o.	0.2 ± 0.1	1.6 ± 0.8	1.9 ± 0.4	9.8 ± 1.8	15.5 ± 1.3	62.8 ± 4.2
TMA	i.p.	0.2 ± 0.0	1.7 ± 0.1	1.9 ± 0.8	13.2 ± 5.8	11.5 ± 1.0	62.8 ± 6.9
TMAO	p.o.	1.7 ± 0.3	12.9 ± 1.1	0.1 ± 0.0	0.7 ± 0.2	12.5 ± 1.3	58.2 ± 2.4
TMAO	i.p.	0.2 ± 0.1	1.8 ± 0.8	0.1 ± 0.0	0.5 ± 0.1	15.8 ± 3.8	71.2 ±25.3
TMAO	i.v.	0.2 ± 0.0	1.5 ± 0.2	0.1 ± 0.0	0.4 ± 0.1	13.2 ± 1.3	64.9 ± 6.

dose: TMA p.o. 1.14mM/kg body weight, TMA i.p 0.89 mM/kg b.w., all TMAO 1.0mM/kg b.w.
analysed by head-space gas chromatography, TMAO after reduction to TMA (Barrett *et al.* 1991; Simenhoff *et al.*, 1977)

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42P P-GLYCOPROTEIN POSSESSES ALLOSTERICALLY COUPLED DRUG ACCEPTOR SITES

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P-glycoprotein (P-gp) is a 170 kDa ATPase which pumps a diverse range of cytotoxics out of tumour cells rendering them multidrug resistant (MDR). Drugs which reverse MDR by preventing cytotoxic drug efflux, such as verapamil can overcome drug resistance of humans with lymphoid malignancies which express P-gp, albeit with cardiac toxicity. P-gp can efflux a wide range of cytotoxics, including anthracyclines (e.g. doxorubicin), vinca alkaloids (e.g. vinblastine) epipodophyllotoxins (e.g. etoposide) and peptides (e.g. actinomycin D). The 'hydrophobic vacuum cleaner model', is the leading hypothesis to explain the wide chemical diversity of P-gp substrates (Endicott and Ling 1989).

We have used the well characterised human lung cancer cell line H69 LX4 which overexpresses P-gp, as a source of plasma membranes. Binding of [^{3}H]-vinblastine (8-16 Ci/mmol) was performed in 50 mM Tris HCl/0.1 mM PMSF pH 7.4 in a volume of 0.25 ml at 23°C with saturable binding defined by 30 uM unlabelled vinblastine. Bound and free [^{3}H]-vinblastine were separated by rapid filtration through Whatman GF/C filters. All values are means of three experiments, where relevant ± s.e.mean.[^{3}H]-Vinblastine bound to 35 ± 8 pmol/mg of protein with a Kd of 9.3 ± 2 nM, employing [^{3}H]-vinblastine concentrations as high as 100 nM there was no evidence for the presence of low affinity sites. Binding was inhibited completely by the cytotoxics (Ki values with s.e.mean in parentheses) vincristine (620 ± 65 nM) actinomycin D (3000 ± 1500 nM), doxorubicin (18000 ± 4500 nM) and MDR reversing agents, verapamil (600 + 200 nM) and Ro 11 2933 (28 ± 14 nM). In contrast the 1,4-dihydropyridine MDR-reversing agents nicardipine (85 ± 38) and nifedipine (5000 ± 2000) inhibited only 80-85 % of saturable binding. Blockade of the association reaction between [^{3}H]-vinblastine and P-gp by 30 uM vinblastine or by 600-fold dilution revealed a dissociation rate constants for [^{3}H]-vinblastine-P-gp complexes (k_{-1}) of 0.034 ± 0.001min^{-1} (n = 7) and 0.026 ± 0.002 min^{-1} respectively. If the association reaction is blocked with a combination of 30 uM vinblastine and 10 uM nicardipine the k_{-1} increases to 0.39 ± 0.11 min ($P < 0.01$). If the association reaction is blocked with dilution in the presence of 30 uM nifedipine then k_{-1} increases to 0.16 min^{-1}. This effect was not restricted to MDR-reversing agents, doxorubicin at 100 uM increased k_{-1} 10-fold relative to 600-fold dilution.

Theoretically drugs which act as competitive inhibitors of [^{3}H]-vinblastine binding to P-gp should not alter the dissociation rate constant of [^{3}H]-vinblastine complexes. We have shown that the 1,4-dihydropyridine MDR-reversing agents and the anthracycline cytotoxic doxorubicin accelerate the dissociation of [^{3}H]-vinblastine-P-gp complexes (i.e. act as negative heterotropic allosteric regulators) and therefore must act at a drug acceptor site(s) other than the vinca-alkaloid-selective binding site of P-gp. Thus P-gp has at least two drug acceptor sites, each of which will have its own structure activity series. This may well explain the wide range of chemical entities recognised by P-gp, the concept of P-gp as a 'hydrophobic vacuum cleaner' can be discarded. This fundamental finding has significant import for the design of drugs to reverse MDR.

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43P IS THE INTRAVENOUS ROUTE THE ONLY THERAPEUTIC ROUTE FOR MONOCLONAL ANTIBODIES?

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Much interest has been shown recently in the potential of monoclonal antibody therapy, especially with the introduction of "humanised" antibodies of reduced immunogenicity. Little is known of their pharmacokinetics and distribution when given by routes other than i.v. To determine the bioavailability and half-life of CB0010 - a murine IgG1 isotype human anti-tumour necrosis factor monoclonal antibody made at Celltech, we performed the following experiments. Male SD rats (200-250g) were anaesthetised (xylazine/ketamine) and catheterised (jugular vein and caudal artery) for drug administration and blood sampling respectively. These were externalised through the neck, connected to a counterbalance harness, and the animals left to recover overnight. CB0010 (2mg.kg^{-1}; 150μL, 0.9% saline) was given either i.v., i.p., i.m., per rectal. (p.r.) or s.c. and blood samples taken over the next 6 hours for serum CB0010 measurement to monitor α-phase distribution. In a separate experiment, uncatheterised rats were given CB0010 i.v., i,m., i.p., s.c. or p.r. and blood samples taken over 21 days by direct venepucture to monitor β-phase. CB0010 was measured in serum by a competition ELISA. Results are mean ± s.e. mean. Data was analysed by ANOVA and Students t-test as appropriate. Difference was assumed at $P<0.05$.

ROUTE	t½ (h)	V (ml)	Cl (μl.h^{-1})	AUC (mg.ml^{-1}.h)	F (%)
i.v. (0-6h)	8.42 ± 1.1	39.65 ± 3.9	3397 ± 261	0.187 ± .016	100
i.v. 0-21 d	204.8** ± 16.5	19.88* ± 1.98	67** ± 40	9.16** ± .56	100
i.m. 0-21 d	182.8** ± 33.5	27.42 ± 3.98	107**# ± .10	5.85**# ± .54	63.9**# ± 5.9
i.p. 0-21 d	217.5** ± 14.3	27.68 ± 2.48	88**# ± 4.6	6.97**† ± .35	76**# ± 3.8

t½, ½ life; V, volume of distribution; Cl, whole body clearance; F, bioavailability. n=4-7. **P<0.001, *P<0.01 vs i.v. 0-6h, # P<0.02, † P<0.05 vs. i.v. 0-21d.

Only i.v. administration showed any appreciable serum levels by 6 hours. There are clearly two phases of distribution, the first being completed within the first 6 hours. CB0010 was not detectable after p.r. administration at any time-point. Serum CB0010 after s.c. was variable and showed no clear pattern of distribution. There is a clear α and β-phase distribution with i.v. CB0010. The similar t½ for i.v., i.m. and i.p., but the lower volume of distribution and higher maximum serum concentration (not shown) for i.v. against i.m. or i.p. suggest a slower release into the circulation, but equivalent kinetics once equilibrum has been reached. These results suggest that i.v. is not the only potential route of administration for monoclonal antibodies.

44P INTERACTIONS BETWEEN ENDOTHELIAL CELL CALCIUM AND ATRIAL NATRIURETIC FACTOR IN BOVINE PULMONARY ARTERY CELLS

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Calcium-calmodulin has been suggested to have a role in regulating atrial natriuretic factor (ANF) stimulation of cGMP production in various cell types (Sekia, 1991), and in turn ANF itself has been reported to inhibit certain calcium-dependant events in endothelial cells such as EDRF release (Hogan, 1989). In this study we used an *in vitro* model of the pulmonary endothelium to investigate (i) the role of Ca^{2+} in the regulation of ANF-stimulated cGMP production and (ii) the modulation of intracellular Ca^{2+} by ANF.

Bovine pulmonary artery endothelial cells (BPAEC), obtained from the Dept. of Surgery, Washington University (St. Louis) were maintained and subcultured as previously described (Redmond, 1990). Following preincubation of cell monolayers with 0.5 mM isobutylmethylxanthine (IBMX) ± the agent of interest for 20 min at 37°C and further incubation with rANF$_{(99\text{-}126)}$ for 5 min at 37°C, reactions were stopped by aspiration. Cellular cGMP was extracted with 0.1M HCl for 1 hr at room temperature and quantified, after acetylation, by radioimmunoassay. Intracellular Ca^{2+} was measured in cells loaded with the Ca^{2+} sensitive fluorescent dye indo-1 (2 μM), for 40 min at 37°C. Excess extracellular dye was removed by centrifugation at 160 x g for 6 min. The resuspended cells (~1x10^6 cells/ml) were kept at 22°C and warmed to 37°C at least 5 min before use. Indo-1 fluorescence was assayed using a FACStar flow cytometer with UV excitation at 251-364 nm. Indo-1 emission was collected using blue (491-500 nm) and violet (400-410 nm) band pass filters.

The ability of the system to register manipulations in cell Ca^{2+} was first assessed: A23187 (1 μM) produced a time dependant increase in intracellular Ca^{2+} which was maximal at ~60% above control levels and was maintained over at least 100 s. Subsequent treatment with 2 mM EGTA produced a fall in intracellular Ca^{2+}. The actions of bradykinin were next investigated and at 10 μM this peptide produced a steady increase in intracellular Ca^{2+} sustained over at least 120 s and reaching a maximum of ~55% above control. This effect was abolished in the presence of 2 mM EGTA. A 15-20% fall in intracellular Ca^{2+} was recorded after treatment with 0.1 μM rANF$_{(99\text{-}126)}$ and the latter also inhibited the bradykinin-stimulated rise in intracellular Ca^{2+}. Finally, ANF-stimulated cGMP production was significantly inhibited by pretreatment of cells with bradykinin (1, 5 μM) or with 4 bromo A23187 (5 μM).

In summary, these results support a role for calcium in the regulation of ANF stimulation of intracellular cGMP production. In addition, ANF appears to produce a fall in intracellular Ca^{2+} which may act as a negative feedback mechanism to control release of EDRF.

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45P EFFECTS OF POTASSIUM CHANNEL OPENERS ON TRACHEAL SUBMUCOSAL GLAND SECRETION AND EPITHELIAL TRANSPORT

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Potassium channel openers (KCO's) relax bronchial smooth muscle *in vivo* and *in vitro* (Black & Barnes, 1990), but their effects on the airway mucosa have not been studied. We have examined the effect of the KCO's Ro 31-6930, 2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl) pyridine-1-oxide, and BRL 38227, (-)-6-cyano-3,4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl)-2H-1-benzopyran-3-ol), on ferret tracheal submucosal gland secretion (using lysozyme as a specific marker of serous cell secretion) and the epithelial transport of albumin produced by the secretagogues methacholine and phenylephrine. Air-filled tracheas (Webber & Widdicombe, 1987) were mounted laryngeal end down in organ baths and bathed with Krebs-Henseleit buffer containing bovine serum albumin (BSA, 4mg.ml^{-1}) and FITC-BSA (40μg.ml^{-1}) on the submucosal side. Secretions were removed every 30min from the trachea and analysed for lysozyme and albumin (fluorescence).

There was no baseline secretion from the trachea, so the outputs of lysozyme and albumin in the unstimulated trachea were considered to be zero. On addition of methacholine (20μM) or phenylephrine (100μm) the lysozyme and albumin outputs increased. On continued application of the secretagogues the outputs fell but reached maintained levels significantly higher than in control (unstimulated) periods. The maintained lysozyme outputs produced by methacholine (n=14) and phenylephrine (n=5) were 97 ± 15 and 171 ± 63ng.min^{-1}, and the maintained albumin outputs were 2.4 ± 0.7 and 5.9 ± 2.0μg.min^{-1} respectively. Ro 31-6930 (1nM - 10μM) inhibited methacholine (-30.5 ± 5.2 to -60.1 ± 4.0%, n=8) and phenylephrine (-29.9 ± 7.9 to -55.5 ± 10.2%, n=5) induced lysozyme outputs, but had no significant effect on the albumin output with the secretagogues (-14.7 ± 8.9 to -23.8 ± 11.8%, n=7; and -22.8 ± 12.5 to -25.5 ± 10.4%, n=4). BRL 38227 (10nM - 10μM) also inhibited methacholine-induced lysozyme output (-8.3 ± 12.7 to -65.5 ± 7.0%, n=6) but had no effect on the albumin output to methacholine (-2.6 ± 11.7 to -13.7 ± 13.0%).

Thus, the KCO's Ro 31-6930 and BRL 38227 inhibit muscarinic cholinergic-induced, and Ro 31-6930 inhibits α-adrenergic-induced submucosal gland serous cell secretion. They have no significant effect on active epithelial albumin transport. The maximum inhibition of lysozyme ouput with the KCO's was 60-65% (irrespective of the secretagogue used), which may suggest a population of serous cells that release lysozyme but are not responsive to these KCO's.

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46P INHIBITION OF HISTAMINE-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN BOVINE TRACHEAL SMOOTH MUSCLE BY BRL 38227 AND ISOPRENALINE

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Activation of phosphoinositide (PI) hydrolysis by spasmogenic stimuli (e.g. histamine) plays an important role in pharmacomechanical coupling in tracheal smooth muscle. The K^+-channel opener BRL 38227 (BRL) and the β-adrenoceptor agonist isoprenaline (ISO) both inhibit histamine-stimulated PI hydrolysis in this tissue (Challiss et al., 1992a), the former exerting its action independently of changes in cellular cyclic nucleotide concentrations (Challiss et al., 1992b). A possible common mechanism of action of BRL and ISO is the ability of these agents to cause membrane hyperpolarization and consequently limit Ca^{2+} entry via voltage-operated Ca^{2+}-channels. Here the consequences of altering extracellular K^+ concentration on the ability of these agents to inhibit histamine-stimulated PI hydrolysis has been investigated.

Bovine tracheal smooth muscle (BTSM) slices (300 x 300 μm) were pre-incubated, labelled with [^{3}H]inositol and incubated as described previously (Challiss et al., 1992a). Additions of BRL and/or ISO were made 15 min prior to, or 10 min subsequent to histamine (100 μM) addition. All incubations were for 30 min following histamine addition. As indicated, KCl was added to alter extracellular K^+ concentration ($[K^+]_e$) 5 min prior to histamine addition. Incubations were terminated, neutralized and [^{3}H]$InsP_x$ separated chromatographically as described previously (Challiss et al., 1992a).

Exposure of BTSM slices to 100 μM histamine for 30 min resulted in a 25-35 fold increase in [^{3}H]$InsP_x$ accumulation. Addition of maximally effective concentrations of ISO (10 μM) and BRL (5 μM) prior to histamine challenge caused respectively 43.3 + 5.8% (IC_{50} 194 + 10 nM) and 59.8 + 2.3% (IC_{50} 327 + 23 nM) inhibitions of the [^{3}H]$InsP_x$ response, whilst co-addition of BRL and ISO resulted in a more profound (88.2 + 0.9%) inhibition. Similar inhibitory actions were observed when ISO and/or BRL were added subsequent to histamine challenge. The inhibitory effect of BRL (5 μM) was significantly attenuated by glibenclamide (1 μM), whereas 10 μM glibenclamide had no significant effect on the ISO inhibition. Elevation of $[K^+]_e$ to 65 mM slightly decreased the histamine-stimulated [^{3}H]$InsP_x$ accumulation (by 10.5 + 2.0% relative to the response at $[K^+]_e$ = 4.7 mM), and completely abolished the inhibitory effects of BRL and/or ISO. The inhibitory action of ISO was dramatically attenuated at 25 mM K^+, whereas 45 mM K^+ was necessary to cause a similar attenuation by BRL of the histamine-stimulated PI response.

These data implicate the hyperpolarizing actions of BRL and ISO in their inhibitory effects on histamine-stimulated PI hydrolysis in BTSM. However, the additive nature of the BRL/ISO inhibition, the differential sensitivity to glibenclamide and the differences exhibited with respect to the effects of elevating $[K^+]_e$ suggest that these agents act via distinct mechanisms to cause membrane hyperpolarization and subsequent inhibition of PI turnover.

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47P K^+-CURRENTS RECORDED FROM CULTURED CELLS ISOLATED FROM OVINE TRACHEAL SUBMUCOSA

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The electrophysiology of tracheal smooth muscle has been studied in great depth and the effect of potassium channel openers (KCO's) on this tissue is well documented (Black & Barnes,1990) with a view to their possible role in the treatment of respiratory diseases. Very little work has been directed to study other cells of the airways, which could be affected by the KCO's. We have investigated the electrophysiology of cultured cells obtained from the submucosal layer of ovine trachea and the effect of the KCO Ro 31-6930, 2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl) pyridine 1-oxide on these cells. Ovine tracheae were obtained from an abattoir, the epithelial cell layer was removed and the underlying submucosa dissected from the trachea wall. The cells of the submucosa were dissociated with a cocktail of enzymes and plated on collagen coated coverslips and maintained in HF12/DMEM+10%FCS culture medium at 37°C in 5%CO_2 in air for up to three weeks (Tournier et al,1990).
The whole cell recording technique was used to voltage clamp the cultured cells at a holding potential of -70mV. Cells were depolarised at a maximum frequency of 0.1Hz by 300ms voltage step commands, net outward currents were activated at potentials positive to -30mV. At a clamp potential (V_C) of +30mV the mean net outward current was 0.36±0.04nA (n=11). The voltage-activated outward currents did not decay significantly during the voltage step commands. Depolarising the holding potential for 30s to values between -100mV and -10mV inactivated the outward current evoked at V_C of +30mV. The mean voltage for 50% steady-state inactivation of the outward current ($V_{0.5}$) was -42.8±5.6mV (n=6). The voltage-activated outward currents observed in this study had components sensitive to the K^+-channel blockers 4-aminopyridine (500μM) and tetraethylammonium (5mM).
The novel KCO Ro 31-6930 possesses smooth muscle relaxant properties (Paciorek et al,1990). Application of Ro 31-6930 (1μM) enhanced the outward current activated at +30mV in 4 of 7 cells giving a mean additional current of 0.49±0.2nA (n=4). The effect of Ro 31-6930 was also observed as an additional inward current of -0.2±0.13nA at V_C of -150mV when the cells were hyperpolarised to potentials negative to E_K.
Our study suggests that a variety of K^+-currents can be activated in cultured submucosal cells and some of these are stimulated by Ro 31-6930, an opener of glibenclamide-sensitive K^+-channels. These K^+-channels maybe therapeutic targets for drugs which reduce airway mucus secretion (Griffin & Webber,1992).

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48P CHARYBDOTOXIN- AND APAMIN-SENSITIVE POTASSIUM CURRENTS IN SMOOTH MUSCLE CELLS ISOLATED FROM BOVINE TRACHEALIS

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The low excitability typical of the trachealis smooth muscle membrane in several species has been attributed to a strong outward rectification mediated by K^+ channels (Muraki *et al.*, 1990). Recent experiments have demonstrated the presence of both Ca^{2+}-activated and Ca^{2+}-independent K^+ channels in bovine trachealis smooth muscle cells (Green *et al.*, 1991). We have utilised several specific K^+ channel antagonists in order to characterise the different components of the K^+ current in freshly isolated bovine trachealis smooth muscle cells.

Single airway smooth muscle cells were isolated from bovine trachealis using papain. Membrane currents were recorded using the conventional whole cell patch clamp technique. The pipette (internal) solution contained (in mM) 135 KCl, 0.5 $MgCl_2$, 0.1 EGTA, 2.0 ATP, and 10 HEPES. The external solution contained 135 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose and 10 HEPES; both solutions were buffered to pH 7.4.

K^+ currents were elicited from a holding potential of -70 mV with depolarising steps beyond -40 mV. The outward current demonstrated little inactivation over 600 ms. The current activated smoothly at potentials negative of 0 mV; with larger depolarisations the current became noisy.

Charybdotoxin (100 nM), a blocker of large conductance Ca^{2+}-activated K^+ channels, reduced the overall current at +70 mV by approximately 40%, and also markedly diminished current noise. The charybdotoxin-sensitive current activated positive of 0 mV, and showed no evidence of inactivation. TEA (2 mM) another relatively specific blocker of Ca^{2+}-activated K^+ channels, had an effect similar to that of charybdotoxin. Apamin (1 μM), which has been shown to block small conductance Ca^{2+}-activated K^+ channels, blocked the current at +70 mV, measured at 300-600 ms, by 15-20%, but had a larger effect on the early current. The apamin-sensitive current activated near -30 mV. The effects of TEA and apamin were additive. 4-aminopyridine (4-AP, 5 mM), which has some specificity for delayed rectifier K^+ currents, inhibited the current at +70 mV by 25-30%. The 4-AP sensitive current activated near -30 mV. Current blockade by 4-AP and apamin was not additive, suggesting a degree of overlap in their effects.
These results suggest that there may be two types of Ca^{2+}-activated K^+ channels in bovine trachealis smooth muscle cells. The charybdotoxin- and TEA-sensitive current appears to activate at more positive potentials than the apamin-sensitive current, which shows some inactivation. This latter current may also be sensitive to 4-AP. The relative contribution of these different currents types to outward rectification remains to be established.

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49P THE NOVEL BENZOPYRANOL POTASSIUM CHANNEL ACTIVATOR BRL55834 ACTIVATES TWO POTASSIUM CHANNELS IN BOVINE AIRWAY SMOOTH MUSCLE

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BRL 55834 shows a significantly greater potency against airway smooth muscle compared to its vasodilator properties *in vivo* (Bowring *et al.*, 1992) and is airways selective *in vitro* relative to BRL 38227 (Taylor *et al.*, 1992a). We have therefore, compared the effects of these compounds on the electrophysiology of isolated bovine airway smooth muscle cells.

Single airway smooth muscle cells were dispersed from fresh bovine trachealis with papain, allowed to settle on a clean glass cover slip, and viewed through an inverted stage microscope (Zeiss). The cells were gently flushed with PSS at room temperature, and only those cells with a smooth surface and spindle shaped appearance were used. Currents were recorded using patch clamp in both whole cell and inside out configurations (Axopatch 1c, Axon Instruments). For whole cell patch clamp the pipette (internal) solution contained 2mM ATP, 0.1 mM EGTA (no added Ca^{2+}), 10mM HEPES and 140mM KCl (pH 7.4). The external solution contained 50mM [K^+] PSS (Ca^{2+} free). For single channel recordings the pipette (external) solution contained 140 mM KCl and no Ca^{2+}. Initially the bath solution was normal PSS (Ca^{2+} free). Once a successful membrane patch had been obtained the bath solution was exchanged for an intracellular solution containing 140 mM KCl, 5 mM EGTA, 10 HEPES and 2 mM $MgCl_2$.

Both BRL 38227 (10μM) and BRL 55834 (1μM) resulted in significant increases in current. The current/voltage relationship of the BRL 38227 induced current was linear, whereas that of the BRL 55834 induced current was not, with increasing currents at more positive potentials. This implies that BRL 55834 is activating either a different, or an additional current. At least two channel types were observed during single channel analysis, both had reversal potentials similar to the calculated equilibrium potential for K^+. Like BRL 38227 (Collier *et al.*, 1991) BRL 55834 activated a low conductance (44.4 ± 3.6 pS) K channel which was blocked by ATP (2mM) and partially blocked by glibenclamide (10μM). Unlike BRL 38227, however, BRL 55834 also induced an increase in open probability (mean control Po 0.021 ± 0.006; BRL 55834 (1μM) 0.055 ± 0.007; $p<0.005$, $n=6$) of a large conductance channel (243 ± 7 pS) similar to that described by Green *et al.*, (1991). The mean increase in Po in individual experiments was 343 ± 91%. This channel was blocked by charybdotoxin (100nM) and activated by Ca^{2+}. Quinidine (100μM) gave a flickery block, whereas glibenclamide (10μM) had no blocking effect.

These results suggests that the novel KCA BRL 55834 not only activates a small conductance, ATP- and glibenclamide- sensitive K^+ channel, (also activated by BRL 38227), but also a larger conductance channel with propertiies similar to that of the Ca^{2+}-activated K^+ channel (and not activated by BRL 38227).

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50P INHIBITION OF SERUM-INDUCED PROLIFERATION BY PHORBOL ESTER AND PROTEIN KINASE INHIBITORS IN GROWTH-ARRESTED CULTURED RABBIT TRACHEAL SMOOTH MUSCLE CELLS

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A marked increase in airway smooth muscle (ASM) mass is one of the most characteristic pathological changes seen in chronic severe asthma. Both hyperplasia and hypertrophy may contribute to this increase (Dunnill *et al.*, 1969; Heard & Hossain, 1973), but the cellular and biochemical pathways underlying these changes are unknown. We have investigated the potential contribution of the protein kinase C (PKC) and protein tyrosine kinase (PTK) pathways in controlling proliferation of cultured rabbit ASM cells. Rabbit tracheal ASM cells, cultured according to established techniques (Twort & van Breemen, 1988) were seeded at $2x10^4$cells/well into 24 well cluster plates containing 0.5ml Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS). After 24 hours cells were rendered quiescent by incubating for 72 hours in a defined serum-free DMEM containing insulin (1μM), transferrin (5μg/ml) and ascorbate (100μM). Growth was initiated by replacement of the serum-free media with DMEM-10%FCS in the presence or absence of the drug under investigation. Proliferation was assessed by three independent methods (Hirst *et al.*, 1992): coomassie blue protein determination, production of the formazan product from the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and by direct haemacytometry.

Prolonged exposure of cultured rabbit ASM to the novel PKC inhibitors, Ro31-8220 (1nM-10μM) and Ro31-7549 (10nM-10μM) (Davis *et al.*, 1989) resulted in concentration-dependent inhibition of proliferation in response to 10% FCS over a seven day period ($n=3$-4). Growth as assessed by each assay was almost completely abolished at 10μM of each inhibitor, Ro31-8220 being approximately 3-fold more potent. Continued exposure of the cells to the PKC activator 4ß-PMA (0.1nM-1μM) during stimulation of growth by 10%FCS, resulted in concentration-dependent inhibition of serum-induced proliferation ($n=4$).
In a further four experiments, incubation of the cells with the specific protein tyrosine kinase inhibitor, ST638 (0.1-100μM) (Shiraishi *et al.*, 1989) resulted in concentration-dependent inhibition of serum-induced proliferation ($n=4$). Growth was markedly suppressed (approx. 80% of the control) at 100μM ST638. Staining of cells with trypan blue did not occur with any the compounds investigated, even at the higher concentrations, indicating that the cells were still viable.
These preliminary observations appear consistent with the involvement of both PKC and PTK in the control of rabbit ASM cell proliferation in response to serum.

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51P INHIBITION OF SEPHADEX-INDUCED LEUKOCYTE INFILTRATION INTO GUINEA-PIG LUNGS BY RAPAMYCIN

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Eosinophils are believed to play a key role in the pathophysiology of asthma. Intravenous injection of Sephadex beads to guinea pigs has previously been shown in our laboratory to increase the migration of eosinophils to the lung (Maghni et al., 1991). We have examined the effect of rapamycin, an immunosuppressive drug, isolated from *Streptomyces hygroscopicus* (Vezina et al., 1975) on the influx of eosinophils and other leukocytes into guinea-pig lung following the injection of Sephadex beads.

Dunkin-Hartley guinea pigs (300-400g) were injected intravenously with Sephadex beads (G50, superfine, 24mgkg^{-1}). Animals were sacrificed 24 h later and bronchoalveolar lavage (BAL) was performed. Rapamycin (Wyeth Ayerst, 0.001-5 mgkg^{-1}) was injected by the intramuscular route 2 h prior to Sephadex injection. Control animals received vehicle (cremophor el and ethanol). Comparative studies using cyclosporin (5 mgkg^{-1}) and dexamethasone (1 mgkg^{-1}), administered at 2 and 1.5 h respectively before Sephadex injection, were also performed. Total and differential cell counts in BAL fluid were assessed.

Injection of Sephadex beads into guinea pigs significantly increased the eosinophil count in BAL from 3.45±0.62 (naive, n=5) to 32.08±2.04 x10^6 (n=10) and the neutrophil count from 0.08±0.05 x10^6 to 16.06±2.04 x10^6. In addition, the number of lymphocytes (0.20±0.08 x10^6) and macrophages (18.26±1.34 x10^6) was significantly increased to 3.75±0.43 and 28.47±2.99 x10^6 respectively. Rapamycin (0.001-5 mgkg^{-1}) dose-dependently reduced the increase in differential leukocyte number recovered in BAL fluid following injection of Sephadex beads. This inhibition of total leukocyte number by rapamycin was related to a decrease in eosinophil, neutrophil, lymphocyte and macrophage number. At the highest dose of rapamycin (5 mgkg^{-1}) the eosinophil, neutrophil, lymphocyte and macrophage counts were significantly reduced to 11.65±0.82, 7.24±1.40, 1.87±0.29 and 23.13±1.90 x10^6 respectively (Dunnett's t-test, $p<0.05$, n=7). In addition, cyclosporin significantly inhibited both the eosinophil and lymphocyte influx into lung tissue ($p<0.05$, n=4) while dexamethasone only significantly inhibited the lymphocyte infiltration. ($p<0.05$, n=3).
These results demonstrate that rapamycin is a potent inhibitor of lung cell infiltration induced by Sephadex beads. This immunosuppressive drug may prove to be useful in the treatment of asthma where lung cell infiltration is a characteristic feature.

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52P SOLUBLE CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ISOENZYMES FROM HUMAN TRACHEAL SMOOTH MUSCLE

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There is increasing evidence that cyclic nucleotide phosphodiesterase (PDE) inhibitors exhibit the required pharmacology to be effective in the treatment of bronchial asthma through their ability to exert both a bronchodilator and an anti-inflammatory influence (see Giembycz & Dent, 1992). This has arisen principally from the realisation that there are at least five mammalian PDE families that differ in a number of physical and biochemical ways in particular their sensitivity to inhibitors. Although the PDE isoenzymes present in airways smooth muscle of a number of species have been identified and characterised, little is known of the biochemistry of the isoenzymes present in human airways. In this abstract the PDE isoenzymes resolved from the soluble fraction of human tracheal smooth muscle (HTSM) is reported and their properties characterised.

HTSM was obtained from donors for heart transplantation and the soluble fraction from epithelium-denuded tissue subjected to anion-exchange chromatography over Q-Sepharose (Giembycz & Barnes, 1991). Fractions were assayed subsequently for cyclic AMP and cyclic GMP hydrolytic activity as described previously (Giembycz & Barnes, 1991).

Anion-exchange chromatography of HTSM resolved four peaks of cyclic nucleotide hydrolytic activity. The first peak of activity that was eluted from the column had the characteristics of a PDE V; it preferentially hydrolysed cyclic GMP (K_m = 6.2 µM) over cyclic AMP (K_m > 100 µM) and was selectively inhibited by zaprinast (K_I = 700 nM) and MY 5445 (IC_{50} = 1.2 µM). The second peak of activity preferred cyclic GMP as substrate (K_m = 46 µM). However, when cyclic AMP (K_m = > 100 µM) was used its hydrolysis was potentiated six-fold by micromolar concentrations of cyclic GMP indicating the presence of a PDE II. Cyclic AMP was the preferred substrate for the PDE present in the final two peaks of activity that were eluted from the column. The first of these displayed kinetics and inhibitor sensitivities indicative of a PDE IV: it preferentially hydrolysed cyclic AMP (K_m = 4.7 µM) over cyclic GMP (K_m = 59 µM) and was selectively inhibited by rolipram (IC_{50} = 1.3 µM) but not by SK&F 94120 or zaprinast. Studies with selective PDE inhibitors suggested that the final peak of activity contained a mixture of a PDE III (~ 25%) and PDE IV (~ 75%). In the presence of 100 µM SK&F 94120 to inhibit PDE III fully, the K_m of cyclic AMP and cyclic GMP for the PDE IV was 6 µM and 81 µM respectively. This activity was potently inhibited by rolipram (K_I = 1.4 µM), denbufylline (IC_{50} = 737 nM) and Ro-20,1724 (IC_{50} = 14.6 µM).

The results of these experiments indicate that HTSM contains at least four soluble PDE isoenzymes: a PDE II, PDE III, PDE IV and PDE V. Consistent with data reported recently by Shahid *et al.*, (1992), multiple PDE IV isoenzymes may be expressed by HTSM although proteolysis of a single PDE IV variant cannot be excluded at this time.

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53P EFFECT OF ISOENZYME-SELECTIVE CYCLIC NUCLEOTIDE PHOSPHODIESTERASE INHIBITORS ON HUMAN TRACHEAL SMOOTH MUSCLE TONE

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In the soluble fraction of human tracheal smooth muscle (HTSM) four Ca^{2+}/calmodulin-independent PDE isoenzymes have been identified which have the characteristics of a PDE II, PDE III, PDE IV and PDE V (Giembycz *et al.*, 1992; Cieslinski *et al.*, 1988). Despite this biochemical classification, however, the functional consequences of PDE isoenzyme inhibition in this tissue have not been systematically studied. In this abstract we report the results of experiments designed to evaluate the potential spasmolytic activity of a number of isoenzyme-selective PDE inhibitors on HTSM tone.

Tracheal smooth muscle strips were obtained from donors for heart transplantation and set up for isometric tension recording using well established methods. The ability of SK&F 94120 (PDE III-selective), rolipram and denbufylline (PDE IV-selective) and zaprinast (PDE V-selective) to relax spontaneous and methacholine (MCh; 1 μM and 10 μM)-induced tone was then evaluated.

Table I: pIC_{50} Values of Isoenzyme-selective PDE Inhibitors for Relaxing Spontaneous and MCh-induced Tone of Human Trachealis

PDE Inhibitor	*Spontaneous*	*Methacholine (1 μM)*	*Methacholine (10 μM)*
SK&F 94120	5.96 ± 0.12 (6)	6.05 ± 0.27 (6)	5.54 ± 0.17 (6)
Rolipram	> 4.00 (6)	6.11 ± 0.32 (6)	4.13 ± 0.56 (6)
Denbufylline	5.55 ± 0.31 (5)	4.67 ± 0.13 (5)	4.50 ± 0.11 (5)
Zaprinast	5.14 ± 0.17 (3)	4.52 ± 0.27 (7)	4.00 ± 0.01 (4)

All of the drugs examined relaxed HTSM in a concentration-dependent manner. Consistent with that reported in other tissues the relaxant potency of the PDE inhibitors was inversely related to the degree of MCh-induced tone (Table I). In tissues that were pre-contracted with both 1 and 10 μM MCh the rank order of potency was: rolipram > SK&F 94120 > denbufylline > zaprinast (Table I). A similar rank order of potency was obtained when the PDE inhibitors were examined on spontaneous tone (Table I) except that rolipram was essentially inactive (8.9 ± 10.7% relaxation at 100 μM).

It is concluded that selective inhibitors of PDE isoenzymes III, IV and V are effective relaxants of HTSM and that their actions are subject to functional antagonism when MCh is used as spasmogen. At the present time we can provide no explanation why rolipram in unable to relax spontaneous tone in HTSM but it is likely to be due to a property of rolipram other than PDE inhibition since denbufylline and other PDE IV-selective inhibitors (unpublished observations) were spasmolytic under identical conditions.

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54P FUNCTIONAL AND BIOCHEMICAL CHARACTERISATION OF PDE ISOENZYMES REGULATING INHERENT TONE IN HUMAN PERIPHERAL AIRWAYS

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Isolated human airways exhibit spontaneous, prostaglandin- and histamine-independent inherent tone which is relaxed by ß-adrenoceptor agonists (Ito *et al.*, 1989). The biochemical action of ß-adrenoceptor agonists, generally an elevation of intracellular cyclic AMP levels through the activation of adenylyl cyclase, can be mimicked by inhibition of phosphodiesterase (PDE) enzymes, which catalyse the metabolism of cyclic nucleotides. There are currently known to be five classes of PDE isoenzyme which differ in their substrate specificity, sensitivity to endogenous cofactors/inhibitors and pharmacological agents, and tissue distribution. Of these, the low K_m cyclic GMP-inhibited (PDE III), low K_m cyclic AMP-specific (PDE IV) and cyclic GMP-specific (PDE V) are sensitive to selective inhibitors including SK&F 94120 (PDE III), rolipram (PDE IV) and zaprinast (PDE V). In this study, the effects of these agents on the inherent tone of human peripheral airway smooth muscle were assessed in order to determine the involvement of cyclic nucleotides in the regulation of this tone and to identify PDE isoenzymes present within the airway smooth muscle. The isoenzyme activities (and their subcellular distribution) of the muscle were also identified through assessment of their sensitivity to selective inhibitors, cyclic GMP and calmodulin.

Human peripheral airways (inner diameter 2-5 mm) were obtained from patients undergoing thoracotomy for lung cancer. Functional investigations were performed with rings in a superfusion system as described (Coleman & Nials, 1989). PDE isoenzyme activities were determined in cytosolic and particulate fractions of tissues homogenates, as described (Schudt *et al.*, 1991).

Airway tone was relaxed concentration-dependently by the non-selective PDE inhibitor IBMX (EC_{50}: 2.9 μM, n= 14), the mixed PDE III/IV inhibitor zardaverine (EC_{50}: 207 nM, n=8) and the selective PDE III inhibitor SKF 94120 (EC_{50}: 1.4 μM, n=15). The selective PDE IV inhibitor rolipram and the PDE V selective drug zaprinast caused only a fractional relaxation (32% (n=26) and 47% (n = 20), respectively, at 10 μM). PDE-catalysed cyclic AMP hydrolysis in both cytosolic and particulate fractions was inhibited by selective PDE III, IV and mixed III/IV inhibitors; cGMP hydrolysis was sensitive only to PDE V inhibitors. cAMP hydrolysis in the cytosolic fraction only was stimulated by cyclic GMP while the activity in both fractions was stimulated by calmodulin in the presence of Ca^{++}. Through analysis of these inhibitory/stimulatory patterns, tissue-specific mean activities of all 5 PDE isoenzymes could be calculated: PDE I, 11.2; PDE II, 12.7; PDE III, 18.7; PDE IV, 29.8; PDE V, 29.2 pmol min^{-1} mg $protein^{-1}$ (n = 11). Although there was some heterogeneity of absolute PDE activities between individuals and measurements, the relative proportions of isoenzymes were found to be constant. Inherent tone in human peripheral airways is most effectively relaxed by drugs that inhibit PDE III and IV. The quantity of a particular PDE isoenzyme does not predict the ability of its specific inhibitors to relax human peripheral airways.

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55P INHIBITION OF HUMAN ALVEOLAR MACROPHAGE RESPIRATORY BURST BY THEOPHYLLINE: ASSOCIATION WITH PHOSPHODIESTERASE INHIBITION

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Selective inhibitors of the low K_m, cyclic GMP-independent cyclic AMP phosphodiesterase (PDE IV) have been reported to suppress the activation of various inflammatory cells from a range of species, including guinea pig peritoneal and human blood eosinophils, human blood neutrophils and mouse and guinea pig peritoneal macrophages (see Giembycz & Dent, 1992). However, Ro 20-1724 - a selective PDE IV inhibitor - failed to affect the release of superoxide anions, thromboxanes or granule enzymes by human alveolar macrophages (AM) stimulated with opsonised zymosan (OZ) or antibodies to cell-bound IgE (Fuller *et al.*, 1988). In order to determine whether human AM are susceptible to functional inhibition by drugs acting on PDEs other than the type IV isoenzyme, the actions of the non-selective PDE inhibitor, theophylline, were assessed on OZ-stimulated oxygen radical generation by AM as well as the enzymatic hydrolysis of cyclic AMP by AM homogenates.

AM were obtained at routine bronchoscopy by bronchoalveolar lavage of the middle lobe or lingula with 5 x 20 ml normal saline. Cells were purified by differential centrifugation over discontinuous Percoll density gradients and fractions of > 90% purity (mean 93.5 $\pm$ 1.3%, n = 21) were used for subsequent experiments. OZ (1 mg 10^6 cells^{-1} 1.5 ml^{-1})-stimulated hydrogen peroxide (H_2O_2) generation and cyclic AMP PDE activity were measured as described (Dent *et al.*, 1991). Cyclic AMP was measured in trichloroacetic acid extracts of AM by radioimmunoassay, as described (Yukawa *et al.*, 1990).

The selective PDE IV inhibitors, rolipram and denbufylline, were weak inhibitors of human AM PDE (28 $\pm$ 3.8% and 30 $\pm$ 3.4% inhibiton at 10 uM, respectively, n = 3) while rolipram was also a poor inhibitor of OZ-stimulated H_2O_2 generation (22 $\pm$ 8.8% inhibition at 10 μM, n = 3). In contrast, theophylline caused a large concentration-dependent inhibition of both PDE activity (IC_{50}: 501 $\pm$ 58 μM; 92 $\pm$ 1.2% inhibition at 10 mM; n = 3) and OZ-stimulated H_2O_2 generation (IC_{50}: 476 $\pm$ 130 μM; n = 7). These IC_{50} concentrations caused an approximate 40 - 50% increase in AM cAMP content. 8-Phenyltheophylline, which has no PDE inhibitory activity, had no significant effect on H_2O_2 generation (10.4 $\pm$ 13.4% inhibition at 100 μM, n = 4, N.S. c.f. 28.0 $\pm$ 5.4% inhibition by theophylline at 100 μM, n = 7, $p < 0.05$). The adenylyl cyclase activator, forskolin, also caused a concentration-dependent inhibition of OZ-stimulated H_2O_2 generation (IC_{50}: 5.5 $\pm$ 2.3 μM, n = 3) but exhibited no synergy of action with theophylline in inhibiting OZ-stimulated H_2O_2 generation: the effect of the combination of drugs was no greater than the sum of their individual effects. It is concluded that theophylline suppresses AM function through inhibition of a PDE activity to which PDE IV makes little contribution but that there is no interaction between theophylline and forskolin, suggesting that the two drugs may elevate cAMP levels within distinct cell compartments.

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56P THE ACUTE EFFECTS OF INFUSIONS OF AMPA, NMDA AND MUSCIMOL INTO THE BASAL FOREBRAIN ON CEREBRAL FUNCTION IN THE RAT

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The nucleus basalis magnocellularis (NBM) region of the basal forebrain provides the major extrinsic cholinergic innervation of the neocortex. Excitotoxin infusions into the basal forebrain have previously been used to investigate the functional consequences of disrupting NBM-cortical projections, using tests of learning, memory and attention and by assaying cholinergic marker levels (Dunnett *et al.*, 1991).

In this study we have used [^{14}C]2-deoxyglucose *in vivo* autoradiography (Sokoloff *et al.*, 1977) to gain further insight into the effects of such interventions on cerebral function. Changes in local cerebral glucose use (LCGU) were assessed after unilateral infusions of the excitatory amino acids N-methyl-D-aspartate (NMDA, 1.5nmol) and α-amino-3-hydroxy-4-isoxazolepropionate (AMPA, 60nmol), or the GABA agonist muscimol (0.015nmol) into the NBM region of the basal forebrain in conscious rats. (Doses used have previously been shown to impair performance in behavioural tests of cerebral function after basal forebrain infusions - Muir, Everitt and Robbins, unpublished data). Saline vehicle was simultaneously infused into the corresponding region of the contralateral hemisphere. LCGU values were measured 1h after infusion of the agonists into the NBM.

Table 1 Agonist Infusions into the Basal Forebrain Resulted in Widespread Alterations in LCGU in the Hemisphere Ipsilateral to Infusion

Cortical Areas	Vehicle	AMPA	Vehicle	NMDA	Vehicle	Muscimol
Prefrontal	62 ± 3	45 ± 4**	56 ± 4	46 ± 4*	64 ± 3	60 ± 5
Frontal	68 ± 6	53 ± 5**	55 ± 3	52 ± 3	67 ± 3	63 ± 3
Sensory Motor	69 ± 7	56 ± 5**	64 ± 3	72 ± 7	73 ± 5	73 ± 5
Sub-Cortical Areas						
Ventromedial Thalamus	73 ± 4	74 ± 7	62 ± 5	77 ± 4**	70 ± 4	80 ± 2*
Entopeduncular Nucleus	39 ± 4	55 ± 5**	34 ± 4	62 ± 6**	39 ± 2	42 ± 2
Substantia Nigra compacta	42 ± 5	52 ± 5*	40 ± 2	46 ± 5*	43 ± 2	50 ± 4*

Values expressed as mean ± s.e. mean LCGU (µmol/100g/min; n=5 for each treatment group). Significant differences between treatment-infused and vehicle-infused hemispheres were assessed using Student's paired t-test:- *p<0.05, **p<0.01. There were no significant differences between the contralateral hemispheres of the three treatment groups (ANOVA).

Excitatory amino acid infusions into the NBM reduced LCGU in cortical regions receiving NBM efferents. AMPA induced more widespread cortical hypometabolism than NMDA, supporting suggestions that AMPA shows greater selectivity for cholinergic neurones in the basal forebrain (Dunnett *et al.*, 1991). All infusates caused alterations in LCGU in sub-cortical structures associated with striato-pallidal projection pathways. Results show that the sequelae of these infusions are not restricted to the NBM and its association areas. Thus, these widespread changes in cerebral function should be taken into consideration when interpreting the consequences of basal forebrain interventions.

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57P SECOND MESSENGER RESPONSES TO THE ISOMERS OF 1-AMINOCYCLOPENTANE-1,3-DICARBOXYLATE IN GUINEA-PIG CEREBRAL CORTICAL SLICES

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We have recently reported on the ability of a racemic mixture of 1R,3S- and 1S,3R-ACPD (1-aminocyclopentane-1,3-dicarboxylate) to stimulate phosphoinositide turnover, enhance A_{2b} adenosine receptor-stimulated cAMP accumulation and inhibit forskolin-stimulated cAMP accumulation in guinea-pig cerebral cortical slices (Alexander *et al.*, 1990; Alexander & Curtis, 1992; Cartmell *et al.*, 1992). In this report we describe the potency of the four stereoisomers of ACPD in assays of these excitatory amino acid (EAA) receptor(s).

Accumulation of [^{3}H]inositol phosphates and [^{3}H]cAMP from [^{3}H]inositol or [^{3}H]adenine pre-labelled slices was conducted on at least three separate occasions as previously described (Alexander *et al.*, 1990; Alexander & Curtis, 1992; Cartmell *et al.*, 1992).

Of the four stereoisomers (1R,3R-, 1R,3S-, 1S,3S-, and 1S,3R-ACPD) only 1S,3R- and 1S,3S-ACPD significantly stimulated accumulation of [^{3}H]inositol phosphates at concentrations up to 100 μM (EC_{50} values of 36.5 ± 7.6 and 75.9 ± 4.3 μM; and maximal responses of 9073 ± 386 and 5207 ± 1084 dpm over basal, respectively). Similarly, the accumulation of [^{3}H]cAMP stimulated by the adenosine analogue 5'-N-ethylcarboxamidoadenosine (NECA, 10 μM) was enhanced by 1S,3R- and 1S,3S-ACPD with EC_{50} values of 18.8 ± 3.8 and 37.5 ± 7.7 μM, respectively (maximal stimulations of 628 ± 162 and 317 ± 45 % NECA response). In comparison, 100 μM concentrations of 1R,3R- and 1R,3S-ACPD enhanced the NECA response by less than 40 %. Forskolin-stimulated accumulation of cAMP was inhibited by 1S,3R-, 1S,3S- and 1R,3R-ACPD with IC_{50} values of 2.1 ± 0.3; 0.89 ± 0.08; 237 ± 30 μM; and maximal inhibitions of 85 ± 2; 96 ± 2 and 88 ± 4 %, respectively. 1R,3S-ACPD was less potent, inhibiting the forskolin response by 51 ± 11 % at 1 mM.

The similar rank order of potency for the isomers of ACPD active at the "metabotropic" EAA receptors linked to phosphoinositide turnover and potentiation of the NECA response further strengthens the hypothesis of a causative linkage between the two responses. The distinct potency order at the receptor mediating an inhibition of forskolin-stimulated cAMP accumulation implies the involvement of a separate receptor subtype, possibly the $mGluR_2$ subtype recently cloned from rat brain (Tanabe *et al.*, 1992).

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58P INHIBITION OF INCREASED LOCOMOTOR ACTIVITY IN THE MOUSE AS A MARKER OF N-METHYL-D-ASPARTATE (NMDA) RECEPTOR ANTAGONISM

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Inhibition of NMDA-induced convulsions in mice has become a well established method of determining *in vivo* antagonist activity at the NMDA receptor complex. This is largely as a result of the observation that relatively high doses of NMDA (300mgkg^{-1}, sc) or NMDLA (500mgkg^{-1}, sc) rapidly induce a consistent and quantifiable behaviour syndrome in the mouse. Soon after injection hypoactivity, lasting about 3.5 mins, is followed by a period of increased locomotor activity or wild running, usually lasting <1.5 mins. This wild running behaviour precedes clonic convulsions (duration approx. 2.8 mins) which then develop into convulsions of a tonic nature, with death following seconds later. (eg Tricklebank *et al*, 1989 ; Koek *et al*, 1990 and own data). The ability of a compound to delay or abolish clonic and/or tonic convulsions, within a given time period, is taken as an indication of its potency as an antagonist at the NMDA receptor. We propose that by measuring an earlier, pre-convulsive response to NMDA the same information can be obtained, but under conditions which produce much less distress to the animals. The feasibility of using wild running as a means of assessing NMDA antagonist activity was investigated.

Groups of at least 8 mice were treated with one of 5 standard antagonists (injected iv in 0.9% saline) 15 mins prior to NMDA (300mgkg^{-1} s.c.) and observed for a further 15 mins. The latency to onset of wild running was recorded and the presence or absence of this behaviour was used to determine an ED_{50} (the probit analysis estimated dose at which 50% of the mice have failed to display wild running by 15 mins post NMDA injection - Table 1). Animals were killed (by cervical dislocation) at the first signs of clonic activity or at 15 mins post NMDA administration, whichever was earliest.

Table 1 Inhibition of NMDA induced wild running and comparison with reported anti-convulsant values from similar studies

Compound	Onset to wild running ED_{50} (mgkg^{-1}, iv with 95% confidence limits)		Inhibition of tonic convulsions (published data) ED_{50} (mgkg^{-1}, iv with 95% confidence limits)	
MK-801	0.12*	-	0.22	(0.15 - 0.34, Tricklebank *et al*, 1989)
CPP	1.27	(0.76 - 2.10)	4.51	(3.19 - 6.43, Tricklebank *et al*,1989)
CGS19755	1.47	(1.00 - 2.18)	-	(no studies directly comparable)
L687414	19.09	(8.50 - 42.89)	19.7	(limits not given, Saywell *et al*, 1991)
Ketamine	22.19	(13.48 - 36.54)	46.36	(33.04 - 67.51, Tricklebank *et al*, 1989)

At least 4 doses of compound were used to estimate the ED_{50}. As expected, all 5 compounds protected against NMDA induced wild running in a dose-dependent manner. Moreover, the rank order of potency agrees with that reported in the literature for the antagonism of tonic convulsions. The present data suggests, therefore, that distinctive and early pre-convulsive behavioural effects, such as wild running, can be the basis for a useful screen for identifying NMDA receptor antagonists *in vivo*. *Estimated ED_{50}

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59P THE NEUROPROTECTIVE EFFECTS OF A COMBINATION OF MK-801 AND NBQX IN FOCAL ISCHAEMIA

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The excitatory amino acids glutamate and aspartate are thought to be involved in the neuronal degeneration resulting from a period of cerebral ischaemia (Choi, 1988). In a rat model of focal cerebral ischaemia the non-competitive NMDA antagonist MK-801 and the α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA)/kainate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX; Gill and Lodge, 1991) have been demonstrated to be neuroprotective. These compounds protect against infarction in the penumbral zone of a focal lesion. Therefore we were interested in investigating the neuroprotective effects of a combination of these two antagonists in the same rat model of focal ischaemia.

Male Sprague-Dawley rats (340-380g) were anaesthetised with a mixture of 2% halothane, 30% oxygen and 70% nitrous oxide and subjected to permanent occlusion of the left middle cerebral artery (MCA) as described by Gill and Lodge (1991). The animals were allowed to survive for 24 h following which time they were perfused using 2,3,5-triphenyltetrazolium chloride (TTC) and then fixed using 10% formalin in saline. Following perfusion with TTC normal areas stained red whereas ischaemic areas remain white. The brains were cut into 9 coronal slices each being 1.5mm in thickness and photographed.

The areas of damage from each slice were mapped onto scale diagrams and measured using an image analyzer. These areas of ischaemic damage were used to determine the total volume of ischaemic damage in each brain. These same slices were also processed for histological analysis. MK-801 (0.1mg/kg, i.v., N=15) was administered immediately after MCA occlusion, NBQX (10mg/kg, i.v., N=15) was administered at the same time as MK-801 and a second dose was given 30 min later. For the combination group (N=15) the same doses of MK-801 and NBQX were administered whilst the control group (N=15) received saline and vehicle.

Permanent MCA occlusion with 24 h survival resulted in ischaemic damage in the dorsolateral cortex and in the caudate nucleus. The mean (±s.e.mean) volume of ischaemic damage in the hemisphere and cortex of the control animals was 169.8±9mm^3 and 126.1±8mm^3 respectively. MK-801 administered alone resulted in significant ($P<0.01$) protection against hemispheric (22%) and cortical (27%) ischaemic damage. NBQX administered alone was not neuroprotective with volumes of hemispheric and cortical ischaemic damage of 144.5±6mm^3 and 104.0±6mm^3 respectively. The combination treatment of MK-801 and NBQX also resulted in a significant ($P<0.01$) reduction of hemispheric (19%) and cortical (25%) ischaemic damage. There was no protection against caudate damage with any of the treatments.

In conclusion combination therapy with MK-801 and NBQX resulted in slightly less protection against infarct volume. Therefore combination of an NMDA and an AMPA/kainate antagonist did not result in a synergistic neuroprotective effect.

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60P NEUROPROTECTIVE PROPERTIES OF PHENYTOIN IN A MOUSE MODEL OF FOCAL CEREBRAL ISCHAEMIA

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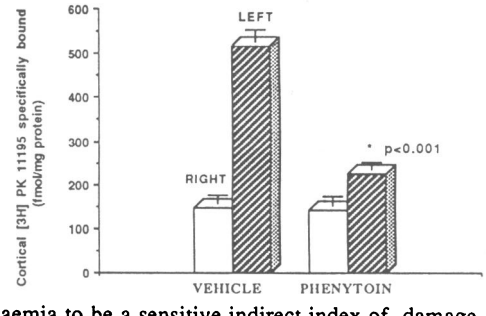
In models of cerebral ischaemia several different approaches have been described which interfere with the precipitous sequence of events leading to a failure of ion homeostasis, neuronal depolarisation and ischaemic cell death including the proposed use of calcium antagonists and NMDA antagonists (Siesjo, 1988). In the present series of experiments we have assessed an alternative neuroprotective strategy by using phenytoin, a compound thought to be anticonvulsive on the basis of its ability to selectively attenuate neuronal excitabilty due to an interaction with neuronal Na^+ channels (Rogawski & Porter, 1990).
Adult male mice (Swiss CD-1, 30-50g) were anaesthetised with pentobarbitone (100 mg/kg i.p.) and the left middle cerebral artery (MCA) exposed by a burr-hole craniectomy and coagulated by bipolar diathermy. Ischaemic damage was assessed in membranes derived from ipsi- and contralateral cortices at various time points post-ischaemia using the ligand [^{3}H]-PK 11195 as previously described (Benavides *et al.*, 1990). This ligand is a marker of peripheral-type benzodiazepine (PTB) sites present on non neuronal cells such as microglia and macrophages which are markedly elevated in brain tissue following cerebral ischaemia. The density of PTB sites increased as a function of time and was maximal at 7 days post-ischaemia (Table 1). This increase was significantly reduced by phenytoin (28 mg/kg i.v.) administered 30 min and 24 hr post-ischaemia compared to vehicle control ($p<0.001$, Figure 1).

TISSUE	n	Kd (nM)	Bmax (fmol/mg protein)
ISCHAEMIC LEFT CORTEX			
Sham control (7 days)	5	0.21 ± 0.05	170 ± 28
24 Hr	5	0.15 ± 0.13	208 ± 60
72 Hr	6	0.18 ± 0.13	462 ± 49*
7 days	5	0.20 ± 0.05	746 ± 28**
NON ISCHAEMIC RIGHT CORTEX			
Sham control (7 days)	5	0.20 ± 0.07	154 ± 32
24 Hr	5	0.27 ± 0.11	152 ± 42
72 Hr	6	0.20 ± 0.10	238 ± 34
7 days	5	0.18 ± 0.07	186 ± 25

Table 1. Temporal increase in [^{3}H] PK 11195 saturation binding parameters.
* $p<0.05$, **$p<0.01$ (ANOVA, Dunnett's t test) compared to sham control.

Figure 1. The protective effect of phenytoin in mouse MCA occlusion model



These data demonstrate the increase in PTB site density following cerebral ischaemia to be a sensitive indirect index of damage. The efficacy of phenytoin in this model, at an effective anti-convulsant dose (McNamara *et al.*, 1989), indicates that a reduction of neuronal excitablity might prove to be a useful neuroprotective mechanism for agents interacting with neuronal Na^+ channels.

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61P IS THERE A REAL POLYAMINE ANTAGONIST?

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Overactivation of the NMDA receptor macrocomplex is thought to contribute to the neuronal injury associated with conditions such as ischaemia, epilepsy and neurodegenerative diseases (Uematsu *et al.*, 1991). Several laboratories have reported evidence which suggests that polyamines may be positive modulators of the NMDA receptor complex acting through a distinct binding site (Williams *et al.*, 1991, Ogita & Yoneda, 1990). Recently there has been much interest in the development of novel polyamine antagonists. Among the compounds proposed as antagonists at the polyamine site are arcaine, 1,10-diaminodecane, diethylenetriamine and ifenprodil. Injection of 100 μg of the polyamine spermine into the cerebral ventricles of mice produces tremor which worsens with time and eventually terminates in tonic convulsions, usually within 8 hours of administration (Anderson *et al.*, 1975). In the present experiments the ability of the putative antagonists to prevent these behavioural effects was assessed. The results obtained show that arcaine and 1,10-diaminodecane were the only agents which gave any protection against spermine induced convulsions with arcaine being the most effective. Maximum inhibition was found with 25 μg icv of arcaine. A higher dose (50 μg) also gave some protection but was less effective. 1,10-diaminodecane, 30 μg icv, showed a small effect, but the antagonism was lost at a dose of 40 μg icv. These effects are illustrated by a reduction of the spermine response at 5.5h to a level of 58 ± 10% ($p < 0.01$, $n = 25$) and 65 ± 15% ($p < 0.01$, $n = 19$) by 25 and 50 μg arcaine respectively, and to 72 ± 14% ($p < 0.1$, $n = 12$) by 30 μg 1,10-diaminodecane. In the light of these findings arcaine and 1,10-diaminodecane may be partial agonists rather than antagonists at the polyamine site. Ifenprodil and diethylenetriamine were ineffective at inhibiting spermine induced convulsions. The lack of effect of ifenprodil may indicate that the convulsions are not mediated through the NMDA site. It is concluded that none of the compounds tested are effective polyamine antagonists. However, the finding of some antagonism by arcaine and 1,10-diaminodecane does suggest that an effective antagonist with therapeutic potential may eventually emerge.

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62P MECHANISM OF THE ANTICONVULSANT ACTION OF DEXTROMETHORPHAN ANALOGUES IN RAT BRAIN

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Dextromethorphan (DM), a non-opioid cough suppresant, has recently been discovered to possess anticonvulsant and neuroprotective properties (Tortella *et al.*, 1989); however, its mechanism of action has not yet been elucidated. DM binds to high affinity sites in guinea-pig and rat brain, and with lower affinity to the NMDA receptor-associated phencyclidine (PCP) site. DM is rapidly metabolised to dextrorphan (DX), which could mediate the anti-convulsant and neuroprotective properties of DM *in vivo*.

To investigate further the mechanism of the anticonvulsant action of DM, a series of (+)-3-substituted-17-methylmorphinans have been synthesised (Table 1). These compounds are either not expected to be metabolised, or to do so at a reduced rate as compared to DM. Anticonvulsant potency of the DM analogues was assessed using the rat supramaximal electroshock (MES) model (Tortella *et al.*, 1986) and compared with the binding profile at the DM and PCP sites in rat brain membranes, using [^{3}H]DM (Craviso *et al.*, 1983), and [^{3}H[thienylcyclohexylpiperidine ([^{3}H]TCP; Monahan *et al.*, 1989) respectively.

DM, DX and compounds, 5, 14 and 15 possessed potent anticonvulsant activity with ED_{50} values in $\mu molkg^{-1}$ (95% confidence limits), of 109 (54-211), 12 (3-69), 76 (49-116), 17.4 (9-33.2) and 9.4 (5.5-15.9) respectively, and providing 70-100% protection. Compounds 7, 16 and 17 were less potent and gave only partial protection (30-40%) against seizures at the highest doses used (40-50$mgkg^{-1}$). Compounds 3, 6, 8, 9 and 11 were inactive at these doses. All of the analogues with the exception of 3 and 5 (>10μM) bound with appreciable affinity to the [^{3}H]DM site in rat brain membranes, with 14 (0.42 ± 0.06μM) and 15 (0.88 ± 0.18μM) being equipotent with DM (0.59 ± 0.12μM), and the other compounds ranging from 1-4μM. For compounds with demonstrable *in vivo* activity, there was a good correlation between anticonvulsant activity and potency at the DM binding site in rat brain. The lack of anticonvulsant activity of 6, 8, 9 and 11 may be due to lack of brain penetration or differences in DM receptor sub-types. With the exception of DX, 5 and 8, all the compounds were more than 10-fold less potent at the PCP site than the DM site. It is concluded that binding to the DM site may mediate the anticonvulsant activity of the DM analogues 7, 14, 15, 16 and 17 and that metabolism to DX may not necessarily be required for DM to produce its effects *in vivo*.

Table 1: 3-substituted-17-methylmorphinans

DM - OCH_3	8 - Cl
DX - OH	9 - NCS
3 - 2-phenyl-4-quinazolinyloxy	11 - H
5 - NH_2	14 - OCH_2CH_3
6 - $NHCH_3$	15 - $OCH(CH_3)_2$
7 - $N(CH_3)_2$	16 - $OCH_2CH_2CH_2CH_3$
	17 - OCH_2Ph

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63P GLUTAMATE-INDUCED CHANGES IN NICOTINAMIDE ADENINE DIPHOSPHONUCLEOTIDE DIAPHORASE ACTIVITY IN PRIMARY CULTURES OF EMBRYONIC RAT GLIA DERIVED FROM CEREBELLUM AND CEREBRAL CORTEX

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Nicotinamide adenine diphosphonucleotide (NADPH) diaphorase histochemistry labels a number of discrete populations of neurones throughout the CNS. Interest in this enzyme has been renewed recently by evidence suggesting that NADPH diaphorase is the same as nitric oxide synthase (NOS) and that NOS fully accounts for NADPH diaphorase staining (Hope *et al.*, 1991, Dawson *et al.*, 1991, Bredt *et al.*, 1991). However the function of this enzyme in the brain has remained elusive.

NADPH diaphorase histochemistry was used to examine the localisation and activity of this enzyme in primary cultures of embryonic rat glia. Cultures were incubated in a reaction mixture containing the tetrazolium salt nitro blue tetrazolium (NBT) and nicotinamide adenine diphosphonucleotide (NADPH) as a substrate at 37°C for 15-30 min. After staining for NADPH diaphorase, cultures were fixed and counterstained with antibodies to confirm the identity of the cells. Hoechst 33258 was used to stain nuclei for counting purposes.

Most of the glial cells had the morphological appearance of type I astrocytes, with 20% staining for glial fibrillary acidic protein (GFAP). Thirty percent of GFAP-positive cells (22 DIV) were NADPH diaphorase-positive, although NADPH diaphorase staining did not correlate with the expression of GFAP since 35% of the GFAP-negative cells were also NADPH diaphorase-positive. Some non-neuronal cells also stained for fibronectin, however these did not contain NADPH diaphorase.

The effect of 500 μM glutamate applied to the culture medium for 5 min was examined after 24 hours. Glutamate caused an increase in the number of glial cells staining for NADPH diaphorase (from 30.1 ± 2.9% to 55.6 ± 4.4% in cortical cultures(N=8), and from 32.7 ± 0.3% to 56.3 ± 0.6% in cerebellar cultures (N=8)). The increase in NADPH diaphorase activity was independent of $[Ca^{2+}]_o$ and could not be blocked by MK801 (10 μM). Biochemical quantification of the effect of glutamate on NADPH diaphorase activity, using homogenates of glial cultures confirmed these findings. Pre-incubation with 1 μM dexamethasone blocked the glutamate-induced increase in the number of NADPH diaphorase-positive glial cells, although dexamethasone alone did not have any effect.

These results show that NADPH diaphorase is expressed in a sub-population of glial cells in culture. Such staining in glial cells has not been previously reported. NADPH diaphorase activity in glial cells can be altered by exogenous application of glutamate. This action of glutamate is independent of $[Ca^{2+}]_o$ and can be blocked by dexamethasone but not by MK801. In light of recent evidence showing that NADPH diaphorase is an NOS, these results suggest that there is an inducible form of NOS present in glial cells.

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64P REGIONAL EFFECTS OF DIZOCILPINE ON DOPAMINE AND ITS METABOLITES IN RAT HIPPOCAMPUS AND STRIATUM

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Dizocilpine is a potent antagonist at the N-methyl-D-aspartate (NMDA) receptor associated ion channel. Dizocilpine induces a characteristic motor syndrome in rodents which is considered to be mediated by the dopaminergic system. The effect of dizocilpine on striatal extracellular dopamine (DA) has been studied using the technique of intracerebral dialysis, but there has been no consensus on the effect of this drug on this parameter. Thus increases, decreases and no change in dialysate DA have been seen after dizocilpine treatment. In the present study we have examined the effect of dizocilpine on extracellular DA and its metabolites DOPAC and HVA in the striatum and ventral hippocampus using intracerebral microdialysis, and in addition concurrently monitored dizocilpine induced circling.
Male Wistar rats (280-350g) were anaesthetised with chloral hydrate (400 mg/kg) and concentric dialysis probes were implanted into the striatum or ventral hippocampus. The following day rats were dialysed with arteficial cerebrospinal fluid and samples collected every 30 min. Injections of saline or dizocilpine (0.25 or 0.50 mg/kg, i.p.) were given 120 min after sample collection. DA, DOPAC and HVA were analysed using HPLC with electrochemical detection.

TREATMENT	DIALYSATE DA, DOPAC AND HVA (pmols/10μl)					
	STRIATUM			HIPPOCAMPUS		
	DA	DOPAC	HVA	DA	DOPAC	HVA
SALINE	0.36 ± 0.02	19.2 ± 1.7	25.4 ± 2.6	0.06 ± 0.01	0.32 ± 0.11	0.50 ± 0.06
DIZOCILPINE (0.25 mg/kg)	0.36 ± 0.02	23.8 ± 2.6	20.8 ± 1.5*	0.25 ± 0.02	0.53 ± 0.24	1.01 ± 0.17*
DIZOCILPINE (0.50 mg/kg)	0.26 ± 0.03*	27.1 ± 1.4*	17.1 ± 1.4*	0.57 ± 0.06*	0.45 ± 0.11	1.72 ± 0.34*

Table 1. Effects of dizocilpine on extracellular DA and its metabolites. Figures show maximal changes, generally seen after 60 min, except in the case of striatal HVA when data is from 150 min after drug. Mean ± S.E.M, n = 6 (* $p<0.05$ v saline).
It is apparent that dizocilpine has regionally specific effects on DA in the extracellular space, as well as distinct effects on the concentrations of its metabolites. In addition to the neurochemical changes observed dizocilpine induced intense circling behaviour at both doses used, in spite of the decrease in extracellular DA observed in the striatum. Since an imbalance in striatal extracellular DA in the striatum is considered to lead to circling in rodents it seems likely that the damage induced by the unilateral dialysis probe implantation underlies the circling behaviour observed in this and other dialysis studies using dizocilpine.

65P EFFECTS OF CLOZAPINE ON BEHAVIOURAL RESPONSES TO THE NEW SELECTIVE D_1 DOPAMINE RECEPTOR AGONIST A 68930: INVOLVEMENT OF A D_1 RECEPTOR SUBTYPE?

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Clozapine is a widely researched atypical antipsychotic agent which differs from typical neuroleptics in demonstrating both superior efficacy and fewer extrapyramidal side effects (Kane et al., 1988). Unlike typical neuroleptics, which block D-1 and D-2 dopamine receptors non-selectively or else block D-2 receptors selectively, clozapine has only modest affinity for both D-1 and D-2 receptors yet may exert some preferential attenuation of D-1 receptor-mediated function (Murray & Waddington, 1990). To further clarify these issues, we have compared the effects of clozapine on behavioural responses to the new selective D-1 agonist A 68930 (Daly & Waddington, 1991) and the selective D-2 agonist RU 24213 (Daly & Waddington, 1992a).

Using previously described procedures (Daly & Waddington, 1992b), rats (n = 8 per group) were challenged with A 68930 (0.25 mg/kg s.c.) or RU 24213 (15.0 mg/kg s.c.) after pretreatment with clozapine (4.0-36.0 mg/kg s.c.) or vehicle, and resultant behavioural responses assessed; the selectivities of these agents for D-1 vs D-2 receptors were confirmed in radioligand binding studies using ^{3}H-SCH 23390 and ^{3}H-spiperone, respectively. Sniffing and vacuous chewing responses to A 68930 were not significantly antagonised by clozapine, and indeed clozapine tended to enhance the vacuous chewing response; however, clozapine readily blocked (-98%, $P<0.01$) intense grooming induced by A 68930. The sniffing (-31%, $P<0.01$) and locomotor (-41%, $P<0.05$) responses to RU 24213 were antagonised only modestly by these same doses of clozapine.

These results suggest that clozapine exerts only modest antagonism of D-2-mediated function at doses which can essentially abolish D-1-mediated function. However, this action of clozapine was exerted only on intense grooming but not on vacuous chewing, and our previous studies have suggested that these two responses to A 68930 are mediated via distinct 'D-1-like' receptor subtypes (Daly & Waddington, 1992c). Thus, clozapine may preferentially attenuate function mediated by a subtype of D-1 receptor, though apparently at a level beyond the D-1 receptor recognition site.

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66P A77636, A FULL D_1 DOPAMINE AGONIST, REVERSES MPTP-INDUCED MOTOR DEFICITS IN COMMON MARMOSETS

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The D-1 partial agonist SKF 38393 does not reverse parkinsonian motor deficits in MPTP treated primates (Nomoto et al., 1985). However, another D-1 partial agonist CY 208-243, was effective in this model (Temlett et al., 1988). Recently, a centrally active full D-1 agonist, A77636, ([1R,3S],3-(1-adamantyl-3-,4-dihydro-5,6-dihydroxy-1H-2-benzopyran hydrochloride) was described (Kebabian et al., in press). In binding studies A77636 was some 32-fold more selective for the D-1 than the D-2 receptor. A77636 was more potent than dopamine in stimulating adenylate cyclase in rat striatum. We now report on the actions of A77636 in MPTP treated common marmosets.

Three months before administration of A77636, adult common marmosets were treated with MPTP (10mg/kg over 5 days) until a clear parkinsonian state developed. The severity of the parkinsonian symptoms during A77636 treatment was rated using a scale of 0 (normal) to 14 (marked motor deficits). Motility was measured automatically and some experiments were video recorded. Subcutaneous administration of A77636 (0.3-3.0 µmoles/kg) increased motor activity in a dose-related manner. There was a decrease in motor disability scores at 1.0 or 3.0 µmoles/kg. Similarly, oral administration of A77636 (4.0-25.0 µmoles/kg) produced a reversal of both motor deficits and disability scores. Some salivation but no obvious nausea or vomiting was observed and no other abnormal behavioral changes were found following oral or subcutaneous administration of A77636. Repeated oral administration of A77636 (4.0 µmole/kg on days 1, 3, 5 and 7) produced some tolerance to the locomotor enhancing actions of the drug with a corresponding increase in disability scores.

Table 1 :Total locomotor counts or disability scores (± s.e.m, n=4) for A77636 (0.3-3.0 µmoles/kg, subcutaneously) in 10hr or (4.0-25.0 µmoles/kg, orally) in 30hr . (*$p<0.05$, compared to vehicle : Student's t-test).

Subcutaneous	Vehicle	0.3	1.0	3.0
Locomotor Counts	5,129 ± 1,079	8,908 ± 2,077	13,525 ± 1,045*	21,314 ± 1,476*
Disability Scores	10.0 ± 0.4	7.4 ± 0.3	5.4 ± 0.15*	2.9 ± 0.2*
Oral	**Vehicle**	**4.0**	**8.0**	**25.0**
Locomotor Counts	8,955 ± 1,352	21,006± 6,006*	28,944 ± 5,398*	30,329 ± 5,104*

Oral or subcutaneous administration of A77636 produced a prolonged, dose-dependent reversal of MPTP-induced motor deficits in common marmosets. These results may indicate a role for D-1 dopamine receptor agonists in the treatment of parkinsonism.

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67P ANTIMUSCARINIC ACTIVITY INCREASES THE FUNCTIONAL EXPRESSION OF D_1 RECEPTOR ANTAGONISM

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The specific D_2 receptor antagonists, (-) sulpiride and raclopride inhibit apomorphine-induced climbing (ACT; a D_1/D_2 effect) in MF1 mice at doses which fail to inhibit SK & F 38393-induced grooming (a D_1 effect) in C3H/He/mg mice (a strain of mice in which a high incidence of grooming is induced by SK & F 38393). Preliminary experiments indicated that the relative expression of the D_2 or D_1 behaviours could be modulated by antimuscarinic agents. We have now examined in more detail the effect of antagonists for these receptors, either alone or in combination, in ACT and SK & F 38393 - induced grooming paradigms.

Both grooming and ACT were observed over a 20 min period essentially as described for ACT by Broekkamp et al., 1990. In grooming experiments, apomorphine was substituted by SK & F 38393 (20mgkg^{-1}) and the total time spent grooming recorded.

Table 1 Inhibition of apomorphine-induced climbing or SK & F 38393-induced grooming by test compounds

Compound	$ED_{50}\pm$s.e.mean in ACT (mgkg^{-1})	$ED_{50}\pm$s.e.mean in Grooming (mgkg^{-1})
SCH 23390	0.013±0.002	0.017±0.002
(-) Sulpiride	6.5±1.1	>>22 (Grooming increased)
Raclopride	0.34±0.06	>>1.0
Chlorpromazine	0.34±0.09	1.20±0.71
Chlorpromazine + Atropine	0.88±0.14	0.93±0.02
Clozapine	2.81±0.11	1.57±0.12
Atropine	Climbing increased (1-10mgkg^{-1})	2.41±0.19

The combination of atropine (1mgkg^{-1}) and chlorpromazine (0.22mgkg^{-1}) decreased grooming by 53±4%, this was a significantly greater ($p<0.001$) inhibition than observed with either compound alone (25±6% and 6.5±4.2%, respectively).

These results indicate that additional antimuscarinic activity enhances the functional expression of D_1 receptor antagonism, at least in a drug such as chlorpromazine. The anti-muscarinic activity also decreases the ability of chlorpromazine to antagonise ACT, presumably by attenuating D_2 function in the striatum. The combination of antagonistic activity at D_1 and D_2 receptors, together with significant muscarinic antagonism, may contribute to the atypical profile of compounds such as clozapine, and further emphasises the potential role of D_1 antagonism in an atypical antipsychotic.

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68P A ^{123}I-IBZM SINGLE PHOTON EMISSION TOMOGRAPHY STUDY OF *IN VIVO* DOPAMINE RECEPTOR OCCUPANCY IN TYPICAL ANTIPSYCHOTIC RESPONDERS AND NONRESPONDERS

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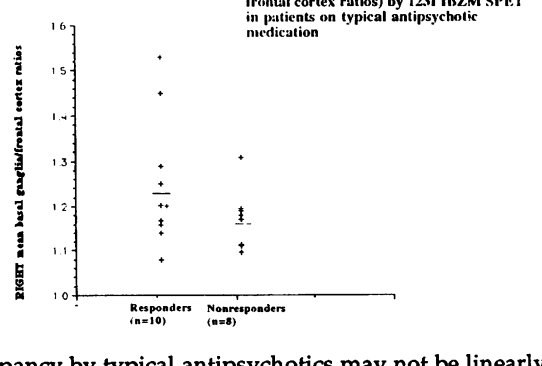
Clinical response to typical antipsychotics has been indirectly correlated with D2 receptor blockade in vivo and in vitro (Johnstone et al, 1978, Peroutka et al, 1980). We now assess this relationship directly in vivo by comparing D2 receptor occupancy determined semiquantitatively with the radioiodinated substituted benzamide ^{123}I-3-iodo-6-methoxybenzamide and single photon emission tomography in two groups of schizophrenic patients on medication, one responsive (n=10) and the other poorly responsive (n=8) to typical antipsychotics. The mean BPRS score in the responders (38.9) was significantly lower than that of the nonresponders (55.2) ($p=0.005$). The degree of occupancy was not significantly different between the two groups (see figure for example).



This suggests that the relationship between D2 receptor occupancy by typical antipsychotics may not be linearly related to clinical response and that there may be subgroups of schizophrenic patients in whom D2 receptor blockade by antipsychotics is unrelated to clinical response.

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69P A SINGLE PHOTON EMISSION TOMOGRAPHY STUDY OF *IN VIVO* DOPAMINE RECEPTOR OCCUPANCY FOR TYPICAL ANTIPSYCHOTICS AND THE ATYPICAL DRUG CLOZAPINE IN SCHIZOPHRENIA

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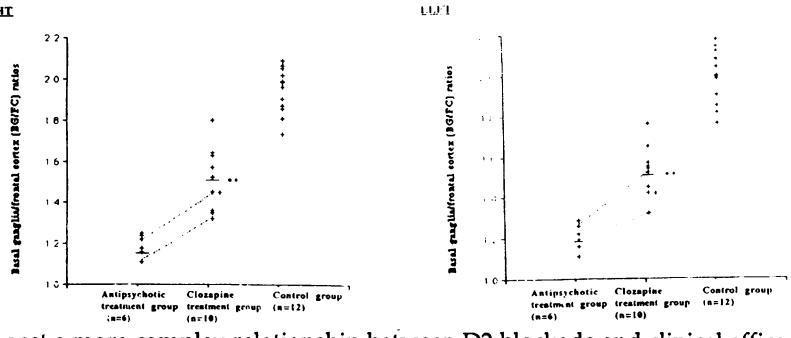
In vitro comparisons of receptor binding with clinical dosimetry suggest that the degree of D2 receptor blockade linearly correlated with antipsychotic action (Peroutka et al, 1980). This observation remains one of the most convincing arguments for the dopamine hypothesis of schizophrenia (Kerwin, 1992). However this relationship has never been tested in vivo in patients. We have used a group of poorly responsive patients to test this hypothesis using a semiquantitative determination of D2 occupancy in vivo by single photon emission tomography of the substituted benzamide ^{123}I-3-iodo-6-methoxybenzomide in two groups of patients on typical antipsychotics (n=6) or on the atypical antipsychotic clozapine (n=10) as well as age and sex matched controls (n=12). The patients on typical antipsychotics showed poor therapeutic responsiveness despite marked D2 receptor blockade. However significant improvement occurred in a similar group of poorly responsive patients on clozapine, but at a much lower level of D2 blockade (see figure for example).



These findings suggest a more complex relationship between D2 blockade and clinical efficacy than previously thought.

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70P THIOPERAMIDE, A SELECTIVE HISTAMINE H_3 RECEPTOR ANTAGONIST, INHIBITS AMPHETAMINE- AND APOMORPHINE-INDUCED LOCOMOTOR ACTIVITY IN THE MOUSE

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Histamine (HA), when administered centrally to rodents, reduces spontaneous locomotor activity (Alvarez & Guerra, 1982) and inhibits methamphetamine-stimulated locomotion (Onodera & Ogura, 1981). The H_3 receptor is a presynaptic receptor which modulates not only the release of HA in the central nervous system (CNS) but several other neurotransmitters in the CNS and periphery. HA levels in the CNS are increased by the selective H_3 receptor antagonist, thioperamide, but reduced by the selective H_3 receptor agonist (R)-α-methylhistamine (RAMH) (Arrang et al., 1987). In order to assess the role of this receptor on locomotor activity (LMA) in the mouse, we have examined the effect of administration of thioperamide and RAMH on LMA induced by a number of stimulants.

Male CRH mice (18-22g) were placed in individual activity boxes. Thirty min later animals were pretreated with RAMH and/or thioperamide (Thio) ip. After a further 30min, amphetamine (Amph; 1mgkg^{-1}), apomorphine (Apo; 2mgkg^{-1}) or cocaine (Coc; 5mgkg^{-1}) was administered sc. LMA (as photocell beam breaks) was then recorded for 90min. The data are expressed as the mean LMA score ± sem over this time period.

Thioperamide (0.2-10mgkg^{-1} ip) or RAMH (0.3-20mgkg^{-1} ip) had no effect on spontaneous LMA. However, thioperamide (2 and 10mgkg^{-1} ip) inhibited, by approximately 50%, the LMA stimulated by amphetamine or apomorphine, but not cocaine (Table 1). The inhibitory effect of thioperamide (2mgkg^{-1} ip) on amphetamine hyperactivity was completely reversed by RAMH (20mgkg^{-1} ip): mean LMA score±sem (n=23/24) for vehicle (Veh)=1152±107, Amph=2568±194, Thio/Amph=1786±203*, Thio/RAMH/Amph=2570±243$^{\#}$ (*p<0.05 vs Amph; $^{\#}$p<0.05 vs Thio/Amph).

Table 1: Effect of thioperamide on stimulant-induced LMA

	Mean LMA score ± sem 90 min after drug ip			
Drug sc	Veh	Thio [10]	Thio [2]	Thio [0.2]
Veh	900±54 (34)	817±75 (33)		
Amph [1]	*1770±97 (36)	$^{\#}$1245±69 (34)	$^{\#}$1350±74 (36)	1710±90 (33)
Veh	967±72 (27)	931±82 (28)		
Apo [2]	*1590±59 (30)	$^{\#}$1350±74 (28)	$^{\#}$1083±70 (28)	
Veh	841±117 (9)			
Coc [5]	*2229±337 (8)	3049±232 (8)	2234±323 (8)	

Unpaired t-test:
* p< 0.05 vs Veh/Veh
# p< 0.05 vs Veh/Stimulant
[mgkg^{-1}]
(number of mice)

We conclude that antagonism of central H_3 receptors inhibits the effects of some locomotor stimulants. All the stimulants used are thought to enhance dopaminergic neurotransmission. Further studies are thus required to elucidate why the effects of amphetamine and apomorphine are sensitive to H_3 receptor antagonism and those of cocaine are not.

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71P INCREASED IRON IN THE NEUROMELANIN-CONTAINING NEURONES IN THE SUBSTANTIA NIGRA ZONA COMPACTA IN PARKINSON'S DISEASE

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Degeneration of the neuromelanin-containing neurones in the substantia nigra zona compacta (SNPC) in Parkinson's disease (PD) is associated with an increase in the bulk concentration of iron in the SNPC (see Dexter et al., 1991) and it has been suggested that oxidative stress may be a cause of cell death in PD (see Boyson, 1991). We have used wavelength dispersive X-ray microanalysis (WDS) to determine the level of iron in identified, unfixed, unstained neuromelanin-containing SNPC neurones in PD and control patients.

Snap frozen, unfixed blocks of the midbrain were obtained from 8 PD patients (mean age 66 years ± 11) and from 8 age-matched control patients (mean age 68 years ± 11). SNPC neuromelanin-containing neurones were precisely located using secondary electron and cathodoluminescence images. Iron and sulphur levels were determined using WDS with background subtraction, in 12 neurones and 6 areas of surrounding neuropil for each case. Sulphur was used as an internal reference element. Iron counts were significantly increased in both the SNPC neurones and neuropil in the PD patients (Table 1). No significant differences were observed between the sulphur levels in the neurones or neuropil in the PD and control patients (Table 1).

Table 1. WDS microanalysis of iron in the substantia nigra pars compacta in PD and control patients

Area	Fe Counts/100s		S Counts/100s	
	Control	PD	Control	PD
Neuronal cell body	1,554 ± 388	2,726 ± 333*	29,411 ± 2,542	30,735 ± 2,742
Neuropil	985 ± 281	1,678 ± 438*	18,027 ± 971	17,807 ± 927

Values represent mean ± SD. *P<0.01 Mann-Whitney U test.

The increased neuronal content of iron in the SNPC in PD patients is consistent with free radical mediated oxidative stress playing a role in the pathogenesis of PD.

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72P CHLORMETHIAZOLE PREVENTS 5-HYDROXYTRYPTAMINE LOSS IN RAT BRAIN FOUR DAYS AFTER MDMA ADMINISTRATION

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Administration of chlormethiazole (CMZ) prevents the neurotoxic consequences of high dose methamphetamine administration, protecting striatal dopamine and hippocampal and cortical 5-HT neurones from neurodegeneration (Green et al, 1992). We now report that CMZ also protects against the neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA).

Male Lister Hooded rats were injected with CMZ (50mgkg^{-1}i.p.) 5 min before MDMA (20mgkg^{-1} i.p.) with a further dose of CMZ being given 55 min after the MDMA. Four days later the concentration of 5-HT and 5-HIAA in cortex and hippocampus was measured by h.p.l.c. with electrochemical detection. Appropriate saline injected control animals were also examined.

Treatment with chlormethiazole prevented the loss of 5-HT and 5-HIAA content resulting from MDMA administration (Table 1). Protection against 5-HT loss was also observed in the hippocampus (but not cortex) when a single dose of CMZ (100mgkg^{-1} i.p.) was given 20 min after injection of MDMA (20mgkg^{-1}) (Table 1). In this experiment CMZ prevented both the 5-HT mediated behavioural changes induced by MDMA and the sustained hyperthermia which also occurs (approx 2.2°C above controls).

Table1 Effect of CMZ on MDMA induced neurotoxicity

Injected	Tissue 5-HT content		Tissue 5-HIAA content	
	Hippocampus	Cortex	Hippocampus	Cortex
Saline	323 ± 9(8)	314 ± 14 (8)	325 ± 7(8)	218 ± 17(8)
CMZ	369 ± 18(6)	346 ± 21(6)	327 ± 16(6)	212 ± 11(6)
MDMA	234 ± 14(16)**	227 ± 16(16)**	249 ± 13(16)**	150 ± 7(16)**
MDMA/CMZ (1)	325 ± 13(6)N.D.	294 ± 13(5)N.D.	288 ± 16(6)*	182 ± 10(6)N.D.
MDMA/CMZ(2)	311 ± 14(6)N.D.	265 ± 11(6)**	348 ± 12(6)N.D.	193 ± 7(6)N.D.

Results (in ng indole g^{-1} tissue) shown as mean ± s.e.mean (n). (1) Injection of CMZ (50mgkg^{-1}) 5 min before and 55 min after MDMA. (2) Injection of CMZ (100mgkg^{-1}) 20 min after MDMA. Different from saline: **p<0.01; *p<0.05. N.D. = not different from saline injected.

These data therefore provide further evidence for the neuroprotective action of chlormethiazole against the neurodegeneration of monoamine pathways in brain induced by certain substituted amphetamines.

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73P [^{3}H]PD 140376: A NOVEL RADIOLIGAND FOR CCK-B/GASTRIN RECEPTORS

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Selective cholecystokinin receptor antagonists such as the dipeptoid CI-988 (Horwell et al.,1991) and the benzodiazepine derivatives Devazepide (L-364,718) and L-365,260 (Chang et al.,1986;1989), has greatly facilitated differentiation of CCK receptors into two major subtypes (CCK-A and CCK-B/gastrin). To date [^{3}H]L-364,718 and [^{3}H]L-365,260 represent the only antagonist radioligands available for labelling the CCK receptor subtypes. In this study we describe the binding characteristics of the dipeptoid [^{3}H]PD 140376 (specific activity 51 Ci/mmol, 4-amino-phenyl butanoic acid, b-[[3-(1 H-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-,[R-(R*,S*)]) to CCK-B receptors.

Guinea pig cortex (GPC) was homogenised in 20 vols ice-cold 50mM Tris-HCl (pH 6.9 at 22C) and centrifuged twice at 40,000g for 15 minutes with resuspension in buffer in between. For inhibition studies, membranes (0.1-0.2mg protein) were incubated in assay buffer (composition in mM: Hepes 10, NaCl 130, KCl 4.7, $MgCl_2$ 5, EGTA 1, and 0.25mg/ml bacitracin, pH 7.4 at 22C) containing 0.3nM [^{3}H]PD 140376, for 40 minutes at 22C, with varying concentrations of test compounds. Non-specific binding (20-30% of total binding) was defined by 1μM CCK8s. Incubations were terminated by filtration under vacuum onto GFB filters and membranes washed with 3 x 3ml ice-cold Tris-HCl. The results shown represent means of data obtained from at least 3 separate experiments.

Association studies yielded monophasic curves (k_1 = 3.04 x 10^8 $M^{-1}min^{-1}$) and showed that equilibrium was reached within 30 minutes. Dissociation curves were also monophasic, with a k_{-1} of 0.028 ± 0.002 min^{-1}. The kinetic K_D (0.094 ± 0.013 nM) agreed closely to the K_D obtained from saturation studies (0.11 ± 0.02nM), and with the K_i for the inhibition of[^{3}H]PD 140376 binding by PD 140376 itself. Linear Scatchard plots indicated binding to a single saturable site with a Bmax of 119 ± 15 fmol/mg protein. The agonists CCK8s, CCK8us, gastrin, pentagastrin, CCK4, and caerulein all displaced [^{3}H]PD 140376 binding in GPC with K_i values of 0.12, 29, 25, 22, 190 and 2.6 nM respectively. Similarly, the CCK-B/gastrin receptor antagonists L-365,260, CI-988, and PD 140376,inhibited binding with high affinity (K_i values 1.5, 1.7, and 0.16 nM respectively). In contrast, the CCK-A selective receptor antagonists lorglumide, Devazepide and PD 140548 (Horwell et al.,1991a) possessed lower affinity (K_i values 360, 79 and 400nM respectively). These results correlate well with [^{125}I]BHCCK8s binding to CCK-B receptors in GPC, suggesting that [^{3}H]PD 140376 is binding to the same site. In support of this, autoradiographical studies showed that the distribution of [^{3}H]PD 140376 binding sites in sections of guinea pig brain was similar to distribution of [^{125}I]BHCCK binding sites.

In summary, [^{3}H]PD 140376 is a novel radioligand which binds with extremely high affinity and selectivity to the CCK-B/gastrin receptor, thus providing a useful and alternative tool to the benzodiazepine derivative [^{3}H]L-365,260.

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74P ALUMINIUM INHIBITS MUSCARINIC AGONIST-STIMULATED INOSITOL 1,4,5,-TRISPHOSPHATE PRODUCTION AND CALCIUM RELEASE IN PERMEABILISED SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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Aluminium causes an encephalopathy, anaemia and osteodystrophy in long-term renal dialysis patients exposed to aluminium-rich diffusate and antacids (Alfey, 1984). Aluminium has also been implicated as contributing to the cause of Alzheimer's disease (Candy et al., 1986). The mechanisms by which aluminium could exert such a veriety of effects remains uncertain. However, it has been hypothesised that aluminium could affect the phosphoinositide-mediated signalling pathway by binding to vicinal phosphate groups on inositol 1,4,5-trisphosphate ($InsP_3$), thus altering the kinetics of its metabolism and binding to its receptor (Birchall & Chappell, 1988). Here we describe studies using electropermeabilised human SH-SY5Y neuroblastoma cells in which we have determined the effect of Al^{3+} on carbachol-induced $InsP_3$ production and Ca^{2+} release.

Results of experiments using a Ca^{2+} electrode showed that $AlCl_3$ (1-1000μM) had no effect on $InsP_3$-induced Ca^{2+} release but that >30μM $AlCl_3$ significantly reduced carbachol-induced Ca^{2+} release. In experiments in which carbachol-induced $^{45}Ca^{2+}$ release was assessed, the IC_{50} for Al^{3+} was approximately 90μM. The EC_{50} of carbachol as a stimulus of $^{45}Ca^{2+}$ release (1.9 x 10^{-6}M) was not altered by 100μM $AlCl_3$. However, maximal release of $^{45}Ca^{2+}$ dropped from 64% (control) to 24% in the presence of 100μM $AlCl_3$. There was no significant effect of $AlCl_3$ (up to 1mM) on $InsP_3$-induced $^{45}Ca^{2+}$ release. $AlCl_3$ also inhibited $InsP_3$ production with an IC_{50} of approximately 15μM. 100μM dimethyl hydroxypyridin-4-one (CP20), a potent aluminium chelator (K_s=31), was able to abort and reverse (after 1/2hr incubation) the effects of $AlCl_3$ on both Ca^{2+} release and $InsP_3$ production.

These data suggest that, in permeabilsied cells, the effect of aluminium on the phosphoinositide-mediated signalling pathway is at the level of phosphatidylinositol 4,5-bisphosphate hydrolysis. This may reflect interference with receptor-G protein-phospholipase C coupling (e.g. through a competition of Al^{3+} with Mg^{2+} at the GTP-binding site of the G protein) or an interaction with the lipid vicinal 4,5 phosphates.

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75P THE AFFINITY OF 8-CYCLOPENTYL-1,3-DIPROPYLXANTHINE AT A_1 ADENOSINE RECEPTORS OF GUINEA-PIG CEREBRAL CORTEX

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In guinea-pig cerebral cortical slices, adenosine analogues stimulate cAMP accumulation (through A_{2b} receptors) and selectively potentiate histamine-stimulated phosphoinositide turnover (through an unidentified adenosine receptor, A_x; Alexander *et al.*, 1989). We have previously observed that the antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) exhibited ten-fold selectivity at these receptors (K_i A_{2b} 131 nM & A_x 12 nM; Alexander *et al.*, 1989). The present report describes an examination of the affinity of DPCPX at A_1 adenosine receptors of guinea-pig cerebral cortex.

Accumulation of [^{3}H]cAMP in [^{3}H]adenine-pre-labelled guinea-pig cerebral cortical slices was assayed as previously described (Alexander *et al.*, 1989). Radioligand binding of [^{3}H]DPCPX was carried out over 90 minutes at 20 C in 50 mM Tris buffer, 1 mM EDTA, 0.01 % Triton X-100, pH 7.4 containing 1-2 U/mL adenosine deaminase, defining non-displaceable binding with 1 mM theophylline. Radioligand bound to a 30 000 g particulate preparation from cerebral cortex was collected by rapid vacuum filtration over Whatman GF/B filters. At least three separate preparations were used for affinity determinations.

The adenosine analogue N^6-cyclopentyladenosine (CPA) inhibited forskolin (30 μM) -stimulated cAMP accumulation in cerebral cortical slices in a concentration-dependent manner (IC_{50} 16 ± 1 nM). The presence of DPCPX shifted the concentration-response curve to CPA to the right in an apparently parallel fashion to allow estimation of the apparent affinity constant of 6 ± 1 nM. Analysis of saturation isotherms of [^{3}H]DPCPX binding to cerebral cortical membranes indicated a single binding site with high capacity (B_{max} 1560 ± 278 fmol/mg protein) and with high affinity (K_d 4.2 ± 0.4 nM). DPCPX displaced [^{3}H]DPCPX (2-3 nM) binding with high potency (K_i 4.4 ± 1.9 nM) in an apparently monophasic manner. The agonist CPA displaced [^{3}H]DPCPX in a biphasic manner, with calculated K_i values of 7.3 ± 2.5 and 450 ± 87 nM, the latter site constituting 65 ± 8 % of [^{3}H]DPCPX binding.

We conclude that the affinity of DPCPX for A_1 receptors of guinea-pig cerebral cortex is comparable in both functional and radioligand binding assays. The similarity of affinities for DPCPX at functional A_1 and A_x receptors, together with the lack of evidence for multiple antagonist radioligand binding sites, raises the possibility that the two receptors are identical, although it is also likely that two binding sites of such similar affinities would not be distinguished. The similarity of the affinities of CPA in displacing [^{3}H]DPCPX appears to align the high affinity site (7 nM) with the A_1 receptor (16 nM) and the low affinity site (450 nM) with the A_x receptor (410 nM; Hill & Kendall, 1987). It is tempting to speculate therefore that whereas the antagonist DPCPX is unable to distinguish between these receptors, the agonist CPA may be useful to define these receptors in radioligand binding studies. However, the question of the identity of the A_x receptor must await further investigation.

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76P CAN Ni^{2+} BE USED TO DEFINE THE COMPONENT DEPENDENT ON Ca^{2+} ENTRY IN HISTAMINE-INDUCED INOSITOL PHOSPHATE FORMATION IN BRAIN?

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Histamine-induced inositol phosphate (IP) formation in mouse and rat cerebral cortex is strongly dependent on the extracellular concentration of Ca^{2+} (Alexander *et al.*, 1990). The same is true, but apparently to a lesser extent in guinea-pig cerebral cortex and cerebellum. The analysis of these responses would be aided if it were possible to selectively block Ca^{2+} entry. The utility of divalent cations such as Ni^{2+} for this purpose is uncertain, since they are effective inhibitors of [^{3}H]-mepyramine binding to histamine H_1-receptors in homogenates of guinea-pig cerebellum (Treherne *et al.*, 1991). However, it is not certain that non-cell-penetrant ions such as Ni^{2+} will inhibit histamine binding to H_1-receptors in intact tissues. The apparent lack of a Ca^{2+} influx component in histamine- and carbachol-induced IP formation in HeLa cells at extracellular Ca^{2+} concentrations above 0.3 mM (Arias-Montaño & Young, (1992) offers the opportunity to test whether Ni^{2+} inhibits H_1-receptor function in intact cells.

HeLa cells were cultured and labelled with [^{3}H]-inositol as described previously (Bristow *et al.*, 1991). Incubations with histamine or carbachol in the presence of 30 mM Li^+ were for 30 min. Cross-chopped slices of regions of guinea-pig or rat brain were incubated with [^{3}H]-inositol and 10 mM Li^+ before addition of 1 mM histamine and further incubation for 60 min. Incubations were terminated by addition of 10% perchloric acid and [^{3}H]-inositol phosphates separated by anion-exchange chromatography. Inhibition of [^{3}H]-mepyramine binding to rat cerebral cortical homogenates was measured as described by Treherne *et al.* (1991).

Ni^{2+} inhibited histamine-induced [^{3}H]-IP_1 accumulation in slices of rat cerebral cortex and guinea-pig cerebral cortex and cerebellum with IC_{50}s of 0.4 and 1 mM in rat and guinea-pig, respectively. The effect of 1 mM Ni^{2+} on the histamine concentration-response curve in rat cerebral cortex was to reduce the maximum response, consistent with non-competitive inhibition. Ni^{2+} also inhibited [^{3}H]-mepyramine binding to homogenates of rat cerebral cortex, the dependence on radioligand concentration being consistent with allosteric inhibition (IC_{50} with 0.5 nM [^{3}H]-mepyramine 0.5 mM). In HeLa cells Ni^{2+} (1 mM) had no significant effect on the concentration-response curve for carbachol-stimulated [^{3}H]-IP_1 formation. However, 1 mM Ni^{2+} caused a parallel displacement to the right of the concentration-response curve for histamine. The curve was displaced further by increasing the concentration of Ni^{2+} to 2 and 3 mM.

The inhibition by Ni^{2+} of histamine-induced [^{3}H]-IP_1 in HeLa cells, compared with the lack of effect on the response to carbachol, which shows the same pattern of Ca^{2+}-dependence, strongly suggests that Ni^{2+} exerts an inhibitory action on H_1-receptor function by acting at a site accessible from the extracellular medium. This makes it unlikely that Ni^{2+} can be used to define the Ca^{2+} entry component in histamine-induced IP formation in brain tissues.

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77P ENDOGENOUS LIPOCORTIN-1 MEDIATES DEXAMETHASONE INHIBITION OF INTERLEUKIN-1-INDUCED NEUTROPHIL ACCUMULATION *IN VIVO*

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We have recently described lipocortin-1 (LC1) and dexamethasone (Dex) as potent inhibitors of interleukin-1 (IL-1) elicited neutrophil (PMN) accumulation into the mouse air-pouch (Perretti and Flower, 1992). In the present study we have investigated the possibility that endogenous LC1 could mediate this Dex effect. We have optimized the conditions for the appearence of anti-LC1 activity in the blood of mice after treatment with a polyclonal sheep antiserum (50 μl s.c.) by using a specific ELISA (Goulding et al., 1989). A peak of anti-LC1 activity was found at 24h (titre 39,000) with levels remaining high for approximately 150h after which they declined rapidly becoming undetectable by 650h post injection. Air-pouches were formed on the back of male Swiss mice (20-22g) by injection of 2.5 ml of air on day 0 and day 3 (Perretti and Flower, 1992). Antibodies prepared against human LC1 were injected on day 5 (50 μl s.c. for non-immune, pre-immune and specific anti-LC1 sheep sera; 100 μg s.c. for mouse IgG and monoclonal antibody (mAb) 1A and 1B, Biogen, Cambridge, MA). After 24h, Dex (5 μg) was administered i.v., and 20 ng IL-1β (I.R.I.S., Siena, Italy) given locally 2h after the steroid. PMN accumulation was evaluated 4h following treatment with the cytokine by staining in Turk's solution the lavage fluids (1). IL-1β-induced migration (6.30 $\pm$ 0.56 x10^6 PMN per mouse, mean $\pm$ S.E., n=23) was reduced by 52% after Dex treatment (3.03 $\pm$ 0.33 x10^6, n=12, $p<0.01$, ANOVA followed by Bonferroni test). In mice pretreated with the sheep anti-LC1 serum Dex inhibition was significantly reduced (5.95 $\pm$ 0.55, n=12, $p<0.05$ vs. Dex-group). In non-immune and pre-immune sheep serum groups the steroid still exerted significant inhibition. Similarly, mAb 1B significantly attenuated the Dex effect (5.04 $\pm$ 0.66, n=10, $p<0.05$ vs Dex-group) whereas normal murine IgG and mAb 1A did not. Both sheep anti-LC1 serum and mAb 1B recognized murine LC1 species (36kD) in PMN collected from the pouches as evaluated by Western Blot analysis, while mAb 1A did not. In conclusion, it appears that in this model LC1 mediates the inhibitory action of Dex, and therefore this protein could be the second messenger of steroid effects on leukocyte migration in other acute inflammatory models.

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78P OXIDISED LOW-DENSITY LIPOPROTEIN INHIBITS ENDOTHELIUM-DEPENDENT RELAXATION THROUGH ACTIVATION OF PROTEIN KINASE C

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Endothelium-derived relaxing factor (EDRF) activity in isolated vascular rings is inhibited by oxidised low-density lipoprotein (LDL), but the mechanism is not clear. We have previously demonstrated that phorbol esters, acting through protein kinase C (PKC) inhibit agonist-induced EDRF release (Smith *et al.*, 1990). In the present study the specific PKC inhibitors Ro 31-8220 (3-[1-{3-amidinothio}propyl]-3-indolyl)-4-(1-methyl-3-indolyl)-1H-pyrrole-2,5-dione methanesulphonate) and Ro 31-7459 (3-[1-(3-aminopropyl)indolyl]-4-(1-methylindolyl)-1H-pyrrole-2,5-dione hydrochloride) (Davis *et al.*, 1988) were used to investigate whether oxidised LDL exerts its inhibitory effects on EDRF release through activation of PKC.

LDL obtained from hypercholesterolaemic patients by apheresis was isolated and copper oxidised as described by Jessup *et al.* (1990). Maximal acetylcholine (ACh)-induced relaxation of rabbit aortic rings *in vitro* (58.8±3.82%, m±s.e.mean, n=15) was significantly ($p<0.001$) inhibited by preincubation for 30 min with 1 mg ml^{-1} oxidised LDL (8.5±2.05%, n=6) but not by similar preincubation with 1 mg ml^{-1} native LDL (59.6±1.03%, n=8). The inhibitory effect of oxidised LDL was abolished by pretreating the tissues for 15 min with Ro 31-8220 (10^{-5} M) (ACh-induced relaxation 64.8±6.94%, n=5, $p<0.001$ cf oxidised LDL alone; $p>0.9$ cf ACh alone). Ro 31-7459 pretreatment (10^{-5} M, 15 min) was less effective, but significantly reduced the inhibitory effect of oxidised LDL (ACh-induced relaxation 27.7±3.84%, n=3, $p<0.01$ cf oxidised LDL alone). Ro 31-8220 or Ro 31-7459 pretreatment did not affect relaxations to ACh in the absence of LDL. These results indicate that oxidised LDL may, like PDB, inhibit EDRF activity through stimulation of PKC.

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79P DEXAMETHASONE INHIBITS ENDOTOXIN-INDUCED VASCULAR PERMEABILITY AND NITRIC OXIDE SYNTHASE IN THE RAT INTESTINE

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The enhancement of intestinal vascular damage induced by acute exposure to endotoxin by the nitric oxide synthase (NOS) inhibitor, N^G-monomethyl-L-arginine (L-NMMA), and its reversal by the NO donor, S-nitroso-N-acetyl penicillamine (SNAP), suggests a protective role for constitutive NOS in the intestinal microvasculature (Hutcheson et al, 1990; Boughton-Smith et al, 1990). Endotoxin can, however, over several hours induce a calcium-independent NOS which has been associated with endothelial cytotoxicity (Palmer et al, 1992). The induction of NOS in a variety of tissues is inhibited by the anti-inflammatory corticosteriod, dexamethasone (Radomski et al 1990; Salter et al 1991). Therefore, in the present study, the action of endotoxin in producing vascular permeability and NOS induction in the jejunum and colon and the effects of dexamethasone have been investigated.

E. coli lipopolysacharide (LPS, 3 mg kg^{-1} i.v.) was administered to rats (male 225-275 g) under halothane anaesthesia . Intestinal vascular damage was assessed by the vascular leakage of [^{125}I] - human serum albumin (500nCi), administered immediately before LPS (Hutcheson et al, 1990). NOS activity was determined, in the cytosol of tissue homogenates (10^3xg, 20 min), as the conversion of [^{14}C]-L- arginine (27nCi, 10 min, 37°C) to citrulline (Salter et al, 1991). LPS induced a time-dependent increase in plasma leakage in the colon and jejunum which after 5h was 76±11 & 30±6 µl plasma g^{-1} tissue, respectively, (mean±s.e.mean, n=12, P<0.01). NOS activity in control colon and jejunum of 102±6 & 25±8 pmol g^{-1} tissue (n=12), which was abolished *in vitro* by L-NMMA (300µM, P<0.01), was also inhibited by 1 mM EGTA (91±5% & 88±12% inhibition respectively, P<0.05). LPS markedly increased NOS activity in the colon and jejunum by 226±48% & 316±92% respectively (n=14, P<0.01).However, the increased intestinal NOS activity, which was abolished *in vitro* by L-NMMA, was not significantly reduced by incubation with EGTA. Pretreatment (2h) *in vivo* with dexamethasone (1 mg kg^{-1} s.c.) prevented the increase in both vascular permeability and calcium-independent NOS activity induced by LPS (n=6, P<0.01), whilst having no effect on the calcium-dependent NOS activity.

In contrast to acute endotoxin shock, where the colon was not affected (Hutcheson et al., 1990), prolonged LPS exposure therefore produces increases in vascular permeability in both the jejunum and in the colon. The induction of a calcium-independent NOS that accompanied the vascular leakage may reflect the involvement of increased NO production in the intestinal vascular damage produced by LPS. Furthermore, the vascular protection by dexamethasone *in vivo* may depend on inhibition of NOS induction.

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80P THE NATURE OF ENDOTHELIAL CELL-DEPENDENT VASORELAXATIONS INDUCED BY N^G-HYDROXY-L-ARGININE

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Endothelium-derived relaxing factor (EDRF) has been identified as nitric oxide (·NO) derived from the guanidino group of L-arginine (L-Arg; Palmer et al., 1988). N^G-hydroxy-L-arginine (L-HOArg) is an enzymatic intermediate between L-Arg and ·NO (Stuehr *et al.*, 1991). Wallace *et al.* (1991) have found L-HOArg to be a potent endothelium-dependent vasorelaxant in vessels with an intact endothelium. We (Zembowicz *et al.* (1991) have recently shown that L-HOArg releases from cultured endothelial cells a relaxing factor, the formation of which is not blocked by inhibitors of nitric oxide synthase (NOS). We have investigated the vasorelaxant properties of this factor using cultured bovine aortic endothelial cells as donor and rabbit aortic strips mounted in the Vane cascade as detector tissues.

The relaxations induced by L-HOArg (10 or 30 µM) were potentiated in the presence of superoxide dismutase (SOD; 10 U/ml; n=6). N^G-nitro-L-arginine (L-NO_2Arg; 10 or 30 µM; n=6) did not block those relaxations, whereas oxyhaemoglobin (Hb; 1-10 µM; n=4), given in the same experiment, did. When EC were perfused with calcium-free Krebs' solution, there was no change in the relaxations evoked by L-HOArg (n=3). In experiments using EC cultured in the presence of dexamethasone (1 µM), the relaxations caused by L-HOArg were not different from control (n=4).

We conclude that: (a) L-HOArg causes endothelial-dependent relaxations which are potentiated by SOD and blocked by Hb, but not L-NO_2Arg; (b) L-HOArg is metabolised by EC via a pathway which does not involve an inducible enzyme, is not dependent on extracellular calcium and is not blocked by the inhibitors of NOS. **(This work has been supported by a grant from Glaxo Group Research Ltd.)**

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81P ACTIVATION OF CONSTITUTIVE AND CYTOKINE-INDUCIBLE NO SYNTHASE ACCOUNTS FOR THE REDUCED PRESSOR RESPONSE TO NORADRENALINE IN ENDOTOXIN SHOCK

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The role of nitric oxide (NO) and the importance of the induction of NO synthase (NOS) in the development of vascular hyporeactivity to noradrenaline (NA) was investigated in a model of circulatory shock induced by endotoxin (lipopolysaccharide, LPS) in anaesthetized rats.

Mean arterial blood pressure (MAP) and heart rate were measured via a carotid arterial cannula in rats anaesthetized with sodium thiopental (120 mg/kg). Calcium-dependent and calcium-independent NOS activity was measured in the presence of NADPH, calmodulin and tetrahydrobiopterin by the conversion of ^{3}H-arginine to ^{3}H-citrulline in homogenates from several organs from vehicle- and LPS-treated rats.

E. Coli LPS (10 mg/kg; i.v. bolus) caused a rapid (within 5 min), fall in MAP (to 60±6 mmHg, n=7, $p<0.01$). At 30, 60, 120 and 180 min after LPS, pressor responses to NA (1 μg/kg i.v.) were significantly reduced (17±1 and 13±3 mmHg at 30 and 60 min, respectively, vs. 32±3 mmHg in control; n=7. $p<0.01$). The reduction was reversed by N^{G}-nitro-L-arginine methyl ester (L-NAME, 1 mg/kg i.v), a potent inhibitor of NO synthesis (to 31±3 mmHg 60 min after LPS, n=7, $p<0.01$). In contrast, L-NAME did not potentiate the NA-induced pressor responses in control animals (n=6). Dexamethasone (3 mg/kg i.v. 60 min prior to LPS), a potent inhibitor of NOS induction, did not alter control pressor responses to NA, but significantly reduced the LPS-induced fall in MAP at 15-180 min after LPS (n=7, $p<0.05$). However, dexamethasone did not affect the development of the LPS-induced hyporeactivity to NA in the first 60 min (14±1 mmHg at 60 min; n=7). Indomethacin (5 mg/kg i.v., 20 min prior to LPS) did not alter the LPS-induced changes in MAP or the hyporeactivity to NA in the first hour (13±2 mmHg; n=6). At 30 min after LPS application, there was no induction of NOS in brain, liver, spleen, kidney, mesentery, aorta, heart or lung, as measured by the activity of the calcium-independent enzyme. Even at 60 min, only the aorta showed increased activity for the induced NOS. At 180 min, there was a substantial increase in calcium-independent NOS activity in the lung, spleen, liver and aorta. This NOS induction was significantly reduced by dexamethasone (n=4, $p<0.05$). Furthermore, dexamethasone-pretreated animals maintained higher blood pressures and exhibited more pronounced pressor responses to NA than controls (n=5, $p<0.05$).

Our results confirm that LPS enhances the formation of NO in the anaesthetized rat and that this is responsible for the hyporeactivity to NA. However, this NO-mediated hyporeactivity to NA precedes in the early phase of LPS shock the induction of NOS. The early hyporeactivity must be due to activation of the constitutive NOS, whereas, after 180 min a marked NOS induction occurs, which may be responsible both for the maintainance of hypotension and vascular hyporeactivity. Thus, inhibitors of both the constitutive and the inducible NOS may be useful in the treatment of the vascular hyporeactivity in septic shock.

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82P LPS INDUCES NITRIC OXIDE SYNTHASE IN THE CULTURED VASCULAR SMOOTH MUSCLE BY A MECHANISM WHICH IS INDEPENDENT OF TNF AND IL-1

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Nitric oxide (NO) synthesis is induced in vascular smooth muscle cells (VSM) by various immunostimulants including bacterial lipopolysaccharide (LPS),tumor necrosis factor (TNF) and interleukin-1 (IL-1); this process requires protein and mRNA synthesis and is responsible for vascular dysfunctions, including hypotension. Since LPS-induced hypotension has been attributed to release of TNF and IL-1, we sought to distinguish whether either of these cytokines may mediate LPS-induction of NO synthesis in VSM. We also investigated whether these agents induce NO synthase through common or independent signalling pathways.

Induced nitric oxide synthase in cultured rat aortic smooth muscle cells was determined by 2 methods: (1) western blot analysis using polyclonal antibodies directed against purified cytosolic NO synthase from LPS-treated rat aortic smooth muscles, and, (2) colorimetric assay of nitrite production by the method of Griess. Nitrite synthesis was found to reflect activity of the arginine-NO pathway since its production was arginine-dependent, blocked in a dose-dependent and arginine-reversible manner by NG-substituted arginine analogues and associated with the production of an NO-like factor which oxidizes ferrous-myoglobin.

Neither nitric oxide synthase activity nor protein were present under basal conditions in VSM. However, after a lag of 6-8 hrs various cytokines were found to markedly induce NO synthase. Notably, synergistic actions between cytokines were observed for induction of NO synthase activity in VSM. Rat recombinant interferon-τ (IFN; 1-1000 ng/ml) did not elicit NO synthesis when administered alone, but increased maximal LPS-induced NO production by 2-3-fold and reduced the EC50 for LPS from 10 to 1 μg/ml. Similarly, IL-1α induced NO synthesis (EC50 of 3 ng/ml) and IFN (50 ng/ml) increased maximal IL-1α-induced NO synthesis by 2-3-fold. Whereas TNFα alone did not induce NO synthesis at concentrations up to 1 μg/ml, in the presence of IFN (50 ng/ml) TNF caused a dose-dependent induction of NO synthesis with an EC50 of 0.1 ng/ml. Synergistic interactions were also observed with combinations of LPS + IL-1α, LPS + TNFα, and IL-1α + TNFα. These synergistic actions suggested that the individual cytokines do not work via a common pathway. Direct verification of independent pathways was obtained with inhibitor studies. IL-1 receptor antagonist (IL-1ra), a selective endogenous inhibitor of IL-1 receptors, abolished the induction of NO synthesis in VSM by IL-1, but did not attenuate NO synthesis when induced by either LPS or TNF. Similarly, anti-TNF antibodies were found to completely block induction of NO synthesis by TNF, but have no effect on NO synthesis when induced by LPS or IL-1. Whereas both LPS and IL-1 caused release of TNF from VSM by a mechanism which was potentiated by IFN, TNF release (< 50 pg/ml) was significantly lower than that necessary for the degree of NO synthase induction observed.

The present studies indicate that LPS, IL-1 and TNF each elicit induction of NO synthesis in VSM by independent signalling pathways. Although these pathways are proximally distinct, it is likely that they subsequently converge on a common pathway for induction of NO synthase gene expression. The point at which these pathways converge for induction of NO synthesis awaits identification. (Supported by NIH grants HL46403 and HL34215)

83P NITRIC OXIDE SYNTHASE INHIBITORS PREVENT THE DELAYED VASODILATOR RESPONSE TO ULTRAVIOLET LIGHT IN THE RAT

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Nitric oxide, synthesized from L-arginine by the enzyme nitric oxide synthase, mediates in part the delayed vasodilation caused by the injection of endotoxin in rat skin (Warren et al., 1992). Exposure to ultraviolet light irradiation causes delayed onset erythema and we tested if this response involves nitric oxide synthase using the inhibitors N^G-nitro-L-arginine methyl ester (L-NAME), N^G-monomethyl-L-arginine (L-NMMA) and cannavanine (CAN), which act as false substrates for the arginine-nitric oxide pathway.

Male Sprague-Dawley rats were anaesthetized with hypnorm (fentanyl citrate/fluanisone) 0.3 ml/kg i.p. The dorsal skin was shaved and depilated. Eight sites were marked out on the dorsal skin, 4 per flank, using a black paper stencil. Each of the 8 symmetrical holes in the stencil was 6 mm diameter. One hour later baseline skin red cell flux was measured at the 8 marked sites using a laser Doppler flow meter. With the paper stencil in position to shield the remaining skin, 7 sites were exposed to ultraviolet B (UVB) irradiation for 8 min and one site was covered to act as control. UVB irradiation was 20 W cm^{-2} with the lamp held 15 cm from the skin. The sites were injected 17.5 h later with the inhibitors (at 10 and 100 nmoles per site), or buffer as control, and blood flow measured again at 18h. All experiments were performed in a temperature controlled laboratory. Results are the mean ± s.e.mean for 6 animals.

Table 1 % Change in basal blood flow at 18h (doses are nmoles per site)

Non-irradiated site	20±5	UVB + control	128±18
UVB + L-NAME 10	18±13	UVB + L-NAME 100	9±19
UVB + CAN 10	86±47	UVB + CAN 100	63±19
UVB + L-NMMA 10	104±20	UVB + L-NMMA 100	50±19

All three inhibitors of nitric oxide synthase suppressed the delayed erythema response to UVB in a dose dependent manner ($P < 0.05$ in each case, ANOVA). These data suggest that endogenous nitric oxide contributes to the inflammatory response to UVB irradiation.

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84P NO SYNTHASE ACTIVITY IN THE LUNG IS SPECIES-DEPENDENT

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Nitric oxide (NO) is produced by several cell types including endothelial cells and neurones by constitutive, calcium dependent NO synthases. A large proportion of the endothelial cells in the body are located in the lung. However, little is known about NO synthase isoforms in the lung. Here we investigated the distribution of NO synthase isoforms in the lungs from various species.

Bovine, ferret, rat and canine lungs were removed and immediately frozen in liquid nitrogen before being homogenized with an Ultra Turrax tissue homogenizer. Soluble and particulate fractions were prepared as previously described (Forstermann et al., 1991) and NO synthase activity was measured by the conversion of [^{3}H] L-arginine to [^{3}H] L-citrulline (Bredt and Snyder, 1990). Homogenates of lungs from the various species contained the following rank order of NO synthase activity ferret> bovine> rat> canine. The values were 9 ± 2, 0.5 ± 0.2, 0.3 ± 0.1 and 0 pmol citrulline/mg/min (n=3 for each),for each species respectively. As bovine and ferret lungs contained the highest NO synthase activity, lungs from these species were used for partial-purification of the enzyme. Following ultracentrifugation of the ferret and bovine lung homogenates the cytosolic fractions were applied to a 2'5' ADP sepharose column and NO synthase eluted as previously described (Bredt and Snyder, 1990). The eluted material (ADP-cytosol) was then assayed for enzyme activity. The membrane fractions were incubated with CHAPS (1 mM) and the solubilized protein applied to a 2'5' ADP column and the eluted material (ADP-CHAPS) similarly assayed for NO synthase activity. Results are shown in table 1.

Table 1

pmol /mg/min	homogenate	cytosol	ADP-cytosol	pellet	CHAPS	ADP-CHAPS
bovine	0.65	0.4	395.8	7.2	12.6	2646
ferret	9.8	10	3011	7.1	14.6	2066

(Results represent the mean of duplicate determinations from individual lungs, similar results were seen in 2-3 separate lung preparations)

Western blot analysis using polyclonal antibodies raised against NO synthase purified from rat brain (Schmidt et al., 1992) was also performed. A band of approximately 155 Kd molecular weight was recognized in the crude cytosol from rat brain and ferret lung but not in the cytosol from rat, bovine or canine lung or in the particulate fraction from any of the species used. These results show that at least two isoforms of NO synthase exist in the lung. The soluble isoform was found to be antigenically similar to the soluble enzyme in rat brain whereas the particulate isoform remains to be characterized.

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85P MODULATION OF CHOLINERGIC NEUROTRANSMISSION BY NITRIC OXIDE IN HUMAN AIRWAY SMOOTH MUSCLE

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Human airway smooth muscle possesses an inhibitory non-adrenergic non-cholinergic (i-NANC) neural innervation which may be mediated by nitric oxide (NO)(Belvisi *et al.*, 1992). In guinea-pig trachea, endogenous NO modulates cholinergic neurotransmission by a post-junctional mechanism (Belvisi *et al.*, 1991; Brave *et al.*, 1991). We have investigated whether endogenous NO modulates cholinergic neurotransmission in human airways, *in vitro*, using L-N^G-nitroarginine methyl ester (L-NAME) and L-N^G-monomethyl arginine (L-NMMA) selective and potent inhibitors of NO synthase.
Tracheal strips and bronchial rings were obtained from donors for heart transplantation and set up for isometric tension recording using well established methods. Contractile responses to electrical field stimulation (EFS: 40V, 0.5ms, 1, 4, 32Hz for 15s every 4min) and exogenous acetylcholine (ACh) were obtained and the effects of L-NAME and L-NMMA investigated. For measurements of ACh release, tracheal strips were incubated with [^{3}H]-choline whilst undergoing EFS (40V, 0.5ms, 4Hz for 45min). These tissues were washed by superfusion with Krebs containing hemicholinium-3 for 2hrs and then samples were collected at regular intervals for liquid scintillation counting. The effect of L-NAME on responses to EFS (40V, 0.5ms, 4Hz for 3min) was investigated. Throughout all experiments Krebs contained indomethacin (10^{-5}M) and propranolol (10^{-6}M).
L-NAME (10^{-7}-10^{-4}M) had no effect on resting tone in trachea, but increased cholinergic contractile responses to EFS (4Hz) in a concentration-dependent manner [L-NAME 10^{-4}M increased responses by 65.1+14.2% (n=8, $p<0.001$)]. This was partially reversed by L-arginine (L-arg, 10^{-3}M) but not by D-arginine (D-arg, 10^{-3}M); L-arg (10^{-3}M) and D-NAME (10^{-4}M) had no effect. This enhancement was maximal at 4Hz and was reduced at 1Hz (22.5+6.9%, n=4, $p<0.05$) and 32Hz (13.8+5.4%, n=4, $p<0.05$). L-NMMA (10^{-4}M) also enhanced the 4Hz response (27.5+5.1%, n=4, $p<0.05$). L-NAME (10^{-4}M) had no effect on cumulative concentration response relationships to exogenously applied ACh (10^{-9}-10^{-2}M). Measurement of ACh release evoked by EFS was shown to be frequency-dependent as well as sensitive to tetrodotoxin (3x 10^{-6}M). EFS (40V, 0.5ms, 4Hz for 3min) was shown to increase release by 60.9+13.6% (n=4, $p<0.05$). L-NAME (10^{-4}M) did not significantly increase this release. Furthermore the enhancement of the cholinergic contractile response to EFS (4Hz) by L-NAME (10^{-4}M) or L-NMMA (10^{-4}M) was reduced in segmental and subsegmental bronchi.
L-NAME and L-NMMA both enhanced cholinergic contractile responses in human trachea. L-NAME, but not D-NAME, produced a concentration dependent enhancement that was partially reversed by L-arg but not by D-arg,and was not due to increased ACh release. The effects of L-NAME and L-NMMA were less marked in segmental and subsegmental airways. These results suggest that endogenous NO modulates cholinergic neurotransmission by functional antagonism of ACh at the level of the airway smooth muscle and that the contribution of this modulation becomes less evident in smaller airways.

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86P THE EFFECTS OF NITRIC OXIDE AND NITROVASODILATORS ON SPONTANEOUS ELECTRICAL AND MECHANICAL ACTIVITY IN THE RABBIT DISTAL COLON

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Intracellular microelectrode recordings have implicated nitric oxide (NO) in inhibitory neurotransmission from non-adrenergic, non-cholinergic (NANC) nerves in gastrointestinal smooth muscle (Dalziel et al, 1991). This communication describes the effects of sodium nitroprusside (SNP), glyceryl trinitrate (GTN) and isosorbide dinitrate (IDN), which release NO, and of NO itself, on spontaneous electrical and mechanical activity in a tissue, the rabbit distal colon (RDC; Smith and Muir, 1991), proposed to release NO following inhibitory nerve stimulation. Simultaneous, spontaneous electrical and mechanical activity was recorded, extracellularly from unstretched longitudinal strips (2cm x 0.3cm) of RDC freed from mucosa and perfused (3ml/min) with Krebs solution (37±1°C) in a Golenhofen apparatus (Golenhofen and v.Loh, 1970). Spontaneous activity comprised bursts (~6 in each) of spikes (2-4/min,0.2-0.6 mV) and contractions (2-4g amplitude). Increases in tension (0.1-3g) and decreases in temperature (37-27°C) each enhanced, while Ca^{++} withdrawal,by replacement with Mg^{++}, or diltiazem (10^{-5}M) each abolished electrical and mechanical activity. SNP,GTN and IDN (10^{-6}-10^{-3}M) each increased the frequency of spike discharge (by up to 600%); this now consisted of a continuous regular discharge of single spikes. The frequency of contractions was increased (by up to 600%); the amplitude reduced. SNP was most potent and increased spike amplitude (by up to 400%). Similar, but less enduring effects were produced by NO (10^{-4}M; Gillespie and Sheng,1988). The effects of SNP were inhibited by oxyhaemoglobin (10^{-4}M) and mimicked by 8-bromo cyclic GMP (8-brcGMP,10^{-4}M). The effects of SNP were not enhanced by 8-brcGMP. BRL 38227 (10^{-6}M) which abolished all activity was synergistic with SNP (10^{-6}M). The Ca^{++} channel agonist, BAY K 8644 (10^{-7}M), overcame the effects of SNP (10^{-5}M). The results suggest that in addition to their ability to hyperpolarize individual cells and relax smooth muscle, nitrovasodilators enhance the frequency and amplitude of spontaneous electrical activity and increase the rate of contractions. The underlying mechanisms may arise from an activation of guanylate cyclase and a reduction in Ca^{++}-mediated contraction. The reduced cytosolic Ca^{++} level may be sensed by the cell thus increasing the frequency of channel opening to allow additional entry of Ca^{++}.

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87P INVESTIGATION OF PURINES AND NITRIC OXIDE AS PUTATIVE NEUROTRANSMITTERS IN RABBIT ISOLATED ANOCOCCYGEUS

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Recent evidence supports a role for nitric oxide (NO) as an inhibitory non-adrenergic non-cholinergic (NANC) neurotransmitter in rabbit isolated anococcygeus (Graham & Sneddon, 1992). However, part of the NANC response is resistant to NO synthase inhibition, and may be due to another neurotransmitter. The possibility that this portion of the response may be mediated by purines (Burnstock *et al.*, 1978) was studied using selective P_1 and P_2 purinoceptor antagonists. Membrane hyperpolarizations induced by NANC nerve stimulation have also been studied.

Adult, male rabbits were killed by i.v. injection of an overdose of pentobarbitone. The anococcygeus muscles were set up for measurement of isometric tension in Krebs solution at 36.5 ± 0.5°C. NANC responses were measured after addition of atropine (10^{-6}M), guanethidine (10^{-5}M) and histamine (10^{-6}M). Relaxant responses were obtained to nerve stimulation at 1Hz, 0.5ms, supramaximal voltage for 20 seconds at 2 minute intervals. Data were expressed as percentage reduction in tone (mean ± s.e.mean). Statistical analysis was by Student's paired t-test.

Addition of exogenous adenosine ($5x10^{-5}$M) produced a reduction in tone of 27.7 ± 3.9% and 8(p-sulphophenyl)theophylline (8-SPT) reduced these relaxations to adenosine to 4.9 ± 2.2% ($p<0.001$,n=11). This concentration of 8-SPT had no effect on NANC nerve mediated responses (82.6 ± 6.8% in controls, 86.7 ± 6.1% after 8-SPT, n=11). Exogenous ATP ($5x10^{-5}$M) produced 72.0 ± 5.3% reduction in tone and this was significantly reduced in the presence of 8-SPT to 40.6 ± 6.0% ($p<0.001$, n=11). The P_2 antagonist suramin (10^{-4}M) had no effect on the relaxations produced by exogenous ATP (84.2 ± 3.7% in controls, 83.0 ± 5.0% in the presence of suramin, n=6) or exogenous adenosine (41.0 ± 5.8% before and 55.3 ± 7.2% after suramin, n=6). Suramin had no effect on responses to NANC nerve stimulation, even after long periods of incubation. (88.3 ± 2.5% before and 89.1 ± 2.0% after suramin, n=9).

To investigate electrical events associated with NANC relaxations intracellular microelectrode recordings were made. In the presence of guanethidine and atropine the mean resting membrane potential of the smooth muscle cells was 47.2 ± 4.5mV. Trains of stimuli produced a frequency dependent hyperpolarization of the smooth muscle cells, e.g. 20 pulses at 20Hz produced a mean hyperpolarization of 16.2 ± 3.1mV in control cells (n=18). The presence of L-nitro arginine (NOARG) significantly reduced the magnitude of the hyperpolarization to 8.2 ± 3.1mV (n=6, $p<0.01$)

These results support the proposal that NO is involved in both the electrical and mechanical responses of the rabbit anococcygeus to NANC nerve stimulation. Although exogenous ATP and adenosine caused relaxation of the muscle, the results with P_1 and P_2 purinoceptor antagonists did not support their involvement in NANC responses. The ineffectiveness of suramin in blocking relaxations induced by exogenous ATP may reflect a suramin insensitive P_2 purinoceptor.

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88P THAPSIGARGIN DIFFERENTIATES BETWEEN EDRF AND PROSTACYCLIN RELEASE INDUCED BY SHEAR-STRESS OR AGONISTS

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The agonist-mediated simultaneous release of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI_2) is initiated by a mobilization of ($[Ca^{2+}]_i$). It is, however, as yet unclear how this increase in $[Ca^{2+}]_i$ relates to the physiologically more important, release of EDRF induced by shear stress. Recently the tumour-promoting sesquiterpene lactone thapsigargin was shown irreversibly to inhibit the re-uptake of Ca^{2+} into inositol-1,4,5-triphosphate($InsP_3$)-sensitive and insensitive Ca^{2+} stores by blocking the Ca^{2+}-ATPase located in the endoplasmic reticulum of various cells, including platelets and polymorphonuclear cells (Thastrup *et al* 1990, Takemura *et al* 1991). By employing this Ca^{2+}-ATPase inhibitor, we have now investigated further the effects of changes in intracellular Ca^{2+} homoeostasis on the release of both EDRF and PGI_2 from bovine aortic endothelial cells (BAEC) grown on microcarrier beads. The cells (2 ml corresponding to 6 x 10^7 BAEC) were packed into a jacketed chromatography column and perfused (5 ml/min) with warmed (37°C), oxygenated (95% O_2/5% CO_2) Krebs' solution. The effluent superfused an endothelium denuded rabbit aortic ring preconstricted to 2-3g tension with 10 nM U46619 (9α,11α-methaneoepoxy-prostaglandin $F_{2\alpha}$) for the detection and quantification of EDRF release. PGI_2 release was determined by radioimmunoassay for 6-keto-prostaglandin $F_{1\alpha}$ and changes in $[Ca^{2+}]_i$ were monitored with Fura-2/AM loaded BAEC in suspension. EDRF release induced by shear stress was defined as the degree of relaxation (16.7 ± 3% of induced tone; n=6) caused by an infusion of superoxide dismutase (SOD; 10 U/ml) through the column of BAEC (t.c). This relaxation was further enhanced (10-25%; n=6) by bolus injections of agonists such as ADP (9 nmol), ionomycin (60 pmol), or poly-L-lysine (550 pmol). Infusions t.c of thapsigargin (1 μM) caused a sustained release of EDRF (29.8 ± 8.1% relaxation; n=5) which declined after stopping the infusion. Thereafter, the agonist-induced release of EDRF was abolished whereas the shear stress-induced release remained unaffected (n=5). The EDRF activity after both types of release was blocked by infusions t.c of haemoglobin (10 μM; n=3). The basal release of PGI_2 from the BAEC (1.04 ± 0.27 ng/min; n=5) was significantly enhanced (3-6 ng/min) by bolus injections t.c of any of the three agonists. Infusions of thapsigargin t.c also caused a substantial increase in PGI_2 release (5.04 ± 1.99 ng/min), but thereafter, as with EDRF, challenges by the agonists failed to cause any further rise in PGI_2 release. The resting $[Ca^{2+}]_i$ level in Fura-2/AM-loaded BAEC (5 x 10^5 cells/ml) was 70-100 nM. ADP (1 μM) produced a typical biphasic change in $[Ca^{2+}]_i$ i.e a marked transient (< 2 min) rise (peak 400-1000 nM) followed by a plateau phase (200-300 nM) lasting for more than 10 min (n=4). In contrast ionomycin (30 nM) caused a slower but more sustained rise in $[Ca^{2+}]_i$ to 400-500 nM (n=4) whereas thapsigargin (1 μM) elicited an initial sharp increase in $[Ca^{2+}]_i$ (peak 800-1200 nM) which declined slowly and remained elevated throughout the experiment at approximately 500 nM. Subsequent exposure to ADP or ionomycin produced no further increases in $[Ca^{2+}]_i$ (n=4). It appears, therefore, that by emptying the $InsP_3$-sensitive and insensitive Ca^{2+} pools, thapsigargin selectively blocks the agonist-stimulated release of EDRF and PGI_2, suggesting a fundamental difference in the control by Ca^{2+} of release of both substances by shear stress or agonists. *(Supported by a grant from Glaxo Group Research Ltd)*

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89P OPPOSITE CHANGES OF ADRENOCEPTOR- AND MUSCARINE-RECEPTOR-LINKED PROSTACYCLIN SYNTHESIS BY THE AORTA AND BLADDER OF THE DIABETIC RAT

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Although diabetes mellitus (DM) is associated with diminished vascular prostacyclin (PGI_2) synthesis, enhanced synthesis of PGI_2 by other tissues (e.g. urinary bladder [UB]) has been reported (Jeremy *et al.*, 1986a). In turn, PGI_2 synthesis by the aorta and UB of the rat is stimulated by activation of α-adrenoceptors and muscarine receptors, respectively (Jeremy *et al.*, 1985, 1986b). In order to investigate this area further, the effect of experimental DM in the rat on adrencoceptor- and muscarine receptor-linked PGI_2 synthesis was studied. Intracellular second messenger systems were studied following stimulation of PGI_2 synthesis with phorbol ester (PE; a protein kinase C [PKC]) activator; Ca^{2+} ionophore A23187 (A23187) and thapsigargin (both elevate intracellular Ca^{2+}, activating phospholipase A_2 [PLA_2]); and arachidonate (AA; substrate for PGI_2 synthesis).

DM was induced in male Sprague Dawley rats with streptozotocin (65 mg/kg, i.v.). After 8 weeks, rats were killed and aortae and UBs excised. Aortae were cut into 1 mm rings and UBs into approximately 2x2 mm segments and processed for assessment of agonist-stimulated PGI_2 synthesis as previously described (Jeremy *et al.*, 1985,1986b). PGI_2 synthesis was stimulated in the aortae with adrenaline and in the UBs by acetylcholine (Ach). PGI_2 release was measured by radiommunoassay of 6-oxo-$PGF_{1\alpha}$, and dose-response curves compiled.

Transformation of data to % maximal response revealed a marked right shift in the adrenaline-PGI_2 dose-response curve in aortae from DM rats (EC_{50} = 4.1 x 10^{-6} M) compared to controls (EC_{50} = 5.4 x 10^{-7} M). In contrast, there was a marked left shift in the Ach-PGI_2 dose-response curve in UBs from DM rats (EC_{50} = 5.8 x 10^{-7} M) compared to controls (EC_{50} = 2.2 x 10^{-6} M). In the aortae from DM rats there was also a marked right shift in the PE-PGI_2 dose-response curve (EC_{50} = 1.4 x 10^{-6} M) compared to controls (EC_{50} = 1.9 x 10^{-7} M), whereas there were no differences in the EC_{50}s of thapsigargin, A23187 or AA. In the UBs there were no differences in the EC_{50}s of PE, thapsigargin, A23187 or AA between diabetic and control rats.

These data indicate that: (i) reduced PGI_2 synthesis coupled to adrenoceptors in the aorta of the diabetic rat may be due to diminished PKC activity and not to Ca^{2+} mobilising systems, PLA_2, cyclooxygenase or PGI_2 synthase; and (ii) the diametrically opposite effect of DM on muscarine receptor-linked PGI_2 synthesis consolidates that changes in PGI_2 are organ-specific. What determines these marked differential changes in the activity of different receptor types in DM warrants further investigation.

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90P A COMPARISON OF THE INHIBITORY EFFECTS OF PROSTANOID EP_2 RECEPTOR AGONISTS AND β_2-ADRENOCEPTOR AGONISTS ON HUMAN MYOMETRIUM FROM PREGNANT DONORS

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β_2-adrenoceptor agonists are used clinically to inhibit premature labour, but they are not very effective (Calixto & Cimas, 1984), and have high side-effect liability (Besinger *et al.*, 1991). E-series prostanoids can both contract and relax human isolated myometrium, contraction being mediated by EP_1- and EP_3-receptors, and relaxation by EP_2-receptors, (Senior *et al.*, 1991). In the present study, the tocolytic activities of two selective prostanoid EP_2-receptor agonists, butaprost (Gardiner, 1986) and AH13205 (Nials *et al.*, 1990) have been compared with PGE_2 and the β-adrenoceptor agonists, isoprenaline (ISO), salbutamol (SAL), terbutaline (TERB) and ritodrine (RIT), on human isolated myometrium.

Human myometrium from consenting pregnant donors (elective Caesarean sections at term, non-labour), was superfused with Krebs solution (2ml min^{-1}) containing 2.8μM indomethacin, and oxygenated with 95% O_2/5% CO_2 at 37°C (Massele and Senior, 1981). Each agonist was infused at a rate of 0.02ml.min^{-1} over a range of concentrations (0.1nM-10μM) for 15 min. Inhibition was seen as a decrease in frequency and/or amplitude of spontaneous contractions. Effectiveness was expressed in terms of potency (minimum concentration significantly inhibiting myogenic activity), and recovery time (period of inhibition following cessation of agonist infusion until return of control myogenic activity).

All agonists except RIT, inhibited spontaneous contractions in a concentration-dependent manner and none of the agonists caused spasmogenic effects. PGE_2, butaprost, ISO and TERB significantly inhibited myogenic contractions at 0.1μM, whereas AH13205 and SAL had no effect at concentrations <1μM. The mean recovery times (min ± SEM) following a 10μM infusion of agonist were: butaprost (> 120, n=7), AH13205 (89.0±11.8, n=11), SAL (73.6±15.2, n=9), TERB (73.3±16.6, n=9), ISO (71.5±15.9, n=8) and PGE_2 (34.1 ± 16.4, n=5). RIT was inactive (n=9).

Both EP_2-receptor and β_2-adrenoceptor agonists, with the exception of RIT, inhibit spontaneous uterine contractions in human myometrium from pregnant donors. The selective EP_2-receptor agonist, butaprost is the most effective of the agonists tested. The fact that EP-receptor agonists cause inhibition of myometrial activity without excitatory effects could have clinical implications for the prevention and/or treatment of premature labour.

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91P CHARACTERISATION OF THE PGE RECEPTOR ON HUMAN PROMYELOCYTIC LEUKEMIA (HL-60) CELLS DIFFERENTIATED WITH DIMETHYLSULFOXIDE INTO NEUTROPHIL-LIKE CELLS

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Human promyelocytic leukaemia (HL-60) cells can be induced to differentiate into mature granulocytes by exposure to dimethylsulfoxide (DMSO) (Collins *et al*, 1978). Functionally, these cells behave in a similar way to peripheral blood neutrophils, responding to formyl methionine leucine phenylalanine (FMLP) with chemotaxis, adherence, phagocytosis and enhanced enzyme release (Collins *et al*, 1979). Since activation of human neutrophils is inhibited by PGE_2 and D_2 (Ney & Schror, 1991), the aim of this study was to determine if HL-60 cells differentiated with DMSO express receptors for PGE_2 and D_2, and to characterise the subtype of the EP receptor involved.

HL-60 cells were grown in suspension cultures in RPMI 1640 medium containing L-glutamine, 10% foetal calf serum, penicillin (50 u/ml) and streptomycin (50 µg/ml). Differentiation was induced by seeding cells at a concentration of 2×10^5 cells/ml and culturing for 6 days in medium containing 1.2% DMSO. Cells were harvested by centrifugation, washed twice, and resuspended in Hank's buffer containing 0.25 mM isobutylmethylxanthine (5×10^6 cells/ml). After incubation for 10 min at 37°C, the reaction was quenched with ethanol, the samples extracted and cyclic AMP levels measured by a protein-binding assay as described (Armstrong *et al*, 1985).

Table 1 Increases in cyclic AMP (pmol/5 x 10^6 cells) in differentiated HL-60 cells (mean ± s.e.mean, n=4-5)

Concentration (µM)	Cyclic AMP levels (pmol/5 x 10^6 cells) PGE_2	misoprostol	butaprost	ZK 110841
0	57.5 ± 6.2	44.6 ± 8.7	51.5± 8.75	54.2 ± 5.0
0.1	173.4 ± 21.2	160.0 ± 47.5	62.1± 15.7	83.6 ± 16.6
1.0	390.6 ± 126.2	488.7 ± 153.7	115.9± 43.5	158.6 ± 50.7
10.0	576.5 ± 193.7	585.0 ± 158.7	237.5± 95.4	193.5 ± 66.2

Differentiated HL-60 cells stimulate adenylate cyclase in response to both PGE_2 and the DP agonist ZK 110841 (Ney & Schror, 1991), with PGE_2 requiring ten fold lower concentrations for threshold activation. This is similar to our results with human neutrophils, looking at inhibition of chemotaxis. However, initial studies with neutrophils found less cyclic AMP to be synthesised (basal neutrophil levels of 13.5 ± 1.4 stimulated to 24.4 ± 3.1 pmol/5 x 10^6 cells, with 10 µM butaprost, n=4). The order of potency of EP agonists was PGE_2 = misoprostol > 11-deoxy PGE_1 (252.5 at 10 µM, n=2) = butaprost = AH13205 (337.5 at 10 µM, n=2), suggesting this is an EP_2 receptor.

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92P CHARACTERISATION OF THE PGE RECEPTOR ON HUMAN MACROPHAGE-LIKE CELLS THROUGH BINDING STUDIES CONDUCTED ON A PURIFIED PLASMA MEMBRANE PREPARATION

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Prostaglandin (PG) E_2 exerts an anti-inflammatory effect through inhibition of lymphokine production by normal human peripheral blood lymphocytes (Rappaport & Dodge, 1982). In particular, it has been suggested that PGE_2 acts as a negative feedback inhibiting interleukin (IL) -1 production by an adherent macrophage cell population, and consequently IL-1 induced IL-2 production by lymphocytes (Bonta & Parnham, 1982). We have conducted binding studies in order to characterise the subtype of PGE receptor present on the macrophage. Human promyelocytic leukaemia (HL-60) cells grown in suspension in RPMI medium containing 10% foetal calf serum, differentiate into macrophage-like cells when exposed to tetradecanoyl phorbol acetate (16nM), causing 80% differentiation within 24 h (Rovera *et al.*, 1979). Plasma membranes prepared from differentiated HL-60s by homogenisation, were layered onto a 0.9 M/1.2 M sucrose density gradient and centrifuged for 2 h at 112,700 g, 4°C. The band at the interface of 0.9 M/1.2 M sucrose was further centrifuged for 40 min at 304,000 g, 4°C and the pellet suspended in Tris-HCl buffer (50 mM pH 7.4). In displacement studies, bound and free radio-ligand were separated by filtration, after incubation with 2 nM [^{3}H] PGE_2 for 1 h at room temperature in 50 mM Tris buffer, pH 7.4, containing 2.5 mM $MgCl_2$. Specific binding was estimated as 70% of total (non-specific determined at 10 µM PGE_2), and represented 587 ± 58 dpm.

Table 1 Displacement of 2 nM [^{3}H]-PGE_2 from human macrophage-like plasma membranes (mean ± s.e.mean, n=3-4)

Competing ligand (nM)	% of specific [^{3}H]-PGE_2 bound 2.25	10	22.5	100	10000
PGE_2	87 ± 6	65 ± 3	35 ± 4	17 ± 6	0
11-deoxy PGE_1	96 ± 6	65 ± 10	48 ± 6	21 ± 7	-3 ± 1
Butaprost	99 ± 6	81 ± 4	86 ± 5	90 ± 5	68 ± 2
AH 13205	-	-	-	101 ± 3	74 ± 7
Sulprostone	99 ± 2	95 ± 2	92 ± 4	96 ± 3	78 ± 1
AH 6809	-	-	-	-	92 ± 1

Of the 4 EP_2-agonists tested, only 11-deoxy PGE_1 (IC_{50} of 25 ± 11 nM) displaced binding in a manner comparable to PGE_2 itself (IC_{50} of 16 ± 2 nM), butaprost and AH 13205 not significantly displacing ^{3}H-PGE_2 until concentrations of 10µM . A similar lack of activity of AH 6809 (EP_1-receptor antagonist; pA_2 ~ 7.0) and sulprostone suggests that the receptor is not of the EP_1 or EP_3 subtype. These results may reflect a further subtype of the EP receptor.

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93P MECHANISM OF POTASSIUM EFFLUX DURING LOW FLOW ISCHAEMIA IN PERFUSED GUINEA-PIG HEARTS *IN VITRO*

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The contributions of ATP sensitive K^+ (K_{ATP}) channels and lactate coupled efflux to K^+ loss from ischaemic myocardium are unclear (Crake et al., 1987; Kantor et al., 1990). We have compared K^+ and lactate release during 4-6 minutes of low flow ischaemia in perfused guinea-pig hearts, the period of most rapid K^+ loss in this model (Gwilt et al., 1992). The effects of blocking K_{ATP} channels (glibenclamide; GLIB) and inhibiting lactate formation (2-deoxyglucose; 2DG) or transport (alphacyano-4-hydroxycinnamate ;ACHC); De Hemptinne et al., 1983) were studied. During four consecutive periods of low flow ischaemia (flow reduced by 95% 30 min apart), K^+ efflux remained constant, while lactate efflux declined by about 50%. Glibenclamide (1-3μM) significantly reduced K^+ efflux and further reduced lactate efflux ($P<0.01$). In studies involving two ischaemic periods, pre-drug values (1st ischaemic period) of K^+ or lactate efflux did not differ significantly between treatment groups, allowing direct comparison of the effects of drug and vehicle (dimethyl sulphoxide; DMSO) treatments applied during the second ischaemic period (Table 1).

Table 1:	DMSO(n=6)	GLIB(3μM,n=10)	ACHC(4mM,n=5)	GLIB+ACHC(n=12)	2DG (10mM, n=7)	GLIB+2DG (n=7)
K^+ EFFLUX	0.57±0.03	0.27±0.04**	0.16±0.04**	0.19±0.03**	0.34±0.04*	0.25±0.03**
LACTATE EFFLUX	0.66±0.02	0.37±0.05**	0.26±0.06**	0.17±0.05**	0.10±0.02**	0.02±0.02**

Means ± s.e.mean (μmol.g^{-1}). * $P<0.01$; ** $P<0.001$ vs DMSO value (t-test corrected for multiple comparisons).

All drugs reduced K^+ and lactate efflux, but glibenclamide combined with ACHC or 2DG did not further reduce K^+ efflux significantly compared to glibenclamide alone, so that these agents may have reduced K^+ efflux via a similar mechanism. During a single 30 min ischaemic period, VF occurred in 6/7 control hearts vs 1/6 glibenclamide (3μM) treated hearts ($P<0.05$, Fisher's Exact test). A marked secondary peak of K^+ loss occurred in control hearts at the time of onset of VF but not in 7 glibenclamide treated hearts in which VF was spontaneous in one heart or electrically induced in six hearts (0.22 ± 0.05 vs 0.03 ± 0.01 μmol.g^{-1}, $P<0.001$). There was no accompanying change in lactate efflux, so that K_{ATP} channels may have been involved in this K^+ efflux. In this model, lactate efflux was sensitive to glibenclamide and linked to potassium efflux in contrast to previous studies. The use of glibenclamide as a selective K_{ATP} blocker during myocardial ischaemia should be viewed with caution in the absence of measurements of metabolic parameters.

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94P K^+ CHANNEL BLOCKERS, K^+ EFFLUX AND VENTRICULAR FIBRILLATION IN ISCHAEMIC PERFUSED GUINEA-PIG HEARTS

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During myocardial ischaemia myocytes lose K^+ ions into the extracellular space, thought partly to be due to the opening of K_{ATP} channels (Janse & Wit, 1989), which may increase vulnerability to ventricular fibrillation (VF; Kantor et al., 1990). We have studied the effects of K^+ channel blockers on K^+ efflux and VF in isolated guinea-pig hearts perfused retrogradely at constant flow (perfusion pressure 50mmHg) paced at 3.5-4.0 Hz. In initial studies, four successive 6 min periods of "ischaemia" (i.e. flow of perfusate was reduced by 95%) 30 min apart were used. K^+ efflux (derived from perfusate K^+ concentrations measured using a K^+ electrode) in the presence of drugs or dimethyl sulphoxide (DMSO) vehicle (added cumulatively to the perfusate) was compared to predrug values using paired t-tests. In later experiments, flow was reduced by 95% for a single 30 min period. The effects of drugs and vehicle on K^+ efflux and the incidence of VF were compared using unpaired t-tests and Fisher's Exact test respectively.

K^+ efflux in 11 DMSO treated hearts remained stable over four 6 min ischaemic periods, while glibenclamide (0.3-3μM) reduced K^+ efflux from 1.33 ± 0.14 μmol.g^{-1} to 0.82 ± 0.11 μmol.g^{-1} ($P<0.05$, n=8). Glipizide, another sulphonylurea, was without significant effect at 3μM but reduced K^+ efflux from 1.18 ± 0.14 μmol.g^{-1} to 0.90 ± 0.18 μmol.g^{-1} at 10μM and 0.83 ± 0.13 μmol.g^{-1} at 30μM ($P<0.05$, n=8). Phentolamine (10μM) reduced K^+ efflux from 1.26 ± 0.08 μmol.g^{-1} to 0.79 ± 0.10 μmol.g^{-1} (n=5), though this effect did not achieve statistical significance. UK-66,914, a selective blocker of delayed rectifier K^+ channels (Gwilt et al., 1991), did not reduce K^+ loss, (1.07 ± 0.06 μmol.g^{-1} predrug vs 1.02 ± 0.14 μmol.g^{-1} at 10μM, n=4). The incidence of VF during 30 min of ischaemia was reduced ($P<0.05$) from 11/11 hearts (vehicle control) to 1/6 by 3μM glibenclamide, to 1/5 by 30μM glipizide and to 0/4 by 10μM phentolamine but not by 3μM UK-66,914 (4/5 hearts exhibited VF). No drug significantly reduced cumulative K^+ efflux over 30 min of ischaemia.

K_{ATP} channel blockers reduced K^+ loss in early myocardial ischaemia and prevented VF during ischaemia in guinea pig hearts during low flow ischaemia. In this model, however, suppression of VF was not clearly associated with reductions of K^+ efflux over longer periods of ischaemia, in contrast to studies described in other models (Kantor et al., 1990). Mechanisms other than K_{ATP} activation appear to contribute to K^+ efflux under these conditions (Gwilt et al., 1992).

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95P ISCHAEMIA AND REPERFUSION INDUCED CHANGES IN RAT MYOCARDIAL PERIPHERAL TYPE BENZODIAZEPINE RECEPTORS

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Pharmacological studies have suggested a functional relationship between peripheral type benzodiazepine receptors (PBRs) and L-type calcium channels (Bolger *et al.*, 1989). In view of the role of calcium in ischaemia and reperfusion-induced myocardial injury, the regional distribution of PBRs was investigated in an *in vivo* rat model of left coronary artery (LCA) occlusion and reperfusion.

Male Sprague-Dawley rats (250-350g) anaesthetised with pentobarbitone underwent 30 min snare occlusion of the LCA (I) followed by 5 min (5R) and 30 min (30R) reperfusion. Successful reperfusion was confirmed by infusion of disulphine blue dye. In the lesioned animals, the risk area was in the anterolateral (AL) region and was confirmed by glycogen depletion on Periodic Acid Schiff staining. After overdose of pentobarbitone, hearts were excised, rinsed in ice cold heparinized Kreb-Henseleit buffer and immediately frozen in isopentane (-35°C). Longitudinal, unfixed 20μm cryostat sections were mounted on acid-washed, gelatin coated slides. Pairs of serial sections were incubated with 1nM [3H] PK-11195 (a specific PBR ligand) for 30 min at room temperature, with one section of each pair incubated with 10μM unlabelled ligand to determine non-specific binding. Radioligand binding was terminated by three 5 min washes in Tris-HCl buffer at 4°C (de Silva *et al.*, 1991). The sections were then exposed to [3H]-Hyperfilm for 1 week together with [3H]-polymer standards (Amersham). The film was developed and fixed and the images scanned and digitised. Regions of interest were drawn on images of specific [3H]PK- 11195 in the epicardium (epi) and endocardium (endo) and the mean grey level values were converted to specific [3H] PK-11195 uptake in pmol/mg tissue.

Table 1 - Data are expressed as mean ± SD for specific binding of [3H] PK-11195 to regions of rat myocardium (pmol/mg tissue).

	S (n=3)	I (n=5)	5R (n=3)	30R (n=4)
AL epi	0.12±0.04	0.18±0.04	0.17±0.05	0.10±0.02
AL endo	0.06±0.03	0.09±0.02	0.11±0.06	0.09±0.02
AL endo:epi	0.52±0.12	0.51±0.09	0.71±0.43	0.94±0.21

In sham-operated control animals (S), a transmural gradient in binding was observed, with significantly higher binding in the epi- than endocardium ($p<0.001$) but there was no gradient in binding from base to apex of the heart. This transmural gradient was also observed in the I and 5R groups. Absolute levels of PBR binding were elevated in both the endo- and epicardium in the I and 5R groups compared to group S ($p<0.05$). In the 30R group the endocardial PBRs remained elevated when compared to group S ($p<0.001$) but the epicardial PBRs decreased significantly compared to groups I and 5R ($p< 0.001$), resulting in the elimination of the transmural gradient in PBRs. The data suggest that acute myocardial ischaemia induces a transmural upregulation of PBRs which gradually reverses upon reperfusion. These preliminary data suggest that further investigations of the role of PBRs in myocardial ischaemia and reperfusion are warranted.

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96P THE I_{K1} BLOCKER, RP58866, IS AN EFFECTIVE ANTIFIBRILLATORY AGENT IN ISOLATED RABBIT HEARTS

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RP58866 is a selective blocker of the inwardly rectifying K+ current (I_{K1}), as shown by Escande *et al.*, 1989. It prolongs refractory period and suppresses atrial and ventricular arrhythmias in dog (Mestre *et al.*, 1989). In rat, 3μM RP58866 prolongs QT interval and suppresses ventricular arrhythmias (Rees & Curtis, 1991). In the present study we examined the effects of 3μM RP58866 on QT interval and on the incidence of reperfusion-induced ventricular tachycardia (VT) and ventricular fibrillation (VF) in rabbit.

Rabbit hearts (n=12/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 vehicle control or 3μM RP58866. 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). Arrhythmia scores were also calculated (no arrhythmias =0, ventricular premature beats =1, bigeminy/salvos =2, VT= 3, VF= 4; arrhythmias defined according to the Lambeth Conventions, Walker *et al.*, 1988).

Table 1. Incidence of reperfusion-induced VF and VT

	VF	VT	QTc Pre-drug	QTc Isch. -1 min	QTc Isch. +15 min
Control	8/12	12/12	0.63±0.02	0.62±0.02	0.59±0.02
3 μM	1/12*	6/12*	0.62±0.02	0.73±0.02*	0.74±0.03*

RP58866 (3 μM) decreased the incidence of reperfusion-induced VF and VT and prolonged QTc (*$p<0.05$). RP58866 also significantly decreased the reperfusion arrhythmia score (3.7±0.1 and 2.6±0.2 in control and drug-treated hearts respectively). These findings are consistent with those from rat (Rees & Curtis, 1991), and therefore show that specific I_{K1} blockade may represent an important new antiarrhythmic mechanism.

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97P ADENOSINE AND AN A_2 AGONIST, CGS21680, REDUCE ARRHYTHMIAS AND SUPPRESS WHITE CELL ACCUMULATION IN THE PORCINE ISCHAEMIC MYOCARDIUM

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White cell accumulation within the ischaemic myocardium may contribute to the extension of damage following a myocardial infarction. Adenosine, released in response to ischaemia, acts as a vasodilator, an action which may reduce arrhythmias, and inhibits the adhesion of leukocytes to vascular endothelium. The aim of this study was to assess the effects of adenosine and a selective A_2-adenosine agonist, CGS21680 (2-[4-(2-carboxyethyl)phenethylamino]-5^1-N-ethylcarboxamido adenosine hydrochloride; Jarvis et al, 1989) on the severity of arrhythmias and white cell accumulation in an anaesthetized pig model of acute myocardial infarction.

Large white/Landrace cross breed male pigs (28-40 kg) were sedated with azaperone, anaesthetized with chloralose (100 $mgkg^{-1}$ i.v.) and prepared for LAD occlusion. Following surgery animals were randomly allocated into three drug treatment groups: i) Control (n=12), ii) Adenosine (150 μgkg^{-1} i.l.v.; n=6) and iii) CGS21680 (0.25 μgkg^{-1} min^{-1} i.l.v.; n=6). Drug infusions, given into the lumen of the left ventricle to minimise peripheral vasodilatation, were commenced 10 minutes prior to LAD coronary occlusion and maintained for the duration of the experiment. Autologous white cells, labelled with Tc-99m, were injected intravenously 5 minutes prior to occlusion in 6 of the control pigs and 5 minutes post-occlusion in the remaining 6 control pigs and all drug-treated pigs. Area at risk was determined by Thallium-201 given i.v. 5 min post-occlusion. The pigs were sacrificed after 2 hours of coronary occlusion and the hearts excised. Paired blocks of myocardium were taken from the same anatomical sites within normal and ischaemic tissue for gamma counting. During the period of coronary occlusion all standard haemodynamic variables were continuously recorded on a computerised data logging system and ischaemia-induced arrhythmias were recorded from a lead II ECG. In cases of ventricular fibrillation, hearts were defibrillated by direct cardioversion.

Infusion of both adenosine and CGS21680 caused an immediate fall in blood pressure (42% and 31% respectively) which was maintained for the duration of the experiment. CGS21680 also caused a sustained increase in heart rate (from 92±10 beats min^{-1} prior to infusion to 131±11 beats min^{-1} just prior to occlusion). The total number of ventricular arrhythmias during the first 30 min of coronary occlusion was reduced by both adenosine (126±28) and CGS21680 (155±38) compared to controls (246±26; $P<0.05$ for both interventions). The incidence of ventricular fibrillation was also reduced from 92% in controls to 57% and 50% in pigs given adenosine and CGS21680 respectively. In all pigs ventricular ectopic activity ceased after 30 min and hearts remained in sinus rhythm for the duration of the experiment. Thallium uptake in the ischaemic region of all hearts was in the range 11-23% of normal uptake. Accumulation of Tc-99m labelled white cells in control pigs was increased in the ischaemic zone compared to normal tissue (235±28% in pigs given labelled cells prior to occlusion; 178±38% in pigs given cells after occlusion). White cell accumulation was markedly reduced by both adenosine (39±10%) and CGS21680 (45±6%). The results of this study show that adenosine and an A_2-agonist are both effective against ventricular arrhythmias and white cell accumulation in the ischaemic myocardium.

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98P IMPORTANCE OF ADENYLATE CYCLASE-MEDIATED VASODILATION IN THE RABBIT INCREASES WITH DECREASING VESSEL SIZE

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We compared the vasodilator activity of four agonists which stimulate adenylate cyclase with the vasodilator activity of nitroprusside (NP), which acts via guanylate cyclase. The responses were measured in the rabbit macro- and micro-circulation using aorta, coeliac artery and skin microvessels. The agonists used to stimulate adenylate cyclase were the neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP38), the ß-agonist isoprenaline, and the prostaglandins PGE_1 and PGE_2.

Aortic and coeliac artery rings were studied with the endothelium removed and were pre-contracted with phenylephrine in an in vitro superfusion bioassay system. Drugs were given in the concentration range 10^{-10}-10^{-4} M and 4-10 rings were used at each drug concentration. In aortic tissue the following vasodilator potencies (-log EC_{50}, 100% = maximum response to NP) were observed: NP 7.0, PACAP38 6.8, isoprenaline 6.3; PGE_1 and PGE_2 were weak vasoconstrictors (agonists were significantly different from each other, $P<0.05$ ANOVA, except for PACAP38 and NP). In coeliac artery rings the vasodilator potencies were: PACAP38 6.6, PGE_1 6.6, NP 6.5, PGE_2 4.9, and isoprenaline 4.3 (PGE_2 and isoprenaline were each significantly different from the other 3, $P<0.05$).

In a further experiment, the microcirculation vasodilator response to intradermal injections was assessed by measuring skin blood flow in vivo with a laser Doppler flow probe. Male New Zealand White rabbits were anaesthetized with sodium pentobarbitone 30 mg kg^{-1} i.v. and the dorsal skin shaved and depilated. Experiments were carried out in a temperature controlled laboratory and repeated x4 per rabbit in a balanced site pattern with 4 animals per drug dose. Test agents and control buffer were injected in 100 μl volumes and assessed at 30 min. The agonists were significantly different from each other ($P<0.05$ in each case), except for the lack of a significant difference between isoprenaline and PGE_2. Comparative vasodilator potencies (-log moles per site of the dose required to increase basal red cell flux by 75%) were: PACAP38 13.0, PGE_2 10.7, isoprenaline 9.7, PGE_1 9.1, NP <7.

Nitroprusside was the most potent vasodilator tested in rabbit aorta but was the weakest when tested in the microcirculation. PGE_1 and PGE_2 were vasoconstrictors of the aorta, of intermediate effect in the coeliac artery, but potent vasodilators of the microcirculation. The results confirm the vasodilator activity of PACAP38 (Warren et al., 1991) and in addition show it to be particularly potent in the microcirculation. In the rabbit, the importance of adenylate cyclase-mediated vasodilation increases with decreasing vessel size.

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99P HYDROGEN PEROXIDE IS THE MEDIATOR OF ENDOTHELIAL BARRIER DYSFUNCTION INDUCED BY XANTHINE OXIDASE AND HYPOXANTHINE

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During ischaemia, anaerobic catabolism of adenosine triphosphate leads to an intracellular accumulation in endothelial cells of hypoxanthine. On subsequent reperfusion, endothelial barrier dysfunction may result through conversion, by xanthine oxidase, of hypoxanthine to xanthine and then to uric acid (Shasby *et al.*, 1985). Superoxide anion is formed in the process of these conversions and undergoes subsequent dismutation to hydrogen peroxide with possible further conversion, driven by the ferric iron-catalysed Fenton reaction, to hydroxyl radical (Kvietys *et al.*, 1989). It is unclear, however, which, if any, of these species is responsible for the dysfunction seen.

Endothelial barrier function was assessed using an *in vitro* model (Gudgeon & Martin, 1989), by measuring, over a 90 min 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$\pm$0.8% (n=30). Neither hypoxanthine (0.2 mM) nor xanthine oxidase (20 mU ml^{-1}) when added alone had any effect on albumin transfer across BAEC, but a combination of these stimulated albumin transfer to 18.2$\pm$1.8% (n=6). This increase in albumin transfer was not affected by superoxide dismutase (6000 U ml^{-1}), or the hydroxyl radical scavengers, mannitol (15mM), dimethylthiourea (10mM) and N-(2-mercaptopropionyl)-glycine (1mM), but was abolished by catalase (3 U ml^{-1}). Addition of ferric chloride (50 μM) failed to enhance this increase whilst addition of deferoxamine (500 μM), an iron-chelator, failed to inhibit it. Dithiothrietol (1mM), a scavenger of the hypochlorous anion, also failed to inhibit this increase. Hydrogen peroxide (0.1 - 30 mM) itself increased albumin transfer across BAEC, exhibiting a biphasic concentration-response curve, the lower peak of which (0.1 - 0.3 mM) equates with the concentration of hydrogen peroxide theoretically produced using hypoxanthine and xanthine oxidase at the above concentrations.

The data show that the endothelial barrier dysfunction induced by hypoxanthine and xanthine oxidase is not mediated by either superoxide anion, hydroxyl radical or hypochlorous anion. The ineffectiveness of ferric chloride and deferoxamine further exclude any involvement of hydroxyl radical generation through the Fenton reaction. The effectiveness of catalase, and the close correlation between the results for hypoxanthine/xanthine oxidase and hydrogen peroxide itself, indicate that hydrogen peroxide is likely to be the sole mediator of endothelial barrier dysfunction induced by hypoxanthine and xanthine oxidase.

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100P ISCHAEMIA/REPERFUSION MODIFIES THE EFFECTS OF ENDOTHELINS AND VASODILATORS IN ISOLATED RAT HEARTS

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Endothelins (ETs) may be involved in the development of myocardial infarction (Watanabe et al 1990). Ischaemia / reperfusion increases ET-1 binding to cardiac membranes (Liu et al 1990) and it has been shown that ET-1 -induced coronary vasoconstriction is enhanced after ischaemia (McMurdo et al 1991; Neubauer et al 1991). The present study investigates the effects of 30 minutes of ischaemia followed by reperfusion on the vasodilator and constrictor components of responses to ET-1,-2, and -3, and of its effects on responses to other dilators.

Hearts from male Wistar rats were perfused at 10 ml min^{-1} with gassed (95% O_2:5% CO_2) Krebs Henseleit solution at 37°C, and subjected to 30 minutes of global ischaemia, produced by cessation of flow, and reperfused, temperature being maintained by superfusing with 95% N_2:5% CO_2 - gassed Krebs Henseleit solution at 37°C. Control hearts were perfused for a time-matched period. Perfusion pressure, heart rate and isometric tension were recorded and drugs were administered by bolus injection.

Following ischaemia, vasoconstrictor dose response curves to ET-2 (1-200pmol) and ET-3 (10-1000pmol) were shifted to the left, accompanied by a loss of the initial dilator component of the response; mean maximal dilations were reduced by 98% (n=5) & 95% (n=6) respectively. There was no significant effect on constriction to ET-1 (0.1-200pmol) although its vasodilator effect was reduced by 70% (n=7).

The dose response curves to bradykinin (0.1pmol-10nmol), sodium nitroprusside (0.01-300nmol), adenosine (0.1-1000nmol) and papaverine (1-1000nmol) ($n \geq 4$) were significantly shifted to the right by ischaemia ($P < 0.05$). The vasodilator effect of verapamil (1pmol-30nmol) was also significantly altered ($P < 0.05$, n=5).

Ischaemia / reperfusion alters the vasodilator response of coronary vessels. The reduced response to papaverine, sodium nitroprusside and adenosine suggests that smooth muscle is directly affected. The vasoconstrictor effects of ET-2 and -3 may be enhanced as a result of loss of opposing vasodilation.

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101P ENDOTHELIN STIMULATES VASCULAR SMOOTH MUSCLE PHOSPHOLIPASE D: ATTENUATION BY TYROSINE KINASE INHIBITOR ST271

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Endothelin 1 (ET-1) is a peptide produced by the endothelium and acts on ET_A receptors on vascular smooth muscle (VSM) cells to stimulate mitogenesis as well as contraction. ET_A receptors on VSM are known to be linked to phospholipase C (Wilkes & Boarder, 1991). Recently it has been reported that ET-1 acting on fibroblasts can stimulate tyrosine kinase(s) (Force et al., 1991) and phospholipase D (MacNulty et al., 1990). Furthermore tyrosine kinase activity has been implicated in the regulation of phospholipase D (Uings et al., 1992). Both these activities are potentially important for the mitogenic activity of ET-1 on VSM cells. Here we investigate the ET-1 stimulation of phospholipase C on A10 rat aorta VSM cells.

A10 cells were grown to confluence in 24-well plates, and loaded with ^{32}Pi (0.25 MBq/ml) for 24h. Cells were then incubated with 50mM butanol for 10 min prior to and during the stimulation period of 3 min. Where appropriate the protein kinase C inhibitor Ro 31 8220 and the tyrosine kinase inhibitor ST271 (Uings et al., 1992) were present for 20 min and 30 min respectively prior to and during the stimulation. After separation of lipid products on thin layers, formation of [^{32}P]phosphatidylbutanol ([^{32}P]PBut) indicated phospholipase D activity, while [^{32}P]phosphatidic acid represented phospholipase C activity.

ET-1 dose dependently stimulated the formation of both [^{32}P]phosphatidic acid and [^{32}P]PBut, indicating a stimulation of phospholipase C and phospholipase D respectively. The stimulation of [^{32}P]PBut reached a maximum at 3 min. Ro 31 8220 (10μM) reduced the ET-1 (100 nM) stimulated [^{32}P]PBut formation by about 60%. Phorbol myristate acetate (100nM) stimulated [^{32}P]PBut to the same level as ET-1. The relatively selective tyrosine kinase inhibitor ST271 attenuated ET-1 stimulated [^{32}P]PBut accumulation (e.g. control, 350 $\pm$ 105; ET-1 (100nM), 1572 $\pm$ 65; ST271 (100μM), 712 $\pm$ 147; ET-1 + ST271, 911 $\pm$ 313; cpm of [32P]PBut, mean $\pm$ SEM, n=3). However, ST271 did not affect agonist stimulated [^{32}P]phosphatidic acid formation.

These results show that: (1) ET-1 stimulates phospholipase D in A10 vascular smooth muscle cells; (2) This phospholipase D stimulation is, in part, protein kinase C dependent; (3) Stimulation of phospholipase D, but not of phospholipase C, is attenuated by the tyrosine kinase inhibitor ST271. This suggests that tyrosine kinase activity links the ET receptor to phospholipase D and raises the possibility that ET-1 stimulation of tyrosine kinase activity is involved in regulation of vascular smooth muscle by ET-1.

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102P ENDOTHELIN (ET) RECEPTORS MEDIATING ET-1-INDUCED CONTRACTION IN GUINEA-PIG AND HUMAN PULMONARY TISSUES

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There is preliminary evidence for distinct ET receptors in airway and vascular smooth muscle (Maggi *et al.*, 1989; Cardell *et al.*, 1992). In the present study sarafotoxin S6c, which has selectivity for the ET_B receptor (Williams *et al.*, 1991), and BQ-123, the potent and selective ET_A receptor antagonist (Ihara *et al.*, 1991), were used to characterize the ET receptors mediating ET-1-induced contraction in guinea-pig and human pulmonary tissues.

Isometric tension was recorded by conventional techniques from strip or ring preparations of human bronchus, human pulmonary artery, guinea-pig trachea, guinea-pig bronchus, guinea-pig pulmonary artery and guinea-pig aorta; in some studies responses in upper, middle and lower regions of guinea-pig trachea were examined. In human bronchus and human pulmonary artery ET-1 (1nM-0.3μM) was a potent and effective contractile agent (pD_2=7.23, n=4, and 8.43, n=3, respectively). BQ-123 (1 or 10μM) potently antagonized ET-1-induced contraction in human pulmonary artery (pK_B=6.74, n=3) but had no effect in human bronchus (n=4). Sarafotoxin S6c (1nM-0.1μM) did not contract human pulmonary artery (n=3) but potently and effectively contracted human bronchus; pD_2=8.41, maximum response=76.7±2.3% of 10μM carbachol, n=3. BQ-123 (3μM) did not antagonize sarafotoxin S6c-induced contraction in human bronchus (n=2). Sarafotoxin S6c (0.1nM-0.1μM) did not contract guinea-pig aorta (n=4) or guinea-pig pulmonary artery (n=6) but potently and effectively contracted guinea-pig bronchus (pD_2=8.24; maximum contraction=63.6±2.6% of 10μM carbachol; n=4). Contractions to sarafotoxin S6c were unaffected by BQ-123 (10 μM, n=4). Sarafotoxin S6c was a much less effective agonist in guinea-pig trachea; maximum contraction=13.9±2.2% of 10μM carbachol, n=4. ET-1 potently contracted guinea-pig trachea, bronchus, pulmonary artery and aorta (pD_2=7.85, 7.41, 8.46 and 8.31, respectively, n=4-6). BQ-123 (1-10μM) antagonized ET-1-induced contractions in guinea-pig artery (pK_B=6.62, n=6) and aorta (pK_B=7.12, n=6) and trachea (pK_B=6.21, n=6) but was without marked effect in bronchus (n=4-6). Appreciable regional differences were observed in the efficacy (relative to carbachol), but not the potency, of sarafotoxin S6c in guinea-pig airways, with a much greater maximum contractile response in bronchus (69.6±2.2% of 10μM carbachol, n=4), or lower trachea (48.5±5.4% of 10μM carbachol, n=4), than in middle trachea (14.4±3.7% of 10μM carbachol, n=4) or upper trachea (19.3±2.5% of 10μM carbachol, n=4). There were minimal regional differences in ET-1-induced contraction or the potency of BQ-123 (3μM) against responses to ET-1 in guinea-pig airways.

These data provide evidence that distinct ET receptors mediate ET-1-induced contraction in human pulmonary artery, guinea-pig pulmonary artery and guinea-pig aorta (ET_A) compared with human bronchus and guinea-pig bronchus (predominantly non-ET_A, perhaps, ET_B). Contractions to ET-1 in guinea-pig trachea appear to involve both ET_A and non-ET_A (ET_B?) receptor subtypes. Furthermore, regional differences appear to exist in the relative distribution of ET receptor subtypes in guinea-pig airways.

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103P BQ123 AND DIFFERENT ISOLATED TISSUE PREPARATIONS REVEAL HETEROGENEITY IN THE RECEPTORS MEDIATING CONTRACTIONS TO ENDOTHELIN-1

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Two receptors that mediate the effects of the endothelins have been cloned and sequenced. These have been classified as ET_A (Arai et al., 1990) and ET_B (Sakurai et al., 1990). The ET_A receptor, which is more selective for endothelin-1 than endothelin-3 has been suggested to mediate contraction, and ET_B which shows no ET-1/ET-3 selectivity to mediate vasorelaxation, possibly via the release of EDRF (Warner et al., 1989). To test whether the ET_A receptor mediates contraction in all tissues we have assessed the effects of the recently reported ET_A receptor-selective antagonist BQ123 (Ihara et al., 1992) on the contractile responses to ET-1 of a variety of isolated tissues.

Rings of guinea-pig bronchi (GPB), rabbit pulmonary artery (RbPA) and rat thoracic aorta (RTA) and rat stomach strips (RSS) were suspended in organ baths under resting tensions of 1-2 g for isometric measurement of contractions. The bathing Krebs' solution contained bacitracin 3 mg L^{-1}, bovine serum albumin 50 mg L^{-1}, indomethacin ($5x10^{-6}$ M), thiorphan (10^{-6} M), captopril (10^{-6} M) and bestatin (10^{-6} M) and was gassed with 95% O_2:5% CO_2 at a temperature of 37 °C. Following an equilibration period of 1 h the tissues were exposed to 150 mM KCl to provide a standard response. After wash out the tissues were incubated with either BQ123 (10^{-5} M; synthesised for this project by Parke-Davis, MI, USA) or vehicle for 20 min before being exposed to cumulative concentrations of ET-1 (10^{-11} to $3x10^{-7}$ M). Each tissue was used for only one curve. Contractile responses to the endothelins were standardised by comparison to the control KCl contraction. In GPB, RbPA or RSS, BQ123 (10^{-5} M) did not effect either the EC_{50}'s for ET-1 (RbPA, $5x10^{-10}$ M; RSS, $2x10^{-8}$ M), the threshold concentrations of ET-1 that produced contractions (GPB, 10^{-9} M; RbPA, $3x10^{-11}$ M; RSS, $3x10^{-10}$ M; RVD) or the maximal responses to ET-1. In contrast, BQ123 shifted the EC_{50} for ET-1 in the RTA from approx. $3x10^{-10}$ M to $1x10^{-7}$ M, and the threshold concentration for contraction from 10^{-10} M to $3x10^{-8}$ M (n=4-8 for each).

These results suggest ET-1-induced smooth muscle contractions are not mediated by a common ET_A receptor. This heterogeneity of contractile receptors indicates that selective antagonists may provide the opportunity to target different tissues or vascular beds.

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104P CHARACTERISATION OF AN ET_A RECEPTOR ANTAGONIST IN THE ANAESTHETISED RAT

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Enhanced plasma levels of endothelin-1 (ET-1) are associated with a variety of cardiovascular diseases such as hypertension, ischaemic heart disease and coronary artery vasospasm. However, it is yet unclear whether ET-1 is involved in the pathogenesis of these disorders. Specific ET receptor antagonists are important tools for assessing the contribution of the ETs to physiology and pathophysiology. At present, two ET receptors have been described, ET_A (Arai *et al.*, 1990) and ET_B (Sakurai *et al.*, 1990). BQ-123 (cyclo(D-Asp-L-Pro-D-Val-L-Leu-D-Trp)) is a specific ET_A receptor antagonist *in vitro* (Ihara *et al.*, 1992). Here we show the inhibitory effects of BQ-123 on the pressor responses to ET-1, big ET-1 and sarafotoxin S6b (SX6b) in the anaesthetised rat.

Male Wistar rats (body weight: 250-400g) were anaesthetised with sodium-thiopental (120 mg kg^{-1} i.p.). The right femoral and left jugular veins were cannulated for drug administration and the right carotid artery for measurement of mean arterial blood pressure (MAP). All animals received the ganglion blocking drug hexamethonium (10 mg kg^{-1} i.v.) . The BQ-123 used in this study was synthesised by Parke-Davis. Statistical analysis was made by Students' paired t-test and by ANOVA.

Resting MAP was 112±3 mm Hg (n=46); this was lowered to 85±2 mm Hg by hexamethonium (10 mg kg^{-1}) (n=46). ET-1 (1 nmol kg^{-1}, i.v., bolus) produced an initial transient depressor response and a subsequent, sustained increase in MAP which reached a maximum of 44±3 mm Hg within 5 min (n=6). MAP returned to control levels within 60 min. BQ-123 (1 or 5 mg kg^{-1}, i.v. bolus) significantly decreased the ET-1 induced rise in MAP to 22±3 mm Hg and 17±3 mm Hg respectively (n=6). While a dose of 0.2 mg kg^{-1} was without effect (36±4 mmHg) (n=5). The ET-1 pressor response was inhibited by 89% when the peptide was given 60 min after the start of a 120 min infusion of BQ-123 (0.2 mg kg^{-1} min^{-1} i.v.). In contrast, the ET-1 depressor response was not affected by BQ-123. The pressor effects of big ET-1 (1 nmol kg^{-1} i.v.) were reduced from 62±5 mm Hg to 22±2 mm Hg by BQ-123 (1 mg kg^{-1}, i.v., bolus) (n=6). Moreover, BQ-123 (1 mg kg^{-1}, i.v., bolus) antagonised the SX6b (1 nmol kg^{-1}, i.v., bolus) induced rise in MAP from 41±5 mm Hg to 5±7 mmHg (n=4).

Thus, BQ-123 inhibits the pressor effects of ET-1, big ET-1 and SX6b in the anaesthetised rat without changing the initial transient hypotension, indicating that the pentapeptide is an ET_A receptor antagonist. Hence, BQ-123 is a useful tool to elucidate the potential importance of ET-1 in cardiovascular disorders associated with enhanced plasma levels of the peptide. ***(This work was supported by the Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co.).***

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105P AN ASSESSMENT OF FLOSEQUINAN'S DIRECT EFFECT ON HUMAN ARTERIAL, VENOUS AND CARDIAC MUSCLE: COMPARISON WITH MILRINONE

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Flosequinan (F) is a novel quinolone undergoing development for treating heart failure. Clinical reports have described both arterial-venous vasodilator and positive inotropic actions for F (Cowley et al., 1984; Burnstein et al., 1990). The goal of this study was to assess the comparative effects of F, BTS 53 554 (BTS; the primary metabolite of F, (Yates, 1991)) and milrinone (M) on isolated human arterial, venous and cardiac muscle. For these experiments, samples of internal mammary artery, saphenous vein and atrial appendage were obtained from patients undergoing open chest surgery. In vitro functional tests were performed as described by Weishaar et al. (1990, 1991). F potently relaxed arterial and venous smooth muscle previously contracted with norepinephrine (NE). At high concentrations, F also exerted a cardiotonic effect on cardiac muscle. Similar results were obtained with BTS. In contrast to F and BTS, the vascular relaxant and cardiotonic responses to M were observed at similar concentrations. The comparative effects of F, BTS and M on human arterial, venous and cardiac muscle are summarized below.

	IC_{25} μM vascular muscle or EC_{25} μM cardiac muscle		
Compound	NE-contracted Arterial Muscle	NE-contracted Venous Muscle	Cardiac Muscle
Flosequinan	0.15	0.80	32
BTS 53 554	6.0	12.0	85
Milrinone	0.82	0.90	1.8

Clinical studies also indicate that peak plasma levels of F and BTS observed in patients (10 and 30μM respectively, Packer et al., 1988) are similar to those that produce 40-50% relaxation of isolated vascular smooth muscle. In contrast, supra-therapeutic concentrations of flosequinan are required to achieve comparable effects on cardiac muscle.

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106P PHARMACOLOGICAL PROFILE OF FPL 66564: THE FIRST ULTRA SHORT-ACTING ANGIOTENSIN-CONVERTING ENZYME INHIBITOR (USACEI) IN THE RAT

R.D. Carr, L. Fraser-Rae, P.G. Killingback[1], C. Hallam[1] & S.T. Harper[1], Departments of Pharmacology & Biochemistry[1], Fisons plc, Pharmaceutical Division, Research & Development Laboratories, Bakewell Road, Loughborough, Leicestershire, LE11 0RH (Introduced by P. Leff)

FPL 66564 (structure given in Figure 1) is the first example of a novel class of compounds, ie. ultra short-acting ACE inhibitors (USACEI).

FPL 66564 is a potent inhibitor of isolated, affinity purified rabbit lung ACE (IC-50 = 5.7 nM) *in vitro* as demonstrated using a method based on the technique of Cushman and Cheung (1971). Adult male Sprague Dawley rats (280-350g; n=5 per dose level of FPL 66564 were anaesthetised (Urethane 1.5g/kg ip) and prepared surgically for measurement of arterial blood pressure, intravenous bolus administration of angiotensin I (150nmol/kg) and continuous intravenous infusion of FPL 66564. FPL 66564 infusion (100-1000 nmol/kg/min) rapidly (t½ = 5-10 mins) caused significant and dose dependent inhibition of angiotensin I pressor responses. Efficacy of FPL 66564 was quantified by measuring the extent of inhibition of pressor responses to angiotensin I obtained during a 30 minute infusion of FPL 66564 relative to pre-infusion responses. The dose of FPL 66564 required to inhibit pressor responses by 70% (ID-70) was found to be 200 nmol/kg/min. Vehicle (10% ethanol in saline) infused at 0.1ml/kg/min had no effect on pressor responses. Recovery from inhibition was rapid upon cessation of infusion; having a t½ of approximately 5-10 minutes irrespective of the dose level of FPL 66564 used.

This profile may enable USACEI to be administered by continuous intravenous infusion to treat hypertensive crisis and acute heart failure in man.

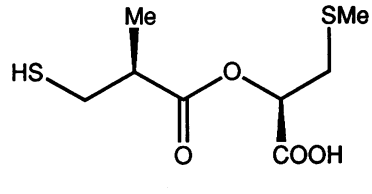

Figure 1: Structure of FPL 66564

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107P EVIDENCE FOR TWO DISTINCT RECEPTORS FOR ATP ON BOVINE AORTIC ENDOTHELIAL CELLS, BOTH LINKED TO PHOSPHOLIPASE C ACTIVATION

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P_2-purinergic receptors are divided into P_{2X} and P_{2Y} subtypes on the basis of rank order of agonist potency. The most potent agonist at the P_{2Y} subtype is 2-methylthio ATP (2MeSATP) and its action is linked to phospholipase C (PLC) activation. A further PLC linked 'nucleotide receptor' has recently been proposed where 2MeSATP has little or no effect but where ATP and UTP are both potent agonists (O'Connor et al., 1991; Brown et al., 1991). We have previously demonstrated heterogeneous responses to P_2-purinergic stimulation when comparing endothelial cells from different domains of the vasculature (Allsup and Boarder, 1990; Purkiss et al., 1992). In this report we provide evidence that there are two, pharmacologically distinct, receptors for 5'-nucleotides linked to PLC activation on the same endothelial cell type.

Bovine aortic endothelial cells (BAEC's) were prepared from collagenase treated aortae and seeded into 24 well NUNC plates and maintained in MEM d-valine with 10% foetal calf serum. Agonist concentration effect (E/[A]) curves were constructed to a range of 5'-nucleotide analogues in BAEC's prelabelled for 48h with myo-(2-[^{3}H])-inositol. Cells were stimulated for 30 min at 37°C in nominally calcium free HEPES buffered (pH7.4) balanced salt solution with 10mM LiCl. Total [^{3}H]inositol phosphates were measured after extraction into chloroform and purification on Dowex-1 (Cl^-).

Agonist stimulation gave the following rank order of potency: 2MeSATP>ADPβS>ATPγS>ATP=UTP>>β,γ-methylene ATP (EC_{50} values: 0.413 ± 0.078, 0.846± 0.4, 11.65 ± 2.9, 16.67 ± 6.67, 32.79 ± 9.4, n=3). ATPγS gave the largest maximal response, approximately equal in magnitude to the sum of the responses to 2MeSATP and UTP. Additivity experiments were carried out with E/[A] curves constructed to UTP and ATPγS in the presence or absence of 100μM 2MeSATP, ADPβS or UTP. There was significant (p=0.01) additivity in the response to UTP when either 2MeSATP or ADPβS were present, due to P_{2Y} stimulation. There was no additivity in the response to ATPγS when UTP was present and both UTP and 2MeSATP caused a small (2.5 fold) rightward shift of the ATPγS E/[A] curve. However, 2MeSATP produced a significant (p=0.05) additivity of the ATPγS maximum.

These results indicate that BAEC's possess two separate 5'-nucleotide receptors linked to PLC activation i.e. P_{2Y} and nucleotide subtypes with 2MeSATP and UTP being the most potent agonists respectively. The results suggest that the PLC mediated endothelial responses to ATP are due to it action via both P_{2Y} and nucleotide receptors.

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

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Brown, H.A., Lazarowski, E.R., Boucher, R.C. and Harden, T.K. (1991) Mol. Pharmacol. 40, 648-655.
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Purkiss, J.R., Wilkinson, G.F. and Boarder, M.R. (1992) Br. J. Pharmacol. Proc. Suppl. (this meeting).

108P REPEATED PAROXETINE ADMINISTRATION IN THE RAT PRODUCES A DECREASED [^{3}H]KETANSERIN BINDING AND AN ANXIOLYTIC PROFILE IN THE ELEVATED X-MAZE

A.K. Cadogan, I.K. Wright, I. Coombs, C.A. Marsden, D.A. Kendall and I. Tulloch[1]. Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham, NG7 2UH, UK. [1]SmithKline Beecham, Coldharbour Rd., Harlow, Essex, CM19 5AD, UK.

Studies with chronic administration of antidepressant drugs investigating adaptive changes in central receptor function have implicated alterations in both β-adrenoceptor (Banerjee *et al.*, 1977) and 5-HT_2 receptor (Peroutka and Snyder, 1980) number and function. However, unlike most other antidepressants, the specific 5-HT uptake inhibitor, paroxetine, has been shown to reduce 5-HT_2 but not β-adrenoceptor number (Nelson *et al.*, 1989). The aim of this study was first to determine the effects of chronic paroxetine administration on 5-HT_2 receptor number and its associated transmembrane signalling system, and second to investigate whether paroxetine has anxiolytic-like effects in the elevated X-maze, a test of anxiety.

Male, Lister hooded rats (initially 200-250g) received an oral injection of either vehicle (1% methyl cellulose, 1ml/kg) or paroxetine (3mg/kg) daily for 21 days. One hour after the last dose of paroxetine the rats were placed on the elevated X-maze and their behaviour monitored for a 5 min period. The percentage time and percentage entries into the open arms, and the actual time on the open and ends of the open arms were used as measures of anxiety. The total number of arm entries was used as an index of locomotor activity. 24 hour after the last dose of paroxetine the rats were killed, their cortices dissected out and sliced (350μm) on a McIlwain tissue slicer. Half the slices were incubated with [^{3}H]myo-inositol, 5-HT and related agonists added to stimulate phosphoinositide (PI) hydrolysis, and then total [^{3}H]inositol phosphates separated by ion-exchange chromatography. The remaining slices were homogenised and membranes prepared for 5-HT_2 receptor binding using [^{3}H]ketanserin.

Rats treated chronically with paroxetine spent significantly more time on the open arms (cont.=34.0±7.0, parox.=51.6±5.5; n=6, p<0.05, 2 tailed Mann-Whitney U-test) and had significantly increased % open:total entries and time (%entries: cont.=27.2±3.2, parox.=36.3±1.6; % time: cont.=14.1±2.1, parox.=21.3±2.4; n=6, p<0.05, 2 tailed Mann-Whitney U-test for both). There was no difference in the number of total arm entries between the two groups. Chronic paroxetine treatment significantly increased basal PI hydrolysis (p<0.05, unpaired Student's t-test) without affecting the 5-HT-stimulated PI response. Preliminary binding data indicate that chronic paroxetine treatment reduces [^{3}H]ketanserin binding (p<0.05, unpaired Student's t-test), the effect being the result of an increased K_D in the paroxetine group (cont. K_D=3.0nM, parox. K_D=4.8nM).

The findings contrast with a previous report of the effects of another 5-HT uptake inhibitor, sertraline, which decreased 5-HT-stimulated PI hydrolysis without altering 5-HT_2 receptor number or affinity (Sanders-Bush *et al.*, 1989). The anxiolytic profile in the elevated X-maze suggests that paroxetine may have use in the clinical treatment of anxiety as well as its established role as an antidepressant.

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Sanders-Bush, E., Breeding, M., Knoth, K. and Tsutsumi, M. (1989). Psychopharmacol. **99**, 64-69.

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G.F. Wilkinson* and M.R. Boarder, Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, LEICESTER. LE1 9HN.

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109P THE HAEMODYNAMIC EFFECTS OF INTRA-PERIAQUEDUCTAL GREY ADMINISTRATION OF 8-OHDPAT ON A CHEMICALLY-INDUCED DEFENCE RESPONSE

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D,L-Homocysteic acid (DLH) administered into the dorsal periaqueductal grey (DPAG) of rats produces behavioural and cardiovascular components of the defence response (Hilton and Redfern 1986). Activation of 5-HT_{1A} receptors within the rat (DPAG) has recently been shown to attenuate the behavioural components of a chemically induced defence response (Beckett et al. 1992). Quantifiable parameters including response duration and number of defensive jumps, elicited by DLH, were significantly reduced by local pretreatment with 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT). The present work was undertaken to investigate the effects of 8-OHDPAT on DLH induced changes in cardiovascular function.

Male hooded Lister rats (200-250g) were anaesthetised with urethane (1.4g/kg i. p.) and the carotid artery cannulated for the measurement of blood pressure and heart rate. Stainless steel guide cannulae were implanted 2mm above the DPAG (AP-7.0, ML+0.2, DV-3.1mm relative to bregma). Percentage changes in basal skeletal muscle blood flow were determined by means of a laser doppler flow probe placed on the gastrocnemius muscle. The response to DLH (8nmols in 250nl) in the DPAG was recorded 10 minutes after intra DPAG pretreatment with either 8-OHDPAT (25nmols in 250nl) or saline (0.9% 250nl). Histological verification was carried out on all microinjection sites. DLH produced an elevation in blood pressure, heart rate and skeletal

Treatment	Blood Pressure Change ($mmHg^{-1}$)	Heart Rate Change (bpm)	Skeletal muscle blood flow (% change from basal)
DLH	+31 ± 4**	+ 64 ± 8 **	+ 62 ± 11
8-OHDPAT	- 18 ± 3**	- 45 ± 6**	- 36 ± 10
8-OHDPAT + DLH	+ 40 ± 4**	+ 70 ± 3**	+ 88 ± 22

Table 1. Haemodynamic effects of dorsal PAG administration of DLH, 8-OHDPAT and 8-OHDPAT prior to DLH (* p<0.05, ** p<.001; paired t-test; n=6)

muscle blood flow. Administration of 8-OHDPAT resulted in a significant fall in blood pressure , heart rate and skeletal muscle blood flow. Subsequent DLH administration produced rises in blood pressure, heart rate and skeletal muscle blood flow (table 1). These results suggest that activation of 5-HT_{1A} receptors within the DPAG produce a depressor effect, while having no effect on the cardiovascular component of defence. This indicates that it is possible to separate pharmacologically the behavioural and cardiovascular components of the defence response.

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Hilton, S.M. & Redfern, W.S. (1986) J. Physiol. **378:** 213-228.

110P DIFFERENTIAL AGE-RELATED CHANGES IN 5-HT AGONIST-INDUCED BEHAVIOURS IN THE HOODED LISTER RAT

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Although there has been extensive research into the neurochemical changes associated with ageing confusion still surrounds the changes induced in the central 5-HT system. The literature contains a variety of conflicting reports (for review see McEntee and Crook 1991). In an attempt to further clarify the situation we investigated age-related changes in central 5-HT_{1A}, 5-HT_{1C} and 5-HT_2 receptor responsiveness in Hooded Lister rats.

Mature (3-4 months, 300-500g) and aged (18-19 months, 500-710g) male Hooded Lister rats were habituated to perspex cages (25 cm long x 18 cm wide x 18 cm high) for 30 minutes prior to treatment with the 5-HT_2/5-HT_{1C} agonist 1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane (DOI), and the selective 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-dipropylamino) tetralin (8-OH-DPAT). Wet dog shakes (WDS) elicited by DOI , (0.1-0.8 mg/kg i.p), were recorded over a 30 minute test period. Flat body posture induced by 8-OH-DPAT, (0.01-0.25 mg/kg s.c.), was scored (0 absent-3 continuous) every 5 minutes over the 30 minute test. Body temperature, taken rectally, was recorded in the DOI and 8-OH-DPAT treated animals immediately prior to injection and at the end of the test period. Animals treated with the 5-HT_{1C}/5-HT_2/5-HT_{1B} agonist m-chlorophenylpiperazine (m-CPP 1.0-10 mg/kg s.c), were injected twenty minutes before being placed into the activity cages where they remained for 5 minutes. Locomotor activity was scored (0 absent-3 continuous).

DOI induced WDS, a 5-HT_2 receptor mediated effect (Fone *et al* 1991), were increased in aged compared to mature rats (n=6/group, total±s.e.m at 0.1, 0.4 and 0.8 mg/kg for mature 1.50±1.05, 3.50±1.35, 6.50±2.60, and for aged 3.00± 1.27, 7.33±1.83, 8.50±3.00). In contrast DOI induced hyperthermia appeared to be less marked in the aged animals (mean change±s.e.m at 0.1 and 0.4 mg/kg for mature +0.66±.37, +0.27±.23, and aged +0.15±.20, -0.15±.17); a hypothermia, significantly different from the response observed in the mature animals ($p<0.05$, 2-tailed Mann Whitney U-test), was seen in the aged group at 0.8 mg/kg (mean change±s.e.m for mature +0.52±.22 and aged -0.27±.16) suggesting an action at an additional receptor subtype. In comparison there was no significant difference between the age groups in the 5-HT_{1A} receptor mediated flat body posture (n=4/saline groups, n=7/drug groups, mean score±s.e.m at 0.01, 0.05, 0.25 mg/kg for mature 1.86±.89, 10.00±1.66, 15.29±.77 and aged 5.14±.98, 10.86±.76, 15.57±1.76) or hypothermia (mean change±s.e.m for mature +0.06±.24, +0.09±.32, -0.54±.28 and aged +0.10±.14, +0.23±.26, -0.41±.20) induced by 8-OH-DPAT. The m-CPP treated animals demonstrated a dose related hypoactivity which was of greater magnitude in the aged compared to mature rats (n=6/group mean score±s.e.m at 1.0, 5.0 and 10.0 mg/kg for mature 1.83±.34, 0.67±.23, 0.17±.18 and aged 1.00±.28, 0.33±.23, 0.00±0.00).

These results suggest that there are differential, age -related changes in the central 5-HT system. There appears to be an increase in 5-HT_2 and 5-HT_{1C} receptor responsiveness while 5-HT_{1A} mediated effects remain unchanged.

L.Robson is a MRC funded student in conjunction with UCB.
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111P INTERACTION OF (R)- AND (S)-ZACOPRIDE AND 5-HT$_3$/5-HT$_4$ RECEPTOR LIGANDS TO MODIFY EXTRACELLULAR LEVELS OF 5-HT IN THE RAT FRONTAL CORTEX

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The isomers of zacopride have been demonstrated to display differential activities to modify extracellular levels of 5-hydroxytryptamine (5-HT) in the rat frontal cortex and behavioural studies in the mouse have indicated that the anxiolytic-like action of (R)-zacopride can be reversed by (S)-zacopride (Barnes *et al.*, 1992; Cheng *et al.*, 1992). In the present study, we investigate the interaction between (R)- and (S)-zacopride and other 5-HT$_3$/5-HT$_4$ receptor ligands to modify the *in vivo* release of 5-HT from the frontal cortex of the freely-moving rat using the intracerebral microdialysis technique as described in Cheng *et al.*, 1992.

Microdialysis probes located in the frontal cortex of male hooded-Lister rats were perfused with artificial CSF. 20-min samples were collected over a 7 hour period and analysed for 5-HT by HPLC-ECD. After four basal level samples (t=0), the animals were then treated with either vehicle (0.9% NaCl) or drugs (all at 100 µg/kg) 5 min prior to the administration of (R)-zacopride ((R)-zac; 10 µg/kg i.p.).

Table 1. The effects of (S)-zacopride, renzapride, ondansetron or vehicle to modify the (R)-zacopride-induced reduction of extracellular levels of 5-HT in the rat frontal cortex.

Treatment (1ml/kg i.p.)	n	Collection time (min) 0	20	40	60	80	100	120	340
Vehicle + (R)-zac	8	101±6	60±10*	63±9*	51±13*	36±10**	38±10**	35±6**	30±10**
Ondansetron + (R)-zac	8	100±2	76±4*	71±3*	65±5*	61±6**	47±5**	49±7**	51±2**
(S)-Zacopride + (R)-zac	8	101±2	96±4	108±5	100±3	110±5	105±3	102±3	95±5
Renzapride + (R)-zac	6	99±5	93±4	97±2	100±4	100±1	95±2	96±3	96±3

Data represent mean±SEM (% of the basal values; 37-63 fmol/40µl sample). Significant reduction compared to the basal levels (one-way ANOVA, followed by Dunnett's t test) is indicated by *P<0.05, **P<0.01. n = no. of animals.

Ondansetron, (S)-zacopride and renzapride failed to modify extracellular 5-HT levels (data not shown). The 5-HT$_3$/5-HT$_4$ receptor antagonists/agonists, (S)-zacopride and renzapride (Baxter *et al.*, 1991), antagonised the reduction in extracellular levels of 5-HT induced by (R)-zacopride. Ondansetron, a selective 5-HT$_3$ receptor antagonist, failed to modify the effect of (R)-zacopride. This may indicate that the ability of (R)-zacopride to reduce the extracellular levels of 5-HT is unlikely to be a result of an interaction at the 5-HT$_3$ receptor and could be a consequence of an interaction with a (S)-zacopride-insensitive site (Barnes *et al.*, 1990). An involvement at the putative 5-HT$_4$ receptor remains to be investigated.

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112P EFFECT OF REPEATED ANXIOLYTIC DRUG ADMINISTRATION ON 5-HT$_{1A}$ RECEPTOR BINDING IN RAT FRONTAL CORTEX AND HIPPOCAMPUS

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Patients treated with benzodiazepines are often difficult to switch to second generation arylpiperazine type anxiolytics such as buspirone (Schweizer and Rickels, 1986). These drugs interact with 5-HT1A receptors and not the GABA-benzodiazepine receptor complex (Peroutka, 1985). It has been reported that chronic administration of alprazolam, but not ipsapirone, increases mouse hippocampal 5-HT1A receptor binding (McMillen *et al.*, 1990). Thus, it has been postulated that benzodiazepines alter 5-HT1A receptors and, as a consequence, alter patient responses to arylpiperazine drugs. We have now examined the effect of repeated administration of the benzodiazepines, diazepam, chlordiazepoxide and alprazolam and the arylpiperazine, buspirone, on rat frontal cortical and hippocampal 5-HT1A receptor binding.

Male CD rats (100-120g; Charles River) were administered anxiolytic drugs (Table 1) or vehicle i.p. once daily for 14 days. Frontal cortices and hippocampi were removed 24 h after the final treatment. Freshly prepared membranes (2.5mg wet weight of tissue/tube) were incubated with [3H]8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) at 8 concentrations (0.1-5nM) for 30min at 25°C. Non-specific binding was defined by 5-HT (1µM). Binding parameters (Kd and Bmax) were determined using weighted non-linear least-squares curve fitting. Statistical comparisons between drug-treated and the appropriate subgroup of controls (pooled data shown in Table 1 for clarity) were performed using Student's t-test (two-tailed, unpaired).

Table 1. Effect of repeated administration of anxiolytic drugs on [3H]8-OH-DPAT binding

	FRONTAL CORTEX Kd	Bmax	HIPPOCAMPUS Kd	Bmax
Pooled Control [n=32-37]	0.51 ± 0.02	136 ± 4	0.60 ± 0.01	348 ± 4
Diazepam (2.5)	0.43 ± 0.02	137 ± 8	0.59 ± 0.04	349 ± 7
Chlordiazepoxide (7.5)	0.44 ± 0.01	132 ± 5	0.56 ± 0.02	366 ± 8
Alprazolam (2.5)	0.46 ± 0.02	138 ± 7	0.64 ± 0.03	359 ± 7
Buspirone (10)	0.62 ± 0.05	142 ± 3	0.69 ± 0.03	344 ± 4

Kd (nM) and Bmax (fmol/mg protein) values ± s.e. mean (n=7-10). Dose mg/kg in parentheses.

[3H]8-OH-DPAT binding was of high affinity and fitted well to a single site binding model. Chronic administration of all three benzodiazepines, including alprazolam, had no effect on the number (Bmax) or affinity (Kd) of 5-HT1A receptor binding sites in rat hippocampus or frontal cortex (Table 1). This finding is in contrast to that of McMillen *et al.* (1990) who reported alprazolam to increase 5-HT1A binding (albeit at a single ligand concentration) in mouse hippocampus. Repeated buspirone administration also had no effect on 5-HT1A sites (Table 1).

Overall the data provide no evidence that benzodiazepine or arylpiperazine anxiolytics alter the number or affinity of 5-HT1A receptors in frontal cortex or hippocampus. These findings do not, however, preclude a change in 5-HT1A function.

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113P EFFECTS OF THE 5-HT$_2$ ANTAGONIST, RITANSERIN, ON BENZODIAZEPINE WITHDRAWAL-INDUCED WEIGHT LOSS IN RATS

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5-HT2 antagonists have been reported to block behavioural effects of precipitated opiate withdrawal (Neal & Sparber, 1986) and of spontaneous cocaine withdrawal (Meert, 1992). We have therefore assessed the effect of ritanserin on spontaneous benzodiazepine (BZ) withdrawal-induced weight loss (Goudie & Leathley, 1990) to assess the generality of ritanserin's effects on drug withdrawal signs.
Groups of female rats (n=7-10/group; 250-350 g at the start of the study) initially received i.p. injections (2 ml/kg) b.i.d. (1100, 1700 hrs) of saline or chlordiazepoxide (CDP). CDP doses increased daily by 2 mg/kg from 10 mg/kg up to a final dose of 30 mg/kg. CDP treatment was maintained for 26 consecutive days (cf. Goudie & Leathley, 1990; 1991a; 1991b; Leathley & Goudie 1992). Over the next 6 days (experimental days 27-32) animals were wither treated (b.i.d. at 1100 or 1700 hrs) with vehicle (20% propylene glycol with a few drops of tartaric acid), or CDP or ritanserin at one of three doses (0.16, 0.63, 2.5 mg/kg). The index of CDP withdrawal recorded was body weight loss.
In CDP-pretreated animals, which were subsequently treated with vehicle over days 27-32, significant weight loss relative to the saline/vehicle treated control was seen [Repeated measures ANOVA, groups $F(1,18) = 21.3$, $p<0.001$]. Post hoc Tukey tests revealed that significant (alpha = 0.05) weight loss relative to control was seen on all of days 28 through 32. The maximal weight loss recorded (on day 29) was 5% of the pre-withdrawal baseline.
CDP withdrawal-induced weight loss was exacerbated by ritanserin in a dose-related fashion. The effect of ritanserin was not statistically significant to the two lower doses, but it was significant at 2.5 mg/kg [Repeated measures ANOVA groups $F(1,15) = 6.32$, $p<0.05$]. Post hoc Tukey tests revealed that significantly (alpha = 0.05) enhanced weight loss relative to the CDP/vehicle group was seen on all of days 30 through 32. The maximal weight loss observed (on day 30) in the 2.5 mg/kg ritanserin group was 8% of the pre-withdrawal baseline. In saline-pretreated animals ritanserin has no significant effect [Repeated measures ANOVA] at any dose on bodyweight over days 26-32. Thus ritanserin potentiated CDP withdrawal-potentiation (i.e. additive effects of CDP withdrawal and of ritanserin treatment).
These data suggest that 5-HT2 antagonists may be of limited value in the treatment of some aspects of the BZ withdrawal syndrome, although a full characterisation of the effects of such drugs on BZ withdrawal requires that a number of different withdrawal signs should be studied.

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114P THE EFFECTS OF BTS 54 505 ON 5-HT AND NORADRENALINE RESPONSES IN THE RAT DORSOLATERAL GENICULATE NUCLEUS

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The dorsolateral geniculate nucleus (dLGN) relays visual information from the retina to the visual cortex, and receives a diffuse serotonergic (5-HT) innervation from the dorsal raphe nucleus and a noradrenergic (NA) innervation from the locus coeruleus. In the rat, ionophoresis of 5-HT results in inhibition of spontaneous or electrically-evoked activity in geniculate relay cells, while ionophoresis of NA results in facilitation of spontaneous or electrically-evoked activity (Rogawski and Aghajanian, 1980). The aim of this study was to determine the effects of the NA and 5-HT uptake inhibitor BTS 54 505, the primary amine metabolite of the putative antidepressant sibutramine HCl (Luscombe *et al.*, 1989), on the monoamine modulation of retinogeniculate input.

Male Lister hooded rats (200-250g) were anaesthetised with urethane (1.3-1.5g/kg, i.p.) and silicone-coated seven-barreled micropipettes were used to record extracellular neuronal discharge activity (Scott and Mason, 1992). The effects of ionophoresed BTS 54 505 (10mM) on 5-HT- (10mM) and NA- (10mM) evoked responses were evaluated from integrated firing rate histograms. The recovery time (RT), i.e. the period required by the neurone to recover by 50% (RT_{50}) from termination of the ionophoretic ejection, was used as an index of the efficacy of the transmitter uptake process. Ionophoresis of 5-HT resulted in inhibition of visually-evoked firing in all cells studied, whereas ionophoretic ejection of NA resulted in a potentiation of visually-evoked firing. BTS 54 505 was ejected at a current (20nA; 120s) that had no significant effect on basal firing rate and ejections of 5-HT or NA were compared before and during BTS 54 505 application. BTS 54 505 prolonged the suppression of firing induced by ionophoresed 5-HT [mean RT_{50}: (control) 13.0 ± 2.0s; (BTS 54 505) 65.7 ± 24.3s; n=6; mean ± S.E.M.] and also prolonged the potentiation of firing by ionophoresed NA [mean RT_{50}: (control) 29.0 ± 1.7s; (BTS 54 505) 130.8 ± 6.7s; n=6; mean ± S.E.M.]. The magnitude of both 5-HT- and NA-evoked responses was unaffected by BTS 54 505 application.

These neurophysiological studies indicate that BTS 54 505 is a potent inhibitor of 5-HT and NA uptake in agreement with previous uptake studies (Luscombe *et al.*, 1989). The observation that the magnitude of 5-HT- and NA-evoked responses was unaffected by BTS 54 505 suggests that elevation of endogenous 5-HT levels by uptake inhibition does not contribute to the attenuation of NMDA-evoked responses by ionophoretic application of BTS 54 505 (Scott *et al.*, 1991).

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115P DIFFERENT EFFECTS OF 8-OH-DPAT ON FOOD INTAKE IN MALE AND FEMALE RATS

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It is well known that the $5HT_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) increases food intake in non-deprived rats (Dourish et al., 1985; Ebenezer, 1992). However, to the authors' knowledge, all the studies on 8-OH-DPAT and feeding have been carried out in male rats, and as the drug has been shown to produce different effects on copulatory behaviour in male and female rats (see Johansson et al., 1991), the present study was designed to investigate whether the effects of the $5HT_{1A}$ agonist on food intake in female rats were similar to those obtained in male rats.

The effects of various doses of peripherally administered 8-OH-DPAT were examined on food intake in 2 different experiments, i.e. on adult male Wistar rats (n=8) (Expt.1), and adult female Wistar rats (n=8) (Expt.2). The experimental procedure was identical for each experiment. The rats had access to food and water at all times in their home cages. The animals were given 3 training sessions on separate days during which they were placed singly in experimental cages where they had free access to food and water, as described previously ((Ebenezer and Pringle, 1992). During the experimental sessions that followed, each animal was injected s.c. with either physiological saline (control) or 8-OH-DPAT (62.5 and 500 µg kg^{-1}) and placed in the experimental cage for 2h. The amount of food consumed by each rat was measured at the end of this period. A repeated measures design was used, with each rat receiving all doses of 8-OH-DPAT and saline in a random fashion. A period of at least 4 drug free days was allowed between successive tests. 8-OH-DPAT increased feeding in male rats from a mean ± s.e.mean control value of 2.5 ± 0.2 g to 4.4 ± 0.4 g ($P<0.01$) at 62.5 µg kg^{-1} and 4.7 ± 0.6 g at 500 µg kg^{-1}. In contrast, 8-OH-DPAT did not have any significant effects on the amount of food consumed by the female rats. The mean ± s.e.mean food intake in the female rats after saline was 2.1 ± 0.4 g, and 2.6 ± 0.4 g and 2.7 ± 0.4 g after the 62.5 and 500 µg kg^{-1} doses of 8-OH-DPAT respectively. The results of the study show that while 8-OH-DPAT increases food intake in non-deprived male rats, the drug does not stimulate feeding in non-deprived female rats. These results thus indicate a possible sex difference in the response of rats to 8-OH-DPAT on food intake. It is of interest to note that results from further experiments in our laboratory have indicated that doses of 8-OH-DPAT up to 1000 µg kg^{-1} have no effect on feeding in female rats (Ebenezer and Tite, unpublished results).

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116P COMPARISON OF THE 5-HT_{1D}-LIKE RECEPTORS IN THE SAPHENOUS VEINS AND CNS OF THE DOG AND RABBIT

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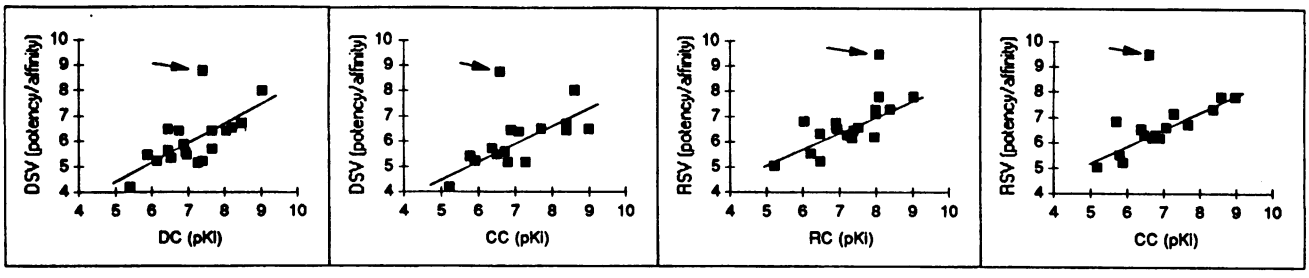
5-HT_1-like receptors mediating contraction of dog (DSV) and rabbit (RSV) saphenous vein share a close pharmacological identity with $5HT_{1D}$ binding site in homogenates of calf caudate (CC, Martin et al 1991, Sumner & Humphrey 1989). Although some ligands discriminate between the vascular receptors and the CC binding site, these differences could be species related (see Hartig et al 1992). Therefore, using a number of agonists and antagonists we have carried out intra-species comparisons of affinity estimates at the vascular 5-HT_1-like receptors and the 5-HT_{1D} binding sites in dog cortex (DC) and rabbit cortex (RC).



The panels above illustrate correlations (excluding the data for methiothepin indicated by arrow) between central 5-HT_{1D} receptor binding affinities (pK_i, CC data from Schoeffter & Hoyer 1990) and potency/affinity estimates at the vascular 5-HT_1-like receptors in dog and rabbit. Agonist potencies are either pK_A or $p[A_{50}]$ (curve midpoint location) and antagonist affinity estimates are either pK_B or pA_2. There were good correlations (with the exception of methiothepin) between DSV and CC (r=0.79) and RSV and CC (r=0.82), although in both cases the slope of the regression was < 1 (DSV: 0.66; RSV: 0.70). Likewise there were good intra-species correlations between central and vascular dog (r=0.78,slope=0.68) and rabbit (r=0.79,slope=0.65) receptors, again with the exception of methiothepin. Therefore, the data demonstrate that the differences between the vascular 5-HT_1-like receptors and the CNS 5-HT_{1D} receptor are maintained within species, suggesting that the receptors in the DSV or RSV are not species homologues of the CC $5HT_{1D}$ receptor but may represent subtypes. Since it has been suggested that the 5-HT_1-like vascular receptors in DSV and RSV can be discriminated (Martin & MacLennan, 1990) it is possible there are multiple $5HT_{1D}$ subtypes as also suggested by Hartig et al (1992).

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117P SPECIES DIFFERENCES IN MULTIPLE [^{3}H]5-CARBOXAMIDOTRYPTAMINE-SENSITIVE (5-HT$_{1D}$-LIKE) BINDING SITES IN VERTEBRATE BRAIN

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(introduced by M.J. Rowan)

Using [^{3}H]5-HT and through the selective masking of 5-HT$_{1A}$ and 5-HT1C receptors, binding sites have been uncovered which display both high (5-HT$_{1D}$) and low (5-HT$_{1E}$) affinity for the 5-HT$_1$-like agonist 5-carboxamidotryptamine (5-CT) (Leonhardt, et al., 1989). 5-CT and sumatriptan produced biphasic displacement of [^{3}H]5-HT in guinea pig striatum (K_i,nM; 5-CT: 0.34 ± 0.01, 190 ± 36; sumatriptan: 7.0 ± 0.4, 343 ± 138) and frontal cortex (K_i, nM; 5-CT: 1.33 ± 0.62, 407 ± 139; sumatriptan: 30 ± 13, 3408 ± 282). To probe the possible heterogeneity in 5-HT$_{1D}$ receptor binding sites, we have eliminated a portion of the complex nature of [^{3}H]5-HT binding (5-HT$_{1E}$) by utilizing [^{3}H]5-CT. [^{3}H]5-CT binding in bovine substantia nigra was rapid, reversible and saturable, displaying high affinity (0.42 ± 0.03 nM) and low non-specific binding (<5%). [^{3}H]5-CT binding was sensitive to guanine nucleotides and labelled a similar number of binding sites as [^{3}H]5-HT (360-400 fmol/mg protein). A linear correlation exists between the potency of competitors (pKi) in displacing both [^{3}H]5-CT and [^{3}H]5-HT. In the corticolimbic region of guinea pig brain, when using a low concentration of [^{3}H]-5-CT for selective labelling of 5-HT$_{1D}$ sites, 5-CT competed in a monophasic manner in both striatum (K_i= 0.68 ± 0.04 nM) and frontal cortex (K_i = 1.85 ± 0.35 nM). However, sumatriptan further differentiated amongst the high affinity [^{3}H]5-CT binding sites in both guinea pig and bovine striatum, frontal cortex and hippocampus, where biphasic displacement curves yielded a high affinity 5-HT$_{1D}$ site, as well as a low affinity sumatriptan-insensitive site. Interestingly, while pigeon brain contains a high density of 5-HT$_{1D}$ receptors (Waeber et al., 1989 a,b); 5-HT, 5-CT and sumatriptan competition for [^{3}H]5-CT binding in optic tectum, brain stem, and telencephalon yielded apparently monophasic displacement curves. These findings have been confirmed autoradiographically demonstrating the localization of multiple [^{3}H]5-CT binding sites in the corticolimbic regions of guinea pig brain, with an apparent lack of heterogeneity in pigeon brain. These results suggest that species differences exist with regard to the multiplicity of 5-HT$_{1D}$-like binding sites in vertebrate brain.

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118P 8-OH-DPAT-INDUCED HYPOTHERMIA IN THE MOUSE MAY BE MEDIATED BY A REDUCTION IN 5-HT RELEASE ONTO POSTSYNAPTIC 5-HT$_{1C}$ RECEPTORS

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8-OH-DPAT-induced hypothermia in the mouse appears to be mediated by the activation of inhibitory 5-HT$_{1A}$ autoreceptors, thereby decreasing 5-HT release (Goodwin et al., 1985; Bill et al., 1991). However, the postsynaptic 5-HT receptor(s) involved in this response have not been identified. We have previously shown that the 5-HT$_{1C/2}$ receptor agonist, DOI, reverses 8-OH-DPAT-induced hypothermia in the mouse (Bill et al., 1990), suggesting the involvement of 5-HT$_{1C}$ and/or 5-HT$_2$ receptors. The aim of this study was to further characterise the postsynaptic 5-HT receptor(s) involved in the hypothermic response to 8-OH-DPAT in the mouse.

Female albino Tuck mice (26-35g; n=8/treatment) were used throughout. In the hypothermia experiments (ambient temperature 21.0 ± 0.5°C) test drugs or vehicle were administered i.p. 20 min before the s.c. injection of 8-OH-DPAT (0.5 mg/kg). Rectal body temperatures were recorded immediately before each drug injection, and at 15 and 30 min after 8-OH-DPAT. This dose of 8-OH-DPAT induced mean ± s.e.mean decreases in body temperature of 2.3 ± 0.4 to 3.0 ± 0.5 °C. The 5-HT$_{1B/1C}$ receptor agonists mCPP, TFMPP and MK212, and the 5-HT$_{1C/2}$ agonist DOI (0.01-1.0 mg/kg), dose-dependently attenuated 8-OH-DPAT-induced hypothermia, with minimum effective doses of 0.03, 0.1, 0.1 & 0.1 mg/kg i.p. respectively. None of the 5-HT$_{1B/1C/2}$ agonists alone had any significant effect on mouse body temperature at doses up to 1.0 mg/kg i.p. In further experiments the potencies of several 5-HT$_{1C/2}$ antagonists to block the attenuation of 8-OH-DPAT-induced hypothermia by mCPP (0.5 mg/kg i.p.) and DOI (0.5 mg/kg i.p.) were compared with their potencies as antagonists of the 5-HT$_2$ receptor mediated head twitch response in mice. In the latter experiments carbidopa (25 mg/kg i.p.) was administered 30 min prior to the co-administration of the antagonists (i.p.) and L-5-hydroxytryptophan (5-HTP; 300mg/kg s.c.). Head twitches were counted over a 4 min period 30 min after 5-HTP administration. The 5-HT$_{1C/2}$ antagonists, mesulergine, ritanserin, mianserin, ketanserin and altanserin dose-dependently and potently blocked the head twitch response to 5-HTP (ID_{50s} were 0.09, 0.05, 0.008, 0.01 & 0.01 mg/kg i.p. respectively). The reversal of 8-OH-DPAT-induced hypothermia in the mouse produced by mCPP or DOI was dose-dependently and significantly ($P<0.05$; ANOVA/t-test) attenuated by mesulergine, ritanserin and mianserin (ID_{50s} against mCPP were 0.12, 0.2 and 0.6 mg/kg i.p. respectively; ID_{50s} against DOI were 0.09, 0.2 and 0.12 mg/kg respectively). In contrast, ketanserin and altanserin (0.03-0.3 mg/kg i.p.), which display some selectivity for the 5-HT$_2$ receptor over the 5-HT$_{1C}$ receptor (Hoyer, 1988), did not significantly attenuate the reversal of hypothermia elicited by mCPP and DOI. Similarly the 5-HT$_3$ receptor antagonist, WAY100289 (Rhodes et al., 1991; 1.0 mg/kg i.p.), and the 5-HT$_{1B}$ antagonist, (-)propranolol (5 & 15 mg/kg i.p.), failed to modify the reversal of 8-OH-DPAT-hypothermia by mCPP. These data suggest that 8-OH-DPAT-induced hypothermia in the mouse may involve a reduction in 5-HT release onto postsynaptic 5-HT$_{1C}$ receptors.

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119P PHARMACOLOGY OF THE 5-HT-INDUCED DEPOLARIZATION OF THE FERRET ISOLATED VAGUS NERVE

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Chemotherapeutic agents such as cisplatin induce vomiting which can be blocked by 5-HT_3 receptor antagonists. In the ferret, this emetic response is thought to be due to the activation of vagal 5-HT_3 receptors (Reynolds et al., 1991). We have used grease-gap recordings from ferret vagus nerve trunks in vitro to determine whether 5-HT_3 receptors are present on these axons.

Ten female ferrets were killed by pentobarbitone (200 mg/kg i.p.). The two cervical trunks of the vagus nerve were excised, desheathed, cut into two equal lengths and set up in three compartment baths for grease-gap recording at 25°C using methods as previously described (Newberry et al., 1991;1992). The agonists were applied via the superfusing medium for 1 min periods with 10-30 min intervals between applications. The agonist application was terminated if the response faded during its superfusion. The pA_2 of (+)-tubocurarine was estimated from $pA_2=\log_{10}(DR-1)-\log_{10}$(molar concentration of (+)-tubocurarine).

5-HT (100 μM) reproduceably induced a fast depolarization of the ferret vagus nerve. The pEC_{50} for 5-HT was 4.9±0.04 (mean±s.e.mean; n=12). This response was mimicked by the 5-HT_3 receptor agonist 2-methyl-5-HT (pEC_{50}=5.2±0.04, n=5) but not by the potent 5-HT_1 receptor agonist 5-carboxamidotryptamine (1 μM, n=3). The maximum response induced by 2-methyl-5-HT was similar to that of 5-HT. The 5-HT response was unaffected by the 5-$HT_{2/1C}$ antagonist ketanserin (1μM, 30 min, n=3) or the selective serotonin reuptake inhibitor paroxetine (1 μM, 30-60 min, n=3). Granisetron (1 nM, 60 min, n=4) reduced the maximum response to 5-HT by 50% but produced little increase in the EC_{50} for 5-HT, a similar situation to that observed on the rat vagus nerve (Newberry et al., 1992) . (+)-Tubocurarine (3 μM, 60 min, n=5) produced a clear rightward shift of the dose response curve to 5-HT , but only reduced the maximum response by 18%. The estimated pA_2 for (+)-tubocurarine (1 μM, n=2; 3μM, n=5) against 5-HT was 6.6±0.1. Against 2-methyl-5-HT, its estimated pA_2 was 6.6±0.1 (n=3).

The data indicate that 5-HT depolarizes the ferret vagus nerve via 5-HT_3 receptors. The 5-HT_3 receptors of the ferret appear to be pharmacologically different from those in mouse, guinea-pig and possibly rat tissues where (+)-tubocurarine has pA_2 values of 8.1, 4.8 and 7.1, respectively (Newberry et al, 1991).

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120P THE PHARMACOLOGY OF RESPONSES EVOKED IN LUMBAR MOTONEURONES OF THE NEONATE RAT SPINAL CORD BY DESCENDING STIMULATION

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We have previously described the actions of 5-HT on the neonate rat hemisected spinal cord preparation and the effects of stimulating descending spinal pathways (Elliott & Wallis, 1991 & 1992). Intracellular recordings were made from motoneurones (MN, L_{4-6}) as before. Descending pathways were stimulated using a fine bipolar electrode on the surface of the ventrolateral funiculus (T_{11-12}). In some experiments, glutamate depolarizations were obtained by pressure ejection (100 mM, 20 psi, 0.1-1s).

Following either repetitive short (0.1 ms) or single long duration (1 ms) supramaximal stimulation, a slow, delayed depolarization was recorded in 23/33 MNs whose amplitude was 5.5 ± 0.5 mV (mean ± s.e.mean). This depolarization was markedly potentiated by cocaine (3-10μM) both in amplitude (10.3 ± 1 mV, n=7) and duration. In the presence of 0.1μM citalopram, a slow depolarization was seen in 21/24 cells (8.9 ± 1 mV). This response was consistently blocked by the 5-HT_2 antagonists, ketanserin (1μM, n=2, 82-91% reduction) and LY 53857 (1μM, n=3, complete block in two, 50% in one). Prazosin (0.1μM) had less consistent effects, but an apparent reduction (≈ 25%) was seen in 3 of 6 experiments. The results suggest that the slow depolarization is mainly due to the release of endogenous 5-HT.

The action of a number of agonists on fast EPSPs evoked by dorsal root (DR) or descending stimulation have been investigated and compared to their effects on glutamate depolarizations. Preliminary experiments indicate that 5-CT (0.1μM) was effective at reducing the fast EPSP following DR stimulation but has little effect on that following descending stimulation, whereas the opposite was the case for noradrenaline (1-10μM, in the presence of 0.1μM prazosin), Baclofen (1μM) reduced both equally. Neither 5-CT or noradrenaline had significant effect on the depolarizing responses to short (0.5 s) pulses of glutamate. A long lasting inhibitory action on DR responses following a single descending stimulus has been detected. The pharmacology of this response is currently being investigated.

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121P THE EFFECT OF OXYGEN ON THE HUMAN UMBILICAL ARTERY

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We have previously demonstrated in the human umbilical artery that an increase in oxygen tension above 2.5% results in a transient contraction due to release of thromboxane (Templeton *et al*, 1991). The procedure of sequential increases in oxygen tension indicated that increasing oxygen tension leads to an increase in the response. However subsequent experiments which were done to examine the effects of selective thromboxane receptor antagonists indicated that repeated exposure to a given oxygen tension led to an increased response. This study was carried out in an attempt to examine whether the oxygen response was time or oxygen tension related. We have previously demonstrated that in 2.5% oxygen the response to 5-HT is mediated via 5-HT_2 receptors, but on increasing the oxygen tension to 20% a 5-HT_1 receptor mediated response is uncovered (MacLennan *et al*, 1989). We therefore wanted to examine whether the 5-HT_1 receptor mediated response requires such a high oxygen tension .
Umbilical arteries were dissected free from surrounding Wharton's jelly and set-up as 1-1.5cm length longitudonal strips in Krebs' solution at 37°C in 2.5% O_2,8% CO_2 balance N_2 under 1-1.5g.wt. The tissues were left to equilibrate for 1.5 hours before experimentation. In all subsequent oxygen changes the CO_2 was maintained at 8% with the balance made up with N_2. We randomised the exposure of the tissues to seven different oxygen tensions (5-50%). We also varied the time between exposures as well as washing out between exposures. In addition we examined the effect of 5,10 & 15% oxygen tension on the ability of the vessels to demonstrate the 5-HT_1 receptor response. Results were calculated as a percentage of the maximum response to oxygen or 5-HT in each individual tissue. There was no correlation between the absolute oxygen tension and the maximum response (coefficient 0.25) but there was a positive correlation (0.87) between the number of exposure to oxygen and the maximum response. Washing the tissues out between exposures had no effect on their ability to contract to oxygen when compared with a control. In addition increasing the time between the exposure to oxygen neither increased nor decreased the size of contraction. Finally we found that the 5% oxygen tension was just as effective at uncovering the 5-HT_1 receptor mediated response as 10 or 15% and the 5-HT_1 response was of the same magnitude as that previously reported for 20%.
In conclusion therefore we have demonstrated that the contraction to increasing oxygen tension seen in the human umbilical artery is not dependent on the oxygen tension but on the number of the exposure. In addition only a small increase in oxygen tension is required to uncover the 5-HT_1 receptor. This would seem to suggest that the response to increasing oxygen tension above 2.5% is initially an all or nothing response which becomes sensitised with time. Since the system works through stimulation of the cyclo-oxygenase enzyme it may be that this enzyme can be induced.

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122P POTENTIATION OF THE RELAXATION RESPONSE TO 5-METHOXYTRYPTAMINE IN THE RAT ILEUM WITH MONOAMINE OXIDASE INHIBITORS

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The relaxation response to 5-hydroxytryptamine (5-HT) in the terminal rat ileum is mediated via a putative 5-HT_4 receptor (Tuladhar et al., 1991) However, in this system 5-methoxytryptamine (5-MeOT) appears to be a very weak agonist (Bockaert et al., 1992). Therefore we have investigated whether the low efficacy of 5-MeOT is due to its inactivation by monoamine oxidase.

Isolated ileal segments (2 cm, taken approximately 3-20 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) containing methysergide (1 µM) and atropine (0.1 µM) under an initial tension of 0.75 g. The tissues were incubated for 30 min with a monoamine oxidase inhibitor (MAOI). The concentration response curves to 5-HT and 5-MeOT were obtained in a cumulative manner using a 2 min contact time. The comparison of the effects of the treatment of MAOI was carried out using paired preparations.

The responses to 5-MeOT were potentiated by the treatment with pargyline (100 µM), a nonspecific A and B MAOI. The degree of potentiation calculated from the EC_{50} ratios was 19. In contrast, the concentration effect curve for 5-HT (pEC_{50}±s.e.mean 6.79±0.09) was not potentiated by the pargyline treatment (pEC_{50}±s.e.mean 6.90±0.04). The monoamine oxidase A specific inhibitor clorgyline produced a similar potentiation (EC_{50} ratio about 12) of the 5-MeOT at a concentration of 0.1 µM. l(-)-Deprenyl (up to 1 µM) was ineffective although at a concentration of 10 µM produced a similar potentiation of the 5-MeOT response (Table 1).

Table 1. Effects of MAOI's on the relaxation response to 5-MeOT in the rat ileum.

	Pargyline		Clorgyline		Deprenyl			
	Control	100 µM	Control	0.1 µM	Control	0.1 µM	1 µM	10 µM
pEC_{50}±s.e.mean	5.12±0.1	6.41±0.08*	5.41±0.22	6.49±0.2*	5.13±0.07	4.97±0.09	5.08±0.07	6.22±0.07*
EC_{50} ratio		19		12		0.7	0.9	12

*Significantly different from the control value ($P<0.05$ paired Student "t" test)

The results indicate that the low efficacy of 5-MeOT to cause relaxation in the rat ileum preparation may partly reflect its inactivation via oxidase enzymes. The potentiation of 5-MeOT but not 5-HT by the MAOI's is probably due to a selective intracellular diffusion of the more lipophilic 5-MeOT. The selectivity of the action of clorgyline indicates a probable involvement of MAO type A in the metabolism of 5-MeOT. The findings emphasise the importance of preventing drug metabolism when using agonists in a receptor characterisation in the gut.

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123P COMPARISON OF THE BRONCHOCONSTRICTION BY 5-HT AND METHACHOLINE IN CONSCIOUS GUINEA-PIGS: A POSSIBLE REFLEX ACTION BY 5-HT

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5HT is commonly used as a bronchocontrictor to demonstrate airway hyperreactivity in anaesthetized guinea-pigs, following bronchial anaphylaxis (Daffonchio, Lees, Payne & Whittle, 1987). Methacholine (MCh) is also a standard bronchoconstrictor used in human studies (Chung & Snashall, 1984). The aim of this study was to compare the effects of inhaled 5HT and MCh in conscious guinea-pigs and to investigate their susceptibility to blockade with atropine.

Bronchoconstrictor responses were measured in unanaesthetized, spontaneously breathing guinea-pigs previously sensitized with 1ml ip of a suspension containing 10µg ovalbumen and 100mg aluminium hydroxide in normal saline. Changes in specific airway conductance (sGaw) were determined by whole body plethysmography (Griffiths-Johnson, Nicholls & McDermott, 1987).

Animals were exposed to nebulized solutions of 5HT or MCh (30, 100 and 300µg/ml in 0.9% NaCl) in a sealed box for 1 min. sGaw was measured before and at 2, 5, 10 and 20 min after exposure. Both agonists caused dose-related bronchoconstriction, as a reduction in sGaw, the peak occurring at 2 min and baseline being restored by 20 min. The doses for a 75% reduction in sGaw (ED_{75}) were 260 and 105µg respectively, for MCh and 5HT, indicating the greater potency of 5HT.

To examine the effect of atropine, guinea-pigs received a control challenge inhalation of either 5HT (500µg/ml) or MCh (300µg/ml) and the following day atropine was administered (ip) ½ to 1½ hr before repeating the inhalation challenge. The inhibitions of the 5HT-induced change in sGaw by atropine, measured at 2 min, were 88.0±12.1, 57.4±27.7 and 53.6±7.5% (n=4) for the 1mg, 100µg and 10µg/kg doses. The blockade of MCh-induced bronchoconstriction by atropine was 69.9±31.4 and 56.6±24.7% (n=4) by 1mg and 100µg/kg, respectively.

Thus a dose-related blockade by atropine of the bronchoconstriction induced by both 5HT and MCh was observed over the same dose range. This suggests that the bronchoconstriction by 5HT is mediated via muscarinic receptors possibly by stimulation of afferent pathways of a vagal reflex, rather than by a direct effect on airway smooth muscle 5HT receptors. The latter mechanism may apply to anaesthetized animals. 5HT may therefore be inappropriate for evaluating airway hyperreactivity in conscious guinea-pigs.

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124P 5-HT CONCENTRATION RESPONSE CURVES IN TERTIARY SURFACE CHORIONIC ARTERIES

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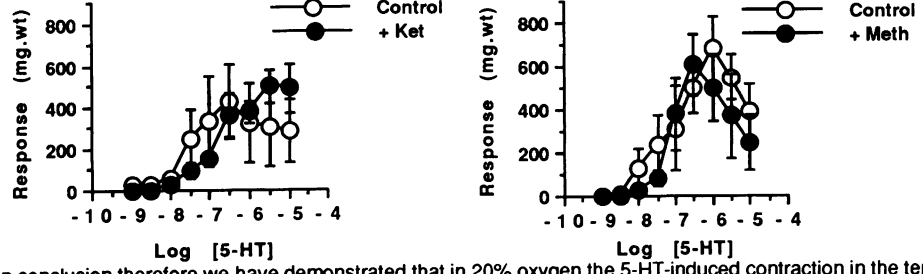
In the human umbilical artery(HUA), 5-HT will produce a contraction in 2.5% oxygen which is mediated via 5-HT_2 receptors. If the oxygen is elevated to 20% this contraction becomes biphasic with the response mediated by both 5-HT_1 and 5-HT_2 receptors (MacLennan *et al* 1989). In a recent study we made a comparisson of the effects of 5-HT in various branches of the umbilical-placental circulation (MacLean *et al* 1992) and found that responses to 5-HT in the tertiary placental arteries in 2.5% oxygen were biphasic with higher concentrations producing a relaxation. This study shows preliminary results investigating the 5-HT receptors involved in the response of this vessel and whether there is an oxygen dependent component.

Human placentas were obtained from term pregnancies. Tertiary surface chorionic arteries(TCA) were identified as the third branching of the umbilical artery after it left the umbilical cord. The vessels were cut into 2-3mm ring segments and suspended in Krebs' under 1500mg.wt. in 2.5% O_2, 8% CO_2 balance N_2 at 37°C for 1.5 hours before experimentation. 5-HT was added cumulatively (1nM-10µM) either alone or in the presence of ketanserin (1nM-0.1µM) or methiothepin (0.1nM-10nM). The tissues were then washed and left for one hour before the oxygen tension was increased to 20% and the procedure outlined above was repeated.

In 2.5% oxygen responses to 5-HT peaked at 1µM and fell from 302±59 to 197±50mg.wt.when increased above this. In the presence of ketanserin(1nM) this fall off of response was reduced and was abolished with 10nM and 1µM. In contrast methiothepin(0.1-1nM) had little or no effect with 10nM almost completely inhibiting all responses, this being consistant with a selective effect at 5-HT_2 receptors. In 20% oxygen, responses to 5-HT were significantly enhanced with the maximum response increasing from 302mg±59 to 672±120mg.wt. The contraction to 5-HT peaked at 0.3µM and then relaxed but to a greater degree than observed in 2.5% oxygen, i.e. fell from 672±120 to 390±85 mg.wt. Ketanserin, at all concentrations, reversed the relaxation seen to 5-HT at the higher concentrations (Fig1a, 10nM ketanserin)). In contrast methiothepin(1nM) had no effect on the relaxation(Fig1b) except for a small reduction at the highest concentration(10nM)



In conclusion therefore we have demonstrated that in 20% oxygen the 5-HT-induced contraction in the tertiary placental artery, like the umbilical artery, the 5-HT response is mediated by 5-HT_2 and 5-HT_1 receptors. In addition activation of these receptors at low concentrations causes a contraction, while higher concentrations produce a relaxation also mediated by 5-HT_2 receptors.

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125P DAU 6285: A PROBE FOR THE 5-HT$_4$ RECEPTOR IN RAT OESOPHAGUS AND MONKEY BLADDER

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DAU 6285 has been reported to act as a silent, competitive antagonist at 5-hydroxytryptamine$_4$ (5-HT$_4$) receptors in several functional systems *in vitro* (Turconi *et al.*, 1991; Baxter *et al.*, 1992). The present study has examined the pharmacology of DAU 6285 in the tunica muscularis mucosae (TMM) of rat oesophagus and on strips of urinary bladder from Rhesus monkeys.

The TMM was set up in Tyrode's solution containing cocaine (30μM), corticosterone (30μM), and methysergide (1μM; see Baxter et al., 1991). Strips of urinary bladder (0.5 x 2.0 cm), taken from either side of the dorsal midline (central region) and dissected free of mucosal and connective tissue, were set up in Tyrode's solution (as above, plus ondansetron, 5μM and indomethacin, 10μM). The preparations were subjected immediately to transmural, electrical field stimulation (20Hz; pulse duration 1 ms; supramaximal voltage; pulse train 5s every 60s). After equilibration for 30 min, the strips were exposed to tetrodotoxin (3μM), followed by washout, and recovery.

In the TMM, DAU 6285 (0.3 to 10μM) evoked parallel, dextral shifts in the concentration-effect curve to 5-HT, yielding a pA_2 estimate of 6.9 (slope of Schild regression = 1.02). The pA_2 estimates for DAU 6285 were independent of incubation time (tested at 1 and 3 h) and agonist. However, DAU 6285 increased the maximum response to 5-HT, 5-MeOT and carbachol; an effect blocked by pretreatment of rats with p-chlorophenylalanine (300 mg/kg, i.p. for 3 days).

In monkey bladder, 5-HT evoked a concentration-dependent inhibition of the electrically-induced contractile responses which was shifted dextrally by DAU 6285 (1μM) with no change in maximum response (pA_2 = 7.0; n=4).

DAU 6285 appears to act as a silent, competitive 5-HT$_4$ receptor antagonist. Enhancement of the maximum response to 5-HT, 5-MeOT and carbachol may result from antagonism of endogenous 5-HT; an effect difficult to see with non-indole 5-HT$_4$ receptor agonists due to biphasicity of their concentration-effect curves (Baxter *et al.*, 1991). In addition, DAU 6285 appears to have exposed an inhibitory 5-HT$_4$ receptor in the bladder of Rhesus monkey.

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126P COMBINED ADMINISTRATION OF A THROMBOXANE ANTAGONIST (ICI 192,605) AND A 5-HT$_2$ ANTAGONIST (ICI 170,809) MARKEDLY REDUCES REPERFUSION-INDUCED ARRHYTHMIAS

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Previous studies have shown that 5-HT$_2$ antagonists can reduce reperfusion-induced arrhythmias (Coker & Ellis, 1987) and that thromboxane antagonists are also antiarrhythmic (Coker & Parratt, 1985). We have now examined the effects of combined administration of both types of drugs to investigate whether this has any additional benefit. Experiments were performed in pentobarbitone-anaesthetized male Wistar rats (238-395g) prepared for occlusion of the left coronary artery as described previously (Coker & Ellis, 1987). A Lead I ECG and carotid arterial blood pressure were recorded continuously and a femoral vein was cannulated for drug administration. Rats were assigned randomly to one of four groups (n = 12 per group): control, ICI 170,809, ICI 192,605 or both drugs. Each rat received a bolus dose of ICI 170,809, 1 mg kg^{-1}, (or its vehicle, acidified water) 10 min before coronary artery occlusion, followed immediately by a continuous infusion of ICI 192,605, 1 mg kg^{-1} min^{-1}, (or its vehicle, alkaline saline) which was maintained for the duration of the experiment. After 5 min of myocardial ischaemia the ligature around the coronary artery was released to allow reperfusion and the resulting arrhythmias were quantified.

Table 1	Heart rate (*beats min^{-1}*)	Systolic BP (*mmHg*)	Diastolic BP (*mmHg*)	VT %	VF %	Mortality %
Control	371 ± 9	95 ± 5	74 ± 6	100	92	67
ICI 170,809	339 ± 12*	94 ± 3	65 ± 4	100	50	33
ICI 192,605	360 ± 10	100 ± 6	70 ± 5	100	58	67
Both drugs	337 ± 14*	80 ± 3*	54 ± 5*	67	33++	17+

*P<0.05 versus control, independent t test; +P=0.036, ++P=0.009 versus control, Fisher's exact test.

Table 1 shows the haemodynamics 10 min after drug administration (i.e. just before coronary artery occlusion) and the incidence of reperfusion-induced ventricular tachycardia (VT), ventricular fibrillation (VF) and the mortality (due to sustained VT or VF). Heart rate was lower in rats which received ICI 170,809, whereas in rats which received ICI 170,809 plus ICI 192,605 both heart rate and blood pressure were lower. Although neither drug alone significantly reduced either VF or mortality, combined administration of both drugs produced marked reductions in both VF and mortality. Further experiments are required to determine whether the increased activity of combination therapy is due to additive effects or a synergistic interaction.

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127P EVIDENCE FOR NITRERGIC VASODILATION IN THE SHEEP MIDDLE CEREBRAL ARTERY

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There is evidence that nitric oxide (NO) is a neurotransmitter in several tissues (eg Li & Rand, 1991; Snyder & Bredt, 1991). The aim of this study was to investigate whether neurally mediated relaxation of the sheep middle cerebral artery involves a nitrergic mechanism.

Sheep heads were obtained daily from an abbatoir and the brain was rapidly excised. The middle cerebral arteries were dissected free, cut into rings 4 mm in length and suspended in Krebs-Henseleit solution at 37°C under their optimum resting force of 1 g. The solution was bubbled with 95% O_2: 5% CO_2. Electrical field stimulation (EFS) was performed with parallel silver wire electrodes, placed either side of the arterial ring. Trains of stimuli (train length 15s, frequency = 0.5-4 Hz, 0.1-0.3 ms pulse width and supramaximal voltage) were delivered every 10 min. The preparations had no functional endothelium as demonstrated by lack of relaxation of the pre-contracted artery on addition of substance P (100 nM) or acetylcholine (1 µM). The data are expressed as the mean ± s.e.m. and n refers to the number of heads used. Statistical analysis was carried out using Students t test. The haemolysate solution was prepared by the method of Bowman & Gillespie (1982).

At optimal resting force, EFS produced a contractile response which was abolished after incubation with guanethidine (5-50 µM) for 45 to 60 min. In the presence of guanethidine and following precontraction with 5-HT (10 µM), EFS produced a relaxant response which was reproducible for up to 10 stimulation cycles. Neurogenic relaxation was significantly reduced by N^G-nitro-L-arginine (L-NOARG; 50 µM) from 50 ± 10% to 19 ± 7% (n = 4, $P < 0.05$), and by haemolysate (1 µl ml^{-1}) from 37 ± 8% to 14 ± 3% (n = 4, $P < 0.05$) of 5-HT-induced tone. The maximum effect of haemolysate was observed within 2-3 stimulation cycles whereas that of L-NOARG took longer to develop (5-11 cycles). The relaxant response was also significantly inhibited by N^G-monomethyl-L-arginine (L-NMMA; 100 µM) by 56 ± 6% (n=5) and was significantly increased by superoxide dismutase (SOD; 150 units ml^{-1}) to 160 ± 12 (n=4) of the control relaxant response. Both the contractile and relaxant responses produced by EFS were abolished by tetrodotoxin (100-300 nM).

It is concluded that EFS excites vasodilator nerves in sheep middle cerebral artery in the absence of endothelium. Neurogenic relaxation was reduced by the NO synthase inhibitors L-NOARG and L-NMMA. Neurogenic relaxation was also reduced by haemolysate (which scavenges NO) and was augmented by SOD (which delays NO breakdown). This evidence suggests that NO is involved in the neurogenic relaxant response of the sheep middle cerebral artery.

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128P INHIBITION OF THE METABOLISM OF PROPRANOLOL BY NICARDIPINE IN RATS: AN *IN VITRO* STUDY

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Nicardipine is a dihydropyridine calcium antagonist which is used for the treatment of angina pectoris and hypertension. It is extensivily metabolised in the liver and only 0,03% of the unchanged drug is eliminated in the urine. Combination therapy of a dihydropyridine calcium antagonist with a ß-blocker is increasingly used and has been reported to be more effective than monotherapy with either drug for the treatment of hypertension and angina pectoris.

We recently reported that, in normotensive volunteers, the co-administration of nicardipine (N) increased the Cmax and area under the curve (AUC) of racemic propranolol(rac-P) (Schoors *et al.*, 1990). These results indicate an interaction at the level of cytochrome P 450 or changes in splanchnic or hepatic blood flow. We therefore studied the interaction of nicardipine with the *in vitro* metabolism of rac-P and R and S-propranolol (r-P and s-P).

Rat hepatocytes were isolated from 3 month old male Wistar rats. 10 µg/ml rac-P was incubated with $1.2.10^6$ cells/ml for 25 minutes. Different concentrations of N (0.1; 0.3; 1 and 3.10^{-5} M) were added. Rac-P was assayed by HPLC, r-P and s-P were assayed by HPLC after derivatisation with a chiral agent. Results were analysed by the Wilcoxon signed rank test.

The results were as follows (n=7):

concentration N added in M	dissapearance rate of rac-P in µM/10^6 cells/min (s.e.mean)	dissap. rate of r-P in µg/ml (s.e.mean)	dissap. rate of s-P in µg/ml (s.e.mean)
0	0.50 ±0.03	0.31 ±0.05	0.22 ±0.02
$0,1.10^{-5}$	0.42 ±0.03	0.26 ±0.04	0.21 ±0.04
$0,3.10^{-5}$	0.31 ±0.02 p<0.05	0.18 ±0.03 p<0.05	0.17 ±0.03 p<0.05
1.10^{-5}	0.26 ±0.006 p<0.05	0.16 ±0.02 p<0.05	0.14 ±0.02 p<0.05
3.10^{-5}	0.22 ±0.01 p<0.05	0.11 ±0.03 p<0.05	0.10 ±0.02 p<0.05

This study indicates that *in vitro* N inhibits dose dependently the metabolism of rac-P and r-P and s-P. There was no significant difference between the metabolism of r-P and s-P, nor between the interaction of N on r-P and s-P, suggesting that the interaction is not stereoselective *in vitro*.

Schoors, D.F., Vercruysse, I., Musch, G., Massart, D.L. & A.G. Dupont, (1990), *Br. J. Clin. Pharmac.* **29**, 497-501.

129P ICI D1542: A POTENT THROMBOXANE A_2 (TXA_2) SYNTHASE INHIBITOR AND RECEPTOR ANTAGONIST

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In vitro ICI D1542 (4(Z)-6-[(2S,4S,5R)-2-[1-methyl-1-(2-nitro-4-tolyloxy)ethyl]-4-(3-pyridyl)-1,3-dioxan-5-yl]hex-4-enoic acid) caused concentration dependent inhibition of arachidonic acid stimulated human platelet microsomal TXA_2 production (IC_{50} mean ± s.e.; 0.018 ± 0.003μM, n=8). In contrast the compound did not potently modify spontaneous or arachidonic acid stimulated release of PGI_2 or PGE_2 from cultured human umbilical vein endothelial cells (IC_{50} > 10μM, n=4).

ICI D1542 (0.005-0.05μM) caused concentration dependent inhibition of U-46619 stimulated human platelet aggregation (apparent pA_2 value = 8.43 ± 0.14, n=8); this antagonism was not competitive as the compound, at a final concentration of 0.1μM caused insurmountable receptor blockade. ICI D1542 was a selective TXA_2 receptor antagonist on human platelets, as the compound, at a final concentration of 100μM did not potently modify either the primary phase of ADP induced aggregation, or the PGE_1, PGD_2 or PGI_2 inhibition of this response.

In vitro ICI D1542 caused profound and selective blockade of U-46619 induced contractions of rat thoracic aorta and guinea pig trachea yielding apparent pA_2 values (mean ± s.e.) of 8.37 ± 0.09, n=4 and 8.29 ± 0.07, n=8, respectively. This antagonism was selective for TXA_2 receptors as the compound at a final concentration of 10μM, did not inhibit noradrenaline induced contractions of rat aorta. Furthermore the drug did not potently modify PGE_2 induced contractions of guinea pig ileum or $PGF_{2\alpha}$ responses of dog iris preparations, yielding apparent pA_2 values (mean ± s.e.) of 5.8 ± 0.14, n=4 and 5.0 ± 0.23, n=4, respectively.

We conclude that in vitro ICI D1542 is both a potent and selective TXA_2 synthase inhibitor and receptor antagonist.

130P THE EFFECTS OF ICI D1542, A THROMBOXANE A_2 SYNTHASE INHIBITOR AND TP RECEPTOR ANTAGONIST, ON COLLAGEN-INDUCED HUMAN PLATELET DEGRANULATION AND AGGREGATION

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ICI D1542 (4(Z)-6-[(2S,4S,5R)-2-[1-methyl-1-(2-nitro-4-tolyl-oxy)ethyl]-4-(3-pyridyl)-1,3-dioxan-5-yl]hex-4-enoic acid) has been shown to be a potent and highly selective inhibitor of thromboxane A_2 (TXA_2) synthase and an equally potent and selective TP receptor antagonist (Brownrigg *et al.*,1992).

The effects of ICI D1542 and aspirin were determined on collagen-induced human platelet aggregation (PA); secretion of beta-thromboglobulin (beta-TG) and TXB_2 formation in citrated (10 mM) whole blood from 6 donors. Drug pre-incubation at 37°C for 10 min was followed by stirring at 1000 rpm for 3 min. PA was quantified by platelet counting and plasma concentrations of beta-TG and TXB_2 determined by radioimmunoassay.

Beta-TG and TXB_2 concentrations before and after stirring (ng/ml, mean ± S.E.mean) were 87 ± 12 and 121 ± 14 and 0.14 ± 0.02 and 0.15 ± 0.02 respectively. Stirring resulted in 34 ± 7% PA. Stirring with collagen (0.3 ug/ml) resulted in ≥ 94% PA, beta-TG release and TXB_2 formation. ICI D1542 effected concentration-dependent inhibition of both PA and beta-TG release (60% and 89% inhibition respectively at 1 uM) which accompanied inhibition of TXB_2 formation (Table 1).

Table 1

	ICI D1542 uM					
	0	0.01	0.03	0.1	0.3	1
%PA	96 ± 1	67 ± 4	55 ± 3	50 ± 15	44 ± 11	38 ± 11
beta-TG	3967 ± 728	2453 ± 606	577 ± 57	553 ± 57	376 ± 51	418 ± 73
TXB_2	10.8 ± 4.5	2.32 ± 0.86	1.02 ± 0.33	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.1

Aspirin (1 mM), with 0.3 ug/ml collagen, inhibited both PA (62 ± 4%, 35% inhibition) and beta-TG release (1030 ± 234, 74% inhibition) to a lesser extent than did ICI D1542 ($P < 0.01$) despite abolition of TXB_2 formation (0.06 ± 0.03). Stirring with collagen (1ug/ml) resulted in 98 ± 1% PA with further beta-TG release (6090 ± 378) and TXB_2 formation (30.8 ± 9.8). ICI D1542 (1 uM) at 1 ug/ml collagen inhibited both PA (74 ± 11%, 24% inhibition) and beta-TG release (1817 ± 83, 70% inhibition) to a lesser extent than at 0.3 ug/ml collagen and aspirin inhibited beta-TG release to a similar extent (1935 ± 155, 68% inhibition) but had little effect on PA (87 ± 4%, 11% inhibition).

ICI D1542 limited both PA and degranulation more markedly than did aspirin. TXA_2 synthase inhibition alone cannot account for this and the greater efficacy of ICI D1542 reflects concurrent and selective antagonism at TP receptors with unopposed formation and action of inhibitory prostanoids.

Brownrigg, N., Garcia, R., Jessup, R. *et al.*, (1992) This meeting.

131P THE PURINE-XANTHINE OXIDASE SYSTEM DOES NOT DEPRESS MYOCARDIAL FUNCTION THROUGH A FREE RADICAL MECHANISM

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In anaesthetised dogs it has been shown that intravenous administration of enzymatic free radical generating systems can reduce indices of myocardial contractility (Prasad et al. 1989) supporting the view the free radicals may play a role in cardiac failure. The aim of this study was to develop a small animal model of free radical-induced cardiac dysfunction in which the effect of drugs with free radical scavenging activity could be readily assessed.

Male Sprague-Dawley rats were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.) and prepared for the measurement of ECG, heart rate, arterial blood pressure and left ventricular pressure (via a Millar Microtip transducer) from which the indices of contractility $dPdt^{-1}$ max and $dPdt^{-1}$ P^{-1} were calculated. Following intravenous injection of a free radical generating system containing purine (0.14 mg kg^{-1}) and xanthine oxidase (2 U kg^{-1}) in the presence of an iron source, EDTA (0.5 mg kg^{-1}) and $FeCl_3$ (0.18 mg kg^{-1}) there were significant falls in mean arterial blood pressure (from 88 ± 2 to 74 ± 1 mmHg), in $dPdt^{-1}$ max (from 5958 ± 288 to 4813 ± 335 $mmHg^{-1}$) and in $dPdt^{-1}$ P^{-1} (from 84 ± 3 to 75 ± $3s^{-1}$; n = 5). These changes were maximal 2 min post-administration with recovery by 10 min. Purine, EDTA and $FeCl_3$ i.v. in the doses described above had no effect but xanthine oxidase alone had similar haemodynamic effects to those of the free radical generating system e.g. arterial blood pressure fell from 101 ± 3 to 89 ± 4 mmHg (n = 5). This was shown to be an effect of the vehicle for xanthine oxidase (2.3 M ammonium sulphate, 1 mM EDTA and 1 mM sodium salicylate in 10 mM sodium phosphate buffer pH 7.8, diluted 1 part in 3 with saline, volume injected, 0.1 ml per 300g body weight) which depressed arterial blood pressure (from 109 ± 16 to 70 ± 11 mmHg; n = 4) over a similar time course to that of the free radical generating system. I.v. administration of a crystalline form of xanthine oxidase (2 - 6 U kg^{-1}) together with purine (0.14 - 7.2 mg kg^{-1}), EDTA and $FeCl_3$ failed to produce any haemodynamic effect in 8 animals. Equivalent concentrations of purine and the crystalline form of xanthine oxidase to those used in the *in vivo* studies were shown to generate 24 ± 4 n mol ml^{-1} of superoxide *in vitro* as assessed by reduction of cytochrome C.

These results demonstrate that in anaesthetised rats xanthine oxidase suspension is not suitable for experiments designed to assess free radical effects on myocardial contractility and the crystalline form of xanthine oxidase is to be preferred. A combination of the crystalline form of xanthine oxidase together with substrate generated free radicals *in vitro* but did not have any haemodynamic effect *in vivo*. This suggests that if free radicals can damage the myocardium, high enough cardiac concentrations of free radicals may not be achieved following i.v. administration.

S Laycock holds an SERC studentship; running costs for the project are provided by Bristol-Myers Squibb.

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132P GLIBENCLAMIDE, BUT NOT UK-66,914, ABOLISHES THE ISCHAEMIA-INDUCED SHORTENING OF THE EFFECTIVE REFRACTORY PERIOD IN PERFUSED GUINEA-PIG HEARTS

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It is thought that one of the main electrophysiological changes that underlie ischaemia-induced arrhythmias is a shortening of the effective refractory period (ERP). The aim of this work was to develop a model in perfused guinea pig hearts in which ERP could be consistently measured and in which the effects of drugs (the delayed rectifier blocker, UK-66,914, Gwilt *et al.*, 1991 and the ATP-sensitive potassium channel blocker, glibenclamide, Escande *et al,*. 1988) could be assessed under normal and ischaemic conditions.

Hearts from Alderley Park guinea-pigs were perfused with Krebs Henseleit solution (gassed with 95% O_2/5% CO_2 and maintained at 36 ± 1°C) via the aorta by the Langendorff method . The hearts were paced at 4 Hz and the ECG recorded. The ERP was measured by the extra stimulus method before and 30 min after the addition of the vehicle (DMSO) or drug to the perfusing solution. The coronary flow was then reduced by 90% and ERP was measured at 5,10,15 and 16 min after the reduction in flow. Measurements of ERP at later time points induced ventricular fibrillation. The results obtained are shown in Table 1.

Table 1. The effects of ischaemia on ERP (ms) in control hearts and those exposed to UK-66,914 (10 μM) and glibenclamide (3 μM)

Group	N	Control	Treatment	5 min	10 min	15 min	16 min post ischaemia
DMSO	11	102 ± 3	103 ± 3	106 ± 6	88 ± 4*	72 ± 3*	71 ± 3*
UK-66,914	4	110 ± 1	142 ± 1**	131 ± 5*	122 ± 8*	95 ± 7*	100 ± 6*
GLIBENCLAMIDE	6	118 ± 4	114 ± 4	115 ± 3	111 ± 3	111 ± 3	111 ± 3

Values are mean ± s.e.mean *,** ($P < 0.05$) indicates significant difference from pre-ischaemia and control values respectively.

In control hearts, ischaemia reduced ERP and this was completely prevented in the presence of glibenclamide. UK-66,914 prolonged ERP prior to and during ischaemia but the ischaemia-induced fall was similar in control and UK-66,914 treated hearts (31 ± 3 vs 30 ± 3% fall at 16 min in control and UK-66,914 treated hearts respectively).

We have demonstrated that in this model ERP can be consistently measured up to 16 min after the introduction of low flow ischaemia and that the opening of ATP-sensitive K^+ channels may be responsible for the shortening of the ERP during ischaemia. UK-66,914 did not prevent the ischaemia-induced reduction in ERP although ERP was lengthened relative to values in control hearts.

D Tweedie holds an MRC CASE award with ICI plc..

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133P THE ROLE OF PHOSPHODIESTERASE INHIBITORS IN REGULATING CYCLIC AMP AND CONTRACTILE FUNCTION IN ISOLATED VENTRICULAR CARDIOMYOCYTES

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Much research effort has been directed over the past decade to development of cardiotonic agents with phosphodiesterase (PDE) inhibiting properties for the effective management of congestive heart failure (van der Leyen, 1989). Although the physiological effects of these compounds have been well documented, their mechanisms of action are still unclear (Nicholson *et al.*, 1991). This study investigated the effect of inhibitors, having different selectivity for PDE isoenzymes, on inotropic activity, as measured by ventricular cell shortening using video microscopy, and on biochemical systems involving cyclic AMP, in ventricular cardiomyocytes isolated from adult Sprague-Dawley rats (200-250 g).

The effects of HN-10200, (2-[3-methoxy-5-methylsulfinyl-2-thienyl]-1H-imidazo-[4,5-c]-pyridine hydrochloride), a novel cardiotonic agent, were assessed in addition to those of sulmazole and enoximone (type III PDE inhibitors), Ro 20-1724 (type IV PDE inhibitor) and IBMX (non-selective PDE inhibitor); forskolin (10μM) was used to potentiate the responses of all the inhibitors tested. HN-10200 exerted a concentration-dependent (10^{-8}M-10^{-4}M) positive contractile effect, which was independent of α- or β-adrenoceptor, or histamine receptor stimulation. The efficacies of the contractile agents were of the order: HN-10200 > IBMX > sulmazole > enoximone (maximum stimulation: 54%, 41%, 38%, and 26% over basal, respectively), while EC_{50} values were 93nM, 64nM, 16nM, and 11nM, respectively). Ro 20-1724 did not have any effect on contractile function.

Due to the low basal turnover rates of cyclic nucleotides in isolated cells, PDE-stimulated accumulation of cyclic AMP was detected only when the levels of cyclic nucleotide were enhanced with forskolin (10μM). HN-10200 and sulmazole had similar concentration-dependent profiles , but these indicated lower potencies than that of IBMX (concentrations of forskolin required to increase cyclic AMP by 4 pmol/mg protein were 13mM, 14mM, and 3mM, respectively). Enoximone failed to increase in cyclic AMP levels, even when stimulated with a maximum forskolin concentration. Furthermore, enoximone attenuated the response of all PDE inhibitors tested; a concentration-dependent inhibition was demonstrated with enoximone in the presence of either Ro 20-1724 or IBMX (10^{-4}M) and forskolin.

In conclusion, the positive contractile effects of HN-10200, sulmazole, and IBMX (type III PDE activity) involve both cyclic AMP-dependent and cyclic AMP-independent mechanisms of action, whilst Ro 20-1724 (type IV PDE inhibitor) increases accumulation of cyclic AMP without effects on contractile function. Enoximone, on the other hand, produces a moderate increase in contraction which is not mediated by cyclic AMP.

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134P THE EFFECTS OF ICI D7288, A NOVEL SINO-ATRIAL NODE MODULATING AGENT, ON GUINEA-PIG ISOLATED ATRIA

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A new class of drugs, the "specific bradycardic agents", has been described. These agents, structural analogues of either clonidine (Bouman et al, 1984) or verapamil (Lillie & Kobinger, 1986), exert a direct effect on the sino-atrial node to cause a reduction of spontaneous beating rate, whilst having minimal effects on myocardial contractility. We have recently identified a novel compound, ICI D7288 (4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride) which has a direct modulatory effect on the sino-atrial node without any negative inotropic activity.

The effects of ICI D7288 have been studied using guinea pig isolated atria. Isolated right atria were impaled on platinum electrodes, for recording the electrocardiogram, and placed in Krebs buffer at 37°C, gassed with 95% O_2 and 5% CO_2. ICI D7288 (0.1 - 10μM) produced a concentration-related reduction in the spontaneous beating rate with a maximum reduction of 57%. These concentrations were without effect on the contractile force of electrically stimulated left atria (square-wave pulses, 2ms duration, 2.5 Hz, twice threshold voltage). The effects of ICI D7288 were not affected by the presence of atropine (1μM).

The positive chronotropic actions of isoprenaline and histamine were not affected by ICI D7288 (1μM, a concentration which reduced the spontaneous beating rate by 40%).

Intracellular recordings from pacemaker cells within the isolated sinus node demonstrated that ICI D7288 selectively reduced the diastolic depolarisation rate by up to 61% at a concentration of 0.3 μM (n=5).

These results demonstrate that ICI D7288 modulates sino-atrial node activity by a mechanism which does not involve the cholinergic, ß-adrenergic or histaminergic receptors. This action, and the absence of a negative inotropic effect, indicate that this agent could be a useful therapy for ischaemic heart disease.

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135P EFFECTS OF LISINOPRIL ON CONTRACTILITY AND ELECTROPHYSIOLOGICAL PROPERTIES IN GUINEA-PIG CARDIAC PREPARATIONS

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Lisinopril (LIS) is an orally active angiotensin converting enzyme (ACE) inhibitor, widely used in the treatment of hypertension and congestive heart failure (Lancaster & Todd, 1988). ACE inhibitors reduced cardiac contractility and very recently the negative inotropic effect of captopril has been attributed to a reduction in L-type Ca current, (I_{Ca}), (Bryant et al., 1991). In this communication, the effects of LIS on cardiac contractility and I_{Ca} have been analyzed.

Guinea-pig atrial and papillary muscles were incubated in Tyrode solution (34ºC). Transmembrane action potentials (AP) were recorded through glass microelectrodes. Slow responses were elicited by isoprenaline (10^{-6}M) in fibres partially depolarized with 27 mM KCl. I_{Ca} was recorded using the nystatin perforated configuration of the patch-clamp technique in guinea-pig ventricular myocytes isolated with collagenase.

In spontaneous right atria, LIS (10^{-8}M - 10^{-5}M) had no effect on sinus rate. However, in both electrically driven left atria (1 Hz) and spontaneous right atria, LIS produced a concentration-dependent inhibition of the amplitude of contractions (IC_{50} = 2.25 ± 0.70 x 10^{-6}M and 1.74 ± 0.91 x 10^{-6}M, respectively. n = 9). In papillary muscles LIS had no effect on the resting membrane potential, amplitude or upstroke velocity ($\dot{V}_{max}$) of the AP, but slightly shortened the action potential duration (APD) measured at 50% and at 90% of repolarization. The effective refractory period (ERP) was also shortened by LIS, and thus, the ERP/APD ratio remained unaltered. In partially depolarized (27 mM KCl) papillary muscles, LIS (10^{-8}M - 10^{-5}M) did not alter the characteristics of the slow APs elicited by isoprenaline (10^{-6}M). Moreover, in single guinea-pig ventricular myocytes LIS (10^{-5}M) had no effect on the peak amplitude of the I_{Ca} (193.9 ± 35.0 pA in control vs 185.9 ± 35.2 pA; $p > 0.05$, n = 5), but inhibited the stimulation-facilitation process of the I_{Ca} during the time course of trains of stimuli.

It is concluded that, in contrast to captopril, the negative inotropic effects of LIS can not be linked to a decrease of the peak I_{Ca}.

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136P ELECTROPHYSIOLOGICAL INTERACTIONS BETWEEN DESIPRAMINE AND IMIPRAMINE ON GUINEA-PIG PAPILLARY MUSCLES

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Desipramine (DMI) is the most important metabolite of imipramine (IMI). Both antidepressant drugs exhibit local anaesthetic properties and inhibit the maximum upstroke velocity ($\dot{V}_{max}$) of the cardiac action potential. In fact, IMI has been classified as a class Ia antiarrhythmic agent (Delpón et al., 1990). The present study was undertaken to : 1) determine the effects of DMI 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, and 2) characterize the electrophysiological effects of the combination of DMI plus its parent compound IMI.

Experiments were performed in guinea-pig papillary muscles (< 1 mm diameter) perfused with Tyrode solution (34ºC). $\dot{V}_{max}$ was obtained by electronic differentiation, using conventionally intracellular recording.

In muscles driven at 0.02 Hz, DMI (5 μM) or IMI (5 μM) alone or in combination had a very small effect on the $\dot{V}_{max}$. DMI alone produced a tonic $\dot{V}_{max}$ block of 3.8 ± 0.3%, IMI alone 3.0 ± 0.4% and the combination 9.7 ± 0.9%. These results indicated that both drugs exhibited a low affinity for the resting state of the Na channel. At 2 Hz, the frequency-dependent $\dot{V}_{max}$ block produced by DMI was 60.4 ± 4.3%, whereas that produced by IMI was 26.1 ± 3.4% and in the presence of the combination 65.3 ± 6.2%. The onset kinetics of frequency-dependent $\dot{V}_{max}$ block induced by DMI was a monoexponential process, the value of K being 0.137 ± 0.01 AP^{-1}, while in the presence of IMI the process was better defined by a biexponential function (K_1 = 0.48 ± 0.04 and K_2 = 0.104 ± 0.01 AP^{-1}). In the presence of the combination, the onset kinetics was also a biexponential function, the values of K_1 and K_2 being 0.640 ± 0.10 and 0.102 ± 0.02 AP^{-1}, respectively. In the presence of DMI or IMI alone, the time constants of recovery of $\dot{V}_{max}$ (τ_{re}) were 14.5 ± 0.1 s and 2.3 ± 0.4 s, respectively, and in the presence of the combination the value of τ_{re} was similar to that obtained in the presence of DMI alone (13.4 ± 1.6 s). In the presence of DMI and IMI alone, the y-intercepts of the slow phase of recovery, which indicated the fraction of the Na channels blocked by the drugs were 54. 9 ± 3.9% and 36.9 ± 2.3%, respectively, whereas in the presence of the combination the fraction was 46.0 ± 1.8%.

These results suggest that DMI can be classified as a class Ic antiarrhythmic agent and that it may be responsible for most of the cardiodepressant effects previously attributed to IMI.

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137P THE ANTIARRHYTHMIC EFFECT OF PRECONDITIONING, IN RAT ISOLATED HEARTS, DOES NOT INVOLVE A_1 ADENOSINE RECEPTORS

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Recent studies have indicated a role for endogenous adenosine, acting through A_1 receptors, as a mediator of the infarct size limiting effect of ischaemic preconditioning in rabbits (Lui et al, 1991). This study examines whether stimulation of A_1 adenosine receptors underlies the antiarrhythmic effect of preconditioning in rat isolated hearts by assessing the effects of adenosine, the A_1 antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine) and the A_1 agonist R-PIA (N^6-1(phenyl-2R-isopropyl)adenosine) on arrhythmias associated with ischaemia and preconditioning.

Isolated Langendorff perfused hearts, perfused at constant flow, were subjected to regional ischaemia by occluding the left main coronary artery. Hearts were randomised into six groups. Groups 1 - 4 were subjected to a single 30 min coronary occlusion and in groups 5 and 6 this was preceded by a preconditioning cycle of 3 min occlusion and 10 min reperfusion. Drugs were added to the perfusion medium as follows: (1) solvent (saline) control; (2) DPCPX 10^{-8}M; (3) adenosine 5 x 10^{-5}M; (4) R-PIA 5 x 10^{-8}M; (5) pre- conditioning solvent control; (6) DPCPX 10^{-8}M. In groups 2 and 6, DPCPX perfusion was started 5 min before the experimental protocol. In groups 3 and 4, drug perfusion was maintained for 3 min followed by 10 min drug-free perfusion to 'mimic' the preconditioning occlusion. The number of ventricular premature beats (VPB) and the incidence of ventricular tachycardia (VT), of ventricular fibrillation (VF) and mortality, during the sustained 30 min occlusion period, are shown in table 1.

TABLE 1

GROUP		n	VPBs	%VT	%VF	%MORTALITY
1	Occlusion.	10	392 ± 107	90	30	30
2	DPCPX + Occlusion	10	843 ± 281	100	60	30
3	Adenosine + Occlusion	6	537 ± 154	100	50	33
4	R-PIA + Occlusion	6	516 ± 217	83	50	0
5	Preconditioning	10	79 ± 28*	40	0	0
6	DPCPX + Preconditioning	10	131 ± 53**	60	10	10

Values for VPBs express total count ± s.e.mean in hearts surviving 30 min occlusion period.
*P < 0.05 compared to control group 1, **P < 0.01 compared to control group 2

These results suggests that preventing activation of A_1 receptors does not attenuate the antiarrhythmic effect of preconditioning, nor does the prior stimulation of these receptors mimic this cardioprotective effect.

L.Piacentini holds a British Heart Foundation studentship

Lui G.S., Thornton J., Van Winkle D.M., Stanley A.W.H., Olsson R.A. & Downey J.M. (1991) Circulation **84**, 350-356

138P THE BK_2 ANTAGONIST, HOE 140, DOES NOT ATTENUATE THE ANTIARRHYTHMIC EFFECT OF ISCHAEMIC PRECONDITIONING IN ANAESTHETISED RATS

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A brief period of myocardial ischaemia and reperfusion, preconditioning, reduces the severity of arrhythmias induced by a subsequent sustained period of ischaemia. One of the possible mechanisms of preconditioning is that it triggers the generation and release of endogenous substances which protect the myocardium against a subsequent, more severe, stress of the same form. Bradykinin (BK) levels in plasma have been shown to be increased during myocardial ischaemia, and it has been shown to be antiarrhythmic in dogs and pigs (Végh et al, 1991; Tobe et al, 1991). The purpose of this study was to examine the hypothesis that BK is a protective agent involved in preconditioning by examining the effects of the selective BK_2-antagonist, Hoe 140, on the anti-arrhythmic effects of preconditioning. The effect of bradykinin on ischaemic arrhythmias without preconditioning was also assessed.

Male Sprague-Dawley rats were anaesthetised with sodium pentobarbitone (60mg kg^{-1} i.p.) and prepared for occlusion of the left main coronary artery. All animals were subjected to a 30min coronary occlusion with or without preconditioning. Preconditioning was induced by a 3min occlusion followed by 10min reperfusion prior to the 30min occlusion. The rats were divided into the following groups: 30min occlusion only - i) Control (vehicle), ii) BK 30ngkg^{-1}min^{-1} i.l.v., iii) BK 1μgkg^{-1}min^{-1}i.l.v.; Preconditioning - iv) Control (vehicle), v) Hoe 140 40μgkg^{-1} i.v., vi) Hoe 140 4mgkg^{-1} i.v. Drug administration was commenced 10 min before starting the experimental protocol and, in the case of infusions, maintained for the duration of the experiment. The total number of ventricular premature beats (VPBs), and the incidences of ventricular tachycardia (VT) and ventricular fibrillation(VF) which occurred during both the preconditioning occlusion (and reperfusion) and the 30min occlusion period were analyzed.

Table 1

		REPERFUSION			30min OCCLUSION		
	n	Nº of VPBs	VT(%)	VF(%)	Nº of VPBs	VT(%)	VF(%)
30min occlusion control	11				1257±270	100	73
BK 30ng kg^{-1} min^{-1} i.l.v.	10				1184±262	100	40
BK 1μg kg^{-1} min^{-1} i.l.v.	11				1349±404	100	73
Preconditioning control	10	91±47	50	0	93±55**	40*	10**
Hoe 140 40μg kg^{-1}	10	140±73	33	10	43±12**	50*	0**
Hoe 140 4mg kg^{-1}	7	84±22	57	0	21±17**#	30*	0**

(i.l.v.: Infusion into the lumen of the left ventricle) *P<0.05, **P<0.01 vs 30min occlusion control; #P<0.05 vs preconditioning control

Preconditioning resulted in a significant reduction in the total number of VPBs, and in VT and VF. Hoe 140 did not reverse this protection. Furthermore, exogenous BK failed to show a similar antiarrhythmic action to preconditioning during a sustained ischaemic period. In conclusion, bradykinin does not appear to be involved in the antiarrhythmic effects of preconditioning in rats.

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139P SUBSTITUTED 4-BENZYLOXYL-NAPHTHYRIDINE DERIVATIVE: A NOVEL, POTENT AND ORALLY-EFFECTIVE ANGIOTENSIN II RECEPTOR ANTAGONIST

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2-Ethyl-6-isopropoxy-4-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methoxy]-1,5-naphthyridine hydrochloride is one of a series of novel substituted 4-benzyloxy-naphthyridine derivatives (Allott et al, in press) which antagonise the pharmacological actions of angiotensin II. This study concerns the *in vitro* effects of the above named compound in guinea-pig tissues and its *in vivo* effects in the rat.

In guinea-pig adrenal gland membranes, the compound displaced (^{125}I)-angiotensin II from its binding sites in a concentration-related manner, with an IC_{50} of 7.9 ± 1.4 nM (n=3). Similarly, the compound was a potent antagonist of angiotensin II-mediated contractions in isolated guinea-pig ileum with an estimated pA_2 of 10.7 (n=4).

Conscious male Alderley Park Wistar rats were prepared with indwelling carotid artery and jugular vein cannulae. Continuous angiotensin II infusion at 1.0 $\mu g\ kg^{-1}\ min^{-1}$ i.v. increased mean arterial pressure by 48.9 ± 1.5 mmHg and cumulative i.v. dosing of the compound inhibited this response with an ID_{50} of 0.4 ± 0.1 $mg\ kg^{-1}$ (n=8). In further experiments, rats prepared as above were given intermittent angiotensin II infusions of 1.0 $\mu g\ kg^{-1}\ min^{-1}$. The compound dosed at 3.0 $mg\ kg^{-1}$ p.o. inhibited the angiotensin II pressor responses by 77.0 ± 8.9% and 60.8 ± 5.3% at 1 and 5 hrs after dosing, respectively (n=8).

Conscious male Alderley Park Wistar rats were prepared with a partial occlusion of the left renal artery, using a platinum clip of 0.25 mm i.d. After 12-14 days, carotid artery cannulae were implanted and, the following day, blood pressure measurements were taken before and for up to 24 hours after administration of the compound (5.0 $mg\ kg^{-1}$ p.o.). Mean arterial pressure was reduced from 176 ± 3.0 mmHg at the start of the experiment to 141 ± 11 mmHg, 3 hrs after dosing the compound (n=5). Blood pressure at 24 hrs after dosing (131 ± 5.7 mmHg) was reduced significantly ($P<0.001$) compared with rats prepared as above but treated with dosing vehicle alone (170 ± 4.0 mmHg, n=13).

The results of these studies are consistent with the conclusion that the substituted 4-benzyloxy -naphthyridine derivative described above is a potent, long-acting and orally effective angiotensin II receptor antagonist. Hence this compound may have therapeutic potential in the treatment of hypertension and heart failure.

Allott, C.P., Bradbury, R.H., Dennis, M., *et al* (In Press) Bioorg. Med. Lett.

140P DEPOLARIZATION INDUCED BY ANGIOTENSIN IN PUTATIVE CARDIAC NEURONES OF RAT STELLATE GANGLIA

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The effect of angiotensin II (AT-II) on the neurones of isolated (left or right) stellate ganglia of young adult rats (3-4 months) were investigated by means of intracellular electrodes. Injection of cobalt chloride through the intracellular electrode was used to reveal the dendritic architecture of the neurone. The distribution and morphology of neurones, whose axons projected into the principal cardiac nerve or into the nerve to the brachial plexus, were studied by back-filling the nerves using cobaltous lysine complex.

Following ether anaesthesia, ganglia and their attached nerves were rapidly excised and transferred to the recording chamber. Ganglia were superfused with oxygenated modified Krebs solution as described by Mo & Wallis (1992). The cardiac nerve (or nerves) were stimulated with suction electrodes and putative cardiac neurones identified by antidromic activation.

Recordings, using glass microelectrodes (35-60 MΩ, filled with K acetate), were obtained from 40 such neurones, which had a resting membrane potential of 60.8±1.1mV, an input resistance of 118.7±10.3 MΩ and a time constant of 11.3±0.5ms (means ± s.e.mean). The cell bodies of these neurones were localised in the ganglion around the entry zone of the cardiac nerve. Five injected neurones showed complex dendritic morphology.

Superfusion of AT-II (1-2μM) for 30 to 60s elicited a membrane depolarization in 32 out of 40 cells. The amplitude of the depolarization varied between 2 to 15 mV and the duration between 3 and 5 min. AT-II caused an increase in input resistance in all of 10 cells tested. The response was made smaller on hyperpolarization of the membrane and disappeared at a membrane potential of -88mV. The extrapolated reversal potential for the depolarization evoked by AT-II, estimated in 6 experiments from I/V curves, was 90.3±4.5mV. In a low Ca^{2+} (0.25mM)/high Mg^{2+} (12mM) Krebs solution, AT-II was still able to elicit a membrane depolarization. Saralasin ([Sar^1, Val^5, Ala^8]-angiotensin II, 1μM), applied to the ganglion for 5 to 10 min, completely and reversibly blocked the AT-II-induced depolarization in 7 cells tested.

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141P RELATIONSHIP BETWEEN INOTROPIC ACTIVITY AND PHOSPHODIESTERASE INHIBITION FOR FLOSEQUINAN, BTS 53 554 AND MILRINONE

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In a previous report, Weishaar et al., (1991) showed that the balanced arterial-vasodilator flosequinan (F) and its primary metabolite BTS 53 554 (BTS) (Yates, 1991) exert a weak cardiotonic effect on cardiac muscle from patients without heart failure. The goal of the present study was to assess the potential that F and BTS produce their effect on cardiac muscle via inhibition of the milrinone-sensitive subclass of cyclic AMP phosphodiesterase (PDE IIIC; isolated from human platelets). PDE IIIC was isolated as described by Weishaar et al. (1986) and enzyme inhibition was assessed at a substrate concentration of 0.2 µM. In addition to F and BTS, the inotropic response to milrinone (M) was also assessed as well as the inhibitory effect of M on PDE IIIC. The results reveal that F and BTS exert little inhibitory effect on PDE IIIC (IC_{50} values = 420 µM for F and 800 µM for BTS). These values are greater than the concentrations of F and BTS required to produce a comparable effect on contractile function of human cardiac muscle (EC_{50} values = 155 µM for F and 420 µM for BTS). In contrast, M exerts a potent inhibitory effect on PDE IIIC activity (IC_{50} value of 1.5 µM) as well as a potent cardiotonic effect on human cardiac muscle (EC_{50} of 8µM). In addition to exerting little inhibitory effect on PDE IIIC activity, F and BTS also exert little effect on other forms of PDE, including i) the calmodulin-stimulated and -insensitive subclasses of cyclic GMP-specific PDE (PDE IB and IC; isolated from bovine aorta and human platelets respectively), ii) the cyclic GMP-stimulated PDE (PDE II; isolated from rat brain), and iii) the rolipram-sensitive subclass of cyclic AMP-specific PDE (PDE IIIB; isolated from rat kidney). These results are summarized below.

	IC_{50} µM				
Compound	PDE IB	PDE IC	PDE II	PDE IIIB	PDE IIIC
Flosequinan	>1,000	>1,000	>1,000	980	420.0
BTS 53 554	>1,000	>1,000	>1,000	700	800.0
Milrinone	210	100	205	24	1.5

Thus, M exerts a potent inhibitory effect on human PDE IIIC, and, at a slightly higher concentration, a potent cardiotonic effect on human cardiac muscle. This relationship was not observed with F or its sulfone metabolite BTS.

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Yates, D.B. (1991) Am. Heart J. **121**, 974-983

142P ELECTROMECHANICAL EFFECTS OF FLOSEQUINAN ON ISOLATED CARDIAC VENTRICULAR MUSCLE

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Flosequinan, a novel arterial/venous dilator agent is currently under development for use in heart failure. Some *in vitro* positive inotropic activity has been reported in a variety of animal species and man (Weishaar et al., 1991) with considerable interspecies variation. The objective of the study was to compare the inotropic activity of flosequinan and its metabolite BTS 53554 (Yates, 1991) with standard inotropes in guinea pig cardiac muscle. Right ventricular guinea pig papillary muscles were isolated, superfused with a $CO_2/NaHCO_3$ buffered Tyrode's solution at 37°C and stimulated at 1Hz. Isometric twitch tension and the rates of contraction and relaxation were measured. Resting and action potentials were recorded with 3M KCl-filled microelectrodes.

All agents exerted a positive inotropic effect, although the magnitude and the range of effective concentrations was variable. Table 1 shows the concentration required to increase force by 50% above control (Inc_{50}). Similar results were obtained if proportional changes to the maximum rates of contraction or relaxation were measured.

Table 1. Inotropic effects of flosequinan, BTS 53554 and other agents. n=no experiments

Drug	Flosequinan	BTS 53554	Milrinone	Sulmazole	Theophylline	Ouabain	Calcium
Inc_{50}	1030µM	176µM	28µM	28µM	700µM	0.22µM	2700µM
n	11	6	10	10	10	10	10

The order of Inc_{50} values was Ouabain<Milrinone≈Sulmazole<BTS 53554<Theophylline<Flosequinan<Ca. The threshold concentrations of flosequinan and BTS 53554 required to increase the force of contraction were between 30 and 100µM. The inotropic action of all agents except calcium (ouabain and BTS 53554 not tested) were attenuated by 3µM carbachol. Carbachol alone induced a small (12±1.7%) negative inotropic effect. Flosequinan (3000µM) had no effect on the resting membrane potential and slightly shortened the action potential (to 94±6%; SD, n=20). Lower concentrations exerted no signficant effect.

Flosequinan and BTS 53554 showed weaker activity than milrinone and sulmazole and less than that described by Gristwood et al. (1992) who used ventricular strips. Abolition of the inotropic actions by carbachol suggests the possibility of cAMP involvement (Endoh, 1980), but the low potency of flosequinan in relation to its clinical plasma levels (≈10µM, Packer et al., 1988) question the relevance of the response in man. Flosequinan exhibited little electrophysiological actions even at concentrations as high as 3000µM.

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143P THE CHARACTERISTIC EFFECTS OF ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) ON MYOCARDIAL CONTRACTION ARE MIMICKED BY INTERLEUKIN-1β

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A recent study has shown the presence in mammalian myocardium of a calcium-independent nitric oxide synthase enzyme with activity inducible by cytokines, e.g. tumour necrosis factor α and interleukin-1 β (IL-1) (Schulz *et al.*, 1992). Induction of the enzyme was preventable by dexamethasone. Using isolated papillary muscle preparations of the ferret we have previously shown that EDRF produced by the endocardial endothelium raises myocardial cyclic GMP and modulates myocardial contractile behaviour in a novel manner, predominantly by reducing contractile twitch duration without major effect on early contractile characteristics, e.g. maximum rate of rise of tension (dT/dt) or unloaded shortening velocity (V_{max}) (Smith *et al.*, 1991). In the present study, we have investigated the effect of IL-1 on contractile behaviour of isolated ferret papillary muscle preparations.

Right ventricular papillary muscles removed from pentobarbitone-anaesthetised ferrets of either sex were prepared and mounted as described previously (Chappell *et al.*, 1986). They were mounted in tissue baths containing Krebs-Ringer solution (1.25 mM Ca^{++}) at 29°C gassed with 95% O_2, 5% CO_2, containing indomethacin 10 μM, acebutolol 1 μM and polymyxin (10 μg/ml). Muscles were stabilized for 3 h before experiments which were performed at lmax, the muscle length at which active tension development was maximal. Results are given as mean (± s.e.mean) percent change from baseline measurements and compared using one way analysis of variance followed by Dunnett's test to isolate differences.

Incubation of the muscles with IL-1 (10 ng/ml) for 3 h (after equilibration), resulted in a characteristic abbreviation of isometric twitch duration (time to peak tension, tPT, -14.0±3.0%; $p<0.01$, n=6) and a slight reduction in peak tension (PT, -6.9±1.6%; p=ns). There were no significant changes in either dT/dt or V_{max}. These effects were identical to those previously noted with EDRF or other cyclic GMP-elevating agents in the same preparation (Shah *et al.*, 1991) and were independent of the endocardial endothelium. In the presence of dexamethasone (3 μM for 3 h) or of the nitric oxide synthase inhibitor L-N-monomethylarginine (50 μM for 30 min), no significant change in tPT or PT was observed after IL-1 treatment (e.g. tPT -3.7±1.4%, -3.5±2.0% respectively, p=ns, n=6).

These effects of IL-1 are best explained by induction of myocardial nitric oxide synthase. Further studies are underway to investigate this possibility.

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144P EFFECT OF DEFIBROTIDE (DEF) ON PLATELET ACCUMULATION IN THE RABBIT

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Platelet aggregation in vivo can be monitored in rabbits by measuring accumulation of ^{111}Indium labelled platelets in the lung or head following administration of platelet aggregating stimuli (May et al, 1990). Twenty min after inducing sustained platelet accumulation in the head by i.c. injection of thrombin (100U/kg), treatment with fibrinolytic agents causes a significant reduction. DEF, a single stranded poly DNA with antithrombotic and profibrinolytic properties is inactive under these conditions (May et al, 1992). Pretreatment with DEF has been shown to protect against renal ischaemic injury in rabbits (Berti et al, 1991). We have therefore investigated the effect of pretreatment with DEF on platelet accumulation in the rabbit.

Male NZW rabbits (2.0-2.8kg) were given a bolus i.c. injection of saline followed by an infusion of saline i.c. for 1h (controls, n=4). Matched animals were treated similarly with DEF, 64mg/kg bolus + 64mg/kg/h for 1h (n=4). Platelet accumulation in the head following i.c. thrombin (100U/kg) was 106.3 ± 4.8% (mean ± sem) above baseline in controls. This was significantly reduced (65.5 ± 8.5%; $P < 0.05$) in DEF animals.

Intravenous thrombin (20U/kg) increased platelet accumulation in the lungs of control animals pretreated with i.v. saline by 32.5 ± 5.8% (n=6). Animals pretreated with DEF (64mg/kg bolus + 64mg/kg/h infusion for 1h) showed significantly reduced responses (7.3 ± 3.7%; n=6; $P < 0.05$). The lung response to thrombin 10U/kg i.v. following i.v. injection of L-NAME (10 mg/kg) was 18 ± 3% in control animals (n=3) and 0 ±1 % in DEF animals (n=3 ; $P < 0.05$). In contrast, the response to i.v. PAF (50ng/kg) did not differ significantly between control (26.5 ±3.6 %; n=3) and DEF (24.0 ±4.2 %; n=3) animals.

These results indicate that the effect of DEF may be selective for thrombin induced responses in this in vivo model system.

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145P EVIDENCE FOR INCREASED PURINERGIC CONTRIBUTION IN HYPERTENSIVE BLOOD VESSELS EXHIBITING CO-TRANSMISSION

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Vasoconstrictions produced by electrical field stimulation of the predominantly noradrenergic rat tail arteries were inhibited by α,β-methylene ATP only in the spontaneously hypertensive rat (SHR), but not in normotensive rats (Vidal et al, 1986) suggesting that the co-transmitter release of ATP may have a more important role in hypertensive than in normotensive conditions. The aim of this study was to examine this in rabbit blood vessels previously shown to exhibit noradrenaline/ ATP co-transmission to different degrees (McGrath et al, 1990; Bulloch & Starke, 1990).

Male New Zealand White Rabbits (2.5 - 3kg) used in this study were divided into two groups. In one group of rabbits hypertension was induced by wrapping the left kidney in cellophane and removing the right kidney . The other group of rabbits (control normotensive group) had their right kidney removed and the left one manipulated but not wrapped. At 6 weeks after surgery, arterial blood pressure was monitored and rabbits were considered to be hypertensive if their conscious mean arterial blood pressure exceeded 100mmHg. Isometric contractile responses of ring segments of isolated rabbit blood vessels to electrical field stimulation (4-32 Hz, 25-40 V, 0.1 ms pulse width, for 1 s and 20s) were recorded. The ileocolic artery and proximal (PSA) and distal (DSA) sections of the saphenous artery were used in this study. The purinergic and adrenergic contributions involved in the sympathetic vasopressor responses produced by electrical field stimulation were separated pharmacologically (prazosin (0.3μM) for α_1-adrenoceptors; rauwolscine (3μM) for α_2-adrenoceptors; α,β-methylene ATP (3μM) for P_{2x}-purinoceptors). In the DSA taken from sham-operated animals responses were almost exclusively noradrenergic (partly α_1- and partly α_2-adrenoceptor-mediated). Analysis of the P_{2x}-purinoceptor contribution to the sympathetic nerve mediated response in the normotensive artery was complicated by the potentiating effect of α,β-methylene ATP on its own. Even after α_1-adrenoceptor blockade no attenuating effect of α,β-methylene ATP was observed. However, in the hypertensive state and in the presence of prazosin sympathetic nerve-mediated responses of the DSA could be significantly attenuated by α,β-methylene ATP. PSA and ileocolic arteries taken from normotensive animals had a larger purinergic contribution than DSA. The P_{2x}-purinoceptor contribution to the sympathetic nerve-mediated response in this artery was observed by the significant attenuating effect of α,β-methylene ATP given in the absence of prazosin. The responses of the PSA and ileocolic arteries taken from hypertensive animals were significantly attenuated by α,β-methylene ATP and showed an increased purinergic contribution to the sympathetic nerve-mediated response in the hypertensive state particularly at higher frequencies and longer pulse durations.Yohimbine (0.3μM) produced significant potentiations of the nerve-mediated response in all arteries studied in both normotensive and hypertensive states. The potentiating effect of yohimbine was most significant at longer pulse durations (5Hz and 10Hz at 20s). The degree of significance of the potentiating effect was lower in the hypertensive arteries.

The results of this study indicate that a difference in blood vessel responsiveness between normo- and hypertensive states exists and could be explained by an increased transmitter release (predominantly ATP) going unchecked in the absence of an ongoing feedback mechanism. Lack of autoinhibition could be the basis for the increased resting blood pressure levels observed in hypertensive animals.
J.M.B. holds a British Heart Foundation Intermediate Fellowship.

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146P EFFECT OF GTP CONCENTRATION ON AGONIST AFFINITY ESTIMATES OBTAINED USING THE RECEPTOR INACTIVATION TECHNIQUE

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According to the steady state ternary complex model (Mackay, 1990), for agonists that act through G protein coupled receptors, the level of GTP in the system can affect estimates of apparent agonist affinities. In this study we have investigated whether GTP concentration affects the apparent agonist affinities obtained using Furchgott's receptor inactivation technique (Furchgott, 1966). We have used NCB-20 cells which express prostacyclin (IP) receptors coupled to the stimulation of adenylyl cyclase. Pretreatment of these cells with iloprost, an IP receptor agonist, causes a reduction in the number of IP receptors, without altering other components of the receptor/effector unit (Kelly et al 1990).

Confluent NCB-20 cells were incubated overnight in the presence or absence of 0.01, 0.1 or 1μM iloprost, washed x3 in phosphate buffered saline and stored at -80°C. Adenylyl cyclase activity and [3H]-iloprost binding in cell homogenates were assayed as described previously (Kelly et al 1990). Iloprost dose-response curves in membranes from control and iloprost pretreated cells were obtained at nominal assay concentrations of 0, 10 and 100μM GTP. Estimates of Ka and receptor loss were obtained at each GTP concentration using Furchgott's receptor inactivation technique and compared with Ka and receptor losses obtained from binding studies performed on the same batches of cells.

From binding studies, pretreatment with 1, 0.1 and 0.01μM iloprost resulted in receptor losses of 80, 44 and 24% respectively, with no apparent effect on iloprost binding affinity. However, Furchgott analysis suggested higher receptor losses; at a nominal assay concentration of 100μM GTP, receptor losses were 82, 80 and 53% respectively and at 0μM GTP the corresponding losses were 80, 80 and 79%. The apparent Ka estimated from binding studies was ≈ 2.1x10-7M (log Ka -6.68 ± -7.22, mean ± sem, n=3). Using Furchgott analysis, the apparent Ka ranged from 1x10-8 at 100μM GTP to 3.8x10-6 at 0μM GTP. There was a trend towards higher apparent Ka values at lower GTP concentrations, but this was not significant.

The estimates of Ka and receptor loss from the Furchgott analysis are substantially different from values obtained from binding studies. Furthermore, it appears possible that GTP levels do influence estimates of receptor loss and apparent agonist affinities in this system. These preliminary results tend to support the steady state ternary complex model.

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147P HEXAMETHONIUM-RESISTANT VAGALLY-EVOKED INHIBITIONS OF GASTRIC SMOOTH MUSCLE TONE IN THE RAT: THE EFFECT OF SHORT-TERM 6-HYDROXYDOPAMINE TREATMENT

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The dorsal motor nucleus of the vagus (DMNx) in the rat contains a population of pre-ganglionic parasympathetic neurones which project to the stomach and are tyrosine hydroxylase immunoreactive (Tayo and Williams, 1988). We have demonstrated vagally evoked gastric relaxations which are resistant to nicotinic antagonists (Carnell *et al*, 1991). Here we examine the effect of 6-hydroxydopamine (6-OHDA) treatment on the response to vagal stimulation.

Rats (350-500 g) were treated over two days with intraperitoneal 6-OHDA (Day 1, 100 and Day 2, 250 mg/kg respectively, dissolved in phosphate buffered saline containing 1% ascorbic acid), or with vehicle alone. On Day 3, the rats were anaesthetized with urethane (1.25 g/kg i.p.) and prepared for the study of the effect of vagal stimulation upon intragastric pressure (IGP). The stomach, cervical vagus and brainstem were then removed and processed for immunocytochemical study using antibodies against tyrosine hydroxylase.

Abundant tyrosine hydroxylase immunoreactive (TH-ir) fibres were observed in the myenteric plexus, within the muscle layers and in association with blood vessels in control groups. TH-ir fibres were observed in the cervical vagus of all animals although these were sparse. No TH-ir fibres or cell bodies were identified in the stomachs of lesioned animals. TH-ir cell bodies and fibres were identified in the A1, A2, the area postrema and in the caudal DMNx of all groups. The gastric responses of treated rats to vagal supramaximal (20 V, 20 Hz, 20 s, 1ms pulse) stimulation were indistinguishable from controls, consisting of an initial contraction, followed by a prolonged relaxation. Hexamethonium (30 mg/kg i.v.) abolished the vagally induced inhibition of mean arterial blood pressure and the initial gastric contraction. However, a vagally evoked inhibition of IGP remained, this being significantly ($P < 0.05$) smaller in lesioned rats (26.0 $\pm$ 6.0%, mean $\pm$ s.e. mean) than in than in the vehicle (44.4 $\pm$ 5.0%) or control (47.8 $\pm$ 4.6%) groups.

These data suggest that the hexamethonium resistant inhibition observed on stimulation of the vagus nerve in the rat involves a significant adrenergic component but also suggests a role for a 6-hydroxydopamine resistant, non-adrenergic pathway.

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148P ACTION OF SYMPATHOMIMETIC DRUGS ON PARASYMPATHETIC SALIVARY SECRETION IN DOGS

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In dog mandibular gland with constant-flow vascular perfusion, concurrent sympathetic nerve stimulation initially enhances but later diminishes salivary secretion induced by parasympathetic nerve stimulation. Both responses are abolished by phentolamine (Lung, 1990a). This study was undertaken to investigate the alpha-adrenergic sub-types involved in the sympathetic influence on parasympathetic salivation.

In sodium pentobarbitone anaesthetized dogs, the mandibular gland was under constant-flow vascular perfusion and salivary secretion (Qs) was measured from the mandibular duct (Lung, 1990b). Parasympathetic stimulation was performed by electrical stimulation of the chorda-lingual nerve (5 V, 1 ms and 4 Hz) or acetylcholine infusion (10ug min^{-1}, i.a.). Sympathomimetic drugs were given intra-arterially at the rate of 0.1 ml min^{-1} after the parasympathetic stimulation had attained a steady salivary response.

Table 1

Treatment	Change in Qs (% control) At 10 s	At 60 s
Nerve stimulation		
+ Phenylephrine HCL (0.25 ug kg^{-1} min^{-1})	+36±7.6*	+8±5.5
+ Clonidine HCL (0.75 ug kg^{-1} min^{-1})	-19±3.6*	-31±3.4*
Acetylcholine infusion		
+ Phenylephrine HCL (0.25 ug kg^{-1} min^{-1})	+20±3.9*	+5±6.0
+ Clonidine HCL (0.75 ug kg^{-1} min^{-1})	-30±5.1*	-57±10.1*

Results are means±s.e.means. *P<0.05, when compared to control flow. Number of animals in each group is 5.

Parasympathetic salivary secretion, induced either by nerve stimulation or acetylcholine infusion, was augmented by phenylephrine but diminished by clonidine. Hence, the sympathetic enhancing effect on parasympathetic salivary secretion is probably exerted via alpha-1 adrenergic mechanism whereas the inhibitory effect is via the alpha-2 adrenergic mechanism. The interaction occurs at postsynaptic level.
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149P CCK-B/GASTRIN RECEPTOR-MEDIATED CHANGES IN INTRACELLULAR CALCIUM IN AR42J CELLS

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The rat pancreatic acinar tumour cell line, AR42J, is known to express functional CCK-A and gastrin receptors (Logsdon, 1986; Watson et al,1991). We have characterised the CCK receptor binding site present in these cells using [^{3}H]PD 140376, a novel radioligand which binds with high affinity to the CCK-B/gastrin receptor (Suman-Chauhan et al.,this meeting) and have investigated CCK-receptor mediated increases in intracellular calcium ($[Ca^{2+}]_i$) levels using the fluorescent indicator Fura-2.

AR42J cells were grown as a monolayer in 200ml plastic flasks in culture medium supplemented with 10% foetal calf serum and 2mM glutamine. Cells were harvested when 70-80% confluent by treatment (1-3min) with 0.05%:0.02% trypsin-edta medium before by centrifugation in an excess of normal culture medium at low speed for 1 min. Cells were then resuspended into the appropriate buffer for either [^{3}H]PD 140376 binding studies (Suman-Chauhan et al.,this meeting) or for calcium measurements. For calcium studies, AR42J cells were sequentially loaded with Fura-2-AM (2.5μM) as required, in calcium-free Krebs-Hepes (composition in mM: Hepes 5, NaCl 118, KCl 4.7, $MgSO_4$ 1.2, glucose 11, $NaHCO_3$ 15 and KH_2PO_4 1.2; pH 7.4 at 22C), containing 25μg/ml BSA, for 1 hour at 37C. Cells were harvested by centrifugation, and resuspended into 2ml buffer in a fluorimetric cuvette. $[Ca^{2+}]_i$ was determined using a Shimadzu RF5001-PC fluorimeter and measuring fluoresence ratios at 505nM with excitation at 340 and 380nM. For each assay, the extracellular calcium concentration in the cuvette was raised to 1.3mM and a baseline recorded before stimulation with pentagastrin (1nM-1μM). Where appropriate, antagonists (2μl) were added 6 min before addition of pentagastrin. At the end, each run was calibrated with the addition of 10μl 10% SDS and 30μl 750mM EGTA/Hepes (pH 8). $[Ca^{2+}]_i$ was calculated as described by Grynkiewicz et al.(1985).

Under the conditions described, total binding of [^{3}H]PD 140376 to AR42J cells was approximately 2000 dpm/1x10^5 cells, 80% of which was specifically displaced by 1μM CCK8s. Scatchard plots were linear, indicating binding to a single site with a K_d value of 0.32 ± 0.032 nM. A comparison of the binding affinities of a range of CCK receptor agonists and antagonists in AR42J cells yielded K_i values comparable to those obtained for [^{3}H]PD 140 376 binding to the gastrin receptor in guinea pig gastric membranes (Meecham et al., this meeting). Surprisingly, no specific binding of [^{3}H]L-364,718 ([^{3}H]-Devazepide) could be detected in this strain of AR42J cells, suggesting that CCK-A receptors were not being expressed. CCK8s and pentagastrin (1nM-1μM) produced concentration-dependent increases in $[Ca^{2+}]_i$ levels with approximate EC_{50} values of between 10 and 30 nM. The presence of L-365,260, CI-988 and PD 140376 (at concentrations 10 times their respective binding affinity), resulted in a parallel rightward shift of the pentagastrin dose response curve, suggesting that these compounds act as competitive antagonists. L-365,260, CI-988 and PD140376 had no effect in the absence of pentagastrin. In agreement with the apparent lack of CCK-A binding sites in these cells, Devazepide (10nM) had no effect on the pentagastrin or CCK-evoked increase in $[Ca^{2+}]_i$.

In summary, AR42J cells provide a suitable functional model for studying CCK-B/gastrin receptor mediated events *in vitro*.

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150P INHIBITION OF NEUROGENIC PLASMA EXTRAVASATION BUT NOT VASODILATION IN THE HINDLIMB OF THE RAT BY THE NON-PEPTIDE NK-1 RECEPTOR ANTAGONIST (±)RP67580

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Sensory afferent nerve fibres in the hindlimb of the rat are known to contain vasoactive neuropeptides, such as substance P and calcitonin gene related peptide, which are released upon electrical stimulation of the nerve fibre to produce vasodilation and plasma extravasation. The non-peptide human NK_1 receptor selective antagonist CP-96,345 has been shown to block this extravasation in rats (Lembeck *et al.*, 1992) and also to have anti-odema effects in inflammatory models in rats (Birch *et al.*, 1992). The non-peptide, perhydroisoindole NK_1 antagonist RP67580 has been shown to be selective for rodent NK_1(rNK_1) receptors whilst its (-)enantiomer RP68651 is essentially inactive at this receptor (Fardin *et al.*, 1992). We have used racemic (±)RP67580 and its active and inactive enantiomers to investigate in non-recovery experiments the role of rNK_1 receptors in mediating neurogenic plasma extravasation and vasodilation in the hindlimb of the anaesthetised rat.

Male Sprague-Dawley rats were anaesthetised with pentobarbitone sodium (60 mg.kg^{-1}, i.p.). In extravasation studies, 10 min. after intravenous injection of either vehicle, (±)RP67580 (10-10000 μg.kg^{-1}), RP67580 (1-1000 μg.kg^{-1}), or RP68651 (300 μg.kg^{-1}), the right saphenous nerve was stimulated at 5 Hz, 2 ms, 25 V for 2 min. The left side was prepared for nerve stimulation but was used as a sham control. ^{125}I bovine serum albumin (70 μCi.kg^{-1}, i.v.) and Evans blue dye (50 mg.kg^{-1}, i.v.) were used as markers for plasma extravasation. Samples of skin from the dorsal surface of the hindpaw were removed 5 min after stimulation, washed, weighed and counted for radioactivity. Results were calculated as the ratio of the extravasation in stimulated/unstimulated side and data are given as the ID_{50} i.e. the dose producing half maximal inhibition of extravasation. In vasodilation studies the saphenous nerve was stimulated (5 Hz, 2 ms, 20 V, for 1 min.) 15 min. before and 15 min. after intravenous injection of (±)RP67580 (1 mg.kg^{-1}). Stimulation-evoked changes in hindlimb superficial tissue blood flow were measured as changes in flux using laser doppler fluxmetry. Results were calculated from the maximum increase in flux produced by stimulation in the absence or presence of antagonist and expressed as %change. Arterial blood pressure was monitored throughout all experiments.

These studies have shown that (±)RP67580 inhibited dose dependently neurogenic plasma extravasation in the hindlimb (ID_{50} 105±4 μg.kg^{-1}). The active (+)enantiomer RP67580 was more active (ID_{50} 10.4±0.3 μg.kg^{-1}) than the racemate and RP68651 was inactive at 300 μg.kg^{-1} , which was the ID_{80} for RP67580. Systemic blood pressure was not significantly affected by any of the experimental manoeuvres. The plasma extravasation resulting from endogenous peptide release after electrical stimulation of the saphenous nerve is thus predominantly rNK_1 receptor mediated. A high dose of (±)RP67580 (1 mg.kg^{-1}) had little effect (-4±14%) on the hindlimb vasodilation produced after electrical stimulation of the saphenous nerve. This suggests that an inhibition of stimulation-evoked vasodilation does not underlie the inhibition of plasma extravasation by RP67580 and that the vasodilation *per se* is not mediated primarily by NK_1 mechanisms.

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151P ENDOGENOUS PEPTIDASES ATTENUATE BRADYKININ-EVOKED ION TRANSPORT IN GUINEA-PIG GALLBLADDER

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Kinins stimulate ion transport in a variety of epithelia (Gaginella and Kachur, 1989). However, electrogenic bicarbonate secretion in guinea pig gallbladder stimulated by bradykinin (BK) is different in terms of sidedness from that described previously in other intestinal epithelia since mucosal addition of the peptide was more effective than addition to the serosal side of gallbladder sheets *in vitro* (Baird and Margolius, 1989). In this study we have attempted to resolve whether the pharmacological action of exogenous kinins is influenced by endogenous peptidases using enzyme inhibitors.

Sheets of gallbladder obtained from Dunkin-Hartley guinea pigs were mounted in Ussing chambers (window area = 0.63cm^2) and bathed in oxygenated Krebs-Henseleit solution at 37°C. Tissues were voltage clamped by continuous application of short circuit current (SCC). Drugs were added either basolaterally to the serosal bathing solution or apically to the mucosal bathing solution. Results are expressed as mean ± s.e.mean; n = 6 throughout. Concentration response curves were compared by analysis of variance.

BK evoked an inward SCC which was not altered by bumetanide (100μM) or amiloride (1mM) but was attenuated ($p<0.02$) by acetazolamide (1mM) confirming that the electrogenic response to kinins in this tissue is, at least in part, due to bicarbonate secretion. BK (0.01 - 30 μM) added mucosally (EC_{50} = 0.4 ± 0.2 μM) was significantly more effective in stimulating SCC than when added serosally (EC_{50} = 18.0 ± 9.2 μM ; $p<0.005$). Another secretagogue (PGE_2) produced identical SCC responses when applied serosally or mucosally. Responses to serosally added BK were enhanced (EC_{50} = 0.9 ± 0.4 μM ; $p<0.001$) in the presence of a cocktail of enzyme inhibitors (MGTPA, 30μM; captopril, 10μM; phosphoramidon, 10μM - which inhibit kininase I, angiotensin converting enzyme and neutral endopeptidase respectively). The concentration response curve to serosal side application of BK in the presence of the enzyme inhibitors was not different from that obtained to mucosal side challenge with BK in non-inhibited preparations. Responses to mucosal addition of BK were not significantly altered by pretreatment with the inhibitor cocktail when compared to the effects of mucosal application of BK in non-treated controls. The enzyme inhibitors alone had no influence on basal SCC.

These results confirm that BK evokes electrogenic bicarbonate secretion in guinea pig gallbladder *in vitro*. Furthermore, we have demonstrated that endogenous peptidases modify responses to BK in this tissue, presumably by degradation of the nonapeptide. This is in keeping with the previously described enhancement of BK induced contractions of guinea pig gallbladder by treatment with peptidase inhibitors (Woods & Baird, 1991). Further investigations will be designed to characterise the enzyme(s) involved.

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152P ACTIVITY OF SR 48968 AT SMOOTH MUSCLE NK_2 TACHYKININ RECEPTORS OF HAMSTER, RAT AND RABBIT SPECIES

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SR 48968, the first non-peptide NK_2 tachykinin receptor antagonist recently disclosed (Emonds-Alt et al., 1992), is characterized by high potency and selectivity toward the NK_2 receptor. In the present study we compared the potency of SR 48968 in antagonizing NK_2 receptor-mediated responses in different smooth muscle preparations from hamster, rabbit and rat, with that of the selective NK_2 receptor antagonists MEN 10,376, R 396 and MDL 29,913. Previously it was shown that these peptide antagonists, along with MEN 10,207 and L 659,877, identify different populations of NK_2 receptors in the mammalian species considered (Maggi et al.,1991; Patacchini et al., 1991; Van Giersbergen et al., 1991). The isolated organs used were: hamster trachea (rings), rat vas deferens (pars prostatica), rabbit pulmonary artery (endothelium-denuded circular strips) and bronchus (epithelium-deprived). Antagonists (15 min incubation) were studied against NKA or [βAla^8]-NKA(4-10) as agonists, in the presence of peptidase inhibitors (thiorphan, captopril and bestatin 1 μM each). Results, expressed as pK_B ± S.E. of the mean (n= 6-8), are reported below.

Table 1	Hamster trachea	Rat vas deferens	Rabbit p.artery	Rabbit bronchus
MEN 10,376	5.6 ± 0.1	6.7 ± 0.1	8.1 ± 0.1	7.8 ± 0.1
MDL 29,913	8.6 ± 0.2	8.5 ± 0.1	7.6 ± 0.1	7.9 ± 0.1
R 396	7.6 ± 0.1	6.3 ± 0.1	5.4 ± 0.1	5.6 ± 0.2
SR 48968	8.5 ± 0.1	8.3 ± 0.1	9.6 ± 0.1	9.5 ± 0.1

The present results confirm the high affinity interaction of SR 48968 with the NK_2 receptors. The higher antagonistsic potency exhibited by SR 48968 in the rabbit compared to the hamster tissue, supports the hypothesis of two heterogeneous forms of the NK_2 receptor, previously recognized by peptide antagonists in these species (Maggi et al., 1991; Patacchini et al., 1991). SR 48968 was competitive at all bioassays, although a depression of the maximal response to NKA, which was neither concentration nor time-dependent, was observed in the rabbit pulmonary artery.

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153P AMLODIPINE-INDUCED INHIBITION OF CALCIUM CHANNEL CURRENTS IN SINGLE SMOOTH MUSCLE CELLS ISOLATED FROM RABBIT EAR ARTERY

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Amlodipine is a novel 1-4 dihydropyridine calcium channel antagonist with a slow onset and long duration of action *in vivo* (Burges et al., 1985) and *in vitro* (Hughes et al., 1991). In voltage clamp studies of cardiac myocytes, the action of amlodipine shows both voltage- and use-dependence (Kass & Arena 1989). In these studies we have examined the effects of amlodipine on calcium channel currents in isolated single vascular smooth muscle cells.

Single cells were isolated enzymatically from rabbit ear arteries as described previously (Benham & Bolton, 1986). Calcium channel currents were measured by whole cell voltage clamp techniques using Ba^{2+} as the charge carrier. Data are presented as means ± s.e.means of n observations.

At a holding potential of -60mV, amlodipine (100pM-100nM) induced a concentration-dependent inhibition of peak calcium channel currents evoked by a 20ms step to +10mV (IC_{50}=0.24nM, n=5). At a more depolarized holding potential of -40mV, amlodipine (10pM-10nM) inhibited the peak current more potently with IC_{50}=0.022nM (n=5). Examination of the current-voltage relationships at both holding potentials showed that amlodipine-induced inhibition was most marked at more depolarized test potentials. In addition amlodipine (1nM), shifted the steady-state inactivation curve in a hyperpolarizing direction with the mid-point of the inactivation curve shifting from -25.5±2.5mV to -46.7±6.1mV (n=4) with no significant change in the slope of the curve (control = 8.5±2, 1nM amlodipine = 10.9±3, n=4). In a further 4 cells the steady-state effect of amlodipine (10nM) was enhanced by increasing the rate of stimulation from 0.1Hz to 1Hz (control = 65±15pA, amlodipine 0.1Hz = 31±6pA, amlodipine 1Hz = 17±4pA).

These studies show that amlodipine is a potent inhibitor of calcium channels in vascular smooth muscle. The interaction of amlodipine with the voltage-operated calcium channel shows both voltage-dependence and use-dependence. The marked voltage-dependence displayed by amlodipine may contribute to the preferential action of this drug on the vasculature seen *in vivo*.

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154P PYRANTEL ACTIVATED SINGLE-CHANNEL CURRENTS IN THE NEMATODE PARASITE *ASCARIS SUUM*

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Several anthelmintic drugs, e.g. levamisole, pyrantel and morantel act as agonists at nicotinic acetylcholine receptors in the nematode parasite *Ascaris suum* producing muscle depolarization and spastic paralysis (Aceves *et al.*, 1970; Van Neuten, 1972; Coles *et al.*, 1975; Harrow and Gration, 1985). A pharmacological profile of this receptor has been obtained (Natoff, 1969; Rozkova et al., 1980 and Colquhoun *et al.*, 1991). The pharmacological differences between the nematode receptors and vertebrate nicotinic receptors are exploited for therapeutic purposes and permit the treatment of nematode infestations.
This present study has investigated the action of pyrantel (0.1-100μM) at the single-channel level using the patch-clamp technique on muscle vesicle membranes (Martin et al., 1991). Pyrantel activated a cation selective channel with conductances levels in the range 11-57pS. Subconductance states were detected. I/V plots were linear. The distribution of open-, closed- and burst-times showed that there were at least two open- and burst-states and three closed-states. At low concentrations (0.1μM) at -75mV the brief openings had a mean open duration of 0.52±0.19ms and the longer opening had a mean open duration of 2.40±0.48ms (Mean±.s.e.,N=8). The open durations decreased on depolarization: at +75mV recordings with 0.1μM produced brief openings with mean open durations of 0.42ms±0.11ms and longer open times with mean durations of 1.63±0.13ms (Mean±s.e.,N=5). Occasionally at 10μM and mostly at 100μM only one open-state was resolved and channel events appeared as a sequence of rapid openings and closings characteristic of a fast flickering open channel-block. With higher concentrations of pyrantel (100μM) in the pipette long closed periods separating clusters of openings thought to represent a desensitised closed state were detected at hyperpolarized and depolarized potentials.
Desensitization has also been observed previously in this preparation with acetylcholine (>25μM) and levamisole (>30μM) (Pennington and Martin, 1990; Robertson and Martin, 1991); a flickering open-channel block has also been observed with levamisole (30-90μM) at hyperpolarized potentials (Robertson and Martin 1991). The therapeutic significance of these two phenomena for anthelmintic use remains to be fully evaluated.

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155P ANALYSIS OF MUSCARINIC INHIBITION IN THE C-CELLS OF BULLFROG SYMPATHETIC GANGLIA

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A variety of agonists hyperpolarize central and peripheral neurones by activating an inwardly-rectifying K^+-conductance (North, 1989). Acetylcholine (ACh) hyperpolarizes atrial cells by a similar mechanism and this is thought to involve a single type of unitary conductance, the 20pS 'I_{ACh}' channel (Clark *et al.*, 1990). In submucosal neurones however, α_2-adrenoceptor agonists, somatostatin and [Met^5] enkephalin activate an inwardly-rectifying K^+ conductance which might involve a variety of unitary conductances including 30-65, 120-160 and 220-260pS channels (Shen *et al.*, 1992). The question therefore arises as to whether the inwardly-rectifying conductance activated by muscarinic agonists in neurones involves one type of unitary conductance, as it does in the heart, or whether ACh, like other agonists, activates multiple unitary conductances. To distinguish between these two possibilities, we studied the action of muscarine on the small (C) cells of bullfrog sympathetic ganglion (*see* Selyanko *et al.*, 1990) using whole-cell and single channel recording techniques. In the presence of 10μM muscarine, the activation curve for the inwardly-rectifying K^+ conductance was fitted by Boltzmann-type kinetics (potential for half activation = -53mV; valency = -2.3). The extent of inward rectification was increased by elevating $[K^+]_o$ but was not influenced by changing $[Mg^{2+}]_i$ or $[Na^+]_i$. Thus, the rectification does not result from the internal blocking of the channel by Mg^{2+} or Na^+ (*see* Vandenberg, 1987). A possible mechanistic interpretation of these results is that some channels are controlled by a voltage-sensitive gate which is opened by hyperpolarization. Alternatively, rectification could involve blockade of the channel by the outward movement of K^+. Muscarine increased channel activity in outside-out patches ($[K^+]_o$ = 20mM; $[K^+]_i$ = 110mM), and activated an inwardly-rectifying K^+ conductance in outside-out macropatches. This indicates close association between receptor and channels which is presumably mediated by 'direct' G-protein coupling. Although muscarine activated a 20pS channel similar to the cardiac I_{ACh} channel in outside-out patches, the activity of at least one other channel, which had a conductance of about 50pS under our experimental conditions, was also increased. In these aspects, the inhibitory action of ACh on peripheral neurones resembles that of adrenoceptor and peptidergic agonists (Shen *et al.*, 1992).

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156P SUBUNIT DEPENDENCE OF 43KDa PROTEIN-MEDIATED CLUSTERING OF NICOTINIC ACETYLCHOLINE RECEPTORS (nAchRs) IN *XENOPUS* OOCYTES

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At the neuromuscular junction nAChRs cluster at the crests of convolutions in the post-synaptic membrane, a process involving cytoskeletal elements including a 43KDa protein (Froehner,S.C. *J.Cell.Biol.* 114:1-7). This localisation must require some interaction between AChR subunits and the 43KDa protein but there is little direct evidence to suggest which subunits are important.

These events were studied by injection of in vitro transcribed mRNAs into *Xenopus* oocytes (Snutch 1988, Sigel 1990). Functional nAChRs were detected using whole cell voltage clamp, whilst clustering was visualised by fluorescein labelled α-bungarotoxin (α-BgT) binding using confocal laser scanning microscopy. Micro-injection of $\alpha_1,\beta_1,\gamma,\delta$, muscle subunit mRNAs. of $<10\mu$m (n=12) . Several species of neuronal ß subunit exist, notably β_2 and β_4. Substitution of the muscle ß transcript with the neuronal β_2 species results in a functional receptor which does not cluster (n=14), whereas substitution with the neuronal species β_4 produces similarly functional receptors that cluster in discrete areas (n=13). Oocytes injected with $\alpha_1,\beta_1,\gamma,\delta$, when incubated with neuronal BgT (100nM for 15 minutes) show a small degree of inhibition (34% $\pm$7% n=6 ACh=10μM) compared to chimeric nAChRs incorporating the β_2 neuronal species which are more sensitive to neuronal bungarotoxin (NBT), (inhibtion =66.8% $\pm$3% n=6 ACh=10μM) or that containing the β_4 specieswhich exibit a small degree of inhibition to NBT (18% $\pm$5% n=5 ACh=10μM). All combinations exhibit a total current block to ACh 10μM when incubated for 15 minutes with 100nM α-BgT. These data suggest that the ß subunit plays a crucial role in the process of receptor aggregation mediated by 43kD protein, and that the source and nature of the ß subunit influences the receptors toxin sensitivity.

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157P FLOW CYTOMETRIC ASSESSMENT OF RENAL CELLULAR INJURY IN RESPONSE TO GENTAMICIN OR HYPOXIA/REOXYGENATION

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There is a need to develop better mechanistic-based screening methods to detect cell injury. Flow cytometry is a powerful new technique in the investigation of cellular injury in single cells (McGlynn, Healy and Ryan, 1991). In this study, we have used the established renal cell lines LLC-PK_1 (of proximal tubular origin) and MDCK (of distal tubular/collecting duct origin) and flow cytometric techniques to investigate cellular injury as induced by gentamicin and hypoxic/reoxygenation conditions.

LLC-PK_1 and MDCK cells were maintained in Dulbeccos modified Eagles medium (DMEM) supplemented with 10% foetal calf serum, 2 mM glutamine, incubated at 37^{o}C in 95% 0_2/5% CO_2 and grown to confluency, in 75 cm^2 Costar flasks. Prior to flow cytometric analysis, cells were dispersed using trypsin-EDTA. Cells were exposed to gentamicin concentrations up to 10^{-2} M for time periods up to 60 minutes. Hypoxia was induced in oxygen-free nitrogen conditions for time points up to 1 hour followed by 1 hour reoxygenation. Cell viability studies were carried out using the fluorescent probes fluorescein diacetate and propidium iodide. Intracellular pH was measured using SemiNapthoRhodaFluor (SNARF-1) fluorochrome. All flow cytometric assays were carried out using a BD FACSTAR Plus Flow Cytometer.

Cell viability was not significantly affected by gentamicin concentrations up to 10^{-2}M for 60 min in both cell types. Hypoxia did not alter cell viability in either cell type but reoxygenation resulted in a significant reduction in cell viability in both cell types. Viability under control conditions was expressed as 100 % in each case. Changes in viability following hypoxia/reoxygenation were as follows; LLC-PK_1 to 81.4% ± 1.3; MDCK to 69% ± 1.4 ($p \leq 0.05$; n=3; in both cell types). Exposure of cells to gentamicin (10^{-2} M) for 60 minutes resulted in significant reductions in intracellular pH as follows: LLC-PK_1 from 7.20 ± 0.07 to 6.70 ± 0.04; MDCK from 7.30 ± 0.04 to 7.08 ± 0.04, ($p \leq 0.005$; n=5; in both cell types). During hypoxia for 60 minutes, changes in intracellular pH in both cell types tended toward acidosis as follows: LLC-PK_1 from 7.15 ± 0.03 to 7.10 ± 0.12; MDCK from 7.20 ± 0.03 to 7.14 ± 0.06 (n=4 in both cell types). These values did not reach statistical significance. A similar trend was observed upon reoxygenation for 60 minutes as follows: LLC-PK_1 to 7.10 ± 0.8; MDCK to 7.09 ± 0.03.

These findings indicate that gentamicin produced alterations in intracellular pH prior to any significant effects on cell viability. The The extent of hypoxia induced or subsequent reoxygenation did not significantly reduce intracellular pH in either cell type. Reoxygenation following hypoxia induced significant reductions in cell viability in both cell types. Flow cytometric analysis can provide a subtle approach to defining mechanisms of cell toxicity in a variety of experimental conditions.

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158P UTP AND ATP STIMULATE BOVINE ADRENAL MEDULLARY ENDOTHELIAL CELLS BY ACTING ON A NUCLEOTIDE RECEPTOR

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We have previously shown that bovine adrenal medullary endothelial (BAME) cells exhibit an "atypical" purinergic sensitivity (i.e. neither P_{2x} nor P_{2y}) (Allsup & Boarder, 1990). Association of similarly distinct purinoceptor mediated responses with sensitivity to UTP has led to the proposal of a 5'-nucleotide receptor (O'Connor et al., 1991). Purinergic stimulation causes phospholipase C (PLC) (Pirotton et al., 1987) and phospholipase D (PLD) (Martin and Michaelis, 1989) activation in large vessel endothelium. Here we present evidence for a 5'-nucleotide receptor on microvascular BAME cells, linked to both PLC and PLD.

BAME cells labelled for 48h with [^{3}H]inositol gave, in the presence of 10mM LiCl, total [^{3}H]inositol phosphate (IP) responses to UTP, ATP and ATPγS (EC_{50} values: 1.66 ± 0.6μM, 13.32 ± 5.6μM and 9.44 ± 0.3μM, n=3). The selective P_{2y} agonist 2-methylthio ATP (2MeSATP) had little effect and the P_{2x} agonist β,γ-methylene ATP was ineffective. No additivity in IP response was seen when concentration effect curves to ATPγS and UTP were constructed in the presence of 10, 30 and 100μM UTP and ATP respectively.

As an index of PLD activation we measured labelled phosphatidylbutanol (PBut) production in cells stimulated in the presence of 50mM butanol. Cells labelled with [^{32}P]P_i for 24h gave similar [^{32}P]PBut responses to stimulation with ATPγS and the protein kinase C (PKC) activator TPA, but a lower response with 2MeSATP. [^{32}P]phosphatidic acid (PA) was also produced with ATPγS stimulation suggesting conversion of PLC generated diacylglycerol (DAG) to PA by DAG kinase. Cells labelled with [^{3}H]palmitate for 48h generated [^{3}H]PBut with the same order of agonist potencies seen for IP responses (UTP > ATPγS = ATP>> 2MeSATP). Removal of extracellular Ca^{2+} reduced ATPγS stimulation of PLD and PKC inhibition with 10μM of the relatively selective inhibitor Ro 31-8220 abolished PLD activation completely.

In conclusion BAME cells express a 5'-nucleotide receptor linked to PLC and PLD. PA, the primary product of PLD, is also generated by the sequential action of PLC and DAG kinase. The PLD response is totally dependent on PKC activation and partially dependent on extracellular Ca^{2+} influx suggesting PLD activation to be down-stream of PLC. The results suggest that ATP regulation of these microvascular endothelial cells is via a nucleotide receptor and not a purinergic receptor.

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159P TREATMENT OF AN EXPERIMENTAL ARTHRITIS WITH INTRA-ARTICULAR ^{90}YTTRIUM, METHOTREXATE OR CHLORAMBUCIL

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The intra-articular (i.a.) injection of radioisotopes for the destruction of proliferative synovium in refractory rheumatoid arthritis was first described by Ansell et al (1963) using colloidal ^{198}Au. More recently, pure ß-emitters such as ^{90}Y have been favoured, although there is evidence that these may damage the articular cartilage (De Vries et al, 1984). Alternatively, cytotoxic agents such as nitrogen mustard, methotrexate or osmic acid have been injected i.a. to effect a chemical synovectomy (Hunneyball, 1986).

In this study we have compared the efficacy of i.a. ^{90}Y, methotrexate and chlorambucil in suppressing antigen-induced arthritis in rabbits. A bilateral arthritis was induced to the knee joints of rabbits as previously described (Foong & Green, 1988) and the arthritis monitored at regular intervals by measuring changes in joint diameter and surface temperature. Single joints were injected with ^{90}Y or cytotoxic drug, usually 7 days after antigen challenge, contralateral control joints being injected with 0.5ml saline. When the rabbits were sacrificed, 8 weeks after induction of arthritis, the knee joints were opened and gross morphological and histological changes assessed blind.

Injection of ^{90}Y silicate 18.5 MBq i.a. caused an initial pro-inflammatory effect, but both joint swelling and temperature were reduced approx. 20% ($P<0.05$; n=5; paired student's t-test) 7 days after injection. However, the beneficial effect did not persist, and the morphological and histological appearance of joints 7 weeks after treatment was not significantly different ($P>0.05$) from control joints. Methotrexate 1mg injected i.a. 7 days after antigen challenge had no significant effect on joint swelling, surface temperature or joint histology. However, methotrexate injected at the time of antigen challenge reduced joint swelling and temperature by 20% ($P<0.05$; n=5), but with little effect on joint histology. Chlorambucil 1mg injected i.a. 7 days after antigen challenge caused no significant initial irritation and suppressed joint swelling and temperature within 3 days of injection ($P<0.05$; n=5). Joint swelling was reduced by 30% for the duration of the study and histological examination of the joints 8 weeks after induction of arthritis also showed a reduction in synovial proliferation and in erosion of cartilage and bone ($P<0.05$). The suppression of an established arthritis by chlorambucil was clearly greater than with the other treatments employed and deserves further study.

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160P PHOSPHORAMIDON-SENSITIVE EFFECTS OF HUMAN BIG-ENDOTHELIN-1 ON PLASMA EXTRAVASATION IN CONSCIOUS RATS

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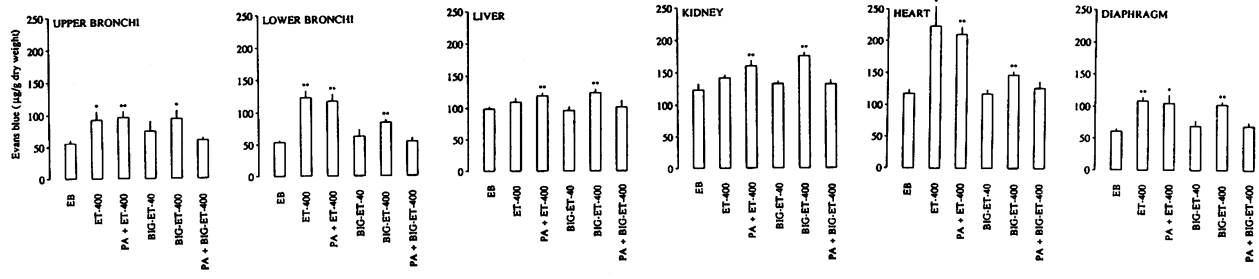
It has been suggested that the precursor of endothelin-1 (ET-1), big-ET-1, induces various pharmacological effects *in vivo* and *in vitro* following an active conversion by a phosphoramidon (PA)-sensitive endothelin-converting enzyme (ECE) (Matsumura *et al.*, 1990; D'Orléans-Juste *et al.*, 1991). In the present study, ET-1 and big-ET-1-induced changes in vascular permeability to serum albumin, in absence or in presence of PA, were measured by extravasation of Evans blue dye (EB) in the upper and lower bronchi, liver, kidney, heart and diaphragm from male Wistar rats, using a modification of the methods described by Filep *et al.* (1991). The animals received bolus injections of EB (20 mg kg^{-1}) in the caudal vein. Big-ET-1 and ET-1, at doses which did not induce significant pressor responses (400 pmol kg^{-1}), were injected with the dye, whereas PA (2 mg kg^{-1}) was administered 5 min earlier. The rats were killed and exsanguinated 10 min following administration of EB.

Big-ET-1 and ET-1 induced a significant increase in Evans blue dye extravasation in most tissues examined (*$P<0.05$; **$P<0.01$; n=5-6). The pretreatment with PA blocked the extravasation induced by big-ET-1 without reducing the effect of ET-1 (Figure 1).

Figure 1. Phosphoramidon blocks big-ET-1 but not ET-1 enhancement of vascular permeability in rat tissues.



We conclude from these results that the increase in vascular permeability induced by big-ET-1 in the above-mentioned tissues follows its conversion to ET-1 by a PA-sensitive ECE.

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161P PHOSPHORAMIDON-SENSITIVE CONVERSION OF HUMAN PROENDOTHELIN *IN VIVO*: EFFECT ON PRESSURE AND PLASMA PROSTACYCLIN

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Endothelin 1 [ET-1(1-21)] is a product of the proteolytic processing of human proendothelin [Big-ET1 (1-38)] by an Endothelin-Converting Enzyme. The in vitro vasoconstrictor activity of Big-ET1 is at least 100 time less than ET-1, whereas in vivo both peptides are equally active to induce pressor effect, indicating that conversion of Big-ET1 to ET-1 is essential for biological activity.

In the current study we have examined the in vivo enzymatic conversion of Big-ET1 by monitoring its ability to elicit pressor response and to promote prostacyclin (PGI_2) synthesis in the anaesthetized (urethane, 1.25 g/kg, i.m), ganglion-blocked (pentolinium: 0.1 mg/kg/min) rats (male, Sprague-Dawley). Mean arterial pressure (MAP) was monitored via carotid artery ; saline or Phosphoramidon (PA) were injected through femoral vein. Big-ET1 or ET-1 vasopressor effects were recorded in absence and presence of PA. Peptides were injected 5 min after bolus injection of PA (10 mg/kg). Plasma levels of PGI_2 (measured by radioimmunoassay, as 6-keto $PGF_{1\alpha}$) following injection of either ET1 or Big-ET1, in presence and absence of PA (10 mg/kg) were also determined. Blood taken via right jugular vein, was collected in tubes containing heparin (0.1 %) and indomethacin (10μM).

The effects of ET-1 and Big-ET1 on MAP and PGI_2 are reported in Table 1. Basal MAP and PGI_2 levels were 53±0.7 mmHg and 80±7.1 pg/100μl plasma (n=31) respectively. Neither of these parameters were affected by PA (54 ± 2.3 mmHg and 80 ± 9.1 pg/100μl plasma; n=12). Big-ET1 and ET-1 induced a similar slow onset, long lasting (> 30 min) increase of MAP.

Table 1: Effects of ET-1 and Big-ET-1 on MAP and PGI_2:

Dose (nmol/kg)	PA (10mg/kg)	MAP (ΔmmHg)	PGI_2 (pg/100μl plasma)
ET-1			
0.25	(-)	6 ± 0.7	228 ± 35*
1.75	(-)	48 ± 1.9	360 ± 63*
1.75	(+)	52 ± 2.2	277 ± 30*
Big-ET1			
1.3	(-)	26 ± 1.2	89 ± 7.1
4.3	(-)	60 ± 1.7	90 ± 14
4.3	(+)	25 ± 3.7°	103 ± 18
12.5	(-)	72 ± 4.0	275 ± 13*
12.5	(+)	24 ± 3.2°	83 ± 4.9°

°p<0.001 vs (-) PA ; * p<0.001 vs basal, non-paired Student's t test (n=8-12)

ET-1 induced a dose-related increase in both MAP and PGI_2 levels. This stimulation was not affected by pretreatment with PA. Big-ET1, at low doses (1.3 and 4.3 nmol/kg), significantly increased MAP with no effect on PGI_2 levels. However, at 12.5 nmol/kg, there was a significant increase in both parameters measured, which were significantly decreased by pretreatment with PA.

These results show that, under our experimental conditions, ET-1 was approximately 2 fold more potent than Big-ET1 on MAP increase, whereas for PGI_2 levels, ET-1 was found approximately 10 times more potent. The increase in PGI_2 observed is most likely not a pressure related phenomenon, because low concentrations of Big-ET1 significantly increased MAP, without affecting PGI_2 levels. This could be attributed to a slow rate of conversion of Big-ET1, and/or to a difference between efficacious ET-1 concentration necessary to activate the receptors coupled to vasoconstriction (smooth muscle) and prostanoid production (endothelium).

The inhibition by PA of Big- ET1-induced in vivo effects (without affecting significantly ET-1 responses), reinforces the concept of a specific PA-sensitive conversion required for biological actions, involving ET-1.

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162P REGULATION OF HISTAMINE-INDUCED INOSITOL PHOSPHOLIPID HYDROLYSIS BY PROTEIN KINASE C IN CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Histamine H1 receptor stimulation increases microvascular permeability in cultured human umbilical vein endothelial cells (HUVEC). The mechanism underlying this effect of histamine is believed to involve stimulation of phosphoinositidase C (Carson et al 1989). In this study we have examined the regulation of histamine induced inositol phosphate formation in HUVEC cells. Total [^{3}H]-inositol phosphates were measured in the presence of lithium (10mM) as previously described (Hawley et al 1992).

In keeping with previous reports (Carson et al 1989) histamine produced inositol phosphate formation in HUVEC with an EC_{50} of 3.7 ± 0.3 μM (n=35). 45 min. exposure to a maximally effective concentration of histamine (0.1mM) produced a 12.4 ± 0.4 fold stimulation (n=85) over basal (unstimulated) levels. The time course of this response was essentially linear. The response to histamine was inhibited by mepyramine (50nM, K_a=3.7 ± 0.9 x $10^{9}M^{-1}$, n=3) indicating the involvement of the H1 receptor subtype in this response.

Prior exposure of HUVEC to histamine for 2h resulted in concentration dependent desensitization (IC_{50} = 0.9 ± 0.2 μM, n=7) of the response to a subsequent challenge with 0.1mM histamine. The maximum reduction observed was 65 ± 2% (n=12, p<0.001) compared with control cells. Prior exposure of cells to forskolin (1μM) or dibutyryl cyclic AMP (1mM) had no effect upon the subsequent inositol phosphate response to histamine (0.1μM-0.1mM). However, prior exposure of cells to phorbol dibutyrate (1 or 10μM) (PdBu) resulted in marked inhibition (maximum reduction 74 ± 2%, n=5, p<0.05) of the response to subsequent challenge with histamine (0.1μM-0.1mM). The inhibitory effect of PdBu (10μM) was itself reversed by preincubation with staurosporine (100nM).

These results indicate that in HUVEC histamine H1 receptor stimulation produces an inositol phosphate response which can be desensitized by prior exposure of cells to histamine. The desensitizing effect of histamine can be mimicked by activation of protein kinase C (PKC) suggesting the possible involvement of PKC in histamine H1 receptor desensitization in these cells.

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163P ANTI-ANGIOGENIC ACTION OF SURAMIN AND HYDROCORTISONE IN RATS

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Suramin is a polysulphonated compound used for several decades as a trypanocidal agent. Recent studies indicate that it can also disrupt mammalian cell function, notably by blocking growth factor/receptor interactions and by inhibiting the activity of enzymes that are critical for cell growth and proliferation. In particular, suramin possesses potent anti-angiogenic activity in several test systems. These observations, together with the finding that clinically achievable concentrations of the drug are toxic to many human tumour cell lines, have prompted clinical investigations of the efficacy of suramin as an antitumour agent. Here we tested the anti-angiogenic effect of suramin and hydrocortisone in rats.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (150-200g) and, using a ^{133}Xe clearance technique (Andrade *et al.*, 1987), neovascularisation was assessed as a function of blood flow through the implants every two days over a period of 14 days. The effects of test substances on angiogenesis were confirmed histologically.

Daily administration of 6 pmol (100 ng) basic fibroblast growth factor (bFGF) into the sponges elicited an intense neovascularisation. This neovascular response was inhibited by co-administration of 3 mg suramin. The 6 min ^{133}Xe clearance values obtained on Day 6, 8, 10 for bFGF group and suramin group were 22.2±0.9, 27.0±0.6, 32.5±1.2, and 14.7±2.4, 20.4±1.2, 24.6±0.3 %, respectively ($P < 0.05$, $n = 4$). Histological studies of sponge sections stained with haematoxylin & eosin or the endothelial cell marker, *Bandeirea simplicifolia* lectin I, isolectin B_4, showed that both the cellular infiltration and neovascularisation in the bFGF-treated sponges were profoundly suppressed by suramin. However, there is a narrow therapeutic window for suramin, 1 mg was not effective but 10 mg appeared to be toxic. The systemic effect of suramin was also investigated. Although a single intravenous injection of 100 mg kg^{-1} on Day 1 after sponge implantation produced no apparent effect, a similar protocol with 200 mg kg^{-1} totally blocked the bFGF-induced neovascularisation. Higher doses led to severe toxicity.

We have previously shown that daily local injection of hydrocortisone (0.5, 5, 50 µg) inhibited the sponge-induced angiogenesis in a dose-dependent manner (Hori *et al.*, 1992). In the present study, daily local administration of 10, 100, 1000 µg suramin produced no apparent effect on sponge-induced neovascularisation. However, 100 µg suramin acted in synergy with a subthreshold dose of hydrocortisone (0.1 µg) to inhibit the sponge-induced angiogenesis. The 6 min ^{133}Xe clearance values obtained on Day 10, 12, 14 for control group and suramin group were 29.0±0.9, 33.3±0.6, 38.1±0.5, and 17.4±0.4, 19.9±0.5, 24.8±2.6%, respectively ($P < 0.01$, $n = 4$).

Thus, these results suggest the use of low doses of suramin and angiostatic steroids could have therapeutic benefit in angiogenic diseases. Work is in progress to establish the anti-angiogenic profile and safety of suramin analogues.

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164P DEMONSTRATION OF MYOFIBROBLAST CONTRACTILITY IN FIBROTIC LUNGS OF CHROMIUM AND METAL-FUME PRETREATED RATS

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Contractile activity in fibrotic lung tissues 'in vivo' and 'in vitro', attributable to myofibroblast cell development, has been cited as a possible cause of respiratory difficulty, e.g. reduced pulmonary compliance, caused by long or short-term inhalation of inhaled occupational pollutants. This has previously been shown to apply to lung-fibrosis provoked by deposition of silica or paraquat toxicity, contractility being evoked characteristically by mepyramine or certain oxidising agents (Olufsen & Hicks, 1990; Hicks *et al.*, 1991). This work has been extended to observe contractile activity in lungs subjected to effects of chromium or chromium-containing metal-fumes.

Female rats (CSE 150-250gm) were pretreated with single intraperitoneal doses of sodium chromate 20mg or staibless-steel (MMA) welding fumes, each in 0.25ml sterile saline. Initial pneumonitis progressed to fibrosis, observable from 4 weeks to 6 months. Animals were anaesthetised with pentobarbitone (30mg/kg) plus alphaxolone/alphadolone injection 1-2ml intraperitoneally and ventilated artificially via a tracheal cannula using a pump producing positive pressure inflations (50-70mm H_2O) at 36 strokes/min. Transmitted transpulmonary pressure fluctuations were monitored, via a saline-filled intrathoracic reservoir, pressures being measured by transducer. Intravenous doses of mepyramine 40-150mg/kg i.v. caused dose-related, reversible reductions of transpulmonary pressure, without bronchoconstriction. This was indicative of reduced dynamic compliance. There were no such effects in untreated, non-fibrotic controlanimals.

Longitudinal pulmonary strips, isolated from lungs of treated animals sacrificed after 4 weeks, were suspended in Krebs solution, gassed with O_2/CO_2 at 37°C, equilibrated to resting tension of 500mg and contractions measured by transducer. Mepyramine (1×10^{-4} to 2×10^{-3}M), sodium tungstate (1×10^{-3} to 1×10^{-5}M) or hydrogen peroxide (1×10^{-5} to 1×10^{-3}M) caused dose-related, repeatable and reversible contractions (100 to 500mg tension) in chromium and metal-fume pretreated tissues, characteristic of myofibroblast contracture.

It was concluded that these results supported the view that the active component of metal-fume induction of myofibroblast development in lung could be attributed to the chromium component

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165P MODULATION OF AGONIST-STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN AIRWAY SMOOTH MUSCLE BY ISOENZYME-SELECTIVE PHOSPHODIESTERASE INHIBITORS

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Increases in cyclic AMP levels in bovine tracheal smooth muscle (BTSM), either by stimulation of formation or inhibition of degradation, have been shown to modulate agonist-stimulated phosphoinositide hydrolysis (Hall et al., 1989). Available evidence suggests that BTSM expresses five phosphodiesterase (PDE) isoenzymic activities (PDE I-V; Shahid et al., 1991) which may contribute to the regulation of cellular cyclic nucleotide concentration, however data supporting a functional role for PDE III are lacking. In the present study, we have investigated the inhibitory effects of rolipram (a PDE IV-selective inhibitor) and Org 9935 (a PDE III-selective inhibitor) on histamine- and carbachol-stimulated PI hydrolysis.

BTSM slices (300 x 300 μm) were labelled with 0.8 μCi [^{3}H]inositol/ml for 24 h at 37°C in minimal essential medium containing 10% newborn calf serum (Offer et al., 1991). For incubations, slices were washed into oxygenated Krebs-Henseleit buffer (KHB) and packed slices (75 μl) dispensed into 400 μl KHB containing [^{3}H]inositol (0.8 μCi/ml) and 10 mM LiCl. PDE inhibitors were added 30 min prior to histamine or carbachol challenge. Incubations were terminated 30 min after agonist addition by addition of 10% (v/v) perchloric acid. Following acid-extraction, [^{3}H]inositol phosphates ([^{3}H]InsP$_x$) were separated using Dowex-1 (Cl^-).

Histamine (100 μM) and carbachol (100 μM) increased [^{3}H]InsP$_x$ accumulation by 7.6 ± 0.4 and 22.4 ±2.6 -fold respectively (basal: 3284 ± 800 d.p.m./mg protein). Rolipram (100 μM) caused a maximal 76 ± 1.4 % inhibition of histamine-stimulated [^{3}H]InsP$_x$ accumulation (IC_{50} for rolipram inhibition: 0.8 μM), whilst a greater inhibitory effect of rolipram was observed in the presence of 100 μM Org 9935 (86 ± 0.7 %). A similar maximal inhibitory effect was elicited by 10 μM isoprenaline (84 ± 5.7 %). Neither rolipram alone, nor rolipram + Org 9935 significantly inhibited the [^{3}H]InsP$_x$ accumulation elicited by a maximally effective concentration of carbachol. However, at lower carbachol concentrations (1-10 μM) rolipram significantly inhibited the [^{3}H]InsP$_x$ response and this inhibition was greater in the presence of Org 9935. Thus the [^{3}H]InsP$_x$ response elicited by 10 μM carbachol was inhibited by 54 ± 4.0 % in the presence of rolipram + ORG 9935. In agreement with previous studies (Offer et al., 1991) 10 μM isoprenaline was ineffective at all concentrations of carbachol (1-100 μM) studied.

These data suggest that co-addition of a PDE III inhibitor can increase the effect of PDE IV inhibition upon histamine- and (sub-maximal) carbachol-stimulated [^{3}H]InsP$_x$ accumulation in this BTSM.

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166P AN EPITHELIUM-DERIVED CYTOSOLIC INHIBITOR OF cAMP-DEPENDENT PROTEIN KINASE ACTIVITY

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Normal human airway epithelial cell chloride (Cl) channels are activated by cAMP-dependent protein kinase (PKA), whereas in epithelial cells of cystic fibrosis (CF) patients Cl channel regulation by PKA is defective (Li et al.,1988). Epithelial cell Cl secretion may also be regulated by membrane-derived arachidonic acid (AA) since it has recently been shown that unsaturated fatty acids such as AA directly inhibit apical membrane Cl channel activity (Anderson et al., 1990) while AA metabolites do not (Hwang et al., 1990). Furthermore recent reports have also shown that hormonally regulated Cl channels in epithelial cells are blocked by a cytosolic inhibitor isolated from human placenta, whose physical properties may be similar to those of unsaturated fatty acids (Kunzelmann et al., 1991). The aim of this study was to characterise the effect of placental cytosol on PKA activity in brush border membrane vesicles (BBMV) prepared from human placental epithelium.

Human term placenta was used as a source of BBMV which were isolated using a method of differential centrifugation and magnesium precipitation. Cytosol was isolated as the supernatant obtained following centrifugation of processed placental homogenate (86,000xg for 35 min). PKA activity was measured using an assay based on the transfer of the γ-phosphate of [γ -^{32}P] ATP to the synthetic substrate kemptide.

Basal activity was stimulated 4 .1 ± 0.01 fold by 1μM cAMP (mean ± s.e.mean n=4). This activity was abolished by placental cytosol and the inhibition was absent from heat treated cytosol (20 min @ 70°-100°C). In separate experiments stepwise dilution of cytosol reduced its inhibitory potency. Fractionation of cytosol was carried out by gel filtration (Sephadex G-10) and the molecular weight of the putative inhibitor estimated to be approx. 48 kDa by SDS-PAGE (15% discontinuous gradient) of fractions containing inhibitory activity. In summary we conclude from this study that human placental cytosol contains an endogenous inhibitor of cAMP-dependent protein kinase activity which appears to be a heat- labile species of molecular weight 48 kDa. Whether or not this inhibitor incorporates bound arachidonic acid remains to be determined. Increased activity of such an inhibitor may be of relevance in CF where failure of PKA to phosphorylate regulatory domains of the protein altered in CF contributes to the blockade of Cl channel function seen in CF epithelia.

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167P MODULATION OF ANF RECEPTOR-EFFECTOR COUPLING IN ENDOTHELIAL CELLS BY PROTEIN KINASE C

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Protein Kinase C (PKC) has been reported to negatively regulate cGMP-mediated atrial natriuretic factor (ANF) signal transduction (Jaiswal et al, 1988). In this study, we have illustrated the presence and distribution of PKC activity in bovine pulmonary artery endothelial cells (BPAEC) and examined the effects of PKC activation on ANF receptor function.

Membrane and cytosolic fractions of BPAEC were prepared and following partial purification by DE-52 cellulose ion-exchange chromatography of both fractions, PKC activity was measured as the transfer of phosphate from γ [^{32}P]-ATP to the exogenous substrate histone III-S in the presence and absence of necessary cofactors. Cellular cGMP was extracted with 0.1M HCl and was quantitated after acetylation, by radioimmunoassay. Specific binding of 0.3nM [^{125}I]-rANF$_{(99\text{-}126)}$ was measured in intact cells and was displaceable by 1μM rANF$_{(99\text{-}126)}$. Results are expressed as mean values ± s.e.m. of n observations.

Stimulated PKC activities in cytosolic and membrane fractions were respectively 3.69 ± 0.7 and 0.27 ± 0.09 pmol phosphate transferred min/mg protein (n=6) illustrating that the majority of enzyme activity (94.9 ± 2.66%, n=6) is found in the cytosolic fraction. Partial purification of the enzyme increased the activity in both fractions by over 80%. Pretreatment of intact cells with 0.1μM PDBu (phorbol 12,13-dibutyrate) for 1 hr caused a reduction of ANF-stimulated cGMP accumulation which was maximal at 10^{-6}M rANF$_{(99\text{-}126)}$ causing an inhibition of 60.6 ± 14.6% (n=3). Investigation of the time course of this inhibition indicated that it was greatest following a 30 min pretreatment with 1μM PDBu. 0.1μM rANF$_{(99\text{-}126)}$ caused a down-regulation of [^{125}I]-rANF$_{(99\text{-}126)}$ binding of 23.2 ± 9.7% (n=6) within 15 min, which returned to normal within 5 hr. Down-regulation of binding was unaffected by 1μM PDBu up to 30 min, but after 24 hr binding was decreased by 70 ± 5.61% (n=6).

These results suggest that activation of PKC in BPAEC negatively regulates the ANF receptor coupled guanylyl cyclase possibly through the phosphorylation of a key regulatory protein of the ANF/cGMP pathway.

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168P THE EFFECT OF ISOPRENALINE, ADMINISTERED AS AN AEROSOL OR AS A DRY POWDER, ON METHACHOLINE-INDUCED BRONCHOCONSTRICTION IN THE ANAESTHETIZED PIG

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The development of large animal models which have similar pulmonary characteristics to humans and to which bronchodilators can be administered directly to the airways is of great interest. The present study describes the bronchoconstrictor effects of methacholine (MCh) in the anaesthetized pig, and compares the effect of isoprenaline, administered as a nebulized aerosol or as a dry powder, on the methacholine-induced responses.

Male, English White pigs (6-10 weeks old) were anaesthetized (ketamine, 20 mg/kg i.m. / sodium pentobarbitone, 12 mg/kg i.v.), intubated and ventilated (20 breaths/min, 10 ml/kg). Pulmonary resistance (R_{pulm}) dynamic lung compliance (C_{dyn}) and mean arterial pressure (M.A.P.) were recorded. Mean pretreatment measurements of R_{pulm} and C_{dyn} were 8.78 ± 2.07 cm H_2O/ l/sec. and 12.67 ± 2.31 ml/cm H_2O respectively. Single doses of methacholine (4-32 µg /kg i.v.) produced dose-dependent increases in R_{pulm} and decreases in C_{dyn}. Changes in lung function were maximum within 1min and returned back to baseline levels within 6 min. Methacholine produced a maximum 561 ± 72 % increase in R_{pulm} and a maximum 69 ± 1 % decrease in C_{dyn} from basal levels (n=4). The pD_{200} (dose to produce 200% increase in R_{pulm}) for methacholine was 13 ± 2 µg/kg.

Isoprenaline administered into the airways as a dry powder co-formulated on lactose (total: 50 mg lactose) or as a nebulized aerosol, 1 min. prior to dosing with methacholine (pD_{200}) produced similar inhibition of the methacholine-induced changes in R_{pulm} and C_{dyn} with a similar duration of action as shown in the table. Lactose alone or nebulizer drug vehicle had no effect on the methacholine airway responses.

TREATMENT		% inhibition MCh-induced responses		
		R_{pulm}	C_{dyn}	Duration (min)
	n			
isoprenaline (7.5 µg/kg, dry powder)	3	60 ± 5	60 ± 1	22 ± 5
isoprenaline (7.5 µg/kg, aerosol)	4	66 ± 10	48 ± 7	20 ± 4

These results demonstrate the bronchoconstrictor properties of methacholine in this large animal model and indicate dry powder formulation and nebulized aerosol are suitable methods for the administration of bronchodilators.

169P ACTION OF HISTAMINE ON NASAL CIRCULATION AND AIRWAY RESISTANCE IN DOGS

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In allergic nasal disorders, histamine is released locally from mast cells in the nasal mucosa resulting in nasal congestion. In dogs, nasal airway resistance is controlled by two venous systems which respond differently to pharmacologically agents (Wang & Lung, 1990; Lung & Wang, 1990). This study was undertaken to investigate the site(s) of action of histamine on the nasal circulation as to unveil the mechanism of its effect on the nasal airway. In sodium pentobarbitone anaesthetized dogs with constant-flow vascular perfusion of nasal mucosa on both sides, nasal airway resistance (Rna), vascular resistance (Rnv), dorsal nasal venous outflow (Qdv) and sphenopalatine venous outflow (Qsv) were measured as described (Lung & Wang, 1989). Histamine dihydrochloride was given intra-arterially at the rate of 0.1 ml min^{-1}.

Table 1

Doses of drug ($ug\ kg^{-1}min^{-1}$)	Rna (% Δ)	Rnv (% Δ)	Qdv (% Δ)	Qsv (% Δ)
0.0005	0	-9±3.3*	0	0
0.005	+3±0.6*	-18±4.2*	-4±1.4*	+4±1.3*
0.05	+18±4.7*	-35±4.0*	-7±2.3*	+17±4.9*
0.5	+60±20.8*	-45±6.5*	-10±2.9*	+33±5.8*

Results are given means±s.e. means of percentage change from controls. *P<0.05. Number of animals in each group is 5.

Histamine increased Rna but decreased Rnv; Qdv was decreased whereas Qsv was increased. The drug dilates both resistance and capacitance vessels and its action on the sphenopalatine venous system is more prominent than on the dorsal nasal venous system. Thus, the histamine-induced increase in nasal airway resistance involves not only dilatation of nasal blood vessels but also a shifting of blood flow from the anterior (dorsal nasal) to the posterior (sphenopalatine) venous system; also a part played by nasal secretion cannot be excluded.

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170P INVOLVEMENT OF SENSORY NERVES IN 15-HPETE-INDUCED AIRWAYS HYPERRESPONSIVENESS IN NORMAL RABBITS

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It has been suggested that airways hyperresponsiveness (AHR) may be induced by similar mechanisms to those which provoke hyperalgesia in peripheral tissues (Adcock and Garland, 1992). Hyperalgesia induced by 15-HPETE is dependent on sensory nerve activation as it is attenuated by BW443C (H.Tyr.D-Arg.Gly.Phe(4-NO_2) Pro.NH_2), a peripherally acting μ-opioid receptor agonist (Follenfant *et al*, 1990). The present study has investigated the ability of 15-HPETE to induce AHR in the rabbit and has evaluated the effect of BW443C on this response.

Male NZW rabbits (2-2.9kg) were anaesthetised (ketamine hydrochloride 35mg/kg i.m. and xylazine 5mg/kg i.m.), intubated and prepared for lung function (Spina *et al*, 1991). Measurements of airways resistance (RL) and compliance (Cdyn) were made following the administration of increasing concentrations of histamine (1.25-160mg/ml). The concentration of histamine to cause a 35% fall in Cdyn (PC_{35}) and a 50% increase in RL (PC_{50}) were calculated. The next day these rabbits were re-anaesthetized, intubated and a polyethylene catheter was inserted into the lungs via the endotracheal tube. One concentration of 15-HPETE (0.1, 0.5, 1.0, 5.0 µg/kg) or vehicle (10% ethanol and 90% saline) were rapidly administered into the lungs via the polyethylene catheter. The following day rabbits were prepared as per day 1 and the new PC_{35} and PC_{50} values were obtained. In some studies rabbits were pretreated with BW443C (1mg/kg or 10mg/kg i.v.) 10 min prior to 15-HPETE administration. Results are expressed as fold differences (FD) between responsiveness to histamine 24h post- compared to 24h pre-15-HPETE exposure, significance was assessed by a 2 way analysis of variance (ANOVA) and a value of $p<0.05$ was considered significant.

The responsiveness to histamine was unchanged 24h after instillation of vehicle (*Cdyn* FD=0.98±0.15; *RL* FD=1.31±0.37; n=6). There was also no difference in responsiveness for 0.1 (*Cdyn* FD=1.45±1.01; *RL* FD=1.5±1.12; n=6) and 0.5µg/kg 15-HPETE (*Cdyn* FD=1.7±0.6; *RL* FD=1.65±1.05; n=6). There was a marked increase in responsiveness to histamine 24h after 1.0µg/kg (*Cdyn* FD=5.77±1.84; *RL* FD=5.45±1.91; n=6) and 5.0µg/kg 15-HPETE increased responsiveness to histamine but only on compliance (*Cdyn* FD=3.89±1.48 $p<0.05$; *RL* FD=2.4±1.32; n=5). The AHR induced by 1.0µg/kg 15-HPETE was still present at 72h post challenge but reponsiveness to histamine had returned to normal by one week post challenge. BW443C at the doses used failed to alter the AHR to histamine induced by 15-HPETE. These results suggest that the AHR induced by 15-HPETE may not be due to stimulation of peripheral sensory nerves.

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171P THE EFFECT OF HEPARIN ON PAF-INDUCED AIRWAY HYPERRESPONSIVENESS AND CELLULAR INFILTRATION IN NEONATALLY IMMUNISED RABBITS

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Heparin is well known as a polyanionic anticoagulant contained in mast cell granules. However, accumulating evidence suggests that heparinoids may also act as anti-inflammatory agents (Sasaki *et al.*, 1991; Ahmed *et al.*, 1992; Seeds *et al.*, 1992). We have investigated the effect of an unfractionated commercial heparin preparation (heparin) and a low-molecular weight heparin ORG 10172 (ORG) on PAF-induced airway hyperresponsiveness and pulmonary cell infiltration in immunised rabbits.

NZW rabbits were immunised neonatally i.p. with *Alternaria tenuis* in Al(OH)3 gel as previously described (Coyle *et al.*, 1990). Airway responsiveness to inhaled histamine was assessed 24 h prior to, and 24 h and 72 h following the administration of PAF (80 μgml^{-1} aerosol for 1 h). Heparin (100 $IUkg^{-1}$, n=6), ORG (100 μgkg^{-1}, n=6) or control saline (0.9%, n=7) was administered intravenously 30 min prior to the PAF challenge. The concentration of histamine required to elicit a 50% increase in airways resistance (RL) (PC_{50}) and a 35% drop in dynamic compliance (C_{dyn}) (PC_{35}) was calculated. Bronchoalveolar lavage (BAL) was performed immediately following the lung function measurements.

Airway responsiveness to inhaled histamine was significantly increased 24 h following PAF challenge in control animals compared with pre-PAF values (RL PC_{50}: 12.1 ± 2.0 $mgml^{-1}$ vs 4.58 ± 0.66 $mgml^{-1}$, $p<0.05$; C_{dyn} PC_{35}: 8.74 ± 1.58 $mgml^{-1}$ vs 3.43 ± 0.8 $mgml^{-1}$, $p<0.05$). Both heparin and ORG significantly inhibited the PAF-induced airway hyperresponsiveness ($p<0.05$). The total cell numbers recovered in BAL were significantly increased 24 h and 72 h following PAF exposure ($p<0.01$), as were the total numbers of eosinophils and neutrophils ($p<0.01$). Heparin and ORG significantly inhibted the PAF-induced influx of eosinophils ($p<0.01$) and neutrophils ($p<0.05$).

These results indicate that unfractionated heparin and low-molecular weight heparin have anti-inflammatory actions in the airways of immunised rabbits.

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172P PAF-INDUCED BRONCHIAL HYPERRESPONSIVENESS IN ANAESTHETIZED GUINEA-PIGS IS DEPENDENT UPON CAPSAICIN-SENSITIVE MECHANISM(S)

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In anaesthetized guinea-pigs, a slow infusion of PAF (600 ng kg^{-1} h^{-1}), but not the carrier molecule bovine serum albumine (BSA 0.25%), induced an increase in bronchial responsiveness to histamine (1 µg kg^{-1} i.v.; increase in pulmonary insufflation pressure before PAF : 4.3 + 0.9 mmHg and after PAF : 11.3 ± 1.5 mmHg, n=5, $p < 0.01$). A complete inhibition of PAF-induced bronchial hyperresponsiveness was obtained in guinea-pigs pretreated with capsaicin (55 mg kg^{-1} s.c. over two days) seven days before the experiments (increase in PIP being 1.33 ± 0.5 mmHg and 1.3 + 0.3 mmHg in control and capsaicin pretreated guinea-pigs, respectively) and in guinea-pigs pretreated with the proposed blocker of capsaicin-operated cationic neural channel Ruthenium Red (Dray, et al., 1990; RR 5 mg kg^{-1} s.c. 1 hour before administration of PAF), the increase in PIP being 14.9 ± 4.5 mmHg and 0.92 ± 0.6 mmHg (n=6, $p < 0.01$) in PAF and RR + PAF treated animals, respectively.

Furthermore, when guinea-pigs were pretreated with the neutral endopeptidase inhibitor thiorphan (1.2 mg kg^{-1} slowly infused i.v. over 10 min), previously shown to enhance the efferent action of capsaicin-sensitive sensory nerves (Maggi et al., 1990) a dose-dependent and significant ($p < 0.01$) enhancement in PAF-induced increase in basal insufflation pressure was observed.

In conclusion, these data suggest that in the anaesthetized guinea-pigs, the functional integrity of capsaicin-sensitive structures is mandatory for PAF-induced bronchial hyperresponsiveness to histamine.

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173P MECHANICAL AND BIOCHEMICAL RESPONSES TO ENDOTHELIN-1 AND ENDOTHELIN-3 IN BOVINE BRONCHIAL SMOOTH MUSCLE

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Endothelin-1 (Et-1) is a potent vasoactive peptide produced from endothelial cells (Yanigasawa *et al.*, 1988), however other cells, including tracheal epithelial cells (Black *et al.*, 1989) also produce this peptide. To assess the importance of endothelins in airway disease, we examined some mechanical and biochemical aspects of the responses to Et-1 and its isomer Et-3 in bovine bronchial smooth muscle. Contractions of rings of bronchi in response to Et-1 and Et-3 were measured isometrically in 10ml organ baths at 37°C under 2g tension in oxygenated Krebs-Henseleit solution. Phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis in response to Et-1 and Et-3 was assessed in small pieces of tissue by measurement of inositol (1,4,5) trisphosphate (IP_3) levels using a specific binding assay (Palmer & Wakelam 1990). Cumulative concentration-response curves (CRCs) were constructed to Et-1. Initiation of contraction occurred at between 10^{-8} and $3x10^{-8}$M and the CRC showed concentration-dependency. Due to financial restraints it was not possible to obtain a maximum response to this compound. Contractions to Et-1 were not altered by atropine (10^{-5}M), potassium chloride (KCl, 30mM), methacholine (MCh, 10^{-6}M) or U46619 (10^{-7}M) or by removal of the epithelium (n=6 in each case). Et-3 initiated a small contraction (0.25-0.5 g wt) at 10^{-10}M which was "all or nothing"; concentrations up to $3x10^{-7}$M failed to evoke a larger response (n=6). Et-1 ($3x10^{-7}$M) evoked a rapid rise in IP_3 levels, maximal at 10s (mean max. increase 306%, $p<0.001$, n=10), returning to baseline by 20s and followed by a second rise, maximal at 60s (mean max. increase 224%, $p<0.001$). This response was unaffected by atropine (10^{-5}M). Et-3 (10^{-10}-10^{-6}M) did not alter levels of IP_3 (n=8).

The potency of Et-1 in bovine bronchial smooth muscle was lower than that observed in vascular smooth muscle (eg Rabbit pulmonary artery, MacLean & McGrath, 1991). This low potency does not reflect a lack of tone in this tissue *in vitro*, since pre-contraction with other spasmogens (KCl, MCh, U46619) failed to alter the CRC to Et-1. Et-1 does not act by release of agents from the epithelium since the response to Et-1 was unaffected by its removal. Et-1 does not act via Et-3 type receptors in this tissue, since the response to Et-3 is very different, both in the contraction and in IP_3 production. Although the pattern of the changes in IP_3 levels evoked by Et-1 resembles that observed for MCh in this tissue (Nally *et al.*, 1992), Et-1 does not act by release of acetylcholine, since pre-incubation with atropine altered neither the contractile nor the IP_3 response to this peptide.The time course for production of IP_3 in response to Et-1 is in accordance with a receptor-operated mechanism initiating contraction, with the early rise in levels of IP_3 preceding the contraction (the second rise was approximately coincidental with the mechanical response). Factors other than those discussed here, such as receptor number may explain the low potency of Et-1 in bovine bronchial smooth muscle.

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174P EFFECTS OF BRL 55834 ON NEURALLY-MEDIATED BRONCHOCONSTRICTION IN THE GUINEA-PIG

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BRL 55834 is a potent potassium channel activator (PCA) which has been shown to be relatively airways selective both *in vivo* (Bowring et al, 1992) and *in vitro* (Taylor et al, 1992) compared to BRL 38227. We have now investigated the effects of BRL 55834 on bronchoconstriction evoked by electrical field stimulation (EFS) in guinea-pig isolated bronchus (GPIB) and by vagal nerve stimulation in the anaesthetised freely respiring guinea-pig.

GPIB were mounted for the recording of isometric tension changes and EFS (0.5ms, 70V pulses at 10Hz, for 10s; Good et al., 1992). BRL 55834 (0.03-1.0μM) inhibited non-adrenergic, non-cholinergic excitatory (NANCe) and cholinergic responses to EFS in a concentration-dependent manner (pIC_{50} vs NANCe responses 6.50 ± 0.08, n=5; pIC_{30} vs cholinergic responses 6.61 ± 0.16, n=5). However, EFS-evoked responses were not completely abolished. BRL 55834 (1μM) had no significant effect on responses to substance P (SP; 1μM) or neurokinin A (NKA; 0.07μM), challenges which were equi-effective with NANCe nerve stimulation, but significantly reduced responses to acetylcholine (6μM), a concentration which was equi-effective with cholinergic nerve stimulation.

Changes in specific airways resistance (R_{aw}) and in dynamic compliance (C_{dyn}) were measured in urethane-anaesthetised guinea-pigs. NANCe nerve-mediated bronchoconstriction was elicited by distal stimulation of both vagi which were sectioned centrally (0.5ms, 5V pulses at 3-7Hz, for 30s), following pretreatment with atropine and propranolol (1mg/kg. i.v.). BRL 55834 (1, 2.5, 5 & 12.5μg/kg. i.v.) produced a dose-dependent inhibition of NANCe nerve-mediated increases in R_{aw}, but had no effect on concomitant decreases in C_{dyn}. Onset of inhibition occurred within 2 minutes, was maximal by 20 minutes post-dose and persisted for at least 60 minutes post-dose. By contrast, mean arterial pressure was only reduced by BRL 55834 at a dose of 12.5μg/kg. i.v. SP (10-20μg/kg. i.v.) and NKA (0.7-1.5μg/kg. i.v.) produced similar increases in R_{aw} to vagal stimulation. BRL 55834 (12.5μg/kg. i.v.) did not significantly reduce responses to these doses of SP and NKA.

As judged by its lack of effect on responses to exogenously-applied tachykinins, the inhibitory effects of BRL 55834 on NANCe nerve-mediated responses may represent a prejunctional site of action. *In vivo*, this is observed at sub-hypotensive doses, confirming the relative airways selectivity of BRL 55834 compared to other PCAs such as cromakalim (Ichinose & Barnes, 1990). These data suggest that since substance P in particular may be pro-inflammatory, in addition to providing direct bronchodilation, BRL 55834 may exert an anti-inflammatory effect in the airways.

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175P EFFECT OF POTASSIUM CHANNEL OPENERS ON GASTROINTESTINAL TRANSIT IN THE MOUSE

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In previous studies (Davies et al., 1991) we examined the relaxant effect of potassium channel opening agents (KCOs) on rat ileum contracted with KCl. Pinacidil (PIN) and BRL 38227 (BRL) caused complete relaxation, whereas minoxidil sulphate (Mx) and diazoxide (DZ) had small or negligible effects on contractions of the ileum, despite their relaxant activity in other tissues (Edwards and Weston, 1990). The present study investigated the effects of these same KCOs on gastrointestinal transit in vivo using the mouse charcoal meal test first described by Janssen and Jageneau (1957).

Following an overnight fast, male BKW mice (25-35g) were dosed orally with a KCO (1-10mg/kg), or carbachol (CARB) (1mg/kg) as a motility enhancer, or morphine (MOR) (1mg/kg) as a motility inhibitor, or appropriate vehicle (saline for CARB and MOR, ethanol for all KCOs except DZ, where DMF was used). Thirty minutes later the mice were dosed orally with 0.3ml of a suspension of 10% charcoal and 5% acacia gum and after a further 30 minutes the animals were killed by a blow to the head followed by cervical dislocation. The intestines, from pylorus to appendix, were removed and the distance which the charcoal front had moved down the small intestine was measured as was the length of the small intestine. Results were expressed as the distance moved by the charcoal front as a percentage of the whole length of the excised small intestine.

Table 1. The effects of KCOs and other agents on charcoal movement in mouse small intestine

	Mean % (sem) distance moved by charcoal (n≥8)								
Agent	CARB	MOR	saline	BRL	PIN	Mx	ethanol	DZ	DMF
1mg/kg	77.6(4.4)*	35.2(2.1)*	54.6(2.6)	27.6(1.1)*	52.8(2.6)	49.2(1.7)	52.3(3.3)	48.9(1.8)	50.0(1.4)
10mg/kg					25.6(2.2)*	30.3(1.6)*	49.0(3.0)	37.7(1.7)	40.8(2.6)

Significantly different from appropriate vehicle *p<0.001

Table 1 shows that the KCOs PIN, BRL and Mx inhibited gastrointestinal transit in the mouse, BRL being more effective than PIN and Mx. The effects of DZ were not significantly different from the effects of its vehicle. These findings are generally consistent with our previous results obtained in the rat ileum in vitro, although Mx appeared more effective in the present model

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176P EFFECT OF CHRONIC EXPOSURE OF CULTURED ENDOTHELIAL CELLS TO LOW DENSITY LIPOPROTEINS ON PHOSPHATIDYLINOSITOL METABOLISM

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Acute exposure of a number of cell types to low density lipoprotein (LDL) cholesterol species stimulates phospholipase C activity (Block et al 1988). However, in vivo cells may be exposed to high levels of cholesterol over long periods. We have therefore examined the effect of prolonged exposure of cultured cells to cholesterol species.

Endothelial and smooth muscle (VSM) cells were prepared from bovine and rabbit aorta respectively by enzymatic digestion. Cells were plated in 1cm diameter multiwells at a density of 10^5 cells/well and grown to confluence (24hr) in the presence of LDL, oxidised LDL (OXLDL) or vehicle. Growth medium was then replaced by M199 for 24 hr, the cells were maintained in M199 +[3H]inositol (0.5uCi ml) for a further 18hr before measuring basal and ATP stimulated [^{3}H] labelled inositol phosphates (IP) and phospholipids (PI). Stimulation, in the presence of LiCl (10mM), was for 30 minutes (Berridge et al 1982). Cells grown in the presence of vehicle were also stimulated acutely with LDL and OXLDL. LDL was prepared from human plasma by sequential ultracentrifugation. Oxidation of LDL was carried out in the presence of $CuCl_2$ (4uM).

Acute exposure (30 min) of endothelial cells to LDL and OXLDL increased IP levels (max 170 ± 50 and 280 ± 66% of basal respectively). In addition endothelial cells grown in the presence of LDL and OXLDL showed a reduced incorporation of [^{3}H] inositol into cell lipids, although neither basal nor agonist stimulated turnover of the pool of [^{3}H] labelled phospholipids was modified by chronic exposure to LDL or OXLDL.

Table: Effect of LDL and OXLDL on Phospholipase C Activity and Incorporation of [^{3}H]Inositol Into the Phosphatidylinositol Cycle

LDL/OXLDL ug/ml	OXLDL Phospholipids % Control	LDL	OXLDL Basal IP % Control	LDL	OXLDL Basal IP % PI	LDL	OXLDL ATP Stimulated IP % PI	LDL
0	100	100	100	100	5.2±0.8	7.6±1.9	17.1±1.4	21.5±2.1
5	86±10	-	133±18	-	8.3±1.4	-	15.9±0.8	-
10	66±12*	99±23	74±15*	104±26	6.7±1.1	8.1±1.8	16.3±2.0	23.1±2.5
20	20±4 *	55±16 *	23±5*	53±13*	7.7±1.1	8.5±2.6	14.4±1.7	21.9±2.1

mean ± SE (n = 5) * Significantly different to control ANOVA

Similar findings were obtained when VSM cells were incubated with OXLDL, 20 10 and 5ug/ml reducing basal IP to 47 ± 10, 71 ± 8 and 111 ± 25 % of control respectively.

Both LDL and OXLDL inhibited [^{3}H] inositol uptake into cells but the effect of OXLDL was consistently greater. Although EDTA 0.1mM was present in medium containing LDL OXLDL would be formed during incubation in the presence of cells and OX LDL may be the main species involved. The reduced uptake of [^{3}H]inositol by the cells may be related to incorporation of OXLDL into cell membranes and reduced membrane fluidity.

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177P WHICH FREE RADICAL/REACTIVE OXYGEN SPECIES IS IMPORTANT IN DAMAGING ENDOTHELIAL FUNCTION?

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Free radicals (FR) and reactive oxygen species (ROS) have been implicated as one of the major factors involved in ischaemia reperfusion injury (Halliwell. 1989). The aim of this study was to identify the primary damaging species involved. Xanthine oxidase (XO) (20mU/ml), hypoxanthine (HX) (250μM), $FeCl_3$ (25μM) and EDTA (37.5μM) were used to generate $O_2^{\cdot}$, H_2O_2, and $OH^{\cdot}$. New Zealand White rabbit aortic rings (2mm) were suspended in an organ bath filled with Krebs bicarbonate buffer at 37°C, aerated with 95% O_2 and 5% CO_2. Rings were constricted with the ED_{50} dose of phenylepherine (PE) and control cumulative concentration response curves to carbachol (C) (10^{-8} - 10^{-5}M) or sodium nitroprusside (SNP) (10^{-8} - 10^{-5}M) were obtained. Rings were then exposed to mercaptopropionyl glycine (MPG), captopril, superoxide dismutase (SOD), catalase, mannitol or vehicle for 5 mins before the addition of either the XO/HX system, H_2O_2, or vehicle for a further 30 mins. Cumulative concentration curves to C or SNP were then repeated. Responses to C and SNP are expressed as a percentage of the PE induced contraction. Due to interexperimental variation, experiments were conducted in such a way that XO/HX data was obtained in parallel with data for XO/HX plus another agent. Values are given as mean ± SD. Comparisons were made using ANOVA ($p < 0.05$).

Exposure to vehicle (30 mins) resulted in a small, significant decrease in maximum response to C (93 ± 10% before vehicle, 83 ± 11% after vehicle, n=9). In all further analysis allowance for this effect was made. Exposure to the XO/HX system resulted in impaired relaxation (83 ± 11% control, 49 ± 20% in the presence of XO/HX, n=49). SH-containing compounds captopril (300μM) and MPG (300μM), which are known to scavenge FR's and the specific $OH^{\cdot}$ scavenger mannitol (20mM), had no protective effect against the XO/HX system (n≥9). SOD (180U/ml) significantly increased the XO/HX induced damage (65 ± 16% in the presence of XO/HX alone, 43 ± 16% in the presence of SOD, n=6). Catalase (1000U/ml) significantly decreased the XO/HX induced damage, however the protection was not complete (28 ± 6% in the presence of XO/HX alone, 65 ± 13% in the presence of catalase, 83 ± 11% control, n=6). A combination of SOD and catalase was able to completely prevent any damage from occuring (27 ± 10% in the presence of XO/HX alone, 80 ± 10% in the presence of SOD and catalase, 83 ± 11% control, n=6). Direct addition of H_2O_2 lead to a similar impairment of relaxation (48 ± 16% in the presence of 500μM H_2O_2, n=18). The effect of a 1 hour incubation was also examined to allow effective distribution of MPG into the intracellular space, to allow for the possibilty of intracellular generation of $OH^{\cdot}$ from H_2O_2. No significantly protective effect was seen. Exposure to vehicle (30 mins) resulted in a small, significant decrease in the maximum response to SNP (107 ± 2% before vehicle, 100 ± 2% after, n=9). Exposure to the XO/HX system resulted in slight inhibition of relaxation at lower concentrations of SNP (10^{-8} - 10^{-6}M), the responses to $3x10^{-5}$M and 10^{-5}M were unaffected.

This data shows that scavenging either $OH^{\cdot}$ or $O_2^{\cdot}$ does not prevent impairment of the response to carbachol. SOD produces H_2O_2 from $O_2^{\cdot}$, this leads to an increase in damage, removing H_2O_2 with catalase decreases damage and H_2O_2 will mimic the effects of the XO/HX system. Thus the primary ROS responsible for causing damage is H_2O_2. The damage to the C induced relaxation is localised to the endothelium as the responses to SNP were not affected in a similar manner.

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178P INHIBITION OF HUMAN LDL CHOLESTEROL OXIDATION

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There is growing evidence that oxidation of low density lipoprotein (LDL) cholesterol is a prerequisite for macrophage uptake and initiation of atherogenesis. The effects of captopril and N-acetylcysteine (NAC) on LDL cholesterol oxidation have been examined.

Blood was collected from healthy female volunteers. LDL was isolated by sequential ultracentrifugation (density 1.019 - 1.063g/cm^3) and was then dialysed for 44-48 hours. Oxidation was initiated with 4uM$CuCl_2$. Following a lag phase there was a rapid increase in diene conjugate formation which was measured spectrophotometrically at 234nm. The duration of the lag phase and the half time (time required for the optical density to reach half of its maximum value) were used as standard measures of resistance to oxidation (Esterbauer et al 1989, Plane et al 1992).

The effects of captopril and of NAC on LDL oxidation were tested. Paired control and intervention experiments were performed (n=6). Friedman two way analysis of variance by ranks was used to compare the effects of captopril and of NAC to control. Results are presented as median (range).

	LAG PHASE (Minutes)	HALF TIME (Minutes)
Control	109 (65 - 157)	123 (76 - 176)
Captopril 1uM	119 (74 - 175)	131 (86 - 190)
Captopril 10uM	209 (168 - 305)	229 (186 - 321) *
Control	98 (83 - 137)	116 (98 - 148)
NAC 1uM	107 (94 - 142)	125 (111 - 151)
NAC 10uM	226 (102 - 395)	244 (116 - 410) *

* Significant at p = 0.01

These results show that oxidation of human LDL is significantly retarded by 10uM captopril and by 10uM NAC. This concentration of captopril is comparable to concentrations achieved therapeutically (Duchin et al 1988). Qualitatively similar effects were seen with 1uM captopril and with 1uM NAC but these failed to reach statistical significance. These findings suggest that the ability of captopril to retard LDL oxidation is due to the presence of a sulphydryl group in its molecular structure (NAC is a sulphydryl-containing compound without ACE inhibiting activity).

The antihypertensive captopril is, therefore, not only 'lipid-neutral' but may also inhibit LDL oxidation. These properties may be relevant to the prevention of hypertension-related atherosclerosis.

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179P BOVINE AORTIC ENDOTHELIAL CELLS IN CULTURE CAN OXIDATIVELY MODIFY LOW-DENSITY LIPOPROTEINS

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Low-density lipoprotein (LDL) has been reported to be oxidatively modified by endothelial cells (EC) derived from rabbit aorta (Henriksen *et al.*, 1983), human arteries, veins or microvessels (van Hinsbergh *et al.*, 1986), and pig aorta and right ventricle (Smith *et al.*, 1992). In contrast, bovine aortic EC have been reported to be unable to oxidatively modify LDL (Henriksen *et al.*, 1983; Morel *et al.*, 1984). Oxidative modification of LDL results in a faster rate of uptake of LDL by macrophages. In the present study we have reexamined the ability of bovine aortic EC in culture to oxidise LDL and enhance its uptake by macrophages.

Normal human LDL was isolated by sequential density ultracentrifugation and radiolabelled with sodium [^{125}iodide], as described by Leake and Rankin (1990). Incubation of the LDL with primary cultures of bovine aortic EC for 24 hours produced inconsistent results, but incubation for 48 hours resulted in a significant ($p<0.01$) 4.6-fold increase in degradation of the LDL by mouse peritoneal macrophages, from 0.87±0.24 (n=3) to 4.01±0.41 (n=3) μg LDL protein mg^{-1} cell protein in 20 h. The increased degradation of LDL by macrophages was completely inhibited by including the antioxidant probucol (5 μM) during incubation of the LDL with the EC indicating that the modification induced by the EC is oxidative. Similar results were observed with subcultures of bovine aortic endothelial cells up to an including the third passage.

These results show for the first time that bovine aortic endothelial cells can oxidatively modify human LDL, but require a longer exposure time than other endothelial cells.

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180P EFFICACY OF IDAZOXAN AT α_1-ADRENOCEPTORS IN RAT AORTA AND SMALL MESENTERIC ARTERY

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Idazoxan is classified as an α_2-adrenoceptor antagonist with a pA_2 of 8.6 and an α_2/α_1 selectivity ratio of ~300 (Chapleo *et al.*, 1981). In rat, idazoxan raises blood pressure (Paciorek & Shepperson, 1983). This has been attributed to partial agonist activity at α_1-adrenoceptors presumably on peripheral resistance vessels. In this study, the effects of idazoxan on resistance arteries have been investigated using a myograph and the results were compared with those obtained on a conduit vessel, the rat aorta (37°C, Krebs-Henseleit buffer, 0.25mM Ca^{2+}).

Small (internal diameter 150-250μm) mesenteric resistance arteries from rats (250-300g) were mounted on a myograph (Mulvany & Halpern, 1977). Single, agonist concentration-effect (E/[A]) curves were obtained by cumulative dosing in the absence and presence of antagonists. The endothelium was removed and both cocaine 30μM and timolol 6μM were present in all experiments.

In the small mesenteric artery (s.m.a.), noradrenaline (NA) was 80-fold less potent than in the aorta ($p[A_{50}]$ 6.2±0.1 and 8.1±0.2, respectively.). Idazoxan (1μM-3mM) did not cause a contractile response in the s.m.a. although 3μM shifted the NA E/[A] curve to the right, without changing the upper asymptote or midpoint slope of the curve, and a pA_2 value of 6.1±0.2 was estimated, consistent with α_1- but not with α_2-adrenoceptor blockade (Chapleo *et al.*, 1981). In contrast, idazoxan caused a dose-dependent contraction of the aorta ($p[A_{50}]$ 6.8±0.1) with a maximum response of 59±2% of the NA maximum. The α_1-adrenoceptor antagonist, prazosin (1-100nM), produced rightward shift of the NA E/[A] curves in aorta, but caused steepening inconsistent with simple competitive antagonism. Without predjudice to mechanism, a pA_2 of 9.55±0.10 was estimated. Similarly, prazosin (3nM) produced rightward shift of the idazoxan E/[A] curve (pA_2=9.6±0.1) with associated steepening. These pA_2 values are consistent with blockade of α_1-adrenoceptors. Combined dose-ratio analysis (Shankley *et al.*, 1988) in aorta, using NA, confirmed that idazoxan and prazosin act syntopically in this tissue.

The loss of potency of NA in the s.m.a. was mirrored by the loss of intrinsic activity of idazoxan in this tissue. These results are consistent with expectations for reduced efficacy (τ) at α-adrenoceptors in the s.m.a. compared with aorta, as might be expected if receptor density was lower in the s.m.a. The increase in blood pressure in rat induced by idazoxan (Paciorek & Shepperson, 1983) may suggest an increase in receptor coupling efficiency *in-vivo* or that an action on the s.m.a. is not involved.

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181P FUNCTIONAL EVIDENCE FOR HETEROGENOUS DISTRIBUTION OF α-ADRENOCEPTOR SUBTYPES IN THE RAT VAS DEFERENS

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We have investigated the relative contributions of α-adrenoceptor subtypes to responses of the epididymal and prostatic thirds of the rat vas deferens to trains of electrical stimuli (ToS, 10Hz, Pulse width 0.8msec for 10 sec). The contribution of individual receptor subtypes to the responses have been determined using Suramin (P_{2x} purinoceptor), Chloroethylclonidine (CEC, α_{1B}-adrenoceptor), Prazosin and Nifedipine. The whole time course of the mechanical response to ToS was recorded using a computerised data collection system, which enabled mean responses from at least 6 tissues to be calculated and plotted.

The whole vas deferens responded to ToS with a biphasic contraction, comprising a rapidly rising 1st phase which peaked at around 0.6s, fell to a nadir (68.9 % ± 5.02 of peak) at 2s and then rose slowly until stimulation ceased (2nd phase). The prostatic third of the vas responded to Tos with a 1st phase component which peaked at 0.5s and then declined to about 21.8% ± 5.52 of the peak after 2s and sustained this level for the remainder of the stimulation. The epididymal third showed a peak response at 1.2s which diminished slightly after 2s but thereafter rose gradually till the end stimulation. (Brown *et al*, 1979).

Suramin (30mins, 1mM) abolished the 2nd phase of the prostatic response and reduced the 1st phase peak by 34.7% ± 3.4. This remaining component was abolished by CEC (10uM). Suramin reduced the 1st phase of the epididymal response by 32.6% ± 2.7, and left a slowly rising 2nd phase. CEC (10uM) then abolished the remaining 1st phase but had little effect on the residual 2nd phase, reducing it by 15.7% ± 2.1. Addition of Suramin (1mM) followed by Nifedipine (10uM) to the epididymal portion obliterated the 2nd phase leaving a residual 1st phase, which was abolished by CEC (10uM). In the prostatic portion, Nifedipine had no significant effect on the component of the response remaining after treatment with Suramin.

We have previously reported that the response of the whole vas deferens to ToS involves contributions from 3 receptors, namely P_{2X}, α_{1A} and α_{1B}(Mallard *et al*, 1992). From the work presented here, it would appear that whilst both portions of the vas deferens respond with an initial phase comprising both Suramin and CEC sensitive components (indicating the presence of P_{2X} and α_{1B} receptors), a Suramin and CEC resistant component is revealed only in the 2nd phase of the epididymal response. This phase is both Nifedipine and Prazosin sensitive, implying that it is mediated via an α_{1A}-adrenoeceptor. No such component is seen in the prostatic portion possibly reflecting a lack of a functional α_{1A} subtype. It would appear that both the α_{1B} and the P_{2X} receptors are present along the whole length of the rat vas but the α_{1A} subtype may only be found in the epididymal region.

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182P α_{1A}-ADRENOCEPTORS IN RAT VAS DEFERENS: THERE, BUT WHERE?

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Mallard *et al.*, (1992) have suggested that α_{1B}- and α_{1A}-adrenoceptors in the rat vas deferens may be localised synaptically and extrasynaptically respectively, the latter requiring increased noradrenaline (NA) overflow after nerve stimulation for activation. To test this hypothesis, we have attempted to induce an α_{1A}-mediated component in the response to single pulse electrical field stimulation (SPFS) by enhancing synaptic overflow of NA by using inhibitors of uptake-1 and uptake-2.

Stripped whole vasa from 350-470g male wistar rats were suspended in a Krebs-filled organ bath kept at 37°C. Following a 45 minute equilibration period and prior to data collection, the tissues were subjected to SPFS until tension responses were reproducible. A computer system controlled SPFS (via parallel platinum wire electrodes) and also collected the digitised tension data. SPFS were delivered at 5 minute intervals and were followed by bath washout and drug re-administration. Results are represented as the mean ± SEM, (n=6) and drug effect quantified by peak tensions of both phases of the biphasic SPFS response.

The uptake-1 inhibitors cocaine, fluoxetine, maprotiline or nortriptyline potentiated the second phase (α_{1B}-mediated) of the response to SPFS to a greater extent than the first phase (P_{2X}-purinoceptor-mediated). Single concentrations of each drug were selected following concentration-response and time-course experiments. The involvement of α_{1B}-adrenoceptors in the potentiated responses was assessed using 1μM chloroethylclonidine (CEC), a selective α_{1B}-adrenoceptor antagonist. CEC was significantly less effective in tissues pretreated with 1μM cocaine or 5μM fluoxetine (% inhibition of the second phase: 35.01 ± 3.99 and 42.31 ± 3.08, respectively compared with 60.92 ± 2.91), but the differences were small, and in all these experiments CEC effected some inhibition (unusual) of the first phase of the response to SPFS. To maximise the opportunity for released-NA to reach α_{1A}-adrenoceptors, an uptake-2 inhibitor was included in the protocol. Concentration-response experiments revealed that 17ß-oestradiol potentiated responses to SPFS to the greatest extent. Tissues were exposed to 500nM cocaine, 10μM oestradiol, 100nM yohimbine (to counter any presynaptic α_2-adrenoceptor-mediated inhibition of NA release) and 1mM suramin (to block the P_{2X}-purinoceptor-mediated first phase of the response). Following this, 5μM CEC was used to block α_{1B}-adrenoceptors and succeeded in almost abolishing the remaining (noradrenergic) response. Nifedipine (10μM) failed to further reduce the residual response.

This evidence provides no support for α_{1A}-adrenoceptors becoming involved in the response to SPFS in the presence of uptake-1 and uptake-2 inhibition, although it may be that insufficient transmitter is released in response to a single pulse to reach the α_{1A}-adrenoceptors even in the presence of both uptake inhibitors.

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183P INHIBITION OF ENHANCED CONTRACTIONS TO UK-14,304 BY YOHIMBINE, PRAZOSIN OR A VASOPRESSIN ANTAGONIST IN RAT TAIL ARTERY RINGS *IN VITRO*

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The existence of post-junctional alpha$_2$-adrenoceptors in isolated vascular smooth muscle can usually only be revealed ("uncovered") when the vessels have been pre-contracted by an agonist, whose action is mediated via a different type of receptor (MacLean & McGrath, 1990). One explanation of this phenomenon is that during the precontraction, not only does the intracellular free calcium concentration increase but cyclic nucleotide synthesis also increases, thereby providing an opportunity for activation of the alpha$_2$-adrenoceptors to inhibit either the synthesis or the inhibitory effects of the cyclic nucleotides and cause a contraction (Aidulis et al, 1992). Such a contraction, though produced by activation of alpha$_2$-adrenoceptors, could be regarded as a continuation of the initial contraction to the precontracting agonist. If this is so, enhanced responses to an alpha$_2$-adrenoceptor agonist, such as UK-14,304, would be antagonised not only by an alpha$_2$-adrenoceptor antagonist such as yohimbine but also by a specific antagonist of the precontracting agonist. This possibility was examined using phenylephrine (PE) and vasopressin (VP) as the precontracting agonists, and prazosin (PRAZ) and the VP antagonist ([β-mercapto-[β, [β -cyclopentamethylenepropionyl^1, O-Me-Tyr^2, Arg^8)-vasopressin (MCVP).

Segments (3-4 mm) of proximal tail artery from Male Wistar rats (150-200 g) were suspended between pairs of stainless steel hooks, inserted into the lumen, in Krebs buffer (37°C), gassed with 95% O_2/5% CO_2. The resting tension on each ring was set to 1 g. After 2 h equilibration, responses to drugs were recorded isometrically. Contractile responses to a standard submaximal concentration of UK-14,304 (50 nM) were examined in rings that had been precontracted with either PE (0.5 μM) or VP (0.4 miu ml^{-1}) and then relaxed with isobutylmethylxanthine (IBMX, 10 μM).

The standard response to UK-14,304 was enhanced in the presence of PE and IBMX by 1732 $\pm$ 541% (mean $\pm$ s.e. mean, 0.05>P>0.01, n=8) and in the presence of VP by 1135 $\pm$ 259% (0.05>P>0.01, n=9). These enhanced responses to UK-14,304 were antagonised either by yohimbine (50 nM) or by a specific antagonist (PRAZ 0.1 μM; MCVP 35 nM) of the precontracting agonist. These results suggest that the "uncovered" responses to UK-14,304 were not enhanced alpha$_2$-adrenoceptor-mediated responses but were continuations of the initial precontractions that had been interrupted by the IBMX. The enhanced response to UK-14,304 was certainly triggered by activation of the alpha$_2$-adrenoceptors but the principal mechanism underlying the contraction caused by UK-14,304 was the mechanism activated by the precontracting agonist.

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184P EFFECTS OF NIFEDIPINE ON RESPONSES MEDIATED BY α_1-ADRENOCEPTOR SUBTYPES

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α_1-adrenoceptors have been subdivided into α_{1A} and α_{1B} based on their affinity for WB4101. (Morrow and Creese, 1986). Han et al.(1987) reported that the calcium channel blocker nifedipine markedly reduces the α_{1A}-adrenoceptor mediated contractions of the rat vas deferens but does not affect the α_{1B}-adrenoceptor mediated contractions of the rat spleen. The present study examines the effects of nifedipine on the α_{1B}-adrenoceptor mediated responses of the guinea pig aorta and rat spleen and the α_{1A}-adrenoceptor mediated responses of the rat anococcygeus muscle, aorta, portal vein and vas deferens. (Mir and Fozard, 1988; Han et al., 1987).

Circular muscle from rat and guinea pig aorta, rat hemispleen and whole anococcygeus muscles, vas deferens and portal veins were set up in aerated Krebs solution at 37°C. Concentration-response curves were obtained to phenylephrine in the presence and absence of 1μM nifedipine. All responses were obtained in the presence of cocaine (3μM), propranolol (1μM) and corticosterone (10μM).

	CONTROL		NIFEDIPINE	
TISSUE	MAXIMUM (g)	EC_{50} (μm)	MAXIMUM (g)	EC_{50} (μm)
Rat Vas Deferens	1.50 $\pm$ 0.16	3.0 (1.9 - 4.7)	-----	-----
Rat Portal Vein	0.90 $\pm$ 0.10	2.3 (0.5 - 11)	-----	-----
Rat Aorta	0.69 $\pm$ 0.09	18.8 (4.4 - 80)	0.35 $\pm$ 0.08**	131 (44 - 388)
Rat Anococcygeus Muscle	6.42 $\pm$ 0.31	1.1 (0.9 - 1.3)	6.49 $\pm$ 0.38	0.8 (0.5 - 1.2)
Rat Spleen	0.44 $\pm$ 0.04	2.4 (1.6 - 3.8)	0.34 $\pm$ 0.03	2.7 (1.6 - 4.6)
Guinea Pig Aorta	0.32 $\pm$ 0.06	4.5 (3.2 - 6.3)	0.37 $\pm$ 0.05*	4.7 (3.1 - 7.3)

Table 1. Mean (n $\geq$ 4) maximum developed tensions ($\pm$ S.E.M.) and EC_{50} values (with 95% confidence limits) for phenylephrine in the absence and presence of nifedipine. (* $P<0.02$; ** $P<0.001$)

Nifedipine abolished responses in the rat vas deferens and portal vein. In the remaining tissues nifedipine had no significant effect on the phenylephrine EC_{50} values. However, the maximum response of the rat aorta was significantly reduced whilst the maximum response of the guinea pig aorta was significantly increased (Table 1). Nifedipine had no significant effect on the responses of the rat spleen or anococcygeus muscle.
The effects of nifedipine on α_1-adrenoceptor mediated responses are therefore variable and are not related to the α_1-adrenoceptor subtype mediating the response.

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185P REGULATION OF CARDIAC α- AND β-ADRENOCEPTORS BY THE SYMPATHETIC NERVOUS SYSTEM

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We have previously shown that chemical sympathectomy with reserpine (Chess-Williams et al.,1987a) or 6-hydroxydopamine (Chess-Williams et al.,1987b) results in a supersensitivity of cardiac ß-adrenoceptor mediated responses without affecting α-adrenoceptor mediated responses. In contrast, Yamada et al. (1980) have reported an increase in rat ventricular α-adrenoceptor sensitivity following 6-hydroxydopamine pretreatment. The present study examines cardiac responses after sympathetic function has been depressed by autoimmunising rats against mouse nerve growth factor (NGF).

Rats (250g) were injected with mouse NGF (7S, 20μg in complete Freund's adjuvant, s.c.) and administered a booster injection (50μg NGF) 4 weeks later. After a further 6 weeks, left atria from NGF and vehicle-treated animals were isolated and set up in aerated Krebs solution at 37°C and paced at 1Hz. Cumulative concentration-response curves to isoprenaline and phenylephrine (in the presence of 1μM propranolol) were obtained in the presence of cocaine (10μM) and corticosterone (10μM).

Serum anti-NGF antibody levels were determined using an enzyme labelled serum immunosorbent assay. Sera from all immunised animals yielded positive results down to a dilution of at least 1:12800; sera from control animals were negative at all concentrations.
Mean (± S.E.M.) ventricular noradrenaline levels (assayed by HPLC) were reduced from 1.03 ± 0.05 to 0.80 ± 0.03μg g^{-1} tissue by immunisation ($P \leq 0.002$).
Left atria from immunised rats were supersensitive to isoprenaline compared to controls, EC_{50} values being reduced ($P \leq 0.005$) from 41.9(17.2-101.9)nM (n=7) to 3.2(0.7-14.6)nM (n=7) whilst maximum increases in developed tensions were similar for atria from control (0.99 ± 0.06g) and NGF-pretreated rats (0.83 ± 0.09g). Inotropic responses to phenylephrine were not affected by immunisation. EC_{50} values and maximum increases in developed tension to phenylephrine were similar in tissues from control [2.9(1.8-4.8)μM; 0.70 ± 0.07g] and immunised animals [2.1(1.0-4.2)μM; 0.79 ± 0.06g].

These results add further support to the hypothesis that atrial ß- but not α-adrenoceptor sensitivity is regulated by the sympathetic nervous system.

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186P PERTUSSIS TOXIN INHIBITION OF α_2-ADRENERGIC ACTIVITY IN HUMAN ISOLATED RESISTANCE ARTERIES

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The presence of postjunctional α_2-adrenoceptors has been demonstrated in human subcutaneous resistance arteries using exogenously applied α-agonists and antagonists (Nielsen *et al*, 1990). How postjunctional α_2-adrenoceptors produce vasoconstriction is not fully understood. This study investigates the involvement of putative second messenger systems ie. G proteins, and phospholipase A_2, with postjunctional α_2-adrenoceptor activation by examining the effect of pertussis toxin (PTX), an inhibitor of the guanine-nucleotide binding regulatory proteins, including G_o and G_i (Boyer *et al*, 1983), and 5,8,11,14-eicosatetraynoic acid (ETYA), an arachidonic acid metabolite, on the α_2-adrenergic response. The effect of N-Nitro-L-arginine methyl ester (L-NAME), a nitric oxide (NO) synthetase inhibitor was also investigated.

Human subcutaneous resistance arteries (internal diameter 225μm-528μm) were mounted as ring segments in a myograph in physiological saline solution at 37°C, bubbled with 95% oxygen and 5% carbon dioxide as described by Mulvany & Halpern (1977). The vessels were then exposed to a high potassium depolarising solution (KDS, 118mM K^+) and 10μM noradrenaline (NA) to assess the vessel viability and the maximal adrenergic response. The arteries were then exposed to 10μM BHT933, an α_2-agonist, to determine the maximal α_2-adrenergic response, followed by 2 hours incubation with 1μg/ml pertussis toxin. This was then followed by 10μM BHT933, KDS and 10μM NA. In some arteries a dose response curve to BHT933 was achieved before and after incubation with pertussis toxin. Similar experiments were performed with L-NAME (10μM), incubated for 30 minutes, followed by a further incubation of 30 mins with ETYA (10μM).

	Control	PTX	Control	L-NAME	L-NAME+ETYA
KDS	100 (n=5)	101.9±1.7 (n=4)	100 (n=13)	120.4±5.3 (n=11)	n.d.
NA	115.8±8.2 (n=5)	106.7±9.0 (n=4)	106.9±8.3 (n=12)	106.1±5.4 (n=8)	n.d.
BHT	69.1±12.7 (n=5)	13.8±3.8** (n=5)	42.6±8.2 (n=13)	63.4±10.3* (n=13)	57.1±13.6 (n=9)

Results are expressed as mean %KDS ± s.e.m. *p<0.05 **p<0.01 according to students t-test analysis. The mean active tension produced by KDS = 2.7±1.1Nm^{-1}

We conclude that the response to the α_2-adrenoceptor agonist BHT933 is produced by interaction with a PTX sensitive G protein, which we suggest to be G_i protein. This is in agreement with cellular work where human α_2-adrenoceptors have been transfected in isolated cells indicating the involvement of multiple G_i proteins in responses to α_2-agonists (Gerhardt & Neubig, 1991). The activation of α_2-adrenoceptors does not appear to involve phospholipase A_2, however, as L-NAME potentiates the α_2-adrenergic response, this indicates an inhibitory role for NO.

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187P CYTOSOLIC HEPARIN INHIBITS NORADRENALINE-INDUCED Ca^{2+} RELEASE FROM INTRACELLULAR STORES IN RAT RESISTANCE ARTERIES

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Contractile agonists such as noradrenaline (NA) can induce Ca^{2+} release from intracellular stores in vascular smooth muscle. This release is thought to be mediated by generation of inositol trisphosphate (IP_3). Heparin has been reported to block IP_3 binding to its receptor thereby inhibiting IP_3-induced Ca^{2+} release (Worley *et al.*, 1987). Caffeine also releases Ca^{2+} intracellular stores, but by a mechanism that does not involve IP_3.

We used a reversible permeabilization technique (Morgan & Morgan, 1984) to introduce heparin (MW ~ 3000) into the cytosol of resistance arteries isolated from rat mesentery. The arteries (internal diameter~200μm) were mounted in a myograph (Mulvany & Halpern, 1977) containing physiological saline (PSS; 2.5mM Ca^{2+}). Vessels were contracted by exposure to depolarising PSS (KPSS; 118mM K^+ substituted for Na^+), NA(10μM) in PSS and Ca-free PSS, caffeine(10mM) in Ca-free PSS and Ca^{2+}(1nM-10mM) under depolarizing conditions (Ca-free KPSS) at 37°C. Subsequently arteries were incubated overnight at room temperature in permeabilizing solution containing heparin(3mg/ml) or no heparin (Control 1). Further controls were performed by exposing the arteries to heparin (3mg/ml) for the same period in the absence of permeabilization (Control 2). After rewarming to 37°C, and recovery from permeabilization, vessels were re-exposed to the same stimulants. Data are means ± s.e.means of n observations.

Table Response to stimulants before and after treatment.

	NA Ca-free PSS(%)		NA PSS(%)		Caffeine(%)		KPSS(N/m)		n
	before	after	before	after	before	after	before	after	
Hep+perm.	9±1	0±0*	92±4	98±4	19±4	17±4	8.2±0.6	7.5±0.3	4
Control 1	17±2	12±2	96±3	96±12	13±2	12±3	8.2±0.4	8.0±0.4	4
Control 2	15±4	14±2	99±7	103±7	10±2	10±4	8.4±0.4	7.7±0.5	4

*Responses are expressed as % response to KPSS before treatment. * indicates p<0.05 by Wilcoxon Signed Rank Test.*

None of the procedures inhibited responses to depolarization or calcium in Ca-free KPSS. When heparin was included in the permeabilizing medium the response to NA was abolished in Ca-free conditions although the responses to caffeine and NA in PSS were unaffected. Permeabilization alone (Control 1) or heparin, in the absence of permeabilization (Control 2), had no significant effect on any response.

These results indicate that a reversible permeabilization technique previously used to load tissues with aequorin can also be used to introduce other large molecules into isolated arteries. Introduction of heparin into rat resistance arteries appears to inhibit noradrenaline-induced release of Ca^{2+} from the intracellular store, probably by acting as an antagonist of IP_3. Intracellular heparin does not deplete the intracellular store sensitive to caffeine, or reduce sensitivity to Ca^{2+}. It is interesting that heparin had no apparent effect on responses to NA when Ca^{2+} was present in the extracellular medium suggesting that the role of the intracellular store is negligible under these conditions.

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188P OXIDISED LOW-DENSITY LIPOPROTEIN DOES NOT INHIBIT ENDOTHELIUM-DEPENDENT RELAXATION BY PROMOTING SUPEROXIDE ANION GENERATION

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The effluent from Krebs perfused aortae of atherosclerotic rabbits has a reduced ability to relax endothelium-denuded rings of pig coronary artery, despite an increased content of nitrogen oxides (Minor *et al.*, 1990), implying enhanced destruction of endothelium-derived relaxing factor (EDRF), perhaps by superoxide anions. Acute exposure of isolated vascular rings to oxidised low-density lipoprotein (LDL) inhibits endothelium-dependent relaxation (Plane *et al.*, 1992). We have investigated whether the acute inhibitory effects of oxidised LDL on EDRF activity in intact rings of rabbit aorta are due to enhanced superoxide anion generation.

Acetylcholine (ACh)-induced relaxation in rabbit aortic rings preconstricted with phenylephrine was inhibited by pretreatment for 30 min with oxidised human LDL (1 mg protein ml^{-1}), reducing the maximum relaxation from 58.8±3.82% (n=15) to 8.5±2.05% (n=6) (m±s.e.mean, $p<0.001$). Native LDL at the same concentration did not significantly alter ACh-induced relaxation. Superoxide dismutase (SOD, 50 u ml^{-1}) did not significantly effect ACh induced relaxation, and did not alter the inhibitory effects of oxidised LDL on ACh-induced relaxation.

Superoxide anion production by rabbit aortic rings (absorbance change (AC)=0.08±0.01, m±s.e.mean, n=8, assayed by SOD-inhibitable reduction of cytochrome C) was not significantly altered by treating the rings for 30 min with 1.5 mg ml^{-1} native (AC=0.065±0.01, n=8) or oxidised LDL (AC=0.097±0.02, n=8). However, superoxide anion production was significantly ($p<0.001$) increased 2.8-fold (0.08±0.01, n=6 to 0.225±0.01, n=6) by treatment with alloxan (0.5 M, 10 min). These results indicate that the acute inhibition of EDRF activity by oxidised LDL is not due to enhanced superoxide anion production.

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189P A COMPARISON OF THE EFFECTS OF VASORELAXANTS IN VASCULAR AND NON-VASCULAR SMOOTH MUSCLE FROM SPONTANEOUSLY HYPERTENSIVE RATS

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Vascular smooth muscle from spontaneously hypertensive rats (SHR) is more responsive to sodium nitroprusside (SNP) than that from Wistar Kyoto (WKY) controls (Sharifi et al, 1990). This study examined the sensitivity of aortic rings from SHR and WKY controls to the vasoconstrictor, phenylephrine (PE), and to the vasorelaxants, isoprenaline (ISO), carbachol (CAR) and SNP. The effects of these drugs on responses of the anococcygeus muscle from SHR and WKY were also examined.

Blood pressure (BP), measured by the tail cuff method, in conscious SHR (194 ± 4 mm Hg (mean ± s.e. mean), n=12) was significantly higher (P<0.001, Student's t-test) than the BP in Wistar (118 ± 3) and WKY rats (126 ± 3, n=12). Aortic rings (3-4 mm) from SHR were heavier (SHR 6.9 ± 0.3 mg, n=20, P<0.001) than those from WKY (4.8 ± 0.2, n=19) and Wistar rats (4.6 ± 0.2, n=32). Aortic rings were suspended under an initial resting tension of 2 g in 25 ml organ baths, containing Krebs buffer at 37°C. Cumulative concentration-response curves to PE (10^{-9}-$3x10^{-5}$ M) and in the presence of the EC_{75} for PE, to ISO (10^{-9}-10^{-5} M), CAR ($3x10^{-9}$-$3x10^{-5}$ M) and SNP ($3x10^{-11}$-10^{-5} M) were obtained in rings from SHR, WKY and Wistar rats. Isolated anococcygeus muscles were weighed and suspended under 1 g resting tension in 25 ml organ baths and cumulative concentration-response curves were obtained to PE (10^{-9}-10^{-5} M) and to SNP (10^{-9}-10^{-5} M).

Aortic rings from SHR were less responsive to PE than rings from the controls. This was reflected in the maximum responses, which were 0.10 ± 0.1 (g mg^{-1}, n=34, P<0.001) for SHR but 0.30 ± 0.1 (n=28) for WKY and 0.31 ± 0.1 (n=43) for Wistar. In contrast, rings from SHR were more responsive to the endothelium-dependent vasorelaxant, CAR, for which the maximum inhibitions were 107 ± 3% (n=15) for SHR and 76 ± 5% (n=16) for WKY rings (P<0.001). Rings from SHR were also more responsive to SNP, which produced a maximum inhibition of 128 ± 3% (n=18) in SHR rings but only 106 ± 2% (n=18, P<0.001) in WKY rings. There was no difference in the responsiveness to ISO in rings from SHR and WKY, in which the maximum inhibitory responses were respectively 111 ± 3% (n=20) and 113 ± 5% (n=18). There was no difference between the responsiveness of anococcygeus muscles from SHR and WKY to either PE or SNP. Aortic rings from SHR were less responsive to the vasoconstrictor, PE, but more responsive to vasodilators such as CAR and SNP, whose actions are mediated via cyclic guanosine monophosphate (cGMP). If this altered responsiveness reflects a homeostatic adaptation to the hypertension, as seems likely since it occurred in vascular smooth muscle and not in smooth muscle of the anococcygeus, it is specific for cGMP-mediated relaxation, since inhibitions to ISO were unchanged.

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190P DIFFERENTIAL PEPTIDERGIC AND NITRERGIC INVOLVEMENTS IN NANC RESPONSES EVOKED BY TRANSMURAL STIMULATION AND DMPP IN THE GUINEA-PIG TAENIA CAECI

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There are several candidates for mediators of the NANC (non-adrenergic, non-cholinergic) relaxations of the guinea-pig taenia caeci (GPTC) including ATP, VIP, PHI (Bywater & Taylor, 1991) with a recent report also suggesting a nitrergic component (Gustafsson et al. 1990). In this study we have attempted to characterise the selective contributions of these transmitters to NANC relaxations induced by transmural nerve stimulation (TS) and also to the ganglion stimulant DMPP.

Taenia caeci muscles were dissected from sacrificed adult Dunkin-Hartley guinea-pigs (300-750 g). Strips of 1.5-2.0 cm were suspended in Krebs solution at 35°C, to which atropine 10^{-6} M was added. Preparations were pre-contracted with histamine 10^{-6} M and transmural nerve stimulation carried out at 5-15 Hz, 100 V for 5 s at 4 min intervals. Relaxant responses are expressed as % of contractile response evoked by histamine.

DMPP (10^{-5} M) evoked a transient relaxation of the GPTC, however, subsequent responses to TS were significantly potentiated (before 45±3% (mean ± SEM) n=20, after 56±3% p<0.05). Relaxant responses to TS and DMPP were abolished by xylocaine (10^{-4} M) but were unaffected by guanethidine (2.5 x 10^{-6}M). Hexamethonium (10^{-4} M) caused a small, transient decrease in TS and abolished responses to DMPP. The NO synthase inhibitor L-NOARG (10^{-4} M) caused insignificant reductions to TS and DMPP (n=5). However, α-chymotrypsin (2 U/ml) abolished the response to DMPP but only reduced TS relaxations from 43±4 to 34±2% (N.S., n=5). However, the combination of L-NOARG plus α-chymotrypsin reduced TS responses from 61±8 to 38±5% (p<0.05; n=5). VIP (0.5 x 10^{-9} M) relaxed the pre-contracted GPTC, however, subsequent responses to TS were significantly potentiated (before 26±1; after 36±1% (p<0.05; n=5). These results indicate that relaxant responses of the GPTC evoked by DMPP are mediated via activation of peptidergic neurones. However, the NANC responses to TS appear to involve both peptidergic and nitrergic components which may act in a synergistic manner. The residual relaxant response to TS which persists in the presence of the inhibitors indicates that another factor(s) is also involved.

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191P THE EFFECT OF NITROVASODILATORS AND CGMP ON Ca^{2+}-INDUCED TENSION RESPONSES AND Ca^{2+}-RELOADING IN THE MOUSE ANOCOCCYGEUS MUSCLE

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Non-adrenergic non-cholinergic (NANC) relaxations of the anococcygeus muscle involve the L-arginine: NO pathway (Gillespie *et al.*, 1989; Gibson *et al.*, 1990), with subsequent activation of guanylate cyclase: cGMP (Mirzazadeh *et al.*, 1991). However, the cellular mechanisms by which cGMP induces relaxation of non-vascular smooth muscle are as yet unclear, and may vary among tissues (Giembycz & Raeburn, 1991). Here, using the mouse anococcygeus muscle, we have examined the effects of nitrovasodilators and cGMP on the contractile elements (using skinned preparations) and on the uptake of Ca^{2+} by the sarcoplasmic reticulum (SR; by determining reloading of Ca^{2+} following depletion in Ca^{2+}-free medium).
Skinned tissues. Anococcygeus muscles from male mice were bathed in a relaxing medium (mM: KCl 130, $MgCl_2$ 5, Tris-maleate 20, Na_2ATP 5, creatine phosphate 10, EGTA 4; 25°C; pH=6.8). Skinning was achieved using either saponin (50μg/ml) or Triton X-100 (1% v:v) for 20 min. pCa-tension curves were constructed, using Ca^{2+}-EGTA buffers, which gave EC_{50} values of 0.71μM and 0.89μM for saponin- and Triton-treated tissues respectively. NO (30μM) and 8-Br-cGMP (200μM) significantly reduced the Ca^{2+}-contractions at pCa of 6.3, 6.0, and 5.0 in saponin-treated tissues, but had no effect on Ca^{2+}- contractions in Triton-treated tissues.
Reloading experiments. Isometric responses (354 ± 17mg) to 50μM carbachol were obtained in normal Krebs solution (Gibson *et al.*, 1990) containing 1μM phentolamine, 1μM verapamil, and 100μM L-N^G-nitro-arginine methyl ester. 2 min after changing to 0Ca: 2mM EGTA Krebs solution the response to carbachol was greatly reduced to 28 ± 4mg, and a second challenge at 20 min elicited no response. The residual response to carbachol at 2 min was taken as a measure of the internal Ca^{2+} store; in support of this, caffeine (10mM) produced a similar degree of contraction (22 ± 5mg). The depleted stores could be refilled by applying a depolarising solution (70mM K^+; 2.5mM Ca^{2+}) for 10 min before again switching to 0Ca: 2mM EGTA Krebs. The residual response to carbachol so-obtained was increased by 63% and 64% ($p < 0.05$ in both cases) by inclusion of 200μM 8-Br-cGMP and 500nM sodium nitroprusside respectively during the reloading period.
Thus, both nitrovasodilator drugs and 8-Br-cGMP relaxed Ca^{2+}-contractions in saponin-skinned (in which the SR remains intact) but not Triton-skinned (in which the SR is non-functional) muscles. Further, both appeared to increase the reloading of Ca^{2+} into previously-depleted internal stores. These results suggest that one mechanism of nitrovasodilator/cGMP-induced relaxation in mouse anococcygeus is increased pumping of Ca^{2+} into the SR.

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192P REDUCED VASODILATOR RESPONSES TO CARBACHOL IN ISOLATED AORTA FROM STREPTOZOTOCIN DIABETIC RATS

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The present study examines the relaxation responses of rat aorta to carbachol, forskolin and nitroprusside, 14 days after the induction of diabetes with streptozotocin. Diabetes was induced in male rats by a single injection of streptozotocin (65mg Kg^{-1}, i.p.). 14 days later, aorta from control or vehicle-treated rats were isolated and segments of circular muscle set up in aerated Krebs solution at 37°C. Tissues were precontracted with phenylephrine (1μM), and relaxation responses obtained to carbachol, forskolin or nitroprusside.

Blood glucose levels were elevated in streptozotocin-pretreated rats (all >16mM) compared with controls (6.9 ± 0.4mM). Responses to phenylephrine were enhanced in tissues from diabetic animals, geometric mean EC_{50} values (with 95% confidence limits) being reduced from 401(161 - 995)nM (n=10) to 10.0(4.0 - 219.0)nM (n=12, $P<0.001$), whilst mean maximum contractile responses (±SEM) were increased from 0.47 ± 0.06g to 0.70 ± 0.05g ($P<0.01$).

Relaxation responses to forskolin (10^{-7} - 10^{-6}M) appeared to be enhanced in diabetic aorta. The maximum relaxation was increased from 0.46 ± 0.04g to 0.69 ± 0.07g ($P<0.01$). However, the greater relaxation was only the result of the greater precontraction obtained to phenylephrine in diabetics since, when expressed as a percentage of the phenylephrine precontraction, responses were similar in control (111.4 ± 6.2%) and diabetic tissues (107 ± 1.9%). In contrast, relaxation responses to carbachol were depressed in diabetic aorta. EC_{50} values were not significantly different between control [0.8(0.63-1.00)μM, n=27] and diabetic animals [1.3(1.1-1.6)μM, n=27], but maximum responses were reduced from 0.43 ± 0.03g to 0.29 ± 0.02g in aorta from diabetic animals ($P<0.001$). This depressed relaxation response was still observed when reponses were plotted as a percentage of the phenylephrine precontraction (60.2 ± 4.1% in diabetics; 77.1 ± 4.5% in controls, $P<0.01$) Relaxation responses to carbachol were endothelium-dependent and were inhibited by N-monomethyl-L-arginine (50μM) equally in control and diabetic animals. Responses to nitroprusside (10^{-7} -10^{-9}) were identical in control and diabetic rats, the maximum relaxations being 101.8 ± 3.9% and 101.6 ± 4.6% of the phenylephrine precontraction respectively.

These results indicate that diabetes of only 14 days duration depresses endothelial-dependent relaxation responses in rat aorta, probably as a result of reduced EDRF release. Our results are different to those reported by Head et al. (1987).

Head, R.J. et al., (1987) Br. J. Pharmacol., 91, 275-286.

193P NITROVASODILATORS POTENTIATE AND INHIBIT SPONTANEOUS ELECTRICAL AND MECHANICAL ACTIVITY IN THE GUINEA-PIG INTERNAL ANAL SPHINCTER (gpIAS)

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Nitric oxide (NO) relaxes and hyperpolarizes non-vascular smooth muscle (Dalziel et al 1991). The effects of sodium nitroprusside (SNP) and of NO itself, have now been examined, on spontaneous electrical and mechanical (E and M) activity in the gpIAS. E and M activity was recorded extracellularly from the Krebs-perfused gpIAS in a Golenhofen apparatus (Golenhofen & v.Loh, 1970). Under 1g stretch, the gpIAS exhibited an almost continuous spike discharge (maximum 0.69 mV) and contractions (maximum 4.3g). SNP, isosorbide dinitrate (IDN) and glyceryl trinitrate (GTN) each produced a qualitatively similar, concentration-dependent change in spontaneous E and M activity (SNP>IDN>GTN). SNP (10^{-8}-$5x10^{-4}$M) altered the continuous discharge to give a more synchronised, intermittent pattern of activity, the amplitude of which was determined by concentration. At 10^{-8}-10^{-6}M, SNP enhanced both contractile strength (maximum 400%) spike amplitude (maximum 300%). At 10^{-6}-$5x10^{-4}$M, the frequency and amplitude of both spikes and contractions were reduced or abolished. BRL 38227 (5×10^{-6}M), Ca^{2+} - free Krebs, or diltiazem (10^{-4}M) each enhanced, whereas membrane depolarization (by increasing $[K^+]o$) reduced the inhibitory effects of SNP. Oxyhaemoglobin (3×10^{-5}M) inhibited and 8-bromo-cGMP mimicked the effects of SNP. BAY K8644 (1.5×10^{-6}M) reversed the inhibitory effect of SNP. NO, (10^{-4}M)(Gillespie and Sheng, 1988) inhibited E and M activity, as did high concentrations of SNP (10^{-4}M). Nitrovasodilators initially enhance spontaneous E and M rhythm in the gpIAS before reducing both. cGMP activation and reduced Ca^{2+} availability by NO-producing vasodilators may stimulate Ca^{2+} entry by way of compensation and account for the initial stimulation. Subsequent membrane hyperpolarization, presumably by Ca^{2+} - mediated K^+ channels, causes relaxation and inhibition of spike discharge.

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194P L-N^G-NITRO ARGININE P-NITROANILIDE (L-NAPNA): A SELECTIVE INHIBITOR OF NITRIC OXIDE SYNTHASE IN THE BRAIN?

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L-N^G-nitro arginine methyl ester (L-NAME) administered i.p or i.c.v causes a centrally mediated, opioid-independent antinociception in three separate models in the mouse (Moore et al., 1991). We have now compared the antinociceptive effect (formalin-induced hindpaw licking; Moore et al., 1991) of L-NAME, L-N^G-monomethyl arginine (L-NMMA) and L-N^G-nitro arginine p-nitroanilide (L-NAPNA) with their ability to inhibit rat cerebellar nitric oxide synthase (NOS) in vitro and to inhibit endothelium-dependent relaxation of the rabbit aorta to acetylcholine (ACh).

Cerebella from Wistar rats (male, 200-280 g) were homogenised (1:4 v/v) in Tris-EDTA (pH 7.4) buffer and centrifuged twice (10,000 g, 30 min; 20,000 g, 20 min). Supernatant was passed over a 0.75 ml column of Dowex AG50WX-8 (Na^+ form) to remove endogenous L-arginine. Aliquots (170 ul) were incubated (45 min, 37°C) with L-arginine (200 uM), NADPH (0.5 mM), $CaCl_2$ (0.75 mM) and 5 ul drug or vehicle. NOS activity measured as nitrite concentration was determined spectrophotometrically by the Greiss reaction (Bredt & Snyder, 1989). L-NAME (IC_{50}, 3.2 $\pm$ 0.6 uM), L-NAPNA (IC_{50}, 1.8 $\pm$ 0.5 uM) and L-NMMA (IC_{50}, 31.6 $\pm$ 5.4 uM) (n=6) inhibited NOS in vitro. L-NAME and L-NAPNA also produced a dose-related reduction in late-phase (but not early phase) paw licking in mice. For example, L-NAME and L-NAPNA (both 50 mg/kg, i.p.) reduced the late phase response by 45.2 $\pm$ 12.3% (n=13) and 40.1 $\pm$ 6.0% (n=11) respectively. L-NMMA (50 mg/kg, i.p.) was inactive. ACh (ED_{50}, 0.07 $\pm$ 0.02 uM, n=12) caused dose-related relaxation of phenylephrine (1 uM) precontracted rabbit aortic rings. L-NAME (1.5 uM) and L-NMMA (5 uM) addition caused a further contraction of aortic rings followed by an inhibition of the vasorelaxant effect of ACh (ED_{50}s, 0.63 $\pm$ 0.06 uM and 0.61 $\pm$ 0.07 uM, n=8, $P<0.05$). In contrast, L-NAPNA (50 uM) did not produce a contraction and did not influence the subsequent vasorelaxant response to ACh (data from 4 experiments).

The present results describe for the first time the NOS inhibitory effect of L-NAPNA and its antinociceptive activity in mice. Interestingly, L-NAPNA appears to have little effect on endothelium-dependent relaxation of blood vessels in vitro.

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195P LOSS OF NERVE-MEDIATED RESPONSES AND NITRIC OXIDE SYNTHASE ACTIVITY IN COLD-STORED ANOCOCCYGEUS MUSCLES FROM MICE

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Non-adrenergic, non-cholinergic (NANC) relaxations of the anococcygeus muscle are blocked by inhibitors of nitric oxide synthase (NOS; Gillespie *et al.*, 1989; Gibson *et al.*, 1990), suggesting that NO or a closely related substance is the NANC transmitter (Hobbs *et al.*,1991). If so, then NOS should be located presynaptically and enzyme activity reduced by denervation. Here, we have examined the effect of cold storage, a procedure recognised to result in denervation of non-intramural nerve fibres (Burnstock *et al.*, 1966), on nerve-mediated responses and NOS activity in the mouse anococcygeus.

Tension responses of mouse anococcygeus muscles were recorded as described previously (Gibson *et al.*, 1990). Some muscles were cold-stored (4°C) in Krebs solution for the appropriate length of time. NOS activity was measured by the conversion of [^{3}H]-arginine to [^{3}H]-citrulline in the presence of cytosolic fractions (Dwyer *et al.*, 1991), using muscles from 12-15 mice for each assay. To record relaxations muscle tone was raised with 50μM carbachol.

Cold storage for 24, 48 and 72h caused a progressive decrease in contractions due to field stimulation of the sympathetic nerves (1-40Hz). By 72hrs, nerve-induced contractions were abolished, as were those to tyramine (up to 10μM), although contractions to carbachol (0.1-100μM) were not significantly altered. The noradrenaline concentration-response curve was displaced 10-fold to the left, presumably reflecting loss of neuronal uptake.
NANC (1-40Hz) relaxations were also progressively reduced by cold storage, the time-course being similar to that with sympathetic stimulation. By 72h, NANC relaxations were absent, but relaxations to NO (1.5-60μM), sodium nitroprusside (0.1-20μM) and 8-Br-cGMP(10-500μM) were unaffected; those to hydroxylamine (1-100μM) were slightly potentiated (3-fold leftward displacement of concentration-response curve).
In control tissues, the conversion of [^{3}H]-arginine to [^{3}H]-citrulline was measured as 99895 ± 12367 dpm/mg protein; after 72h cold storage, this was reduced to 3080 ± 900 dpm/mg protein (mean±s.e.mean; $n>6$; $p<0.05$).

Thus, cold storage for 72h produced a selective loss of nerve-mediated responses (sympathetic and NANC), which was associated with almost complete loss of NOS activity. This would be consistent with a neuronal location for NOS.

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196P INHIBITION OF CYTOCHROME P-450 ACTIVITY BY NITRIC OXIDE *IN VITRO* AND DURING ENDOTOXIN SHOCK *IN VIVO*

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The actions of nitric oxide are mediated through binding to haeme and non-haeme iron in the active centers of target proteins. The avidity of NO for haeme iron is extremely high; NO has an affinity for haemoglobin haeme-iron which is 1,000-fold greater than that of CO. Since cytochrome P-450s are a major family of haemoproteins, we have studied whether NO can inhibit P-450 mediated drug metabolism.

Liver microsomes from rat or chick were used to test whether in vitro exposure to NO attenuates cytochrome P-450 function. NO treatment of microsomes was via an enzymatic generating system prepared from crude cytosol of rat aortic smooth muscle cells which had been pretreated with a combination of LPS (30 μg/ml) and interferon-γ (50 ng/ml) to induce NO synthase. Synthesis of NO was initiated by addition of L-arginine and NADPH, 0.5mM and tetrahydrobiopterin, 10μM. Exposure of ß-naphthoflavone-induced chick liver microsomes to 300 pmol/min NO for 3 min, resulted in an 83% reduction in metabolism of 7-ethoxycoumarin (7-EC) to umbelliferone. Similar experiments with rat liver microsomes demonstrated that phenobarbital (PB)-induced, cytochrome P-450IIB1-dependent O-deethylation of 7-ethoxycoumarin (7-ER) and 16ß-hydroxylation of androstenedione (AD) was significantly (65-68%) attenuated by pre-exposure of microsomes to NO. In contrast, these two metabolisms were not affected when NO synthesis during the 5 min preincubation was blocked by co-treatment with N^G-methyl-L-arginine (10mM).

To test whether sufficient quantities of NO can be produced *in vivo* to inhibit cytochrome P-450, some rats were pretreated with bacterial lipopolysaccaride (LPS; 2mg/kg i.p. 6h beforehand) which induces NO synthase in liver and other tissues. Six groups of 6 Wistar rats were studied: Control, PB-treated, LPS-treated, N^{ω}-nitro-L-arginine methyl ester (L-NAME)-treated, PB+LPS-treated and PB+LPS+L-NAME-treated. Animals which were treated with L-NAME for NO synthase blockade received drug in their drinking water (250mM) ad libitum, commencing 2 days prior to LPS treatment. Livers were assessed for NO synthesis (measured as conversion of [^{3}H]arginine to [^{3}H]citrulline) and liver microsomes were studied for 7-penthoxyresorufin (7-PR) N-dealkylation and AD-16ß-hydroxylation. Whereas NO synthesis was very low in both control and PB-induced liver (0.18-0.41 pmol/min/mg protein), it was substantially induced by LPS (23-56-fold) and this induction was abolished by co-treatment with L-NAME. LPS treatment also resulted in 68% and 87% reduction in both 7-PR and 16ß-AD PB-induced metabolisms respectively. These inhibitory effects of LPS on microsomal metabolism were significantly diminished in rats co-treated with L-NAME, suggesting that LPS-induced NO synthesis mediates, at least in part, inhibition of cytochrome P-450 monooxygenases *in vivo*.

The present study reveals that NO inhibits various forms of cytochrome P-450 in each of two species tested. It is likely that cytochrome P-450 inhibition by NO occurs in vivo during septic shock. NO-mediated suppression of the metabolism of endogeneous and exogenous substances in septic patients may contribute to the clinical manifestations and may be an important consideration for rational drug therapy of this condition. (Supported by a grant from GLAXO and NIH grants HL46403 and ES03606)

197P A COMPARISON OF THE EFFECTS OF L-N^W-NITRO ARGININE METHYL ESTER AND FLURBIPROFEN ON RESPONSES TO VASOCONTRICTORS IN THE PULMONARY CIRCULATION OF THE RAT PERFUSED LUNG

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Humoral substances, such as catecholamines, histamine, 5-hydroxytryptamine (5-HT) can affect the tone of the pulmonary vasculature. Some of these substances have complex effects on the pulmonary vasculature because they act simultaneously on different receptors, which mediate opposing effects. The complexity of their effects may also be due to their ability to release endogenous vasoactive agents such as nitric oxide (NO) which opposes the constrictor effect of 5-HT (Shaw et al, 1992). This study examined the possibility that endogenous prostaglandins, like NO, might influence pressor responses to 5-HT, phenylephrine (PE) and KCl, whose effects were therefore examined in the absence and presence of L-N^W-nitro arginine methyl ester (L-NAME, 0.5 mM) or flurbiprofen (FLB, 2 uM), to inhibit respectively the synthesis of NO and prostaglandins.

Male Wistar rats (230-280 g) were anaesthetised with pentobarbitone sodium (60 mg kg^{-1}, i.p.), heparinised (2000 i.u. kg^{-1}, i.p.) and respired artificially (stroke volume = 2.5 ml, rate = 55 $min.^{-1}$). A stainless steel cannula was inserted through the right ventricle and into the pulmonary artery. The pulmonary vasculature was then perfused at 5 ml per minute for 1 hour with Krebs buffer (35°C, gassed with 95% O_2/5% CO_2) before drugs were administered either as bolus injections or by perfusion to equilibrium.

L-NAME (0.5 mM) slightly increased the basal perfusion pressure. Pressor responses to 5-HT, PE and KCl were enhanced in the presence of L-NAME. The maximum increases in perfusion pressure (mm Hg mean $^+$ s.e. mean) before and after L-NAME were respectively 5.8 $^+$ 1.2 and 18.0 $^+$ 1.8, n=7, P<0.001 (5-HT), 5.0 $^+$ 1.6 and 11.2 $^+$ 1.8, n=8, P<0.001 (PE) and 25.5 $^+$ 1.68 and 34.5 $^+$ 3.0, n=18, P<0.001 (KCl). These results suggest that in the pulmonary vasculature, endogenous NO antagonises responses to vasoconstrictors, whether they act by initiating phosphoinositide hydrolysis (5-HT, PE) or depolarization (KCl). The effect of flurbiprofen depended on how the vasoconstrictors were administered. When 5-HT and PE were administered as bolus injections, flurbiprofen inhibited the pressor responses (72.7 $^+$ 7.4% of 5-HT control maximum, n=6, P<0.05; 78.0 $^+$ 7.7% of PE control maximum, n=7, P<0.05) but when administered by perfusion to equilibrium, flurbiprofen had little or no inhibitory effect. These results suggest that endogenous NO plays a part in regulating pulmonary vascular perfusion but provide no evidence that prostaglandins, particularly prostacyclin, have a similar role.

Shaw, A.M., Pollock, D. & McGrath, J.C. (1992) Br. J. Pharmacol. **105**, 92P

198P ENDOGENOUS NITRIC OXIDE FORMULATION SUPPORTS RENAL BLOOD FLOW AFTER ISCHAEMIA-REPERFUSION INJURY

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Ischemia-reperfusion (I/R) injury in the kidney is associated with a loss of autoregulation, an increase in renal vascular resistance (RVR), a decrease of renal blood flow (RBF) and ultimately acute renal failure. The aim of this study was to determine the formation and the role of nitric oxide (NO) in the recovery of RBF after I/R injury.

Forty-two Wistar-Kyoto rats were anaesthetized with sodium thiopental (120 mg/kg i.p.) and submitted to acute renal ischemia by clamping the left renal artery for 1 h. Mean arterial pressure (MAP) (carotid artery cannulation) and RBF (transonic doppler flow probe) were monitored before renal artery occlusion, and for 2 or 6 h of reperfusion. Kidneys were removed and frozen in liquid nitrogen. The NO synthetase (NOS) activity was measured in vitro in both ischemic and non-ischemic (control) kidneys by measuring the conversion of (^{3}H)L-Arg to (^{3}H)L-citrulline in the presence or absence of calcium. The changes in RBF and renal vascular resistances (RVR) are depicted in table 1.

		Before	30 min	60 min	120 min
I/R	RBF (ml/min)	13±1	8.9±1*	8.7±1*	9±1*
n=6	RVR (mmHg·min/ml)	11±0.5	16±2*	15.7±1*	15±1*
L-NAME	RBF (ml/min)	10.3±1	5.6±0.5*†	4.5±1**††	2.8±0.1**††
n=5	RVR (mmHg·min/ml)	11.3±1	25±2*†	31±2**††	46±1**††
SOP+L-NAME	RBF (ml/min)	11.5±1	6±1*	4.1±1*†	3.2±1**††
n=3	RVR (mmHg·min/ml)	10±1	21±3*	37±8*†	48±9*††

Table 1: Data are expressed as mean±s.e. mean. Significance was determined by paired (*) or unpaired (†) student t test within or between groups respectively. (*) p<0.05, (**) p<0.01, paired t test; (†) p<0.05 and (††) p<0.01 with umpaired t test.

Following I/R injury, RBF was significantly reduced at 30 min of reperfusion and remained reduced during the 2 hours of reperfusion. RVR was significantly increased, while no change was observed in MAP. Neither infusion of L-Arg (1 or 3 mg/kg/min) or D-Arg (1 mg/kg/min), starting 30 min after occlusion, improved the recovery of RBF. Infusion of N^G-nitro-L-arginine methyl esther (L-NAME, 30 μg/kg/min) caused a significant reduction of RBF, which was maximum after 1 hr, in sham operated animals (SOP). Althougth I/R reduced RBF, L-NAME was still able to further decrease RBF. This shows that following I/R NO synthesis in whole or in part is still present to support RBF. No further reduction of RBF was obtained with indomethacin (5 mg/kg i.v.). A calcium-independant (inducible) NOS activity was not detectable in kidney homogenates obtained from either SOP-control rats, or from animals subjected to I/R for 2 (n=4) or 6 h (n=3). Moreover, dexamethasone (3mg/kg i.v., 60min prior to I/R), an inhibitor of the induction of NOS, had no effect on either RBF or RVR in rats subjected to I/R (n=3).

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199P CAPTOPRIL PREVENTS HYPERTENSION DEVELOPED BY INHIBITION OF NITRIC OXIDE SYNTHESIS INDUCED BY CHRONIC TREATMENT OF RATS WITH L-NAME

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EDRF is important in the control of vascular function (Vanhoutte, 1989) and intravenous injections of nitric oxide (NO) inhibitors such as L-NAME induce an acute increase in rat blood pressure (Gardiner *et al.*, 1990). The present study was performed to determine the effect of chronic inhibition of NO synthesis on rat arterial blood pressure and also the effect of captopril on the hypertension induced by chronic L-NAME treatment.

Male rats (250-270g; Wistar) were divided into 3 groups. Arterial blood pressure was determined 3 times a week by the tail-cuff method. L-NAME (70 mg/kg) and captopril (75 mg/kg) were administered orally in the drinking water. Group A (n=12) was treated with L-NAME for 28 days. The animals were then killed (ether) and 5 rats from this group had their kidneys removed for histological study. Group B (n=12) was treated with L-NAME for 28 days and then with captopril for another 10 days; Group C (n=7) was treated with captopril for one day and then with captopril plus L-NAME for another 28 days. The blood pressure of untreated rats did not change over a 28-day period.

Histological studies performed in kidneys of rats treated chronically with L-NAME for 28 days showed ischaemic injuries with obsolescent glomeruli. There was also thickening of the glomerular basement membrane and of the media with marked intimal hyperplasia in 4 rats. The changes in arterial blood pressure (mmHg) induced by L-NAME and captopril are expressed in the Table below.

Group	Control	( ) Days after beginning of L-NAME							Captopril	
		(03)	(05)	(10)	(15)	(20)	(25)	(28)	(05)	(10)
A	102 ± 9	118 ± 2	133 ± 2	133 ± 2	143 ± 2	144 ± 4	148 ± 3	152 ± 2	-	-
B	104 ± 3	122 ± 3	131 ± 2	134 ± 3	133 ± 3	139 ± 2	145 ± 3	153 ± 1	118 ± 2	115 ± 4
C	106 ± 2	109 ± 4	111 ± 3	114 ± 1	117 ± 1	112 ± 2	119 ± 3	115 ± 3	-	-

These results indicate that the synthesis of NO is important for the maintenance of normal blood pressure, and that arterial hypertension (possibly malignant) can be developed by chronic inhibition of NO synthesis. Renin-angiotensin and kinin systems may play a role in the L-NAME hypertension since captopril prevented its development and reduced high levels of arterial blood pressure.

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200P VASODILATION TO HISTAMINE IN THE RAT ISOLATED PERFUSED KIDNEY (IPK) PRECONSTRICTED WITH K^+ IS EDRF(NO)-INDEPENDENT AND MEDIATED BY H_2 RECEPTORS

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Vasodilation in the rat kidney preconstricted with noradrenaline (NA) in response to histamine (HIST) has been associated with EDRF(NO) (Palmer *et al*, 1987) synthesis and/or release (Bhardwaj and Moore, 1988). The aims of the present study were to evaluate the role of EDRF(NO) in the observed HIST-elicited vasodilation in K+-depolarised preparations and to characterise the receptor(s) involved.

Male Wistar rats (250-300g) were anaesthetised with Hypnorm/Hypnovel and the kidneys excised and perfused at 6 ml/min with modified Krebs Henseleit solution (KHS) gassed with 95%O_2/5%CO_2 and warmed to 37°C.

HIST elicited dose-related decreases in renal perfusion pressure (RPP) elevated to 166±6 mmHg with 30mM K+ (ED_{50}=7.5±1.0 nmol, E_{max}=25±2% decrease in RPP, ED_{max}=100 nmol (mean±sem, n=20)). Two submaximal doses of HIST (10,30 nmol) were subsequently employed to investigate the effects of various drug treatments. Responses to HIST were expressed as a percentage of the response to a maximally effective dose of the directly acting vasodilator, papaverine (PAP) (100 nmol). 10 and 30nmol HIST elicited 49±3% and 63±3% of the maximal PAP response, respectively (mean±sem, n=12). Basal RPP in the time-matched control group was 94±5 mmHg (mean±sem, n=12). RPP was elevated with 30mM K+ to 162±5 mmHg (mean±sem, n=12).

Dilation to HIST (10,30 nmol) was not inhibited with L-NAME or L-NOARG (both 300μM). In addition, responses were unaffected by mepyramine (0.8nM) and thioperamide (10nM). In contrast, responses to HIST (10,30 nmol) were significantly attenuated by 22±6% ($p<0.01$) and 16±6% ($p<0.05$) relative to the maximal PAP response, respectively (mean±sem, n=7), in the presence of ranitidine (200nM). Further investigation with ranitidine (10^{-7}-10^{-5}M) yielded pA_2 and pA_{10} values of 6.76±0.23 and 5.84±0.23, respectively (mean±95% confidence interval, n=7).

In conclusion, no role for EDRF(NO) could be established for HIST-elicited dilation in the K+-preconstricted rat IPK. The response under these conditions is most likely mediated by H_2 receptors on the vascular smooth muscle of renal resistance vessels.

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201P DIFFERENCES BETWEEN ENDOTHELIUM-DEPENDENT RELAXATION TO ACETYLCHOLINE AND A23187 IN THE RABBIT ISOLATED FEMORAL ARTERY

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The biological actions of endothelium-derived relaxing factor (EDRF) appear to reflect the release of nitric oxide (NO) which is synthesized from L-arginine by NO synthase (reviewed by Moncada *et al.*, 1991). This pathway can be inhibited by structural analogues of L-arginine such as L-N^G-nitroarginine methyl ester (L-NAME: Rees *et al.*, 1990). However, in porcine coronary arteries responses to A23187 and bradykinin are resistant to blockade by such arginine analogues and by methylene blue and haemoglobin suggesting the release of an additional relaxing factor (Nagao & Vanhoutte, 1992).
In this study acetylcholine (ACh) and A23187-evoked relaxations were compared in segments of the rabbit femoral artery (3-4 mm in length) mounted under 3-4 g tension in organ baths containing Krebs' buffer maintained at 37°C and bubbled with 95%O_2/5%CO_2. Pre-incubation of tissues with L-NAME (100 μM) for 20 minutes completely inhibited ACh-evoked relaxations in tissues pre-contracted with noradrenaline (0.1-1 μM), an effect which was not reversed by L-arginine (100 μM). In contrast, responses to A23187 were unaffected by pre-incubation with L-NAME alone or together with methylene blue (100 μM). However, when potassium (15-20 mM) was used to elevate tone, exposure to L-NAME and methylene blue inhibited A23187-evoked relaxations, and the maximal response was reduced from 81 ± 9% to 56 ± 9% ($P<0.05$). Indomethacin (10 μM), ouabain (500 μM) and glibenclamide (5 μM) had no effect on either ACh or A23187-evoked relaxations in the presence or the absence of L-NAME or methylene blue. In conclusion, these results suggest that the endothelial cell mediators released by ACh and A23187 in the isolated rabbit femoral artery may be different. However, the possibility that the apparant resistance of A23187-induced relaxation to arginine analogues results from more potent release of NO remains to be discounted (Martin *et al.*, 1992).

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202P A COMPARISON OF THREE NITRIC OXIDE SYNTHASE INHIBITORS ON ENDOTHELIUM-DEPENDENT RELAXATION IN RABBIT AORTA AND ON NO SYNTHASE ACTIVITY IN RABBIT CEREBELLUM

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In this study we have compared the effects of L-N^G-monomethyl-arginine (L-NMMA), L-N^G-nitroarginine methyl ester (L-NAME) and L-N^G-nitroarginine benzyl ester (L-NABE) on basal and stimulated release of EDRF from rabbit aorta and the effects of these inhibitors on the activity of constitutive NO synthase isolated from rabbit cerebellum.

The thoracic aorta of male New Zealand White rabbits (2-2.5g) was removed into Krebs buffer containing 0.01mM indomethacin and gassed with 95% CO_2/ 5% O_2 at 37°C. The cerebellum was removed into liquid nitrogen. The cleaned aorta was cut into 2-3mm wide endothelium-intact rings and mounted in 7ml tissue baths for isometric tension recording. A resting tension of 2g was set. The cerebellum was homogenised in liquid nitrogen and then prepared for NO synthase analysis as previously described (Salter *et al.*, 1991). All data are expressed as mean±s.e.mean (n≥6) and compared by fitting concentration-response curves using a least squares minimization procedure.

All tissues were preconstricted with phenylephrine (PE,10^{-6}M) which produced a mean constriction of 3.6±0.3g. The subsequent addition of L-NMMA (10^{-6} to 3×10^{-4}M), L-NAME (10^{-7} to 10^{-5}M) and L-NABE (10^{-7} to 10^{-5}M) to the precontracted rings produced further constrictions of 25.9±2.0, 17.6±0.5 and 33.3±5.9% respectively of that induced by PE alone. The IC_{50}s for L-NMMA, L-NAME, and L-NABE were 2.2×10^{-6}, 2.7×10^{-6} and 2.8×10^{-7}M respectively. Acetylcholine (Ach, 10^{-8} to 10^{-5}M) induced endothelium-dependent relaxations in PE-constricted rings reaching a maximum of 83.9±1.6% of the PE-induced response. This relaxation was inhibited in a concentration-dependent manner by prior incubation for 30min with L-NMMA, L-NAME and L-NABE (concentrations as above) giving IC_{50}s of 1.5×10^{-5}, 7.3×10^{-7} and 7.6×10^{-7}M respectively. The Ach-induced relaxations produced in the presence of the most effective concentrations of L-NMMA (3×10^{-4}M), L-NAME (10^{-5}M) and L-NABE (3×10^{-6}M) were 19.5±2.4, 53.4±5.7 and 8.3±2.1% respectively.

Total NO synthase activity in the cerebellum preparation was 110.62±7.0 fmol of citrulline produced per min per μg protein. The concentrations of L-NMMA,L-NAME and L-NABE which produced 50% inhibition of this activity were 1.1×10^{-5}, 1.2×10^{-5} and 3.2×10^{-6}M respectively. In the presence of the lowest concentrations of L-NMMA, L-NAME and L-NABE used i.e. 10^{-7}M, enzyme activity was 98.04±3.27, 131.96±10.09 and 126.84±4.21% respectively compared with the activity in the absence of the NO synthase inhibitors.

The data presented shows that the order of potency for the inhibition of the basal activity of EDRF and the inhibition of the isolated cerebellar enzyme is L-NABE>L-NMMA>L-NAME. The order of potency for the inhibition of the stimulated activity of EDRF differs however, this being L-NAME>L-NABE>L-NMMA. The order of efficacy for the inhibition of both basal and stimulated activity of EDRF is L-NABE>L-NMMA>L-NAME. For the inhibition of the isolated cerebellar enzyme L-NMMA, L-NAME and L-NABE are equally efficacious. This data demonstrates that the inhibitors differ in their potency and efficacy depending on the context in which they are used. It also shows that care must be taken when interpreting data obtained with the inhibitors at low concentrations, where some may potentiate the action of NO synthase.

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203P D-CPP AND PHENYTOIN PROTECT AGAINST HYPOXIA-INDUCED FAILURE OF SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS *IN VITRO*

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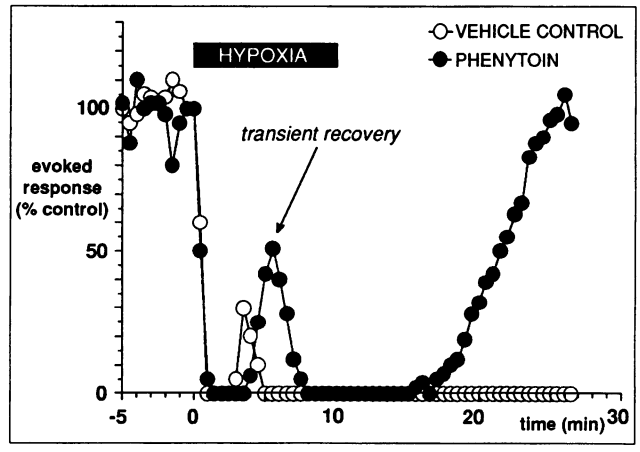
The usefulness of the hippocampus as an *in vitro* model of CNS ischaemia relies on the finding that certain agents with demonstrable efficacy in *in vivo* stroke models also promote recovery of transmission in the slice following a period of hypoxia (e.g., MK-801: Grigg & Anderson, 1990). In the present experiments we have further explored the utility of the hypoxic hippocampal slice model by examining the competitive *N*-methyl-D-aspartate receptor antagonist, D-CPP [3-((RS)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid], and the sodium/calcium ion channel modulator, phenytoin, for their abilities to promote recovery of post-hypoxic function. Both of these compounds confer neuroprotection *in vivo* (D-CPP: Boast *et al.*, 1988; phenytoin: Boxer *et al.*, 1990; Kenny *et al.*, this meeting). (+)MK-801 was used as a reference compound.

Rat hippocampal slices (500 μm thick) were prepared and maintained in artificial cerebrospinal fluid (ACSF) containing 10 mM glucose (gassed with 95% O_2/5%CO_2 at 22 °C). After 1h slices were transferred to the recording chamber and superfused with ACSF containing 4mM glucose at 35 °C. The Schaffer collateral/commissural pathway was electrically stimulated at 0.1 Hz and the CA1 population spike monitored using conventional extracellular recording techniques. Hypoxia was induced by superfusing the slices for 10 min with ACSF gassed with 95% N_2/5%CO_2. Drug or vehicle was applied 20 min before and throughout the hypoxic period.



In control (n=18), vehicle- (n=6) and drug-treated slices, hypoxia resulted in the rapid loss of the evoked response followed by a transient recovery (see figure). Following reperfusion of control and vehicle-treated slices with oxygenated ACSF, synaptically-evoked responses failed to return (up to 60 min post-hypoxia). However, in slices treated with 10 μM D-CPP (n=4), 10 μM (+)MK-801 (n=4) or 20 μM phenytoin (n=6; see figure), evoked responses returned within 20 min of reoxygenation. Furthermore, in both D-CPP- and phenytoin-treated slices the duration of the transient recovery was significantly prolonged (D-CPP: 306 ± 96 s compared to control: 84 ± 8 s; phenytoin: 228 ± 36 s compared to control: 78 ± 24 s). This last finding suggests that D-CPP and phenytoin may slow the spreading depression-like change that accompanies early hypoxia in this brain region (Grigg & Anderson, 1990).

The present data demonstrate that both D-CPP and phenytoin protect the *in vitro* hippocampus against hypoxic insult and confirm the efficacy of (+)MK-801 in this model. These findings also provide further support for the utility of the hippocampal slice in the identification and selection of potentially neuroprotective compounds whose activity *in vivo* may otherwise be obfuscated by more complicated factors.

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204P MODULATION BY POLYAMINES OF [^{3}H]L-689,560 BINDING TO THE GLYCINE SITE ON THE N-METHYL-D-ASPARTATE RECEPTOR FROM RAT CORTEX/HIPPOCAMPUS MEMBRANES

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The suggestion that endogenous polyamines may modulate N-methyl-D-aspartate (NMDA) receptors in the mammalian brain first came when Ransom and Stec (1988) showed that spermine and spermidine enhanced [^{3}H]dizocilpine (MK-801) binding through a site distinct from those recognised by glycine and L-glutamate. Further studies showed that spermine and spermidine increased [^{3}H]glycine binding through an increase in [^{3}H]glycine binding affinity (Ransom and Deschenes, 1990) and that spermine enhanced the affinity of glycine site agonists for [^{3}H]glycine binding without affecting glycine site antagonist affinity (Sacaan and Johnson, 1989). We have investigated the effects of polyamines on the binding of the glycine site antagonist [^{3}H]L-689,560.

[^{3}H]L-689,560 binding to thoroughly washed P_2 membranes from rat cortex/hippocampus was measured using methods described by Grimwood et al (1992). Under conditions where [^{3}H]L-689,560 binding was not at equilibrium (45 min incubation @ 4°C), spermine and spermidine inhibited [^{3}H]L-689,560 binding with IC_{50} values (geometric mean (-SEM,+SEM); n≥6) of 25.9 (21.5, 31.3)μM and 106.0 (92.1, 122)μM, respectively, with a maximal effect (B_{max}; mean ± SEM) of 58.7 ± 2.44% and 41.9 ±3.47%, respectively. The putative polyamine antagonists arcaine, putrescine and diethylenetriamine (DET) and the inverse agonist 1,10-diaminodecane (DA10) had no effect on [^{3}H]L-689,560 binding at 1mM. Arcaine (1mM) antagonised the effects of spermine on [^{3}H]L-689,560 binding while putrescine, DET and DA10 did not. In the presence of 1mM arcaine, spermine inhibited [^{3}H]L-689,560 binding with an IC_{50} value of 72.6 (59.2, 89.0)μM (n=4) with no change in B_{max} (54.3 ± 9.51%). Kinetic experiments revealed that spermine (100μM) slowed down both the association and dissociation rates of [^{3}H]L-689,560 binding. The $t_{1/2}$ values for association (time to reach 50% of equilibrium value) of [^{3}H]L-689,560 binding in the absence and presence of 100μM spermine were 34.0 ± 7.71 min (mean ± SEM; n=3) and 59.7 ± 12.3 min, respectively. The $t_{1/2}$ values for dissociation in the absence and presence of 100μM spermine were 134.1 ± 5.99 min and 288.5 ± 17.5 min, respectively. Saturation experiments revealed that under equilibrium conditions (6h incubation @ 4°C) spermine (100μM) had no effect on the affinity or number of [^{3}H]L-689,560 binding sites.

In conclusion, spermine and spermidine indirectly inhibited the binding of [^{3}H]L-689,560 binding to the glycine site, an effect which was blocked by arcaine, and therefore has the pharmacology associated with the polyamine site on the NMDA receptor. However, the inhibition is due to a change in the kinetics of [^{3}H]L-689,560 binding which is not compatible with a simple allosteric interaction between the polyamine and glycine recognition sites.

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205P THE EFFECT OF NEUROPEPTIDES ON POTASSIUM-STIMULATED RELEASE OF GLUTAMATE FROM RAT STRIATAL SLICES

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Neuropeptide Y (NPY), peptide YY (PYY) and galanin are found throughout the CNS, often co-localised with acetyl choline and/or NADPH-diaphorase (Vincent et al., 1983: Pasqualotto and Vincent, 1991). Their distribution in motor areas has led us to investigate the effects of these peptides on the release of amino acids in the striatum.

Coronal striatal slices (250μm) were cut on a McIlwain tissue chopper and perfused with gassed (95% O_2 / 5% CO_2) aCSF at 0.5ml/min at 37^{o}C. Slices were allowed 60 min to equilibrate and two-min samples of perfusate were collected for the measurement of amino acids. Release was stimulated with three two-min pulses of 45 mM potassium-containing aCSF at 4,16 and 28 min and the neuropeptides were perfused from 10-20 min. Amino acids were assayed by HPLC with flurometric detection.

NPY, PYY and galanin specifically decreased the release of glutamate. Aspartate, GABA, glycine and taurine were not affected. NPY (800nM) decreased release by 59%, PYY (800nM) by 56% and galanin (200nM) by 51% ($p < 0.001$ for each). For NPY the release to the third potassium pulse recovered to control values, however, the effect of PYY and galanin persisted for the third pulse ($p < 0.05$).

It is possible that the specific reduction in glutamate release with NPY and PYY is due to an action at sigma receptors as they both have high affinity for these sites (Roman et al., 1989). We have shown that haloperidol, which has a high affinity for sigma sites, also decreased glutamate release (Ellis and Davies, 1991). There is no evidence that galanin binds to sigma receptors, however, it does activate ATP-sensitive K^+ channels and blocks anoxia-induced depolarizations in hippocampal neurons which are considered to result from glutamate release (Ben-Ari, 1990). These results indicate that NPY and PYY may be the endogenous ligands for sigma receptors and that galanin and possibly NPY and PYY are acting through ATP-sensitive K^+ channels to reduce the release of glutamate.

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206P EFFECT OF TETANUS TOXIN ON EXTRACELLULAR AMINO ACID LEVELS IN RAT HIPPOCAMPUS: AN *IN VIVO* MICRODIALYSIS STUDY

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It has been demonstrated that tetanus toxin produces neuronal damage 7-10 days after microinjection into rat hippocampus (Bagetta *et al.*, 1990a). Since tetanus toxin is known to inhibit GABA release from nerve terminals it has been proposed that the neuropathological effect produced by the toxin may be related to a loss of neuronal inhibition, resulting in a net increase of excitatory input. Furthermore, the neuropathological and behavioural effects resulting from intrahippocampal injection of tetanus toxin can be prevented by the non-competitive NMDA antagonist, dizocilpine (Bagetta *et al.*, 1990b). In an attempt to determine whether a reduction in extracellular GABA occurs we have used the technique of *in vivo* microdialysis coupled with HPLC to measure levels of neuroactive amino acids.

Male Wistar rats (270-300g) were anaesthetized with chloral hydrate (400mg/kg i.p.) and tetanus toxin injected unilaterally into the ventral hippocampus. Concentric dialysis probes, of a construction previously described (Whitton *et al.*, 1991), were implanted 10 minutes after toxin injection. After 24 hours, dialysis was commenced over a two day period. For measurements on day 7, probes were implanted 6 days after the initial microinjection of tetanus toxin. In both cases, hourly dialysate samples were collected (4 per day from each hippocampus) from freely moving animals following an initial 90 minute stabilization period.

During the first 2 days of dialysis, no significant changes in extracellular levels of aspartate, glutamate, taurine and GABA were detected in toxin-treated hippocampi when compared with the contralateral control sides. On day 7 however, whilst the taurine level was not statistically different from the values obtained on days 1 and 2 the mean levels of aspartate, glutamate and GABA in the toxin-treated hippocampi were significantly reduced compared to the control side (Table 1).

Day 7	Hippocampal concentration pmoles/15µl sample (mean ± s.e.m.)		
	Aspartate	Glutamate	GABA
Vehicle Treated	11.0 ± 0.9	122.9 ± 18.6	1.3 ± 0.2
Toxin Treated	7.1 ± 1.9*	68.7 ± 29.6*	0.6 ± 0.2*

Table 1

* $p<0.05$ using paired Student's "t" test

n = 4 rats; 4 samples/rat

The mean concentration of glutamate in vehicle-treated hippocampi on day 7 was 228% and 218% higher than the corresponding values obtained on days 1 and 2 respectively whilst on the toxin-treated side the glutamate levels on days 1,2 and 7 were similar. The GABA and aspartate concentrations in vehicle-treated hippocampi at days 1, 2 and 7 were not significantly different.

In conclusion, 7 days after injection of tetanus toxin when neuronal degeneration is evident (Bagetta *et al.*, 1990b) there was a significant reduction in extracellular GABA and aspartate at the site. The levels corresponded to 49.5% and 64.7% of control basal levels respectively. The significant reduction in GABA levels at the site would support the possibility that a loss of GABA could contribute to the production of neurodegeneration mediated through an unopposed action of endogenous excitatory amino acids. PB is a SERC CASE student with Smithkline Beecham plc.

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207P EFFECT OF ETHANOL ON RESPONSES OF RAT CENTRAL NEURONES *IN VIVO* TO EXCITATORY AMINO ACIDS

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Evidence has continued to mount in recent years, in support of selective antagonism of *N*-methyl-D-aspartate (NMDA)-receptor mediated processes. The involvement of these receptors in the phenomenon of long-term potentiation with its implications in memory and cognition, together with the commonly experienced effects of ethanol intake on these processes have led several groups to investigate a possible link.

Whilst earlier studies indicated a preferential reduction, by ethanol, of quisqualate (QUIS)- and kainate (KAIN)-stimulated Na^+ flux in rat striatal slices (Teichberg *et al.*, 1983), subsequent investigations have shown ethanol to reduce NMDA-activated ion currents in single hippocampal cells (Lima-Landman & Albuquerque, 1989), whilst currents activated by QUIS and KAIN appear much less sensitive (Lovinger *et al.*, 1989). Furthermore, susceptibility of NMDA receptor-mediated neurotransmission has been reported (Lovinger *et al.*, 1990).

Female Wistar rats (200 - 300g) anaesthetised with pentobarbitone Na (50 mg/kg) were used in all experiments. Using seven-barreled glass microelectrodes, the techniques of extracellular recording and iontophoretic amino acid ejection were combined to study the effect of systemic administration of ethanol (25% in 0.9% saline) on responses of neurones in either brainstem or the lumbar spinal cord to NMDA, KAIN, QUIS and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). No apparent differences in pharmacology were observed between brainstem and spinal cells so the data have been pooled.

An intravenous dose of 1g/kg ethanol was tested on all 8 cells resulting in a 9±5% reduction of the peak firing rate in response to NMDA. KAIN responses were reduced by 9±11% and those to QUIS/AMPA by 13±9%. At 2g/kg, the reduction of NMDA responses of 3 cells was 27±6% with similar depression of KAIN and QUIS or AMPA and at higher cumulative doses of 3g/kg and 4g/kg there was still no evidence of selective depression of responses to any of these agonists.

In these experiments we have been unable to obtain evidence to support the findings of other groups suggesting selective antagonism of NMDA receptor mediated processes by ethanol. Differences in the extracellular environment of cells *in vivo* and *in vitro* may account for the lack of corroboration between the results of our study and others.

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208P THE CALCIUM CHANNEL ANTAGONIST, DILTIAZEM, INCREASES THE SEVERITY OF THE ETHANOL WITHDRAWAL SYNDROME

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We have shown that the dihydropyridine class of calcium channel blocking drugs protect against the ethanol withdrawal syndrome (Littleton et al., 1990). Although these compounds have weak anticonvulsant activity in against chemical convulsants, diltiazem, a calcium channel blocker of the benzothiazepine class, increased the convulsion incidence after bicuculline, NMDA or 4-aminopyridine (Watson and Little, 1992). Diltiazem binds at a site on the receptor complex different from the dihydropyridines, but allosterically increases the binding of these compounds, in both peripheral and CNS tissues (Yamamura et al., 1982). High concentrations of diltiazem were needed to affect calcium channels in the CNS (Akaike et al., 1989). In the present study we examined the effects of diltiazem on a mild ethanol withdrawal syndrome.

Ethanol was given by inhalation, 3-6 mg/L, to male TO mice (30-35g) for two weeks. Diltiazem, in saline, was given, i.p., immediately on withdrawal. Handling-induced convulsive behaviour (Littleton et al., 1990) was rated, on a scale 0-4, hourly for 12h. Data are medians and interquartile range; comparison by nonparametric analysis of variance (Meddis, 1984). *P < 0.05, for comparison with ethanol plus saline, between 2h and 6h.
n = 25 - 31 for chronically treated mice, n = 10 for naive mice. Eth. = Ethanol, Dilt = Diltiazem 100 mg/kg.

Chronic/acute	2h	3h	4h	5h	6h	Naive	2h	3h	4h	5h	6h
No eth/saline	1 (0-1)	1 (0-1)	1 (0-2)	1 (0-1)	1 (0-1)						
Eth/saline	1 (1-2)	1 (1-2)	1 (0-2)	1 (0-2)	1 (0-2)	Saline	1 (0-1)	1 (0-1)	1 (0-1)	0 (0-1)	0 (0-1)
Eth/Dilt.*	2 (1-2)	2 (1-2)	2 (1-3)	2 (1-3)	2 (1-2)	Dilt	1.5(1-2)	0.5(0-1)	0 (0-1)	0.5(0-1)	0 (0-1)

Diltiazem, at 100 mg/kg increased the severity of the ethanol withdrawal syndrome. In controls not receiving ethanol, this dose of diltiazem did not increase handling scores. These data, and previous work, show that the central effects of diltiazem differ considerably from those of the dihydropyridine calcium channel antagonists.

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209P SODIUM VALPROATE ALTERS EXTRACELLULAR GABA LEVELS IN THE RAT VENTRAL HIPPOCAMPUS *IN VIVO*

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The anticonvulsant sodium valproate (NaVPA) is extensively used in the treatment of most epilepsies, but no concensus currently exists regarding its mode of action. Previous studies have reported NaVPA to increase whole brain (Godin et al,1969) and synaptosomal GABA (Löscher et al,1985), with some reporting alterations in brain taurine, glutamate and aspartate levels (Patsalos et al,1981). It is reasonable to assume that NaVPA-induced changes in intracellular amino acid turnover might be reflected in the extracellular enviroment and thus may relate to the drugs anticonvulsant action. We have addressed this using in vivo microdialysis to monitor levels of amino acids extracellularly in the rat ventral hippocampus following i.p. injection of NaVPA.

Male Wistar rats (240-300g) were anaesthetised with chloral hydrate (400 mg/kg) and stereotaxically implanted with concentric microdialysis probes into the ventral hippocampus. Following a postoperative recovery period of approximately 18h, perfusion with artficial cerebrospinal fluid was commenced (0.5µl/min) and dialysates collected every 30 min. Injections of saline or NaVPA (100,200 or 400 mg/kg) were given 150 min following the commencement of dialysis and dialysates were analysed for amino acid content using HPLC with pre-column derivitisation. Basal dialysate amino acid levels were found to be 3.0±0.35 (GABA), 33.4±3.7 (glutamate), 101.8±2.5 (taurine), 3.1±0.6 (aspartate) and 43.3±5.2 (glutamine), all values mean pmols ± s.e.mean/10µl dialysate.

TREATMENT	% OF BASAL LEVELS (MEAN ± s.e. mean; n=8 in each group)				
	GABA	glutamate	taurine	aspartate	glutamine
saline	119±14	120±4	107±5	112±20	113±12
100 mg/kg	40±5*	126±10	93±6.5	88.3±9	95.7±14.2
200 mg/kg	141±12	114±7	95.3±2	81±2	110±5.7
400 mg/kg	209±33*	137±8.7	111.2±1.4	101.7±9.5	109.5±13

TABLE 1. Summary of changes in extracellular amino acids following NaVPA treatments. Figures represent maximal changes generally observed 60 min following injection. (*p<0.05 against saline). Results suggest that NaVPA may act specifically to alter extracellular GABA levels in a biphasic manner, dependent on the dose given. These changes are elicited by doses known to protect rodents against seizures. Since the hippocampus is known to be particularly susceptible to epileptogenic phenomena, we believe these data may relate to the mode of action of this drug.

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210P *IN VIVO* MEASUREMENT OF EXTRACELLULAR GABA IN RAT NUCLEUS ACCUMBENS: IMPROVED METHODOLOGY

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GABA transmission in the nucleus accumbens (NAc) is believed to be a critical substrate for the expression of psychostimulant drug action (Willner and Scheel-Kruger). Few attempts have been made to measure release of GABA in the NAc in vivo although such measurements should be possible using microdialysis and HPLC-EC (Kehr and Ungerstedt, 1988). The latter assay is based on detection of an electroactive derivative of GABA produced by a pre-column reaction with *o*- phthalaldehyde (OPA) and *t*- butyl thiol. This derivative has relatively poor stability and suffers from strong and unpleasant thiol odours, but such problems might be avoided by replacement of the thiol agent with sulphite ions (Jacobs, 1987). Here we have utilised such an assay with microdialysis to detect extracellular GABA in the NAc of the anaesthetized rat.

GABA in standards and dialysis samples was derivatised as follows: freshly made OPA reagent (44 mg OPA, 1 ml ethanol, 1 ml 1M sodium sulphite, 18 ml 0.4M boric acid buffer, pH 10.4) was added to samples (10 µl to 1 ml standard, 2 µl to 20 µl dialysis sample) and reacted for 10 sec-60 min at room temperature. Derivatized GABA was separated on a reversed-phase HPLC column (Rainin Dynamax 25 cm C_{18}, 5 µm) using a mobile phase comprised of; 0.1 M $NaH_2PO_4.2H_2O$, pH4.,8, 0.5 mM EDTA and 40% (v/v) methanol. Detection was by a glassy carbon electrode (+0.85 V). Derivatized GABA had a retention time of about 5.5 min. GABA standards (0.4 pmoles) gave a maximal response after a reaction time of 2 min (coefficient of variation was 7.8%) although this diminished little over 60 min. The approximate limit of detection was 0.05 pmol. For microdialysis, a probe (single cannula) was stereotaxically implanted into the NAc of a chloral hydrate anaesthetised rat. Rats were allowed to recover and then reanaesthetized 24 h later. The probe was perfused with artificial cerebrospinal fluid (1.2 mM NaH_2PO_4, 0.27 mM Na_2HPO_4, 140 mM NaCl, 3.0 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 7.2 mM glucose) and 20 µl samples were collected every 20 min.

GABA was readily detectable in the microdialysates (0.497 pmol±0.026, n=16) and stable over several hours. Addition of 100 mM KCl to the perfusion medium for 20 min increased GABA levels by 104±21 % (n=5). Addition of 0.5 mM nipecotic acid, a GABA uptake blocker, to the perfusion medium for a prolonged period also increased GABA (n=5) to levels which stabilised at about 50 % above baseline for several hours. In preliminary experiments (n=4 in each case), GABA output was not reduced by perfusion with calcium-free medium or medium containing tetrodotoxin; calcium removal however reduced the effect of 100 mM KCl by 70%.

In summary, we report a method which is sufficiently sensitive to measure basal extracellular GABA in the NAc in vivo. We are presently attempting to characterise the nature and source of GABA output and its responsiveness to pharmacological manipulation.

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211P AUTORADIOGRAPHY OF GABA RECEPTOR BINDING SITES IN THE DORSAL VAGAL COMPLEX OF THE RAT HINDBRAIN

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In the mammalian hindbrain, the nucleus tractus solitarius (NTS) together with the area postrema and the dorsal motor vagal nucleus (DMVN) collectively constitute the dorsal vagal complex (DVC) from which much of the activity of the autonomic nervous system is integrated. Whilst there is a relative paucity of published reports of possible functional roles for $GABA_A$ and $GABA_B$ receptors specifically within the NTS/DVC, direct evidence does exist for a role for GABA in the maintenance of arterial blood pressure. The pressor responses mediated by injections of nipecotic acid and baclofen into the NTS are significantly higher in spontaneously hypertensive rats compared to normotensive controls (Catelli & Sved, 1988). Conversely, no significant differences were observed between the two strains with respect to muscimol-induced pressor effects. The present study extends initial reports of GABA receptor autoradiography (Bowery *et al.*, 1987; Chu *et al.*, 1990) by describing the distribution of both $GABA_B$ and $GABA_A$ receptors in the DVC. We have previously reported that unilateral nodose ganglionectomy reduces the levels of 5-HT_3 receptor binding in the NTS after a period of 10 days (Pratt & Bowery, 1989). Since this is indicative of presynaptically-located receptors on vagal afferent terminals, this model has been employed to establish the probable neuronal locations of $GABA_B$ and $GABA_A$ receptors in parallel with 5-HT_3 receptor autoradiography. Coronal cryostat-cut sections from the brains of perfused-fixed (0.1% paraformaldehyde) Wistar rats were incubated in 50mM Tris HCl buffer (pH 7.4) containing 2.5mM $CaCl_2$ and [^{3}H]-GABA (50nM). Selective labelling of $GABA_B$ and $GABA_A$ sites was achieved in the presence of isoguvacine and baclofen, respectively. 5-HT_3 receptor sites were visualised using [^{3}H]-BRL 43694, as described by Pratt & Bowery (1989).

5-HT_3 and $GABA_B$ receptor sites predominated in the NTS (393.5 ± 40.4; 90.7 ± 15.9 fmol/mg tissue, respectively, n = 5 rats in each case) with binding also apparent in the DMVN. By contrast, $GABA_A$ binding sites prevailed in the DMNV (154.5 ± 18.9 fmol/mg tissue). Additionally, whilst both 5-HT_3 and $GABA_B$ receptor sites were observed in the nucleus of the spinal tract of the trigeminal nerve, $GABA_A$ sites were absent from this region. Following nodose ganglionectomy, 5-HT_3 sites in the ipsilateral NTS were significantly reduced by 55 ± 5.9% compared to the contralateral side ($p < 0.01$; Student's "t" test, n = 5 rats) which in turn, differed significantly by 42 ± 3.4% ($p < 0.01$) from sham-treated animals. In parallel with these findings, reductions in $GABA_B$ (44 ± 7.4%) and $GABA_A$ (58 ± 5.3%) sites, also occurred in the ipsilateral portion of the NTS and DMVN, respectively. The effects of vagal lesioning appeared to be confined to these brainstem nuclei.

In summary, unilateral lesioning of the vagus nerve reduced the densities of $GABA_B$, $GABA_A$ and 5-HT_3 binding sites on vagal terminals within the DVC, indicative of their presynaptic location on afferent vagal fibres. However, 25% of the three types of binding site remained unaffected ipsilateral to the denervation. This could be attributed to incomplete terminal degeneration or may reflect the existence of postsynaptic sites.

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212P DIFFERENTIAL ACTION OF (+)AMPHETAMINE ON ELECTRICALLY-EVOKED DOPAMINE EFFLUX IN THE RAT CAUDATE PUTAMEN AND NUCLEUS ACCUMBENS

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(+)Amphetamine has actions on dopamine (DA) neurotransmission which induce stereotypy in the caudate putamen (CPu),and locomotor activation in the nucleus accumbens (NAc; Robinson & Becker, 1986). The mechanisms of action of amphetamine are complex, involving uptake inhibition, MAO inhibition and monoamine displacement and depletion(MacMillen,1983). Using fast cyclic voltammetry (Palij et al, 1990), we have compared the effects of (+)amphetamine(10^{-6}M) on electrically evoked DA overflow in 350μm thick rat brain slices incorporating CPu and NAc. Slices were superfused with oxygenated artificial cerebrospinal fluid(1ml/min), at 32°C. Electrically evoked DA overflow was monitored using the following stimulations: single pulse (1p; 0.1ms, 20V), 4pulses at 10Hz(4p/10Hz) and 20pulses at 20Hz(20p/20Hz). The stimulation sequence in each slice was 0min (+)amphetamine; 2.5min (1p); 5min (4p/10Hz); 7.5min (1p); 10min (20p/20Hz), repeated for 40min.

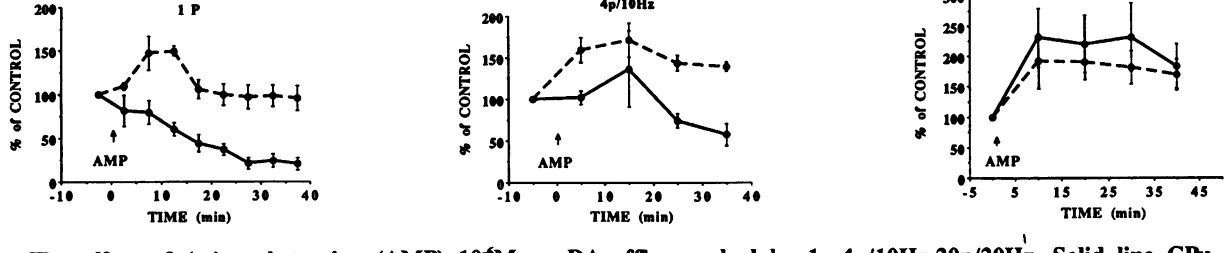

Fig 1 The effect of (+)amphetamine (AMP) 10^{-6}M on DA efflux evoked by 1p,4p/10Hz,20p/20Hz. Solid line CPu, n=4;broken line Nac,n=5 (mean ± s.e.mean)

(+)amphetamine reduced DA overflow evoked by 1p and 4p/10Hz (CPu) but potentiated DA overflow in the NAc evoked by all stimulations and in the CPu at 20p/20Hz. In the NAc amphetamine increased the uptake half time of DA to 1p by 294±72%;4p/10Hz by 331±71% and 20p/20Hz by 361±87%(n=5). In the CPu the respective increases were 643±49%; 984±211% and 870±117% (n=4). The results show that (+)amphetamine has qualitatively and quantitatively differing stimulus dependent and site dependent actions on dopamine overflow in NAc and CPu.

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213P HETEROGENEITY OF THE AFFERENT DOPAMINERGIC INNERVATION TO THE MEDIAL AXIS OF THE RAT NEOSTRIATUM: *IN VITRO* VOLTAMMETRIC DATA

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The primary dopamine (DA) afferents to the neostriatum (CPu) and nucleus accumbens (Acb) are from the A9 and A10 cell groups respectively. A recent *in vitro* study using fast cyclic voltammetry (FCV) at carbon fibre microelectrodes reported strikingly greater DA efflux evoked by short (20 pulse) stimulus trains in the Acb than CPu (Trout & Kruk 1992). Anatomical evidence has, however, shown that some A10 afferent clusters innervate the medial (periventricular) aspect of the CPu (Gerfen 1987). The present study sought to use FCV to locate, identify and measure the size of these clusters.

All experiments were conducted in 350 μm slices of CPu superfused with oxygenated artificial CSF at 32°C. Slices were taken from approximately 9.2 mm vs the interaural line (Paxinos & Watson 1986) i.e caudal to the most posterior extent of the Acb. The recording electrode was placed at a depth of 80 μm below the slice surface while a bipolar tungsten stimulating electrode was located 200 μm ventromedially. DA efflux was evoked using 0.1 ms, 10 mA pulses applied singly (1p) or in 20 pulse trains (20p) at 50Hz. FCV recording parameters were as follows: Scan rate = 480V/s, range -1 to +1.4V vs Ag/AgCl, interscan interval: 500 ms. DA efflux with 1p and 20p stimulations was studied at 104 recording sites throughout the CPu (9 slices).

Single pulse DA efflux (121 ± 10 nM, mean ± s.e.m, n = 104) did not show significant variation throughout the CPu. Twenty pulse DA efflux and 20p:1p ratio, however, was significantly greater in the medial than lateral CPu ($P < 0.05$, Duncans New Multiple Range Test). Concentrating then on the medial CPu, clusters were individually located (20p:1p ratios > 6) and "mapped" on the basis of evoked 20p DA efflux (7 slices, 21-38 sites per slice). The clusters were irregular in outline and around 400 μm across. The DA efflux vs frequency response of the clusters was also different from the CPu "background" (Table 1). The maximum response in the clusters occurred at 50Hz. The corresponding curve for the CPu background was virtually flat. The response in the clusters was similar to that observed by Trout & Kruk (1992) in the Acb, which also peaked at 50Hz.

Table 1: Frequency response of subpopulations of DA neurones in CPu

	10 Hz	20 Hz	50 Hz	100 Hz	200 Hz	500 Hz
"Clusters"	261 ± 66	646 ± 165*	964 ± 142**	764 ± 154*	530 ± 68**	275 ± 43*
"Background"	134 ± 25	165 ± 44	155 ± 42	171 ± 52	169 ± 55	117 ± 43

Peak DA efflux (% of single pulse ± s.e.m., n=4) on 20 pulse trains. * $P < 0.05$, ** $P < 0.01$ cluster vs background

The medial location of these clusters within the CPu more closely matches the anatomical distribution of the A10 afferents of Gerfen (1987) than the more evenly dispersed striosomes. The DA efflux characteristics are also much closer to those reported for the A10-innervated Acb (Trout & Kruk, 1992) than the mainly A9 CPu. We therefore tentatively suggest that these clusters correspond to small (ca. 400 μm) groups of A10 DA neurones within a predominantly A9 CPu. Further studies are in progress to test this assertion.

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214P DIFFERENTIAL SENSITIVITIES OF RAT STRIATAL D_2 DOPAMINE AUTORECEPTOR SITES

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Among mechanisms which regulate dopamine release in the neostriatum, are axon traffic deriving from depolarisation of nerve cell bodies in the substantia nigra and ventral tegmental area, and local control at axon terminals regulated by auto and hetro-receptors (for review see Grace 1991). We have used fast cyclic voltammetry at a carbon fibre electrode to examine the ability of dopamine D2 agonists to inhibit electrically stimulated endogenous dopamine overflow in rat brain slices, using established methods (Trout & Kruk 1992). Rats were killed and 350μm slices of caudate putamen were incubated in artificial CSF at 32°C in a total emersion chamber. A carbon fibre electrode and a bipolar stimulating electrode were placed 80μm into the slice, and single pulse (1p) or repeated electrical stimulations (20 pulses at 10, 20 or 50Hz; (20p/10Hz,20p/20Hz,20p/50Hz)) were applied every 5 min. Fast cyclic voltammetry was carried out using a Millar Voltammeter and the peak current at the oxidation potential for dopamine was monitored at 2Hz using a sample and hold circuit fed into a CD1401 computer interface and a chart recorder.

When dopamine overflow following 1p electric stimulation was compared with overflow following a burst of 20p/50Hz, two populations of sites were identified. In the first, the ratio of 1p:20p/50Hz was 1.5± 0.3 (mean ± s e mean; n=17), in the second the ratio of 1p:20p/50Hz was 4.6± 0.9 (n=11). The effects of the dopamine D2 agonist quinpirole and of apomorphine were examined for their ability to inhibit dopamine release evoked by single pulse electrical stimulation at both types of site. Quinpirole and apomorphine were more potent at high ratio than at low ratio sites. EC50 values (concentration of agonist needed to reduce electrically stimulated overflow of endogenous dopamine following single pulse stimulation by 50%) were quinpirole: 16 ± 4 nM in high ratio sites, and 34 ± 6 nM at low ratio sites (n= 4 and 6 respectively; p=0.035; d.f. 8; unpaired t-test); apomorphine 233 ± 107 nM in high ratio sites, and 458 ± 150 nM at low ratio sites (n = 4 and 5 respectively; p= 0.37; d.f. 7; unpaired t-test).

Other experiments in this laboratory have shown that there are significant differences between the caudate putamen, nucleus accumbens and olfactory tubercle (Trout & Kruk 1992), and anterior and posterior caudate putamen (Patel et al 1992) in their ability to release dopamine following single pulse or bursts of electric stimulation. In the experiments reported here, the brain slice corresponded to anterior caudate putamen (Patel et al 1992). These results add to the body of evidence which shows that regulation of dopamine release in the striatum is not the same at all sites. Further studies are needed to understand the biological implications of these results both in terms of normal physiology and potential therapeutic exploitation of these hetrogeneities.
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215P AGE-RELATED DIFFERENCES IN EVOKED NORADRENALINE EFFLUX AS MEASURED BY FAST CYCLIC VOLTAMMETRY IN RAT BRAIN SLICES

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Fast cyclic voltammetry (FCV) has recently been applied to the detection of noradrenaline (NA) efflux and reuptake in small nuclei of the superfused rat brain slice (Palij & Stamford, 1992). In this study we measured stimulated efflux of NA in the nucleus interstitialis stria terminalis (NIST) in rats of different ages.

Experiments were conducted in 350 µm slices of NIST superfused with oxygenated artificial CSF at 32°C. A carbon fibre microelectrode was positioned at 80 µm depth in the NIST, immediately ventral to the anterior commissure. Bipolar tungsten stimulating electrodes were located 200 µm ventromedially. NA efflux was evoked using trains of 0.2 ms, 10 mA pulses, applied every 10 minutes and monitored using FCV at the following parameters: scan rate 480 V/s, scan range:-1.0 to +1.4 V vs Ag/AgCl, interscan interval: 500 ms. Three age groups (mean ± SEM) were compared: young (5 weeks, 130 ± 8.4g), adult (12 weeks, 307.5 ± 11.8g) and old rats (36 weeks, 575 ± 20.6g). The effect of train duration (20, 40, 60, 80 and 99 pulses at 100 Hz) and frequency of stimulation (50 pulses at 10, 20, 50, 100, 200 and 500 Hz) on NA efflux were examined. The effect of the α_2 antagonist yohimbine (10^{-6} M) was also investigated.

Significant differences were observed between the 3 groups, both in the level of stimulated NA efflux and the response to autoreceptor blockade. Firstly, NA efflux was significantly ($0.01<P<0.05$) greater in young than adult or old rats at *all* train durations and stimulus frequencies tested. Maximal observed NA efflux on the longest train (99 pulses, 100 Hz, 10 mA, 0.2 ms) was 423 ± 48 nM (Young), 135 ± 24 nM (Adult) and 155 ± 26 nM (Old). There were no significant differences between adult and old rats. Secondly, yohimbine (10^{-6} M) elevated NA efflux to a greater extent at lower than higher frequencies in all age groups. Yohimbine also potentiated NA efflux more in young and adult rats than in old animals (see Table 1). There were no significant differences between young and adult rats.

Table 1. Effect of yohimbine (10^{-6}M) on stimulated NA efflux in NIST (% of pre-drug, *P < 0.05, P < 0.01 vs Old group)

	10 Hz	20 Hz	50 Hz	100 Hz	200 Hz	500 Hz
Young	285 ± 45%*	309 ± 27%**	188 ± 19%*	139 ± 2%	118 ± 6%	109 ± 14%
Adult	291 ± 46%*	305 ± 48%*	181 ± 23%	137 ± 16%	131 ± 4%*	112 ± 15%
Old	175 ± 14%	168 ± 21%	132 ± 18%	114 ± 12%	90 ± 13%	86 ± 12%

The results provide evidence in support of age-related differences in both NA efflux and its control by α_2 adrenoceptors. There is a decrease in the stimulated efflux of NA between 5 and 12 weeks of age, possibly reflecting a maturation-dependent diminution of the size of the releasable NA pool. A decrease in the response to the α_2 antagonist yohimbine occurs between 12 and 36 weeks of age, possibly indicating loss or down-regulation of the autoreceptors. It is interesting that the decreases in NA efflux and in yohimbine response are not temporally linked. In conclusion, the results suggest that the younger rats have a greater NA efflux and higher autoreceptor activity.

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216P CONTROL OF NORADRENALINE EFFLUX IN THE RAT NUCLEUS INTERSITITIALIS STRIA TERMINALIS BY α_2 AUTORECEPTORS: *IN VITRO* VOLTAMMETRIC DATA

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Fast cyclic voltammetry (FCV) at carbon fibre microelectrodes has been used to monitor stimulated noradrenaline (NA) efflux in slices of the ventral part of the rat nucleus interstitialis stria terminalis (NIST; Palij & Stamford, 1992). In this study we investigated the effect of α_2 antagonists upon stimulated NA efflux.

FCV was used to monitor stimulated NA efflux in superfused slices of rat NIST as previously described (Palij & Stamford, 1992). Efflux was evoked with trains of 50 pulses (0.2 ms duration, 10 mA constant current) applied at 10 min intervals. Three stimulation cycles were applied, each comprising 6 trains at different frequencies (10, 20, 50, 100, 200 and 500 Hz). All drugs (10^{-6} M) were added to the superfusate after the first cycle.

For trains of 50 pulses, the maximum efflux (271 ± 92 nM) was produced by 100 Hz stimulation. Stimulation with 10, 20, 50, 200 and 500 Hz trains resulted in efflux that was 35.9 ± 4.0, 47.1 ± 4.2, 91.5 ± 2.7, 78.7 ± 3.6 and 33.1 ± 0.7 % of the 100 Hz efflux respectively (n=4). At the lower frequencies (10, 20 & 50 Hz), efflux was less than that at 100 Hz due to released NA activating presynaptic inhibitory autoreceptors. This was demonstrated by the increases in efflux observed with yohimbine and rauwolscine at these frequencies (Table 1). The reduction in stimulated efflux at frequencies above 100 Hz may be due to the inability of the terminals to release NA in response to each pulse when the inter-pulse interval is so short.

Table 1. Frequency dependence of the effect of α_2 antagonists upon stimulated NA efflux in the NIST.

	10 Hz	20 Hz	50 Hz	100 Hz	200 Hz	500 Hz
Control	101.1 ± 2.5	117.8 ± 9.2	101.1 ± 5.3	106.0 ± 4.7	109.5 ± 2.8	107.6 ± 4.4
Yohimbine	216.2 ± 57.6	391.7 ± 63.0*	146.7 ± 4.2**	98.0 ± 15.4	109.8 ± 22.9	117.5 ± 26.5
Rauwolscine	228.1 ± 19.0**	242.5 ± 7.0***	131.9 ± 8.2*	106.3 ± 4.4	103.4 ± 1.2	90.8 ± 5.0*
Prazosin	89.0 ± 8.3	99.3 ± 9.9	95.1 ± 11.7	74.9 ± 4.5**	77.9 ± 7.1*	64.4 ± 12.2

Mean third cycle NA efflux as a percentage of equivalent first cycle response ± s.e.m (n=4). **P<0.05, **P < 0.01,* vs control.****P < 0.001* (unpaired Student's t test).

Yohimbine and rauwolscine, the non-subtype selective α_2 antagonists, increased NA efflux on the lower frequency trains but not on 100, 200 and 500 Hz trains. Prazosin, the $\alpha_1/\alpha_{2B}/\alpha_{2C}$ antagonist, failed to increase efflux at any frequency. The increases produced by yohimbine and rauwolscine confirm that NA efflux evoked by 50 pulses at 10, 20 and 50 Hz is subject to α_2 receptor-mediated autoinhibition. The lack of effect of prazosin at low frequencies suggests that the autoreceptors are not of the α_{2B} or α_{2C} subtypes, in agreement with the study of Limberger et al. (1991) in rabbit occipito-parietal cortex. The decrease in NA efflux observed with prazosin at high frequencies merits further investigation.

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217P MONOAMINE EFFLUX EVOKED BY ELECTRICAL STIMULATION IN RAT BRAIN SLICES CONTAINING SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA

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Fast cyclic voltammetry (FCV) has been used to monitor electrically-evoked dopamine (DA) efflux in several dopaminergic terminal regions (Trout & Kruk, 1992) and 5-hydroxytryptamine (5-HT) efflux in both serotonergic terminal and somatodendritic regions (O'Connor & Kruk, 1991) in rat brain slices. Other techniques have demonstrated DA release from dendrites and/or neurones from regions containing somatodendritic dopaminergic elements (Cheramy *et al.*, 1981). A preliminary study has been made of the feasibility of detecting electrically-stimulated DA overflow in coronal rat brain slices incorporating the substantia nigra pars compacta (SN*c*), pars reticulata (SN*r*) and ventral tegmental area (VTA). The methods used have been described previously (Trout & Kruk, 1992). Efflux was monitored as faradaic current detected at the electrode, monitored at +600 mV on the driving waveform.

Efflux in response to a single electrical pulse (20 V, 0.1 ms), was rarely detectable in any of the regions. Trains of 20 such pulses applied between 10 and 500 Hz resulted in a frequency-dependent efflux profile: in all regions, efflux increased with frequency to a maximum (50 Hz in SN*c* and SN*r*; 100 Hz in VTA), and thereafter declined. The efflux maxima were (mean $\pm$ s.e.mean) 4.89 $\pm$ 0.50 (SN*c*, n = 10), 3.11 $\pm$ 0.56 (SN*r*, n = 9) and 7.01 $\pm$ 1.37 nA (VTA, n = 6). The small voltammetric signals obtained, although showing monoamine characteristics, did not permit the electrochemical identification of the released substance(s). The effects of drugs were assessed on efflux evoked by trains of 20 pulses applied at 50 Hz. Responses were reduced (approximately 70%) in the presence of 0.1 μM tetrodotoxin in all three regions. Nomifensine (1 μM) did not significantly increase evoked efflux in SN*c* (n = 8) or SN*r* (n = 4), whereas fluvoxamine (1 μM) significantly ($P < 0.05$) enhanced efflux in both nigral regions (SN*c* 230 $\pm$ 33% of control, n = 8; SN*r* 212 $\pm$ 35% of control, n = 6). In the VTA, neither nomifensine (n = 6) nor fluvoxamine (n = 5) significantly increased evoked efflux.

In conclusion, under the present experimental conditions, we have been unable to demonstrate DA efflux in any of the somatodendritic dopaminergic regions examined. The increase in voltammetric signal observed in the SN*c* and SN*r*, in the presence of fluvoxamine, suggests that at least part of the signal is a result of endogenous 5-HT efflux released from axon terminals arising from the dorsal raphe nucleus.

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218P A COMPARISON OF CUT AND SPARK-ETCHED ELECTRODES FOR FAST CYCLIC VOLTAMMETRY

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Fast cyclic voltammetry (FCV) has been used to study regulation of monoamine neurotransmitter overflow in vivo and in vitro (Millar, et al., 1985; O'Connor & Kruk, 1992). Carbon fibre electrodes are critical to the success of experiments, but there has been no systematic study comparing method of manufacture to electrochemical characteristics. Until recently, FCV electrodes have been trimmed to working length by mechanical fracture, a process with a high rate of attrition. We present data comparing charging and Faradaic current as a function of electrode length, and method of trimming.

25 carbon fibre electrodes were made as described by (Armstrong-James & Millar 1979). 10 were broken to length by compression fracture of the fibre using forceps, the others were electrically spark-etched to comparable length using a fine tungsten electrode connected to a high voltage source. The length of electrodes was estimated using a reticule on a light microscope. FCV was carried out in vitro using the method described by Millar & Barnet (1988), with Ag/AgCl reference electrode, in 0.1 Molar phosphate buffered 0.9% w/v NaCl solution, pH 7.4 (PBS). The applied potential consisted of 1.5 cycle, 100Hz triangular ramp scanning between -1.0 and + 1.4 V relative to the Ag/AgCl reference electrode at a voltage scan rate of 480Vs-1. The scan was applied at 2Hz and between scans the potential was maintained at 0 V. Signals were fed into a Nicolet 310 digital storage oscilloscope, and measurement made from hard copies of signals.

Charging current of cut and etched electrodes increased with electrode length. Faradaic currents for cut and spark-etched electrodes were comparable. In PBS containing 10^{-6}Molar dopamine, the oxidation peak potentials were 693.7mV $\pm$ 25.3(n=10; cut); 648.4mV $\pm$ 20.0 (n=15; etched). Reduction peak potentials were -335.0mV $\pm$ 21.6 (n=10; cut); -283.7mV $\pm$ 22.1 (n=15; etched); mean $\pm$ s e mean in each case. Spark etched electrodes showed less variance than cut electrodes when the Faradaic current in 10^{-6}Molar DA was measured: (40.7 $\pm$ 9.9;n=10; cut electrodes; 41.3 $\pm$ 4.8; n=15; etched electrodes).

The spark etching process is inherently more reproducible than compression fracture, and results in fewer electrodes being discarded during manufacture. The smaller variance in the Faradaic current may prove advantageous in quantitative studies.

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219P MULTIPLE ACTIONS OF NORADRENALINE ON SYMPATHETIC PREGANGLIONIC NEURONES *IN VITRO*

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The presence of noradrenaline (NA)-containing axon terminals surrounding sympathetic preganglionic neurones (SPN) suggests that NA may have a role in the regulation of SPN activity. We have therefore investigated the effects of NA on SPN in the spinal cord slice preparation. Intracellular and whole-cell recordings were made from SPN in neonate rat spinal cord slices as described previously (Spanswick and Logan, 1990; Pickering et al, 1991).

Superfusion of NA (5 to 50 μM) induced hyperpolarising responses (20μM for 10s; 8.5±1.1mV; 158 ±31s) in the majority of SPN tested (50/60). The response was concentration-dependent, enhanced in the presence of the NA uptake blocker xylamine (1-10μM) and was maintained in the presence of TTX (500nM) suggesting a direct action on the cell. The inhibition was associated with a reduction in neuronal input resistance, was reduced in amplitude with progressive membrane hyperpolarisation and was sensitive to changes in extracellular potassium. A comparison of the effects of some α–adrenoceptor agonists revealed an order of potency for induction of inhibition of SPN: UK-14304 > Adrenaline > NA > Phenylephrine. Inhibitory responses to NA were blocked by the α2-adrenoceptor antagonist yohimbine (200 nM-1μM). These were also reduced by forskolin (50-100μM) and could be blocked by inclusion of GDP-β–S (400μM) in the patch-pipette solution.

NA-induced excitation in SPN (n=10), persisted in the presence of TTX (500nM) and was characterised by depolarisation associated with an increase in neuronal input resistance. The order of agonist potency for induction of excitation of SPN was phenylephrine > adrenaline > NA. In some SPN a biphasic response to NA was apparent, characterised by inhibition followed by excitation. This excitation consisted of the discharge of small EPSPs towards the offset of the inhibitory response. The EPSPs were blocked by the glutamate receptor antagonist CNQX (10μM).

A long-lasting afterhyperpolarisation potential (AHP) can be evoked in SPN following a train of spikes. This AHP is sensitive to both the potassium channel blocker TEA and the calcium channel blocker cobalt, suggesting the involvement of a calcium-activated potassium conductance (Spanswick and Logan, 1990). Superfusion of NA (10-100μM) reduced the amplitude and duration of the AHP and could give rise to an afterdepolarisation potential (ADP).

We conclude that NA inhibits some SPN by activating α2-adrenoceptors. The receptor is coupled to a G-protein and reduces cAMP levels resulting in the enhancement of a potassium current. NA also excites some SPN via an action at α1-adrenoceptors and modulates a calcium-activated potassium conductance. NA indirectly excites some SPN presumably via an action upon an excitatory interneurone, which in turn releases glutamate onto SPN.

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220P LITHIUM-SENSITIVE ACCUMULATION OF A NOVEL INOSITOL PHOSPHATE PRODUCT OF PHOSPHOINOSITIDE TURNOVER IN RAT CEREBRAL CORTEX SLICES

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Hydrolysis of phosphatidylinositol 4,5-bisphosphate by agonist-stimulated phospholipase C (PLC) produces $Ins(1,4,5)P_3$ which is rapidly metabolized by two enzymic routes to either a further putative second messenger $Ins(1,3,4,5)P_4$ or an inactive $Ins(1,4)P_2$. Further metabolism proceeds via a well-established sequence of phosphatases to generate myo-inositol by the action of Li^+-sensitive inositol monophosphatase. We have recently shown that carbachol (CCH) stimulates accumulation of a novel inositol bisphosphate ($Ins(4,5)P_2$) in cerebral cortex slices in the presence of Li^+ (Jenkinson et al., 1992). Here we report that $Ins(4,5)P_2$ accumulates in response to stimulation of PLC by a variety of agents and the magnitude of the accumulation appears to be related directly to the extent of phosphoinositide hydrolysis.

Rat cerebral cortex slices (350 x 350 μm) were pre-incubated for 60 min at 37°C in Krebs-Henseleit buffer equilibrated with O_2/CO_2 (19:1). Packed slices (50 μl) in a final volume of 300 μl were incubated in the presence of [^{3}H]inositol for 60 min (Kennedy et al., 1990). Slices were then challenged with agonist (as indicated below) and incubated for 20 min. Incubations were terminated by addition of $HClO_4$ (300 μl, 10% v/v) and supernatants neutralized by addition of freon/octylamine. [^{3}H]inositol phosphates were separated by h.p.l.c. as described previously (Batty et al., 1989).

Four inositol bisphosphates ($Ins(1,4)P_2$, $Ins(1,3)P_2$, $Ins(3,4)P_2$ and $Ins(4,5)P_2$) were resolved in extracts of CCH-stimulated (1 mM) cerebral cortex slices. Although the latter bisphosphate was a minor late-running h.p.l.c. peak (basal: 76 ± 12 d.p.m. ; +CCH: 1365 ± 81 d.p.m.), its accumulation was dramatically enhanced in the presence of 1 mM Li^+ (+CCH/Li^+: 10338 ± 1123 d.p.m.). Concentration-response experiments have demonstrated that agonist-stimulated $Ins(4,5)P_2$ accumulation is extremely sensitive to enhancement by Li^+ (EC_{50} 94 ± 3 μM), implicating a Li^+ inhibited degradative enzyme involved in its metabolism. A range of agents known to increase phosphoinositide turnover in cerebral cortex also caused small increases in $Ins(4,5)P_2$ levels in the absence of Li^+ and much more dramatic accumulations in the presence of 1 mM Li^+ (fold increase over basal: CCH (1 mM) 140; quisqualate (10 μM) 87; noradrenaline (100 μM) 15; 5-HT (100 μM) 15; KCl (30 mM) 30). The enhancement of $Ins(4,5)P_2$ accumulation in the presence of Li^+ caused by these agents correlated closely with their respective abilities to stimulate total [^{3}H]$InsP_1$ accumulation.

We conclude that $Ins(4,5)P_2$ is generated in cerebral cortex slices in response to stimulation of a variety of PLC-linked receptors. Thus, $Ins(4,5)P_2$ is a novel inositol phosphate product which appears to be produced in proportion to the degree of agonist-induced PLC activation.

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221P THE EVALUATION OF L-CHIRO INOSITOL TRISPHOSPHATE ANALOGUES AS SELECTIVE INOSITOL 1,4,5,-TRISPHOSPHATE 5-PHOSPHATASE INHIBITORS

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D-myo-inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) is a second messenger that mobilises Ca^{2+} from intracellular stores, and mediates some of the actions of a variety of cell-surface receptors. Its effects are, however, relatively short lived, as metabolism by dephosphorylation, catalysed by $Ins(1,4,5)P_3$ 5-phosphatase, or phosphorylation, catalysed by $Ins(1,4,5)P_3$ 3-kinase, remove the second messenger signal and thus serve as potential targets for pharmacological intervention. We describe the properties of four novel synthetic analogues, including L-chiro-inositol 1,4,6-trisphosphorothioate (L-ch-1,4,6 $InsPS_3$) which is a potent and selective inhibitor of the former enzyme.

Experiments investigating Ca^{2+} mobilisation from $Ins(1,4,5)P_3$-sensitive pools were performed as described (Safrany et al., 1991). Briefly, SH-SY5Y human neuroblastoma cells were electrically permeabilised in a cytosol-like buffer and were loaded with $^{45}Ca^{2+}$ for 12-15 minutes before being challenged with $Ins(1,4,5)P_3$ or its analogues. Reactions were stopped after 2 minutes. $Ins(1,4,5)P_3$ mobilised $^{45}Ca^{2+}$ potently (EC_{50} = 0.12μM), L-chiro-inositol 2,3,5-trisphosphate (L-ch-2,3,5 $InsP_3$) and L-chiro-inositol 2,3,5-trisphosphorothioate (L-ch-2,3,5 $InsPS_3$) also mobilised $^{45}Ca^{2+}$ but less potently (EC_{50}=1.4μM and 4.9μM respectively). L-chiro-inositol 1,4,6-trisphosphate (L-ch-1,4,6 $InsP_3$) and L-ch-1,4,6 $InsPS_3$ did not mobilise $^{45}Ca^{2+}$ and did not inhibit $Ins(1,4,5)P_3$-induced $^{45}Ca^{2+}$ mobilisation (<30μM).

L-ch-2,3,5 $InsP_3$ and L-ch-2,3,5 $InsPS_3$ were also found to interact with $Ins(1,4,5)P_3$ 3-kinase, inhibiting $Ins(1,4,5)P_3$ phosphorylation competitively when incubated with a supernatant from rat brain homogenate in the presence of ATP (see Safrany et al., 1991) with K_i values of 0.97μM and 0.82μM respectively. L-ch-1,4,6 $InsP_3$ and L-ch-1,4,6 $InsPS_3$ were without effect at concentrations below 250μM and 30μM respectively.

All four analogues were found to be resistant to hydrolysis when incubated with a human erythrocyte ghost $Ins(1,4,5)P_3$ 5-phosphatase preparation, liberating no inorganic phosphate. It was found, however, that all inhibited the dephosphorylation of $Ins(1,4,5)P_3$. L-ch-2,3,5 $InsP_3$ and L-ch-1,4,6 $InsP_3$ inhibited with K_i's of 7.7μM and 44μM. The introduction of phosphorothioate groups, as discussed previously (Safrany et al., 1991), appears to increase the affinity of $Ins(1,4,5)P_3$ 5-phosphatase for the analogues, L-ch-2,3,5 $InsPS_3$ and L-ch-1,4,6 $InsPS_3$ inhibiting dephosphorylation with K_i's of 0.21μM and 0.3μM.

These data suggest that L-ch-1,4,6 $InsPS_3$ is a novel, selective and highly potent inhibitor of $Ins(1,4,5)P_3$ 5-phosphatase.

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222P C-*fos* IMMUNOCYTOCHEMISTRY IDENTIFIES BRAINSTEM NEURONES INVOLVED IN THE EMETIC ACTION OF LOPERAMIDE IN THE FERRET

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Loperamide-induced emesis in the ferret has recently been described (Bhandari et al., 1992). Loperamide has activity at μ and ∂ opiate receptors (Giagnoni et al., 1983) and its emetic effects are antagonized by naloxonazine, an opiate receptor antagonist with some selectivity for μ receptors (Bhandari et al., 1992). C-*fos* immunocytochemistry is a new technique for identifying metabolically active neuronal pathways with cellular resolution, making it ideally suited for studies on the small nuclei involved with the control of emesis in the caudal brainstem (Reynolds et al., 1991). We have used it to examine the brainstem nuclei mediating loperamide-induced emesis in the ferret.

Adult ferrets were given s.c. injections of saline or naloxonazine (1mg/kg) followed 30min later by saline or loperamide 0.5mg/kg. All animals were observed for emesis for a further 135min and then killed with i.p. sodium pentobarbitone and fixed with 4% paraformaldehyde. The brainstems were sectioned and processed for c-*fos* immunocytochemistry.

No saline treated controls vomited and levels of Fos-like immunoreactivity (FLI) in these animals were low or undetectable (n=4). Animals which received saline followed by loperamide all vomited (n=6) and showed dense FLI in the dorsomedial subnucleus (dmNTS) of the nucleus tractus solitarii (NTS) and much lower levels in the area postrema (AP) and nucleus ambiguus. Pretreatment with naloxonazine prevented loperamide-induced emesis (n=4) and markedly reduced FLI in the brainstem. Four animals which underwent cervical nodosectomy 2 weeks earlier were treated with saline followed by loperamide and all vomited. The vagal lesion did not affect FLI.

Loperamide induces FLI in a highly specific distribution in the dmNTS which is inhibited by naloxonazine but unaffected by cervical vagotomy. It is interesting that almost no FLI is seen in the AP which has been proposed as the site of action of loperamide in causing emesis (Bhandari et al., 1992). FLI identifies cell bodies, so it is possible that the FLI in the dmNTS reflects loperamide activation of receptors located on dendrites of dmNTS neurones which project to the AP.

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223P EFFECT OF THYROTOPHIN-RELEASING HORMONE ON HIGH-AFFINITY CHOLINE UPTAKE IN RAT BRAIN SYNAPTONEUROSOMES

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Cholinergic nerve terminals are characterised by having a sodium-dependent high-affinity choline uptake (SDHACU) mechanism (Kuhar *et al.*, 1973) which is rate limiting for ACh synthesis and indicative of cholinergic neuronal activity. Thyrotrophin-releasing hormone (TRH) enhances central cholinergic systems which have been implicated in cognition and previous evidence (Watson *et al.*, 1990, 1991) indicates that cholinergic antagonists produce learning deficits in rats accompanied by co- and post-training changes in endogenous TRH levels. This study examines the effect of pre-incubation with TRH and TRH analogues on SDHACU on validated rat brain synaptosomes prepared from frontal cortex/hippocampus.

Male Hooded Lister rats (300-500g) were used to prepare combined frontal cortical/hippocampal synaptosomes (Durkin *et al.*, 1981) in 0.32M sucrose. Supernatant aliquots of tissue were diluted in Krebs Tris HCl and incubated with 7.2 to 240 nM [^{3}H] methyl choline chloride (^{3}H-MC) or 'cold' choline (1 mM) at 37°C for 4 min and pre-incubated with either hemicholinium-3 (1, 10 or 100 µM), TRH (10, 100 or 1000 µM) or TRH analogues (3,3'-dimethyl TRH, RX 77368 or L-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-carboxamide, MK 771; 1mM) or following *ex vivo* pretreatment (20 min) with scopolamine (5 mg kg^{-1} i.p.) or saline (0.1 ml kg^{-1}, n = 5 each). Choline uptake was terminated on ice and tissue pellets washed in saline, solubilised in Protosol (50 µl NENC) and tritium content determined by liquid scintillation counting. Non-specific choline uptake was determined with excess cold choline (100 mM) and values converted to molar units per protein content. Metabolic integrity of the synaptosomes was evaluated by estimates of the cytosolic enzyme lactate dehydrogenase, oxygen uptake (electrode) and EM studies, and uptake kinetics were validated by determining the time, temperature, sodium ion and choline concentration parameters for optimal uptake.

The choline uptake inhibitor, hemicholinium-3 demonstrated linear, dose-dependent, competitive inhibition following Schild plot analysis (K_i = 7.5 x 10^{-5} m) of the SDHACU (K_m 1.3 µM; V_{max} 0.47 pmol min^{-1} mg^{-1} protein) in mixed frontal cortex/hippocampus synaptosomes. Pretreatment (-20 min) of the rats with the cholinergic antagonist, scopolamine (5 mg kg^{-1} i.p.) resulted in (the anticipated) enhanced SDHACU which was significantly increased (F = 359.3 P<0.001 ANOVA) with increasing concentrations of ^{3}H-MC. *In vitro* pretreatment (2 min) with TRH resulted in a significant dose-dependent inhibition of SDHACU at 10 (p<0.05, following ANOVA F = 42.6, p<0.001), 100 (p<0.01) and 1000 µM (p<0.005) from a mean ± s.e.mean control SDHACU of 0.010 ± 0.003 (at 7.2nM ^{3}H-MC) and 0.304 ± 0.007 pmol 4min^{-1} mg^{-1} protein (at 240 nM ^{3}H-MC). In contrast, the TRH analogues (RX 77368 and MK 771) were without effect on SDHACU at similar concentrations and conditions.

These results suggest that one mechanism by which endogenous TRH alters the release and turnover of ACh from CNS neurones is via modulation of choline uptake.

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224P ANGIOTENSIN II RECEPTOR SUBTYPES ON BOVINE CEREBELLUM: CHARACTERISATION BY AGONISTS, ANTAGONISTS AND DITHIOTHREITOL

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Angiotensin II receptors have been described in bovine cerebellum . This preparation has been characterised as containing only the AT2 subtype (Bottari et al. 1991) or a mixture of both AT1 and AT2 subtypes, the latter being predominant (Speth, 1991). In the present study, we have further characterised the AT II receptor populations present in cerebellar microsomes, prepared from fresh (BC-Fresh) and frozen (BC-Frozen) bovine brain (-80°C, ≤ 1 week).Competition experiments (25°C, 4 hours) using ^{125}I-[Sar^1, Ile^8]ATII as the ligand, were performed using agonists : Angiotensin III (ATIII) , [pNH$_2$ Phe^6] ATII (AT2 selective) and the antagonists [Sar^1,Ile^8]ATII, DUP 753 (AT1 selective) and PD 123319 (AT2 selective). Displacement curves were constructed in the absence and presence of Dithiothreitol (DTT;10 mM), as an inhibitor of the AT II binding to AT1 subtype.

As shown in Table 1, in BC-F and BC-FR , AT III and [p-NH$_2$Phe^6]ATII yielded biphasic curves identifying both high and low affinity sites.The lower affinity site was abolished in presence of DTT. The AT2 selective PD 123319, and the non-selective [Sar^1,Ile^8]ATII antagonists yielded monophasic displacement curves, apparently binding to only a high affinity site, unaffected by DTT. In the BC-Frozen, competition curves showed DUP 753 binds only to a low affinity site, whereas, in the BC-Fresh, DUP 753 was best fitted with a three-site model (F=5.7). In the presence of DTT, only a low affinity site was recognized .

TABLE 1

Agonists / Antagonists	BC.Frozen Ki H / Ki L (nM)	BC.Frozen Bmax (%)	BC.Frozen Ki-DTT* (nM)	BC.Fresh Ki H / Ki L (nM)	BC.Fresh Bmax (%)	BC.Fresh Ki - DTT* (nM)
AT III	0.079 / 3.6	89 / 11	0.06	0.07 / 8.1	70 / 30	0.04
[pNH2 Phe6] AT II	5.8 / 5420	87 / 13	8.9	3.5 / 4822	61 / 39	7.1
[Sar1-Ile8]-AT II	0.081	100	0.021	0.11	100	0.09
PD 123319	26.0	100	Not Tested	16.0	100	7.0
DUP 753	112,000	100	102,000	0.06/192/104,000	18/20/62	116,000

Results are Ki values calculated with LIGAND. Ki-H: High, Ki-L: Low affinity binding site. Bmax is expressed as % of the total binding. Ki-DTT, Ki value calculated from the displacement curves in presence of DTT (10 mM).

We conclude that bovine cerebellum contains predominantly the AT2 subtype and that the small proportion of AT1-DUP 753 sensitive component was lost upon freezing. ATIII and [pNH$_2$Phe^6]ATII recognize two sites in BC-Frozen, including the low affinity binding site sensitive to DTT. If we assume DTT is selective for ***only*** the AT1 subtype, it would suggest a possible heterogeneity of the AT1 receptor population. On BC-Fresh, the analysis of DUP 753 displacement curves have shown that two high affinity sites, both DTT sensitive, can be recognized by the AT1-selective antagonist. This is not readily explained on the basis that the AT1 subtype exist in different affinity states since the antagonist [Sar^{1}Ile^8]AT II does not differentiate between the two affinity states.Taken together these results support the concept of multiple subtypes for AT1 receptor.

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225P CAPSAICIN DESENSITIZATION OF PERIPHERAL NOCICEPTIVE FIBRES IMPAIRS SENSITIVITY TO ATP

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The modulation of noxious sensory information in the periphery may involve adenosine triphosphate (ATP), since application of ATP to a blister base causes pain. This preliminary study investigated the properties of primary afferent neurones in the neonate rat to determine the contribution of ATP to modulation.

The spinal cord with functionally attached tail was isolated from 1 or 2 day old Wistar rats and the superficial layers of skin carefully removed. The spinal cord and tail were separately superfused with an oxygenated artificial cerebrospinal fluid at 24°C, the spinal cord at a rate of 3.5ml/min and tail at 6ml/min. The activation of peripheral nerve fibres was measured by recording spinal ventral root depolarization in the lumbar region, L_3-L_5, using an extracellular glass micropipette (Dray et al., 1990).

Tissue responsiveness varied between preparations. The depolarization in response to a noxious thermal stimulus was taken both as a measure of tissue viability and as a control parameter against which subsequent depolarizations could be measured. Noxious thermal stimuli, applied to the tail manually from a 2ml syringe at 20 min intervals, produced ventral root depolarization, which were reproducible at 45°C. An interesting feature of the temperature response was that the application of a 50°C stimulus to the tail elicited gradually increasing ventral root responses, suggesting a process of thermal "wind-up" or of nociceptor sensitization. The origin of this phenomenon, be it peripheral or central, has not been investigated in this study.

Peripheral nerve fibres were also activated by ATP. ATP, superfused for 10sec at a known concentration, produced a concentration-dependent depolarization of the ventral root with an EC_{50} of 15.5μM, obtained from pooled data (n=3 to 15). In contrast, adenosine did not elicit ventral root depolarization (n=3), suggesting that the ATP response was not mediated via P_1 purinoceptors and was not, therefore, due to the breakdown of ATP to adenosine.

The tails also responded to the noxious chemical agent, capsaicin. 0.01 to 10μM capsaicin elicited ventral root depolarization. However, when desensitization to capsaicin was induced by the continuous superfusion of 1μM capsaicin for 5 min, thermal sensitivity was maintained while sensitivity to ATP was abolished (n=3). This is in contrast to the results observed by Dray et al. (1989), who suggested that capsaicin desensitization of peripheral nociceptive fibres does not impair sensitivity to other noxious stimuli. Our results suggest that the mechanisms involved in the desensitization of capsaicin receptors may be shared by P_2 purinoceptors.

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226P THE EFFECT OF HISTAMINE RECEPTOR AGONISTS AND ANTAGONISTS ON RESTRAINT-INDUCED ANTINOCICEPTION IN MICE

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It is well established that stress can induce antinociceptive activities in experimental animals. The characteristics of stress-induced antinociception depend upon the type and duration of the stress and on the method by which antinociception is assessed (Kelly, 1982). There is evidence suggesting histamine may act as a central neurotransmitter and that its turnover was altered under physiological stress (Taylor and Synder, 1971). Furthermore, histamine has been shown to be involved in animals' responses to noxious stimulus (Chung, et al, 1984). The present work was undertaken to study the effects of histamine H_1 and H_2 receptor agonists and antagonists on restraint-induced antinociceptive activity in mice.

Male CFLP mice weighing between 20-25g were used. The animals were restrained individually in a cylindrical tube with holes along the whole length for ventilation. Each animal would have only a limited degree of movement inside the tube. The animals were restrained for one hour then tested for latency response to hot plate test at 55°C, at 0, 10, 20, 40 and 60 min post-restraint. The histamine receptor agonists and antagonists were given s.c. 15 min before restraint. Mean hot plate response times were analyzed and compared with the analysis of variance.

Restraint for one hour induced significant antinociceptive activity in mice (10.78 ± 0.85s versus 4.48 ± 0.36s in controls). The antinociceptive activity was significant throughout the one hour period of observation. Prior administration of the histamine H_2 receptor agonist dimaprit (1.5 - 6.0 mg kg^{-1}) further enhanced the antinociceptive activity. Peak antinociceptive activity was 13.56 ± 0.94s versus 10.78 ± 0.85s in saline restraint control ($P<0.01$). Furthermore, the induction of antinociception was antagonised by prior administration of cimetidine (2.5 - 10.0 mg kg^{-1}), a histamine H_2 receptor blocker. The peak antinociceptive activity was reduced to 8.08 ± 0.76s ($P<0.05$). Prior administration of 2-pyridylethylamine (2.0 - 8.0 mg kg^{-1}), a histamine H_1 receptor agonist, or mepyramine (5.0 - 20.0 mg kg^{-1}), a histamine H_1 receptor blocker, did not affect the development of antinociceptive activity induced by restraint. These results suggest that histamine H_2 receptors may be involved in the restraint induced antinociception in mice.

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227P EFFECTS OF THE SUBSTANCE P ANTAGONIST, RP 67580, ON NEURONAL ACTIVITY IN THE RAT LOCUS COERULEUS

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Substance P (SP) is known to excite neurones in a number of brain regions including the locus coeruleus (LC) (Guyenet & Aghajanian, 1977). In addition, morphological studies have revealed SP-reactive fibres in this region of the brain (Pammer *et al.*, 1990). We have used this model to study the effects of the non-peptide perhydroisoindolone SP antagonist, RP 67580, which binds potently and selectively to NK_1 receptors (Garret *et al.*, 1991).

Neuronal activity of identified LC neurones was recorded with extracellular multibarrelled electrodes in rats anaesthetized with chloral hydrate using conventional electrophysiological methods. Substance P, glutamate and acetylcholine were applied to the recorded neurone by micriointophoresis. Substance P antagonists were administered by i.p. injection. The recording sites were confirmed histologically.

RP 67580 depressed the LC neuronal excitation in response to iontophoretically applied SP at doses between 2-4 $mg.kg^{-1}$ i.p.. No concomitant depression of either glutamate- or acetylcholine-evoked activity was seen at these doses. Partial recovery of the depression was seen in most studies. In contrast, the diastereoisomer, RP 68651, had no effect on SP-evoked activity of these neurones at doses up to 8.0 $mg.kg^{-1}$ i.p..

These preliminary studies suggest that RP 67580 has central effects *in vivo*, although at relatively high doses. The bio-availability of the compound and its central sites of action have yet to be fully evaluated. RP 67580, should, however, prove invaluable in studying SP-mediated synaptic transmission.

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228P PHARMACOLOGY OF NEUROKININ RECEPTORS ON NEURONES WITHIN BRAIN SLICES OF THE GUINEA-PIG LOCUS COERULEUS

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This study examines the pharmacology of neurokinin receptors on neurones in brain slices of the guinea-pig locus coeruleus, a brain region which has been implicated in the modulation of noxious sensory input from the spinal cord. Intracellular and extracellular recordings were made from >95 neurones within locus coeruleus brain slices using methods as described for the rat (Henderson *et al.* 1982). The majority of cells were spontaneously active, with a mean firing rate of 1.1 ± 0.2 Hz and membrane potential of –56 ± 3 mV. In 24/33 cells, to which both the NK1 selective agonist substance P methyl ester (SPOMe) and the NK3 selective agonist senktide were applied (1 μM), a depolarisation–mediated increase in action potential firing rate was observed. Of the remaining cells, 18% were selectively activated by senktide, and 9% responded only to SPOMe. The rank order of agonist potency was as follows (mean ± SEM, n = 3 to 5 cells):

	EC_{50} (nM)	Hill Slope	Relative Maximum
senktide	5 ± 1	1.2 ± 0.1	0.67
substance P	70 ± 24	1.0 ± 0.2	0.52
neurokinin B	167 ± 107	0.4 ± 0.1	0.72
neurokinin A	1176 ± 54	0.6 ± 0.1	1.00

Excitations caused by the NK1 selective agonist SPOMe (EC_{50} = 92 ± 15 nM, n = 16), were antagonised by the non–peptide quinuclidine antagonist (±)–CP–96,345 (Snider *et al.* 1991) with a K_B of 34 ± 17 nM (n = 4), but not by (±)–RP–67580 (Garret *et al.* 1991) or the peptide L–668,169 (Williams *et al.* 1988; both 1 μM, n = 5). These data suggest that both NK1 and NK3 receptors are present on most, but not all, cells within the locus coeruleus of guinea–pig brain. Moreover, the NK1 receptors in this brain region are functionally different from NK1 receptors described in other guinea–pig tissues in that they have low affinity for (±)–CP–96,345 and are insensitive to micromolar concentrations of (±)–RP–67580 and L–668,169.

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229P [^{3}H]PD140376 BINDING TO GUINEA-PIG GASTRIN RECEPTORS

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[^{3}H]PD140376 is a novel, antagonist radioligand which binds with high affinity to CCK-B receptors in guinea pig cortex (Suman-Chauhan, this meeting). In order to determine whether CCK-B receptors in the cerebral cortex and gastrin receptors in the fundic mucosa could be differentiated, we compared binding affinities for a range of agonists and antagonists using [^{3}H]PD140376 and membranes prepared from the two tissues.

Guinea pigs were killed and the gastric mucosa was scraped from the stomach fundus. Tissue was homogenised in 20 vols ice cold buffer (20mM Hepes, 1mM EGTA 0.01% bacitracin) and centrifuged at 250g for 3 mins.. The surface layer of mucous was removed and the tissue recentrifuged twice at 40,000g for 15 mins. in fresh buffer. For competition studies, membranes (8 mg/ml wet weight) were incubated for 40 mins at 22°C in assay buffer (mM composition: Hepes 10, NaCl 130, KCl 4.7, $MgCl_2$ 5, EGTA 1, and 0.25mg/ml bacitracin, pH 7.4 at 22°C) with 0.2-0.4 nM [^{3}H]PD140376 in the presence of varying concentrations of test compounds. Non-specific binding was defined by 1μM CCK8s in all experiments. In studies to investigate the effects of guanyl nucleotides on the binding affinity of test compounds, 30μM GppNHp (a stable analogue of GTP) was included in the assay. Reactions were stopped by filtration through GF/B filters.

Under the conditions described, total binding of [^{3}H]PD140376 to guinea pig gastric membranes was 2000-2500 dpm, 50-60% of which was specifically displaced by 1μM CCK8s. Saturation data showed high affinity binding to a single population of non-interacting sites with a K_D of 0.12 ± 0.01 nM and Bmax of 296 ± 44 fmol/mg protein. Affinities (Ki's) obtained for the CCK-B ligands CCK8s, CCK8us, pentagastrin, gastrin$_{1\text{-}17_{us}}$, CI-988, L365260 and Devazepide (CCK-A selective) were 0.18, 8.4, 2.0, 3.2, 0.82, 2.0 and 44 nM respectively and Hill slopes for the agonists were significantly less than unity (-0.65 to -0.82). These affinities were up to 10 fold higher than values obtained in the cortex (Suman-Chauhan, this meeting) though the rank order of potency was similar. This variation may result from differences in receptor coupling as in the presence of 30μM GppNHp, a clear 7-8 fold decrease in agonist affinities was observed in the gastric membranes, together with an increase in the Hill slopes to unity, while the affinities (and Hill slopes) of the antagonists CI-988 and L365260 were unaltered. Similar experiments using homogenates of guinea-pig cortex gave only small (approximately 2-fold) though consistent decreases in the affinity of the agonists and increases in Hill slope values.

[^{3}H]PD140376 selectively binds with high affinity to CCK-B/gastrin receptor in the guinea pig stomach fundic mucosa. These receptors show a similar binding profile to central CCK-B receptors though the greater effect of GppNHp in gastric tissue indicates a stronger coupling of this receptor to a GTP binding protein.

230P SELECTIVE EFFECTS OF THYMIC PEPTIDES ON THE RELEASE OF PITUITARY HORMONES BY RAT ADENOHYPOPHYSIAL TISSUE *IN VITRO*

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Previous studies in this laboratory and elsewhere have indicated a role for hormones of the anterior pituitary gland in the control of the endocrine function of the thymus and, for example, both corticotrophin (ACTH, Buckingham et al, 1992) and prolactin (Dardenne et al, 1989) have been shown to initiate the release of thymulin, a nonapeptide from thymic tissue in vivo and in vitro. This study was undertaken to investigate the reciprocal actions of two well characterised thymic peptides (thymulin, thymosin α1) on the resting and neurochemically evoked secretion of ACTH, prolactin and luteinising hormone (LH) by the rat anterior pituitary gland in vitro. Pituitary tissue was collected post mortem from young, adult male CFY rats (≈ 200g body weight), incubated in conditions described previously (Hadley et al, 1990) and exposed to graded concentrations of the thymic peptides in the presence and absence of a sub-maximal concentration of hypothalamic extracts (HE 0.1/ml). Peptides released into the medium were measured by radioimmunoassay; in some instances the immunoreactive cyclic AMP (cAMP) content of the tissue was also determined.
Thymulin (0.8-40pM) stimulated basal ACTH release (0.8-4pM, $P<0.05$, n=6; 8-40pM, $P<0.01$, n=6, Duncan's test) and in concentrations of 4pM and above potentiated the release of the peptide evoked by HE ($P<0.05$, n=6). In addition, it produced a small but significant rise in the resting but not the neurochemically evoked LH release ($P<0.05$, n=6) and (0.8-40pM) inhibited both the resting ($P<0.01$, n=6) and HE induced release of prolactin ($P<0.05$, n=6). By contrast, thymosin α1 inhibited resting ACTH release (10-100pM, $P<0.01$, n=6), raised basal prolactin secretion (10-100nM, $P<0.05$, n=6) but had no effect on the HE-induced release of either hormone. It also stimulated the basal release of LH (1-100nM, $P<0.05$, n=6) and potentiated markedly the LH response to HE (10pM-100nM, $P<0.01$, n=6). Furthermore, both thymulin (40pM) and thymosin α1 (100nM) produced small but significant rises in the tissue content of cAMP ($P<0.05$, n=6) which were unaffected by denbufylline (10μM), a type IV (cyclic GMP independent, calcium independent, cAMP specific) phosphodiesterase inhibitor which readily potentiates the increases in tissue content of the nucleotide induced by releasing factors of hypothalamic origin (Hadley et al, 1991). These results suggest that thymulin and thymosin α1 exert selective effects on pituitary hormone release in vitro which may be mediated, at least in part, by increases in the intracellular accumulation of cAMP.

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231P PERIPHERAL AND CENTRAL EFFECTS OF DENBUFYLLINE ON NEUROENDOCRINE FUNCTION IN THE RAT

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Previous studies in this laboratory have shown that the alkylxanthine, denbufylline (a selective inhibitor of type IV phosphodiesterase) stimulates the secretion of corticotrophin (ACTH) and corticosterone but reduces prolactin when given i. p. in the rat. It is not yet known whether these effects reflect actions of the drug on the pituitary or whether they are secondary to changes in the release of the hypothalamic hormones which regulate anterior pituitary function. In the present study, we have compared the effects of central and peripheral injections of denbufylline on pituitary function in vivo and examined the effects of the xanthine on the release of pituitary peptides in vitro using methods described elsewhere (Hadley et al, 1990). Radioimmunoassay (luteinising hormone (LH), thyrotrophin (TSH), prolactin, ACTH, corticosterone) and bioassay (ACTH) were used for hormone determinations.

Given peripherally, denbufylline (0.07-0.6mg/kg i.p., 1ml/kg) caused, within 20 minutes, significant ($p<0.05$, n=6) increases in the serum concentrations of immunoreactive luteinising hormone (ir-LH) and ir-corticosterone; serum ir-TSH was unaffected by the drug treatment but in doses of 0.1 and 0.2 mg/kg the xanthine reduced the serum prolactin concentration ($p<0.05$, n=6).

The hormonal profiles obtained when denbufylline (0.04-25μg/kg) was injected into the third ventricle of conscious rats, via an indwelling intracerebroventricular (i.c.v.) cannulae (placed under pentobarbitone anaesthesia), were rather different. Thus, although denbufylline (0.1 and 0.2μg/kg in a volume of 3μl) caused a significant ($p<0.05$, n=5) increase in plasma ACTH and serum ir-corticosterone, it failed to influence ir-LH release and caused a significant ($p<0.05$, n=4/5) increase in the secretion of both ir-TSH (0.05μg/kg) and ir-prolactin (0.2 and 1μg/kg).

In vitro, denbufylline (10μM-1mM) stimulated ($p<0.05$, n=6) the basal release of ir-ACTH and ir-LH but inhibited the release of ir-prolactin ($p<0.05$, n=6). In these and lower concentrations it also potentiated the ir-ACTH and ir-LH responses to a submaximal dose (0.1 HE/ml) of hypothalamic extract ($p<0.01$, n=6) and inhibited the prolactin responses to HE ($p<0.01$, n=6). Inhibitory effects of denbufylline on stimulated release of ir-TSH were also apparent ($p<0.05$, n=6).

The results suggest the hormonal responses to peripheral injections of denbufylline are exerted predominantly at the pituitary level. However, the xanthine may also exert regulatory actions within the hypothalamus which, with regard to prolactin, are not necessarily complementary to the pituitary actions.

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232P NITRIC OXIDE MEDIATES NMDA-STIMULATION OF α-MSH RELEASE FROM RAT HYPOTHALAMUS

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α-Melanocyte-stimulating hormone (α-MSH) synthesised by perikaria in the arcuate nucleus of the hypothalamus is postulated to be a neurotransmitter or neuromodulator. We have demonstrated that the glutamate agonist, N-methyl-D-aspartate (NMDA), stimulates release of α-MSH from rat hypothalamic slices (Wayman & Wilson, 1990) and that changes in levels of endogenous glutamate also modify peptide release (Wayman & Wilson, 1991). In this study we have investigated the role of nitric oxide in mediating NMDA-stimulation of α-MSH release by use of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, Rees *et al.*, 1990), its inactive D-enantiomer D-NAME, and the nitric oxide precursor, L-arginine.

Coronal 250μm thick slices of hypothalamus obtained from 150-200g male Wistar rats were superfused with oxygenated artificial cerebrospinal fluid (aCSF) at 37°C and 0.5ml/min under an atmosphere of humidified 95% O_2/5% CO_2. Two min samples of superfusate were collected commencing 30 min after the start of superfusion, frozen immediately and freeze-dried at -25°C. The dried samples were reconstituted and their content of α-MSH determined by radioimmunoassay (Wilson & Morgan, 1979). The responses to drug treatments were investigated by comparing the total release of α-MSH over a 10 min period following the administration of a 4 min pulse of 10^{-4}M NMDA in the absence and then the presence of the test agent. Responses to the second pulse of NMDA were compared to the second of two sequential NMDA pulses in unmodified aCSF.

Superfusion of hypothalamic slices with a 4 min pulse of 10^{-4}M NMDA resulted in significant ($p<0.001$, one-way analysis of variance) increases in release of α-MSH in all experiments. A second pulse of NMDA in normal aCSF produced a stimulation of α-MSH release that was not significantly different ($p>0.05$) from that to the first pulse. When tissue slices were superfused with 10^{-6}M L-NAME, there was no significant change ($p>0.05$) in basal peptide release, however, NMDA-stimulated release was completely inhibited ($p<0.001$). This inhibition of NMDA-stimulated release of α-MSH by 10^{-6}M L-NAME was reversed by the addition of 10^{-5}M L-arginine, the response to NMDA in the presence of L-NAME and L-arginine not differing from control ($p>0.05$). Treatment of slices with aCSF containing 10^{-6}M L-NAME and 10^{-5}M L-arginine also caused a transient significant increase ($p<0.01$) in basal release of α-MSH. Superfusion of slices with 10^{-6}M D-NAME produced no significant change ($p>0.05$) in either basal or NMDA-stimulated release of α-MSH.

These data demonstrate that the stimulatory action of NMDA on the release of α-MSH from the rat hypothalamus is blocked by specific competitive inhibition of nitric oxide synthase and that the blockade is reversed by an excess of the enzyme substrate. L-Arginine also caused significant increases in basal peptide release, indicating that a shortage of substrate for nitric oxide synthase is normally present in superfused slices of hypothalamus. We conclude that nitric oxide mediates the stimulatory action of glutamic acid on the release of α-MSH from the rat hypothalamus.

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233P PREPARATION OF RELIABLE LOW COST MICRODIALYSIS PROBES

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The technique of *in vivo* microdialysis is well established and readily used to monitor the release of many different types of neurotransmitter in the central nervous system. To date, there is a great variety in both the design of probe and its cost. The aim was to design a concentric microdialysis probe which was easy to prepare, robust, low in cost, and had a high recovery for amine neurotransmitters.

A length of dialysis tubing (~120mm) (Hospal, U.K. Ltd. 300μm o.d., 220μm i.d.), made of acrylonitrile-sodium methallyl sulphonate (M.Wt. cut-off point 20,000 Daltons), was cut and a piece of tungsten wire threaded into the lumen to act as support during the initial preparation of the probe. The dialysis tubing was then inserted (~5mm) into a steel cannula (23G) and secured with epoxy resin. The tungsten wire was then removed, the dialysis tubing cut (~8mm in length), and the epoxy resin allowed to dry for 2 h. A 20mm length of portex-tubing (1.02mm o.d., 0.58mm i.d.) was pushed over the other end (i.e. the end without the dialysis tubing) of the steel cannula and secured in place with cyanoacrylic adhesive. A small hole was then made in the portex-tubing using a 25G needle and fine fused silica-glass capillary tubing (Scientific Glass Engineering) inserted inside the dialysis membrane and pushed through the steel cannula and out through the hole in the portex-tubing. The end of the dialysis tubing was cut to the desired length (4mm), sealed with epoxy-resin and left to dry for 2 h, as was the hole in the portex tubing through which the silica-glass tubing emerges. Dialysis probes were made in batches and stored until used. A video is available that fully illustrates the preparation of the microdialysis probes.

The *in vitro* recovery of indoleamines through the probes was tested by perfusing the probes with artificial CSF (1μl min^{-1}, pH 7.4) in a beaker containing 5-HT and 5-HIAA (both at $2.5x10^{-8}$M in artificial CSF). After 45 min equilibration four 20 min samples were assayed for 5-HT and 5-HIAA using HPLC-ECD. Probes were then placed in artificial CSF, left for 24 h., and then returned to a beaker containing 5-HT and 5-HIAA (both at $2.5x10^{-8}$M) and the recovery of the indoleamines was again determined.

The *in vitro* recoveries of 5-HT and 5-HIAA were 14.2 ± 0.1% (n=9) and 10.4 ± 0.7% (n=6) respectively, and these significantly increased after being placed in artificial CSF for 24 h to: 5-HT: 22.2 ± 2.3% ($p<0.01$, n=7, 2-tailed paired Student's t-test); 5-HIAA: 19.4 ± 4.0% ($p<0.05$, n=4, 2-tailed paired Student's t-test).

These probes have been used successfully *in vivo* to study central indoleamine levels in both freely moving rats (Wright et al., 1991) and guinea-pigs (Lawrence and Marsden, 1991). The increase in *in vitro* recovery observed in the probes after being in contact with CSF for 24h illustrates the difficulty in trying to determine extracellular concentrations *in vivo*.

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234P TREATMENT WITH BRAIN-DERIVED NEUROTROPHIC FACTOR REVERSES DEFICIENT NERVE GROWTH FACTOR mRNA IN SCIATIC NERVE OF STREPTOZOTOCIN-DIABETIC RATS

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Neuropeptide gene expression in sensory neurones is regulated by the classic neurotrophic factor, nerve growth factor (NGF). Studies *in vivo* and *in vitro* have shown that NGF elevates substance P and calcitonin gene-related peptide (CGRP) mRNA and peptide levels. Experimental diabetes in rats is associated with reduced sciatic nerve levels of the neuropeptides, substance P and CGRP. This deficit in neuropetide levels may be explained by a diabetes-induced impairment of neurotrophic support. Specifically, the levels of NGF available to the sensory neurone might be lowered leading to a down-regulation of neuropeptide gene expression. This study was designed to test whether administration of a neurotrophic factor could reverse diabetes-induced deficits in neuropeptides and/or NGF mRNA levels in sciatic nerve. Brain-derived neurotrophic factor (BDNF) was chosen for this pilot study as a member of the NGF family of growth factors, which exhibits many of the physiological properties of NGF.

Male Wistar rats (starting weight 310-360 g) were allotted at random to 4 groups. Two groups were made diabetic with streptozotocin (50mg/kg i.p.). The experiment proceeded for 4 weeks with one diabetic and one control group being treated with BDNF (0.9 mg/rat s.c. three times per week). Treatment with BDNF had no effect on plasma glucose levels, sciatic nerve glucose content, or intake of water and food in control or diabetic rats. Substance P peptide levels, measured by radio-immunoassay, were reduced in diabetic rats (150.1 $\pm$ 40.8 pg/mg nerve protein; n=10) compared to control (244.9 $\pm$ 46.5 pg/mg nerve protein; n=10). Treatment with BDNF failed to reverse the deficit in diabetic rats - control with BDNF (269 $\pm$ 69.2 pg/mg nerve protein; n=10) compared with diabetic rat treated with BDNF (169.7 $\pm$ 44.4 pg/mg nerve protein; n=10). CGRP peptide levels, measured by an enzyme-linked immunoassay, were also reduced in diabetic sciatic nerve, and BDNF failed to reverse this deficit. Total RNA was isolated from sciatic nerve as described by Chomzynski and Saachi (1987) and subjected to Northern transfer. A 450base truncated NGF sense transcript was added at the start of the isolation procedure permitting quantification of NGF mRNA and total RNA yields. Northern blots were hybridised using a mouse NGF cDNA (Pst 1 fragment), hybrids were detected by autoradiography and quantified by image analysis (AI Cambridge). In diabetic rats NGF mRNA levels were reduced (51 $\pm$ 17.06 pg/g nerve wet weight; n=3) compared with control (124.5 $\pm$ 9.75 pg/g nerve wet weight; n=4). Treatment of diabetic rats with BDNF succeeded in reversing the deficit in NGF mRNA levels - control with BDNF (113.1 $\pm$ 27 pg/g nerve wet weight; n=4) compared with diabetic rat with BDNF (123.9 $\pm$ 18.1 pg/g nerve wet weight; n=4).

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235P CAPSAZEPINE DOES NOT BLOCK THE PROTON-INDUCED ACTIVATION OF RAT SENSORY NEURONES

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Capsazepine is a recently discovered competitive antagonist of the sensory neurone excitant, capsaicin, which acts with an apparent K_d of 100-220nM (Bevan et al, 1991,1992). The agonist effects of capsaicin are very similar, if not identical, to those of protons (Bevan & Yeats, 1991) and experiments indicate that capsazepine can block the proton-induced release of calcitonin gene related peptide from sensory nerves (see Santicioli et al, 1992). This latter result is in contrast to our finding that capsazepine had no significant effect on the proton activated efflux of radioactive Rb^+ and guanidinium ions from cultured sensory neurones (Bevan et al, 1992). We have therefore made several types of experiment to re-investigate whether capsazepine can block the activation of sensory nerves by acidic solutions.

Rat isolated dorsal root ganglion (DRG) neurones were studied electrophysiologically under voltage clamp. Acidic solutions (pH5.8) evoked a depolarizing current in a sub-population of DRG neurones. Addition of capsazepine (10μM) to the medium had no significant effect on the amplitude of the proton induced currents. Acidic solutions can also depolarize rat vagal nerve fibres (Rang & Ritchie, 1988) but this effect was not inhibited by capsazepine (10μM), which had no obvious effect on the pH-response curve. The depolarization of vagus nerves by acidic solutions could, however, be inhibited by ruthenium red (5μM), which depressed the amplitude of the response without any significant shift in the pH-response curve. The possible inhibitory effects of capsazepine were also studied with intradermal injection into human volunteers. The degree of pain was rated at 5 second intervals for up to 1 minute after injection of algogenic substances. Intradermal injection of acidic medium (pH5.0 acetate or pH6.0 succinate buffers) evoked a brief sensation of pain that disappeared after about 5-10 seconds. Capsazepine (100pmol in 10μl) had no effect on this pain response although it did show a dose dependent block of the pain evoked by capsaicin. Capsazepine also failed to inhibit the sensation of pain evoked by intradermal injection of either K^+, histamine or bradykinin. Our data therefore support the idea that capsazepine does not inhibit the activation of sensory neurones by low pH solutions. The mechanism by which it can inhibit peptide release in response to low pH remains to be investigated.

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236P STUDIES WITH CAPSAZEPINE ON PERIPHERAL NOCICEPTOR ACTIVATION BY CAPSAICIN AND LOW pH: EVIDENCE FOR A DUAL EFFECT OF CAPSAICIN

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Peripheral nociceptors may be selectively acitivated by capsaicin and by low pH solutions (Bevan & Yeats 1991). Indeed these substances depolarize sensory neurones by a similar ion conductance mechanism. Recently the selective antagonist capsazepine has provided evidence for a specific membrane receptor to capsaicin (Bevan et al 1991; Dickenson & Dray et al 1991). We have therefore used this antagonist to determine whether pH-induced nociceptor activation was also mediated via the capsaicin receptor.

The intact spinal cord and the functionally connected tail were removed from 1-2 day old rats following decapitation. The skin was carefully removed from the tail. The preparation was placed in a chamber and the cord and tail were separately superfused (2-4ml/min) with a physiological salt solution (composition mM: NaCl 138.6, KCl 3.35, $CaCl_2$ 1.26, $MgCl_2$ 1.16, $NaHCO_3$ 21.0, $NaHPO_4$ 0.58, glucose 10; at 24^0C and gassed with 95% O_2/5% CO_2). Peripheral nociceptive fibres in the tail were activated by 10-20sec administrations of capsaicin, by superfusate adjusted to pH 6.4 or 5.5 with hydrochloric acid and by superfusate heated to 50^0C (noxious heat). Spinal ventral root depolarization was recorded from a lumbar (L_3-L_5) root.

Consistent responses were obtained to capsaicin, noxious heat and by the low pH solutions. Capsaicin-evoked responses were consistently and reversibly attenuated by capsazepine (IC50= 350nM). However capsazepine did not affect the responses to noxious heat, to pH6.4 or pH5.5 at concentrations (2-10μM) which completely abolished the responses produced by capsaicin. In addition applications of capsaicin (2μM, 5min) which induced a complete and selective desensitization to capsaicin did not affect responses to low pH. Surprisingly capsazepine did not abolish the responses evoked by prolonged (1-2min) administration of capsaicin (0.2-1.0μM) but delayed the apparent onset of the response from 32±2 to 116±8 sec (n=6, $p<0.01$). Furthermore the initial phase of the capsaicin induced response was completely "protected" from desensitization when capazepine was present (n=8). However following the removal of capsazepine, the preserved capsaicin-induced response was also lost after a further challenge with a desensitizing concentration of capsaicin. Ruthenium red (100nM) attenuated both phases of the capsaicin response.

These data indicate that although capsaicin and low pH activate nociceptors, the effect of low pH is not mediated via a capsazepine sensitive capsaicin receptor site. However a capsazepine resistant effect of capsaicin on nociceptors was revealed. The significance of this is unclear though the possibility of a capsazepine resistant receptor or a non-receptor mediated effect of capsaicin can be considered.

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237P ANTINOCICEPTIVE ACTIVITY OF BRADYKININ B_1 AND B_2 ANTAGONISTS IN TWO MODELS OF PERSISTENT HYPERALGESIA IN THE RAT

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There is substantial evidence suggesting a significant role for bradykinin (Bk) in the activation of nociceptors during **acute** inflammation mediated via a B2 receptor. We have now investigated the involvement of bradykinin receptors in the hyperalgesia produced during **persistent** inflammation in the rat.

Hyperalgesia to mechanical stimulation was induced by injecting female Sprague Dawley rats with 100μl of Freund's complete adjuvant intra-articularly into one knee under enflurane anaesthesia. On the third day, the load (g) that the animals would tolerate on the injected leg was reduced by 40-50% compared with the uninjected leg indicating hyperalgesia (Perkins and Campbell 1992). Thermal hyperalgesia was produced in female Sprague Dawley rats (100g) by exposing one hindpaw to UVA light (intensity maximum 365 nm, 69mwatt/cm^2). Subsequently, the withdrawal threshold to a focused beam of radiant heat applied to the underside of each hind paw was measured.

Mechanical hyperalgesia. On the third day after Freund's adjuvant injection the specific B_2 antagonist D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]Bk (HOE 140, Wirth *et al.*1991) was ineffective in alleviating the hyperalgesia when given intravenously (10nmol-5μmol/kg). The B1 antagonist, desArg^{9}Leu^{8}Bk, however, was effective when given intravenously on day 3 with a 65% reversal of the hyperalgesia at 10nmol/kg iv which lasted for 2-2.5hr. Both the B1 and B2 antagonists administered intra-articularly at 100-500pmol, with the Freund's adjuvant, attenuated (by 60% on day 1) the development of hyperalgesia compared to controls. **Thermal hyperalgesia.** On the first day after UV exposure (day 1) there was a 63% fall in the withdrawal latency, from 19.4±0.4s (n=20) to 7.2±0.5s (n=8, means ± sem). The NSAIDs, ibuprofen (100-500mg/kg sc) and paracetamol (50-500mg/kg sc) both reversed the hyperalgesia on day 1 as did morphine (2-16mg/kg sc) in a naloxone-sensitive manner. DesArg^{9}Leu^{8}Bk also reversed the hyperalgesia when given on day 1 (10nmol/kg iv). In addition, desArg^{9}Leu^{8}Bk partially prevented the development of the hyperalgesia when given intra-plantar twice daily (30-50pmole) until day 1 with an 85% increase in latency at 50pmole. HOE140 was inactive by either route at 10nmol/kg iv and 50pmol intraplantar, respectively. The B1 agonist, desArg9Bk given on day 1 (10nmol/kg iv) increased the hyperalgesia with a 45% reduction in latency, 60min after injection.

The data support the involvement of kinins in the hyperalgesia produced during persistent inflammation and suggest that hyperalgesia is mediated via B1 rather than B2 receptors.

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238P CAPSAICIN DESENSITIZATION IN RAT DORSAL ROOT GANGLION NEURONES IS DUE TO ACTIVATION OF CALCINEURIN

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Capsaicin (cap) selectively activates a cation-specific ion channel in some sensory neurones (Bevan & Szolcsanyi, 1990). Repeated exposure to capsaicin leads to a desensitization of the response which is partly dependent on entry of calcium (Yeats, Docherty & Bevan, 1992). In the present experiments we have attempted to identify the sub-cellular mechanism of this calcium dependent desensitization. Experiments were performed on dorsal root ganglion (DRG) neurones from adult rats. Cells were grown in culture for 1-5 days and then replated on laminin-coated glass coverslips 2-8 hrs prior to recording. Membrane current was recorded by the whole-cell voltage-clamp technique. The patch electrode usually contained (mM): NaCl 130, $MgCl_2$ 1, HEPES 10, $CaCl_2$ 1, EGTA 10, pH=7.4; and the superfusate contained (mM): NaCl 130, $MgCl_2$ 1, KCl 3, $CaCl_2$ 1, HEPES 5, glucose 11, 1% dimethylsulphoxide, pH=7.4.

Cap (500nM) was applied from a u-tube for 5 s at 2 min intervals. When cells were held at -60mV the response was an inward current which desensitized with repeated applications. Most desensitization occurred between the first and second applications and this was followed by a smaller progressive decline in the response with each successive application. Removal of calcium from the superfusate or equimolar replacement of EGTA in the pipette with BAPTA abolished the large initial component of desensitization.

We tried to block the desensitization with non-specific protein kinase inhibitors. Neither staurosporine (1μM) added to the superfusate nor H-7 (5μM) added to the pipette solution reduced desensitization. If anything, desensitization was enhanced by these measures. Okadaic acid, (1μM) a selective inhibitor of protein phosphatases 1 and 2A was without effect. The desensitization was, however, abolished when calcium-calmodulin dependent phosphatase 2B (calcineurin) was blocked by cyclosporin A/cyclophilin complex (14-17 nM) (Liu, *et al.*, 1991). To generate the complex cyclophilin was added to the pipette solution and cyclosporin A was then added to the superfusate. Cyclophilin (17 nM) by itself had no effect on desensitization. In cells which were not dialyzed with a cyclophilin containing solution cyclosporin (50 nM) reduced but did not abolish desensitization.

These data suggest that calcium-dependent desensitization of capsaicin responsiveness is due to activation by calcium of calcium-calmodulin dependent phosphatase 2B. Whether the channel activated by capsaicin is a substrate for this enzyme or whether other intermediates are involved remains to be established.

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239P ARGIOTOXIN-636 INHIBITS Ca^{2+}-DEPENDENT CURRENTS RECORDED FROM CULTURED NEURONES FROM RAT DORSAL ROOT GANGLIA

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Argiotoxin-636 is a polyamine toxin isolated from the venom of orb-web spiders, including those of the genera Argiope. Previously argiotoxin-636 has been found to inhibit Ca^{2+} influx into neurones mediated by the NMDA receptor ion channel complex (Priestly et al 1989). In this study we have investigated the inhibitory action of argiotoxin-636 (0.1 to 100 μM) on neuronal voltage-activated Ca^{2+} currents and Ca^{2+}-activated Cl^- currents. Cultured rat dorsal root ganglion neurones were voltage clamped at -90 mV and Ca^{2+} currents activated by step depolarizations. Argiotoxin-636 (10 μM) inhibited voltage-activated Ca^{2+} currents activated over a wide voltage range. Argiotoxin-636 was not selective for low voltage-activated Ca^{2+} currents, unlike one of the synthetic polyamine funnel web spider toxins (Scott et al 1992). Experiments were carried out on high voltage-activated Ca^{2+} currents evoked by 1 s long depolarizations to -10 mV or 0 mV. Parameters of a single current activated after 3 minutes application of argiotoxin-636 were measured. Argiotoxin-636 (10 μM) inhibited the mean calcium current measured at the peak and end of step and the time for the current to decay by 63%, by 23 ± 6%, 56 ± 10% and 22 ± 7% (n=8; mean ± S.E.M.) respectively. Argiotoxin-636 appeared to have a significant use-dependent component as revealed by the inhibitory effect of the polyamine on 20 currents activated for 30 ms at a frequency of 0.1 Hz. Under control conditions I_{Ca} declined by 15 ± 5% (n=10), but in the presence of argiotoxin-636 I_{Ca} was reduced to a significantly greater extent, 41 ± 13% (n=6, $p<0.05$; independent Student's t test). In addition to inhibiting voltage-activated I_{Ca}, argiotoxin-636 (10 μM) reduced the Ca^{2+}-activated Cl^- tail current, attenuating the current amplitude measured 20 ms after the end of the voltage step command and tail current decay measured at 50% by 56 ± 8% and 78 ± 6% (n=4). Argiotoxin-636 (10 μM) also inhibited the amplitude of the Cl^- currents activated by caffeine-induced Ca^{2+} release. These data suggest that argiotoxin-636 may interact with membrane constituents to reduce the Ca^{2+}-activated Cl^- current by a direct mechanism as well as by reducing Ca^{2+}-influx through voltage-gated Ca^{2+} channels.

We conclude that the polyamine spider toxin, argiotoxin-636, inhibits Ca^{2+} currents in DRG neurones and that several mechanisms of action appear to be involved, including both use-dependent and non-use-dependent components.

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240P THE $GABA_B$ RECEPTOR AGONIST (-)-BACLOFEN AND THE 1,4-DIHYDROPYRIDINE Ca^{2+}-CHANNEL AGONIST Bay K8644 MODULATE THE GTP-BINDING PROTEIN G_o VIA DIFFERENT MECHANISMS

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Previously we have demonstrated an enhancement of the GTPase activity of the GTP-binding protein (G-protein) G_o by both the $GABA_B$ receptor agonist (-)-baclofen, and drugs which act directly on effectors, such as the 1,4-dihydropyridine (DHP) L-type Ca^{2+}-channel agonists (Sweeney & Dolphin, 1992). In the present study, we have investigated whether the observed stimulation of GTPase was due to an increase in the binding of GTP to a G-protein by measuring the binding of a non-hydrolysable analogue of GTP (GTPγ[^{35}S]) to neuronal membranes. Membranes were prepared either from the frontal cortex of adult rats or from cerebellar granule neurones grown in primary culture. The amount of bound GTPγ[^{35}S] was counted in a scintillation counter following an incubation at room temperature in a Tris HCl buffer pH 7.4 essentially as described previously (Avissar et al., 1988). Non-specific binding was determined in the presence of 1μM unlabelled GTPγS or 500μM GTP. Binding of GTPγ[^{35}S] to neuronal membranes was (i) maximal after 20 minutes (ii) largely irreversible, with only 25$\pm$5% (mean $\pm$ s.e. mean, n=5) dissociating from the membranes when the assay was diluted 5-fold with Tris HCl pH 7.4 containing 100μM GTP (iii) dependent on Mg^{2+} ions, where maximum binding was observed in the presence of 10mM Mg^{2+}. Binding of GTPγ[^{35}S] was attenuated by unlabelled GTPγS and GTP in a concentration-dependent manner, the IC_{50} values being 50nM and 2μM respectively, while ATP had little or no effect on GTPγ[^{35}S] binding (IC_{50} = 1mM). Baclofen (10μM) produced a 35$\pm$9% increase in the specific binding of GTPγ[^{35}S] to frontal cortical membranes. In contrast,(+)-Bay K 8644 (10μM), a DHP agonist, had no effect on binding (96$\pm$9% of vehicle which was 0.04% ethanol v/v (6.7mM)). When Scatchard analyses were performed, 10μM Bay K 8644 produced a statistically insignificant decrease in the affinity (K_D) for GTPγ[^{35}S] from 11.1nM under control conditions to 14.2nM, and had no effect on the maximal number of binding sites (B_{max}) (105$\pm$5% of control, P=0.84, paired t-test). Baclofen (10μM) increased the binding affinity to 8.9nM and the B_{max} from 262.12 to 355.65 pmol/mg protein. These effects of baclofen on the K_D and B_{max} corresponded to increases of 31$\pm$2% and 36$\pm$5% of control respectively ($P<0.03$, paired t-test). The stimulatory effect of baclofen on GTPγ[^{35}S] binding was inhibited by pretreatment of frontal cortical membranes *in vitro* or cerebellar granule neurones *in situ* with pertussis toxin, which uncouples the G-proteins G_i and G_o from various receptors. Pretreatment of frontal cortical membranes with anti G_o antibodies also attenuated the effect of baclofen on GTPγ[^{35}S] binding by 60-70%. The results described here suggest that the stimulation of GTPase induced by baclofen may be due in part to an increase in the binding of GTP to G_o (and possibly also other G-proteins), which would result in activation of the G-protein. However, the DHP-induced modulation of the GTPase activity of G_o is likely due to an increase in the hydrolysis of GTP already bound to the activated state of $G_o\alpha$. Thus, L-type Ca^{2+}-channels in the DHP-agonist bound state do not appear to be able to activate G_o, but may play a role in switching off its activity via the hydrolysis of GTP, thereby recycling G_o to its inactive, GDP-bound state.

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241P EFFECT OF DIZOCILPINE AND ENADOLINE ON IMMEDIATE EARLY GENE EXPRESSION FOLLOWING ISCHAEMIA

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Recently we reported that a variety of immediate early genes (IEG) are induced in the dentate gyrus of the gerbil following a brief period of ischaemia (McKibbon *et al.*, 1991). Here we investigate the effects of two drugs known to protect CA1 neurones from degeneration in the gerbil model of global ischaemia (Hayward *et al.*, 1992) in IEG expression.

Ischaemia was induced in female Mongolian gerbils by occlusion of the carotid arteries for a period of 7 minutes under forane anaesthesia. The animals were allowed to recover and sacrificed one hour after the occlusion. Dizocilpine (MK-801; 3 mg/kg) and enadoline (CI-977; 1 mg/kg) were administered 30 min before the occlusion. The controls were sham operated with or without drug treatment. Sections (10µm), at the level of the hippocampus, and sections were hybridised overnight at 42°C with oligonucleotide probes complementary to c-*fos*, c-*jun*, *jun*B and *hsp*70 which were labelled at the 3′ end with [^{35}S]-dATP using terminal transferase. Following stringency washes the IEG mRNA was visualised by autoradiography on XAR film.

Table Effect of drugs on IEG mRNA in dentate gyrus

IEG	ischaemia/saline	ischaemia/enadoline	ischaemia/dizocilpine
c-*fos*	59 ± 3	59 ± 3	39 ± 2*
c-*jun*	94 ± 3	123 ± 5*	72 ± 3*
*jun*B	92 ± 2	126 ± 2*	61 ± 2*
*hsp*70	28 ± 4	0 *	3 ± 2*

Results are measurements of relative optical density from 6 animals expressed as % increase above sham operated controls. * $p<0.05$, Mann Whitney U test.

The table shows that all of the IEG mRNAs are increased in the dentate gyrus, these increases are attenuated by dizocilpine and unaffected or increased by enadoline, although both compounds protected CA1 neurones from degeneration and inhibited the expression of *hsp*70. This is similar to results of Uemura *et al.* (1991) which showed that Fos protein was inhibited by dizocilpine. Further the results emphasise differences in the mechanism of action of the NMDA non competitive antagonist and the kappa opioid agonist as neuroprotective agents in global ischaemia.

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242P COMPARISON OF QUINOXALINEDIONE POTENCY AS DEPRESSANTS OF THE MONOSYNAPTIC ACTIVATION OF SPINAL MOTONEURONES FROM DORSAL ROOTS *IN VITRO*

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6-Nitro-7-sulphamoylbenzo-(f)-quinoxaline-2,3-dione (NBQX) has a potency for displacing α-amino-5-methyl-4-isoxazole-propionate (AMPA) from cerebrocortical membranes which is more than tenfold higher than that of 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX). However, these two quinoxalinediones show only a twofold selectivity between kainate binding sites of cerebrocortical membranes (Sheardown et al, 1990).

In a previous study we have tested the ability of these compounds to antagonize the kainate-induced depolarization of primary afferent C-fibres. These fibres are relatively insensitive to AMPA and thus represent a type of kainate selective receptor (Agrawal & Evans, 1986). No significant difference in potency between CNQX and NBQX was observed at this kainate-sensitive site (Brugger & Evans, 1992) whereas at spinal motoneurones NBQX is fivefold more selective than CNQX against AMPA-induced depolarizations (Watkins et al, 1990).

In the present study CNQX and NBQX have been compared as depressants of the short latency presumed monosynaptic component of the synaptic activation of motoneurones following electrical stimulation of dorsal roots (MSR). Hemisected spinal cords from immature (<6 day old) rats were bathed in a medium containing (mM):- NaCl 118, KCl 3, $CaCl_2$ 1.5, $MgSO_4$ 0.75, $NaHCO_3$ 24, dextrose 12. The medium was maintained at 25°C and gassed with 95% O_2 5% CO_2. The synaptic potential was recorded across a grease seal placed along the ventral root. The corresponding dorsal root (L4 or 5) was stimulated with 0.5 msec supramaximal square pulses at 30 second intervals.

CNQX and NBQX abolished the MSR yielding respective EC_{50} values (nM ± s.e.mean) of 784 ±141 (n=3) and 140 ±12 (n=5). The fivefold higher potency of NBQX indicates that the the monosynaptic spinal reflex is mediated mainly at the AMPA subclass of non-NMDA receptor.

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243P ANTAGONISM OF EXCITATORY AMINO ACID-EVOKED RESPONSE OF RAT SPINAL NEURONES *IN VIVO* BY 2,3-BENZODIAZEPINES GYKI 52466 AND GYKI 53655

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Antagonism of synaptic responses mediated by the kainate/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtype of glutamate receptor by a 2,3-benzodiazepine was first demonstrated by Tarnawa et al. (1989). We have since shown that this compound, 1-aminophenyl-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466) reduces excitation of rat spinal neurones *in vivo* evoked by iontophoresis of kainate and AMPA with less effect on responses to *N*-methyl-D-aspartate (NMDA; Jones and Lodge, 1991). Here, the effects of GYKI-52466 and an *N*-methyl carboxamide-substituted analogue, GYKI 53655, are compared following iontophoretic and intravenous administration, on the increase in action potential firing rate of spinal neurones in pentobarbitone-anaesthetised rats, in response to iontophoretic ejection of kainate, AMPA and NMDA.

The results, expressed as mean percentage reduction in the peak amplitude of responses ± standard error, are summarised in the table below. The figures in brackets denote the number of cells on which each test was made.

Drug	Route	Mean Percentage Reduction		
		AMPA	kainate	NMDA
GYKI 52466	iontophoresis			
	(5-40nA; 4mM in 200mM NaCl)	70±11 (8)	47±10 (7)	17±5 (9)
	intravenous (10mg/kg)	66±8 (6)	33±5 (5)	6±6 (6)
GYKI 53655	iontophoresis			
	(10-40nA; 4mM in 200mM NaCl)	77±7 (9)	69±8 (9)	26±8 (10)
	intravenous (5mg/kg)	45±11 (10)	20±9 (4)	6±6 (9)
	(10mg/kg)	76±7 (10)	52±13 (5)	10±7 (10)

The data presented here show that, *in vivo*, GYKI 52466 and GYKI 53655 both reduce AMPA- and kainate-evoked excitation of rat spinal neurones rather than NMDA-evoked firing. Thus, our findings concur with those of Tarnawa et al. (1989), showing these compounds to be effective non-NMDA antagonists, although the intravenous doses required to achieve substantial depression of responses were higher than those reported for reduction of monosynaptic reflexes in the cat. The results of the intravenous studies with both compounds may indicate a degree of selectivity against kainate-evoked excitation. However, a larger population of cells would need to be studied in order to investigate this.

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244P ANTAGONISM OF NOCICEPTIVE RESPONSES OF RAT THALAMIC NEURONES *IN VIVO* BY NOVEL ANTAGONISTS OF (1S,3R)-ACPD

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We have previously shown that the putative metabotropic glutamate receptor agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (Schoepp et.al., 1990) has an excitatory action on thalamic neurones which appears to be mediated by a receptor which is distinct from the known ionotropic excitatory amino acid receptors and which can be antagonised by (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG) (Eaton et.al., 1992). We have used this novel antagonist, and the related (S)-4-carboxyphenylglycine ((S)-4CPG), to investigate the role of (1S,3R)-ACPD-sensitive receptors in synaptic transmission in the thalamus.

Extracellular action potential recordings and iontophoretic drug applications were made with multibarrel glass electrodes in urethane-anaesthetised rats (Salt & Eaton, 1991). Single neurones were recorded dorsal to, and within, the ventrobasal thalamus. Non-noxious sensory stimulation consisted of a jet of air directed at the neurones receptive field (typically a single vibrissa). Noxious sensory stimulation consisted of tail immersion in water at 52°C (15-60 s). Responses to agonists and sensory stimulation were recorded before, during and after iontophoretic application of (S)-4C3HPG or (S)-4CPG. Both compounds antagonised responses of neurones to (1S,3R)-ACPD but not NMDA, kainate or AMPA. During such selective antagonism, responses to air jet stimulation of 15 and 5 neurones, respectively, were not reduced and, in some cases, these synaptic responses were enhanced. In contrast, when responses of thalamic neurones to noxious stimuli were challenged with (1S,3R)-ACPD-selective currents of either (S)-4C3HPG or (S)-4CPG, such responses were reduced to 38% (±7.6, n=12) and 37% (±3.9, n=3) of control values, respectively. These results suggest that (1S,3R)-ACPD-sensitive metabotropic receptors may be involved in the responses of thalamic neurones to noxious sensory stimuli but not responses to non-noxious stimuli.

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245P 4-AMINOPYRIDINE-INDUCED EPILEPTOGENESIS IN RAT TEMPORAL LOBE *IN VITRO*: EFFECTS OF EXCITATORY AMINO ACID ANTAGONISTS AND ANTICONVULSANT DRUGS

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The rat entorhinal cortex (EC) *in vitro* has a pronounced susceptibility to epileptogenesis evoked by $GABA_A$ receptor blockers or reduction of extracellular magnesium (see Jones and Heinemann, 1991). In *in vivo* and *in vitro* tests the potassium channel blocker, 4-aminopyridine (4-AP) has been shown to be a powerful convulsant (Galvan *et al*, 1982; Yamaguchi and Rogawski, 1992) and the present experiments have used a slice preparation to compare the epileptogenic effects of 4-AP in the EC to the adjacent hippocampus and temporal neocortex (TC) and to determine the role of excitatory amino acid receptors in the mediation of such effects. In addition, the effects of some clinically effective anticonvulsant drugs have been examined. Combined TC-EC-hippocampal slices were cut from the rat brain and maintained *in vitro* at the interface between artificial CSF (34°C) and carbogen gas (95% O_2, 5% CO_2). Epileptiform activity was recorded with extracellular tungsten electrodes placed in CA3 of the hippocampus and the deep layers of the EC and TC.

Spontaneous epileptiform activity started within 20-40 min of perfusion with 4-AP (100 µM). Two types of activity were recorded in the EC; long lasting (range 15-70 sec, mean +/- S.D. 32 +/- 14 sec in a sample of 20 slices) electrographic seizures occurred at regular intervals (85-400 sec, 207 +/- 88 sec) and were interspersed with brief (30-250 msec) single field potential spikes of varying form and amplitude. The long lasting, but not the brief events were synchronized with similar activity in the TC. In CA3, brief (50-100 msec) events comprised of bursts of population spikes recurred at frequencies of 1-3 Hz) and long lasting discharges were rarely recorded (cf Rutecki *et al*, 1987). The NMDA receptor antagonist, D,L-2-amino-5-phoshonovalerate (2-AP5, 30 µM) could abolish (n=4) or reduce the frequency, amplitude and duration of the long lasting events (n=3) in EC and TC. At the same time, the brief events in CA3 were unaffected while those in the cortical areas were reduced (n=2) increased (n=3) or unaffected (n=2). The AMPA/kainate receptor antagonist 6-cyano-7-nitro-quinoxaline-2,3-dione (2-10 µM) always (n=6) abolished the long lasting discharges as well as the brief events in CA3 and often (n=3) those in EC and TC. Phenytoin (50-100 µM) had no effect on the brief activity in CA3 (n=9) but the long lasting seizure events in both EC and TC were abolished (n=6) or much reduced were greatly reduced in frequency (n=3). The brief events in TC and EC were unaltered or increased in frequency and amplitude by phenytoin. Ethosuximide (50-100 mM, n=6) had no effect on either brief or long lasting events in any brain area. Carbamazepine (50-100 µM, n=5) had similar effects to phenytoin whereas sodium valproate (50-100 µM, n=6) was similar to ethosuximide.

Thus, 4-AP evoked epileptiform activity in the EC and TC which differed markedly from that in the hippocampus. The predominant response in the EC was long electrographic seizures which involved activation of both NMDA and non-NMDA receptors. Anticonvulsants with differing clinical profiles, differentially affected the activity in the TC and EC but did not affect that in CA3.

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246P EFFECTS OF COMPETITIVE NMDA ANTAGONISTS, GIVEN CONCURRENTLY WITH ETHANOL, ON THE ETHANOL WITHDRAWAL SYNDROME AND THE DEVELOPMENT OF TOLERANCE TO ETHANOL

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Chronic ethanol treatment causes tolerance, and a withdrawal syndrome (tremor and convulsions). Involvement of NMDA in adaptations to ethanol was suggested by increased hippocampal [^{3}H]MK-801 binding after chronic ethanol (Grant et al., 1990), and blockade of withdrawal signs by NMDA antagonists (Liljequist, 1991). Here we report the effects of competitive NMDA antagonists, CGP39551 and CGP37849, when given during chronic ethanol treatment.

For the withdrawal studies, male TO mice, (30-35g), were given ethanol (4-10 mg/L) by inhalation for 14 days. Controls were kept in identical conditions without ethanol. CGP39551, 5 mg/kg, or saline was given i.p. bidaily during the ethanol treatment, until 24h, 48h, 72h or 96h before ethanol withdrawal. Handling induced convulsive behaviour (HICB, median with interquartile ranges, on a scale 0-5) was scored every hour for 12h following ethanol withdrawal, and the incidence of spontaneous convulsions (Sp. Conv.) noted. The results showed that the addition of CGP39551 during chronic ethanol treatment increased the ethanol withdrawal signs, if sufficient time is allowed for the prolonged anticonvulsant action of CGP39551 to fade. HICB results given below are at 5h into ethanol withdrawal.

Chronic treatment	T	HICB	Sp. Conv.	Chronic treatment	T	HICB	Sp. Conv.
Saline alone	24 h	1 (0-2)	0/15	Ethanol + Saline*a	72 h	2 (2-3)	0/6
Saline alone	72 h	0 (0-0.5)	0/12	Ethanol + CGP39551*b	24 h	1.5 (1-2)	1/14
CGP39551 alone*a	24 h	1 (0-1)	0/15	Ethanol + CGP39551*b	48 h	1 (0.5-2)	2/17
CGP39551 alone	96 h	0.5 (0-1)	0/12	Ethanol + CGP39551*b	72 h	3 (2-4)	4/7**
Ethanol + saline*a	24 h	2.5 (0.5-3)	0/12	Ethanol + CGP39551*b	96 h	4.5 (3-5)	5/8**

T = time between last administration of CGP39551 or saline, and ethanol withdrawal. ** P<0.01 Fishers exact test *P<0.01 over whole 12h; a = cf. saline alone; b = cf. ethanol + saline (nonparametric analysis of variance)

Ethanol tolerance was produced in male Wistar rats (75-100g) by once daily i.p. injections of ethanol, 2 g/kg, with or without CGP37849, 10 mg/kg, for 8 days. Controls received saline. The ataxic effects of ethanol alone were measured using a rotorod apparatus on day 9. Animals were practised on the rotorod during the chronic treatment, the practice being matched for equivalent drugged states. The time they were able to remain on the rotorod on day 9, 24h after the last of the following chronic treatments, were: saline 27 ± 13*; ethanol alone 147 ± 17; ethanol + CGP37849 85 ± 23* (s, mean ± s.e.m., measured 15 min after acute administration of ethanol, 2 g/kg) *P<0.05, compared with ethanol alone, Mann-Whitney U-test. Chronic administration of CGP37849 alone did not alter the effects of ethanol at 24h. Administration of CGP37849 during chronic ethanol treatment therefore decreased ethanol tolerance development.

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247P ADENOSINE INHIBITS THE NMDA RECEPTOR-MEDIATED EXCITATORY POSTSYNAPTIC POTENTIAL IN THE HIPPOCAMPUS

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It was recently shown that the stable adenosine analogue 2-chloroadenosine (CADO) decreases frequency-induced long-term potentiation (LTP) of the responses evoked by stimulation of the Schaffer fibres and recorded in CA1 area, in hippocampal slices of the rat (de Mendonça & Ribeiro, 1990). As it is well established that the activation of one type of glutamate receptor, the N-methyl-D-aspartate (NMDA) receptor, is essential for induction of LTP in this pathway in the hippocampus (Collingridge et al., 1983), we tested in the present work the hypothesis that the adenosine analogue CADO may have an inhibitory effect on the NMDA receptor-mediated excitatory postsynaptic potentials (e.p.s.p.) in the hippocampal CA1 area of the rat.

The experiments were performed on hippocampal slice preparations taken from Wistar rats. Monopolar stimulation was delivered to the Schaffer fibres, and evoked e.p.s.p. extracellularly recorded in hippocampal CA1 area. The NMDA receptor-mediated component of the e.p.s.p. was revealed by applying the antagonist of the glutamate receptor of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 µM), and the antagonist of the gamma-aminobutyric acid receptor of A ($GABA_A$) type, picrotoxin (50 µM), and increasing the intensity of the stimulus (Bashir et al., 1991). The specific NMDA receptor antagonist DL-2-amino-5-phosphonopentanoate (AP5) (20 µM) was applied at the end of the experiments to ensure that the response was entirely mediated by NMDA receptors.

The concentration-response curve for the inhibitory effects of CADO (0.1-1 µM) on the slope of the initial phase of the e.p.s.p. (EC50=0.12±0.03 µM) and the concentration-response curve for the inhibitory effects of CADO (0.05-0.3 µM) on the amplitude of the NMDA receptor-mediated component of the e.p.s.p. (EC50=0.37±0.12 µM) were obtained in the same slices (n=6). CADO was about 3 times more efficient in inhibiting the NMDA receptor-mediated component of the e.p.s.p. than in inhibiting the e.p.s.p..

It is concluded that adenosine may modulate phenomena associated with the NMDA receptor, such as synaptic plasticity and excitotoxicity.

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248P CONTRIBUTION OF THE CATABOLISM OF ADENINE NUCLEOTIDES TO THE FORMATION OF ENDOGENOUS EXTRACELLULAR ADENOSINE IN THE RAT HIPPOCAMPUS

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Ecto-nucleotidases, able to catabolize ATP into adenosine are present at cholinergic nerve terminals in the hippocampus (Cunha et al., 1992). The present work was undertaken to investigate whether the enzymes responsible for the extracellular catabolism of ATP into adenosine contribute to the amounts of endogenous extracellular adenosine that modulate transmitter release and neuronal excitability in the rat hippocampus.

[3H]Acetylcholine (ACh) release was measured in an hippocampal synaptosomal P2 fraction (Cunha et al., 1992). Synaptosomes were loaded with [3H]choline (10 µCi/ml, 0.125 µM) for 5 min, washed and perfused at a flow rate of 0.6 ml/min at 37°C, with sample collection every min to determine tritium outflow. Synaptosomes were stimulated twice (S1 and S2) with veratridine (10 µM) for 2 min with a time span of 10 min. Test drugs were applied 8 min before S2 and the drug effects were expressed by alteration of S2/S1. Purine release was studied in hippocampal slices (400 µm thick) loaded with [3H]adenosine (30 µCi/ml, 0.7 µM) for 45 min. After a 60 min perfusion at a flow rate of 0.5 ml/min at 37°C, groups of 3 slices were field stimulated (40 V, 3 ms, 5 Hz for 3 min) with 3 min fraction collection. 100 µl of each fraction were used for ATP determination by the luciferin-luciferase coupled assay and the remaining was freeze-dried and ressuspended in 100 µl of 100 mM KH_2PO_4. [3H]adenosine was separated by HPLC (Cunha et al., 1992) and the radioactivity determined. Electrophysiological experiments for recording orthodromically-evoked population spikes from the CA1 pyramids were performed with hippocampal slices (Sebastião et al., 1990).

Adenosine deaminase (ADA, 2 U/ml), which converts adenosine into its inactive metabolite inosine, reversibly increased population spike (PS) amplitude (30 ± 7%, n=3). This effect was equivalent to the decrease caused by 4.0 ± 0.7 µM of adenosine on PS amplitude, in the same slices, indicating that the endogenous extracellular adenosine concentration in the CA1 area is near 4 µM. [3H]ACh release from hippocampal synaptosomes was increased (34 ± 4 %, n=5) by the ecto-5'-nucleotidase inhibitor, α,ß-methylene ADP (AOPCP, 100 µM), which prevents the formation of adenosine from ATP. ADA (2 U/ml) increased [3H]ACh release by 89 ± 11 % (n=5). Electrical stimulation of hippocampal slices evoked the release of ATP (45 ± 14 pmol/mg, n=16) and of adenosine (4.4 ± 0.5% of the total [3H]adenosine retained, n=12). This evoked accumulation of extracellular [3H]adenosine was decreased (36 ± 9 %, n=7) in the presence of 100 µM AOPCP.

The results suggest that both ATP and adenosine are released as such and contribute to the amounts of endogenous extracellular adenosine that tonically inhibits [3H]ACh release in the hippocampus.

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249P THE EFFECT OF SALMETEROL ON CYCLIC AMP ACCUMULATION IN A NEURONAL CELL LINE

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Salmeterol (4-hydroxy-α[[[6-[4-(phenylbutyl)oxy]-hexyl]amino]-methyl]-1,3-benzenedimethanol) has been shown to be a potent and selective β_2-adrenoceptor agonist with a long duration of action in airway smooth muscle (Ball *et al.*, 1991). In this study we have compared the effect of salmeterol and isoprenaline on the accumulation of cyclic AMP in a neuronal cell line (B50) derived from the rat nervous system.

B50 cells were cultured in 24 well cluster dishes in Dulbecco's modified eagles medium (DMEM) containing 10% foetal calf serum (FCS) and 2 mM glutamine in humidified air:CO_2 (90:10) at 37°C. ^{3}H-cyclic AMP accumulation was measured in ^{3}H-adenine-labelled cells as described previously (Ruck *et al.*, 1990). Prior to addition of agonists (in 10 µl medium) prelabelled cell monolayers were incubated in Hanks/HEPES buffer, pH 7.4, containing the type IV phosphodiesterase (PDE) inhibitor rolipram (0.1 mM), and where appropriate antagonist drugs, for 20 min. ^{3}H-cyclic AMP was isolated using sequential Dowex-alumina chromatography (Donaldson *et al.*, 1988).

Salmeterol (10 µM; 30 min stimulation) produced a significant increase (3.8 ± 0.2 fold over basal levels; $p < 0.05$; n = 15) in ^{3}H-cyclic AMP accumulation in cultured B50 cells. The response to salmeterol was concentration-dependent yielding an EC_{50} value of 43 ± 11 nM (n = 15). This response to salmeterol was inhibited by the β_2-adrenoceptor antagonist ICI 118551 (K_D 4.8 ± 1.4 nM, n = 3) but not by the β_1-adrenoceptor antagonist atenolol at concentrations up to 100 µM. A similar β_2-adrenoceptor-mediated cyclic AMP response was also obtained in this cell line with 10 µM isoprenaline (7.9 ± 0.9 fold; 10 min incubation; n = 22). The EC_{50} value obtained for isoprenaline was 294 ± 84 nM (n = 22). A direct comparison of the concentration-response curves to salmeterol and isoprenaline indicated that salmeterol produced a maximum response which was only 46.0 ± 4.2 % (n = 6) of that obtained with isoprenaline. 1 µM salmeterol was also able to attenuate the responses produced by high concentrations of isoprenaline (>0.1 µM; 12 min incubation period following simultaneous addition of both agents) yielding an estimate for the dissociation constant of the partial agonist salmeterol of 55.6 ± 28.2 nM (n = 4).

These results suggest that salmeterol has a high affinity, but lower efficacy (relative to isoprenaline) for β_2-adrenoceptors coupled to cyclic AMP accumulation in the B50 neuronal cell line.

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250P ACTIVATION OF β_1- OR β_2-ADRENOCEPTORS OPENS PLASMALEMMAL K^+-CHANNELS IN AIRWAYS SMOOTH MUSCLE: EVIDENCE FROM $^{86}Rb^+$ EFFLUX STUDIES IN BOVINE TRACHEALIS

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The patch clamp studies of Kume et al. (1989) have shown that isoprenaline opens plasmalemmal K^+-channels in rabbit trachealis muscle by a mechanism probably involving activation of protein kinase A and hence phosphorylation of the ion channel. However, salmeterol, an agonist highly selective for β_2-adrenoceptors (Ball et al., 1991), caused no significant hyperpolarisation of guinea-pig trachealis and failed to promote $^{86}Rb^+$ efflux from strips of bovine trachealis preloaded with the radiotracer (Cook & Small, 1992). Such observations prompted us to examine whether the activation of β_1- or the activation of β_2-adrenoceptors provokes K^+-channel opening in airways smooth muscle.

In the present study, CGP 20712A (1μM) caused >100 fold antagonism of noradrenaline in increasing beat frequency in the guinea-pig paired atrial preparation, but failed to antagonise either procaterol or salmeterol in relaxing the histamine (460μM)-contracted bovine trachealis muscle. In contrast, ICI 118551 (100nM) caused more than 60-fold antagonism of procaterol and salmeterol in relaxing the bovine trachealis without antagonizing noradrenaline in increasing atrial rate. Concentrations of 1μM CGP 20712A and 100nM ICI 118551 were therefore assumed to provide selective antagonism at β_1- and β_2-adrenoceptors respectively.
Strips of bovine trachealis were preloaded with $^{86}Rb^+$ prior to monitoring the efflux of the radiotracer as described by Longmore et al. (1991). Isoprenaline (1μM) promoted the efflux of $^{86}Rb^+$ from the strips of trachealis, an effect markedly inhibited by CGP 20712A (1μM), by ICI 118551 (100nM) and by a combination of 1μM CGP 20712A + 100nM ICI 118551. Procaterol (10nM -1μM) also promoted $^{86}Rb^+$ efflux. The stimulant effect of procaterol (100nM) on $^{86}Rb^+$ efflux was markedly inhibited by ICI 118551 (100nM) and, to a lesser extent, by CGP 20712A (1μM). These findings suggest that the activation of either β_1- or β_2-adrenoceptors can provoke the opening of Rb-permeable K^+-channels in the plasmalemma of airways smooth muscle. This conclusion is supported by the results of electrophysiological studies in guinea-pig trachealis (Cook et al., 1992). The failure of salmeterol to hyperpolarise guinea-pig trachealis or to promote $^{86}Rb^+$ efflux from bovine trachealis (Cook & Small, 1992) does not, therefore, simply reflect the great selectivity of salmeterol in activating β_2- as opposed to β_1-adrenoceptors. Rather, it provides a further indication that the relaxation of airways smooth muscle caused by the activation of β-adrenoceptors is not crucially dependent on K^+-channel opening with consequential cellular hyperpolarisation and inhibition of Ca^{2+} influx through voltage-dependent channels.

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251P LONG-TERM EXPOSURE TO NORADRENALINE IN GUINEA-PIG RESULTS IN DOWN-REGULATION OF PULMONARY β-RECEPTORS

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ß-Adrenoceptors regulate many aspects of lung function and may be down-regulated by ß-agonists. However, there are few studies on the long-term effects of ß-agonists on pulmonary ß-receptors *in vivo*. We therefore studied $ß_2$-receptors in guinea pig lung after chronic exposure to a ß-agonist. Guinea pigs were exposed to either noradrenaline (NA, 0.9 mg/kg/h) or vehicle (normal saline containing 1 mM ascorbic acid) for 7 days using an osmotic minipump. The density and affinity of ß-receptors were determined by Scatchard analysis of [^{125}I]-cyanopindolol binding in a lung membrane preparation and $ß_1$- and $ß_2$-receptor subtypes were studied in the presence of 0.1μM ICI 118,551, a selective $ß_2$-receptor antagonist, 0.1 μM CGP 20712 A, a selective $ß_1$-receptor antagonist, respectively. $ß_2$-Receptor mRNA and the transcription factor cyclic AMP responsive element binding protein (CREB) were examined by Northern blot analysis and gel shift assay, respectively. The tissue distribution of $ß_2$-receptors and of $ß_2$-receptor mRNA in lung after NA infusion were determined using receptor autoradiography and *in situ* hybridization. The functional significance of the decrease in $ß_2$-receptor was tested by measuring the relaxation to a $ß_2$-agonist, salbutamol, of spontaneous tone in isolated parenchymal strips.
NA treatment resulted in decreases of 75±9%, 84±4% and 66±9% ($p<0.001$) of total ß, $ß_1$- and $ß_2$-receptors in the lung, compared to control animals. The administration of NA had only minimal effects on the apparent K_d of the receptor for the radioligand. Down-regulation of ß-receptors was associated with a reduction of 38±12% ($p<0.01$) in $ß_2$-receptor mRNA and of 60±6% ($p<0.01$) in CREB-like DNA-binding activity, suggesting that the reduced ß-receptors density is due to reduced gene transcription resulting from reduced activity of a transcription factor. There was a correspondence between the distribution of $ß_2$-receptors and $ß_2$-receptor mRNA localization both in control and in NA treated guinea pigs. NA treatment reduced alveolar $ß_2$-receptors by 70±3% and its mRNA expression by 80±3%. However, NA treatment decreased $ß_2$-receptors in airway smooth muscle and epithelium by 46±8% and 45±7%, and their mRNA expression by 68±4% and 65±4%, respectively. Furthermore, NA treatment reduced $ß_2$-receptors in vascular smooth muscle and endothelium by 47±5% and 44±5%, and their mRNA expression by 80±5% and 76±4%, respectively. This cellular heterogeneity may reflect differences in RNA stability or transcription rate in different lung cells. The decrease in $ß_2$-receptor was accompanied by a significant reduction in maximum relaxation response to salbutamol (NA: 70.8±1.9% vs control: 80.8±1.5%, $p<0.001$). These findings indicate that agonist-induced receptor down-regulation may occur at the level of transcription and may involve a reduction in transcription factors.
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252P Org 20241, A CYCLIC NUCLEOTIDE PHOSPHODIESTERASE INHIBITOR FOR ASTHMA

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Considerable interest has recently been expressed in the potential of isoenzyme selective cyclic nucleotide phosphodiesterase (PDE) inhibitors in the treatment of asthma. In the present study, we examined the ability of Org 20241 (N-hydroxy-4-(3,4-dimethoxyphenyl)-thiazole-2-carboximidamide) to inhibit PDE isoenzymes, to attenuate the contraction of airways smooth muscle and to inhibit the activation of eosinophils.

PDE activities were obtained from bovine tracheal smooth muscle ($n \geq 3$), rabbit heart (n=4) and guinea-pig eosinophils (n=8) using methods previously described (Shahid *et al.*, 1991; Dent *et al.*, 1991). The ability of Org 20241 to attenuate histamine and methacholine contractions was examined in guinea-pig tracheal smooth muscle (n=3). Additionally, the effect of the compound on thromboxane B_2 (n=7; TxB_2) release and H_2O_2 (n=4) generation induced by leukotriene B_4 (LTB_4; 300 nM and 30 nM, respectively) in guinea-pig eosinophils was examined (Dent *et al.*, 1991). All data are mean±s.e. mean.

Bovine tracheal smooth muscle yielded PDE I, PDE II, PDE IV and PDE V. Org 20241 selectively inhibited PDE IV (IC_{50} 6.8±0.8 μM). PDE I, PDE II, PDE III and PDE IV were obtained from rabbit heart, PDE IV and PDE III were selectively inhibited by Org 20241 (IC_{50} 5.1±1.1 and 26.1±5.7 μM, respectively). IC_{50} values for PDE I, PDE II and PDE V were always in excess of 250 μM. The PDE IV-like activity in eosinophils was also inhibited by Org 20241 (IC_{50} 1.7±0.4 μM). Org 20241 relaxed histamine - (1 μmol/l) and methacholine - (1 μmol/l) precontracted preparations of guinea-pig trachea. These agonists produced 57±4.1 and 74.7±3.8% of the methacholine-induced maximum response, respectively. Org 20241 completely inhibited contractions produced by both agonists, PD_2 (-log M) values were 6.2±0.3 for histamine- and 4.9±0.2 for methacholine-contracted preparations, respectively. Org 20241 also attenuated H_2O_2 generation and TxB_2 production induced by LTB_4 (IC_{50} values, 0.39±0.18 μM and 7.38±0.4 μM, respectively).

The data indicate that over the same concentration range Org 20241 inhibits PDE IV (and to a lesser degree PDE III), and relaxes pre-contracted airways smooth muscle and reduces the activation of eosinophils. Such an activity profile suggests that the compound has potential utility in the treatment of asthma.

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253P FURTHER EVIDENCE FOR THE INVOLVEMENT OF NK_2-RECEPTORS IN TACHYKININ-INDUCED CONTRACTION OF HUMAN ISOLATED BRONCHUS

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Previous studies suggest that tachykinin (TK)-induced contraction of human isolated bronchus (HuB) is mediated by NK_2-receptors (see Maggi, 1990). However,these studies have involved agonists of variable selectivity, endogenous peptidase activity has not always been inhibited, and true equilibrium conditions have not been demonstrated. In the present study, therefore, we have compared the effects of substance P methyl ester (SPOMe), GR64349 and senktide, highly selective agonists at NK_1-, NK_2- and NK_3-receptors respectively (Ireland *et al.*, 1991), in the presence of peptidase inhibitors and under equilibrium conditions. We have also tested NK_1 and NK_2-receptor selective blocking drugs on TK-induced contractions of HuB.

Macroscopically normal lung was obtained from patients undergoing resection for carcinoma. Epithelium-denuded HuB rings (1-4mm internal diam.) were suspended in modified Krebs-Henseleit solution at 37° C, containing indomethacin (2.8μM), atropine, mepyramine and propranolol (all 1μM), and gassed with 5% CO_2 in O_2. The inhibitors of neutral endopeptidase (phosphoramidon, 1μM) and aminopeptidase (bestatin, 100μM) were added, and after 15min, cumulative concentration-effect curves to NKA were repeated until sensitivity was constant, after which a curve was constructed to a test agonist.

NKA produced concentration-related contractions of HuB (mean EC_{50} = 2.4 (95% cl = 1.7-3.3)nM, n=23). GR64349 also contracted HuB, but was 2.7 (1.9-3.8, n=4) -fold less potent. SPOMe and senktide (up to 10μM) did not contract HuB, even in the absence of indomethacin, atropine, mepyramine and propranolol. In 45% of preparations, the peptidase inhibitors caused a marked and well-maintained contraction of HuB (293 ± 33mg, i.e. ≃ 20% of NKA max.). We next tested the selective NK-receptor blocking drugs, GR82334 (NK_1, Hagan *et al.*, 1991) (10μM), and MEN10207 and R396 (NK_2, Maggi *et al.*, 1989) (0.3-30μM), against GR64349-induced contractions, in the absence of peptidase inhibition. In these experiments, GR82334 was inactive (pA_2 <5, n=2), whereas both MEN10207 and R396 were active, with pA_2s of 6.7 (6.1-8.4, n=7 from 2 experiments) and 6.1 (5.7-7.5, n=6 from 2 experiments) respectively, and slopes not significantly different from unity.

In summary therefore, NKA and the selective NK_2-receptor agonist, GR64349 potently contract HuB, whereas agonists highly selective for NK_1- or NK_3-receptors are apparently inactive. GR64349 is antagonised by selective NK_2-receptor blocking drugs, but not by an NK_1-receptor blocking drug. These results support the conclusions of others that TK-induced contractions of HuB are mediated solely by NK_2-receptors. In addition, the contractions to the peptidase inhibitors may indicate that endogenous TKs are involved in the regulation of tone in HuB.

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254P THE EFFECT OF ROLIPRAM AND SK&F94120 ON OZONE-INDUCED BRONCHIAL HYPERREACTIVITY TO INHALED HISTAMINE IN GUINEA-PIGS

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Phosphodiesterase (PDE) inhibitors are currently being investigated for the potential treatment of asthma (Torphy & Undem, 1991). A characteristic of asthma is bronchial hyperreactivity (BHR) and models have been designed to examine this feature. Exposure of guinea-pigs (GP) to ozone (O_3) induces BHR to a wide range of spasmogens (Lew et al., 1985), although the mechanisms underlying this effect are unclear. We have investigated the effects of increasing cAMP using inhibitors of PDE IV (rolipram) and PDE III (SK&F94120) on O_3 induced BHR to inhaled histamine in the G.P.

Male Dunkin-Hartley G.Ps. (400-550g) received rolipram, SK&F94120 or solvent (20% ethanol: saline) i.p. After 60 minutes they were exposed to O_3 (3 ± 0.5ppm) for 30 min, control animals breathed room air. G.Ps. were anaesthetised (sodium pentobarbitone 40mg.kg^{-1} i.p.) a tracheostomy performed and artificially ventilated (1ml.100g^{-1}; 60 breaths.min^{-1}). Tracheal flow and transpulmonary pressure were measured and used to calculate resistance (R) and compliance (C). Aerosols were generated via a nebuliser in the afferent arm of the ventilator circuit. Bronchial reactivity was assessed by measuring the change in R and C following 10s inhalation of nebulised solutions of histamine in saline (10-640μg,ml^{-1}). The concentration of histamine required to cause a 200% increase in R (RPC_{200}) and a 50% decrease in C (CPC_{50}) was calculated.

O_3 exposure increased basal R and decreased C in all G.Ps., drug treatment had no effect. (Table 1). BHR was characterised by a leftward shift and an increased slope and maximum of the histamine dose response curve. Rolipram reduced the BHR in a dose dependent fashion affecting both R and C, whereas SK&F94120 had no effect (Table 1). These data indicate that PDE IV is an important regulator of cAMP in G.P. airways and that selective inhibition of PDE IV can reverse ozone-induced BHR.

Table 1: The Effect of Rolipram & SK&F94120 on O_3-induced BHR

	Solvent + air	Solvent + O_3	Rolipram (mg.kg^{-1})+O_3			SK&F94120 (mg.kg^{-1})+O_3
			0.01	0.1	1.0	1.0
n	6	10	6	6	6	6
Basal R ($cmH_2O.L^{-1}.s^{-1}$)	92.4 (8.2)	153 (12.1)*	158(11.2)*	167(19.1)*	164(10.4)*	148 (13.1)*
RPC_{200} (μg.ml^{-1}.10s^{-1})	160 (9.1)	10 (4.1)*	10(3.8)	70(6.8)+	90(7.2)+	12.6 (8.9)*
Basal C (ml.cmH_2O^{-1})	0.97 (0.1)	0.27 (0.1)*	0.31(0.1)*	0.21(0.1)*	0.49(0.2)*	0.31 (0.2)*
CPC_{50} (μg.ml^{-1}.10s^{-1})	59.8 (7.6)	10.8 (3.8)*	14.2(5.1)	24.5(2.8)+	44.2(6.1)+	12.8 (4.9)*

Data is mean (± sem); *p<0.05 compared to solvent + air; + p<0.05 compared to solvent + O_3

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255P ROLE OF VERY LATE ANTIGEN-4 (VLA-4) IN EOSINOPHIL ACCUMULATION *IN VIVO*

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Eosinophil accumulation has been associated with the pathology of several diseases, such as pulmonary eosinophilic syndrome and allergic diseases such as atopic asthma. The mechanisms which control the preferential accumulation of this cell type in such responses remain unclear. Recently, a novel eosinophil adherence pathway involving VLA-4 and VCAM-1 has been described using in vitro assays (Walsh et al 1991; Dobrina et al, 1991; Weller et al 1991). However, the interaction of VLA-4 expressed on eosinophils (but not on neutrophils) with the endothelial cell adhesion molecule VCAM-1 has not been investigated in vivo.

In the present study, we have investigated the role of VLA-4 in an in vivo model of eosinophil accumulation using an anti-VLA-4 monoclonal antibody (mAb) HP1/2. Flow cytometry analysis indicated that mAb HP1/2 bound to guinea pig eosinophils, but not neutrophils, with a saturating concentration of 50μg per 10^6 cells. Accumulation of ^{111}In-eosinophils and leakage of ^{125}I-albumin was measured in a passive cutaneous anaphylaxis (PCA) reaction (Weg et al, 1991) and in response to intradermal PAF, LTB_4, C5a des Arg, zymosan particles and arachidonic acid (AA) as previously described (Faccioli et al, 1991).

In vitro pretreatment of ^{111}In-eosinophils with mAb HP1/2 (50μg/10^6 cells), prior to their final wash and i.v. injection, resulted in a significant inhibition of cell accumulation in all the reactions investigated (e.g. Table 1).

Table 1 ^{111}In-eosinophil x 10^3 ± s.e. mean (n=6-9)/site (*p<0.05)

	Saline/0.1% BSA	PAF 10^{-9}mol/site	LTB_4 $5x10^{-10}$mol/site	Zymosan 100μg/site	PCA
control	1.05±0.17	5.35±1.32	4.03±1.11	4.60±1.07	8.21±1.96
HP1/2	0.45±0.10*	1.62±0.34*	1.38±0.46*	1.13±0.39*	2.65±0.91*

Similarly, intravenous HP1/2 (3mg/kg) inhibited eosinophil accumulation, but did not affect oedema formation. These results indicate that in this model VLA-4 plays an important role in the accumulation of ^{111}In-eosinophils in vivo.

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256P LOCAL EOSINOPHIL ACCUMULATION AND OEDEMA FORMATION INDUCED BY SUPERNATANTS OF HOMOGENIZED CERCARIA OF *SCHISTOSOMA MANSONI* IN GUINEA-PIG SKIN

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Eosinophils play a central role in the pathogenesis of parasitic diseases and in allergic diseases such as asthma. In this report, we describe the accumulation of these cells and local oedema formation in response to intradermal injection of supernatants of homogenized cercaria of *Schistosoma mansoni* (CH) and of medium containing excretion/secretion products of the transformation from cercariae to schistosomulae (CTF). The effects of inhibitors of inflammatory mediators and protease inhibitors are also described.

Eosinophils, induced in the peritoneal cavities of guinea pigs by horse serum, were separated on a discontinuous percoll gradient and labelled with [^{111}In] (Faccioli *et. al*, 1991). The [^{111}In]-labelled eosinophils (5.10^6 cells/animal) and [^{125}I]-human serum albumin were injected i.v. via an ear vein of recipient guinea-pigs followed five minutes later by the i.d. injections of CH (1.0 to 15 μg of protein) and CTF (0.4 to 5 μg of protein) with or without different inhibitors. After a two-hour period, the skin sites were punched out and counted in a gamma-counter. Time-course experiments were also carried out in which the i.d. injections were given 5 minutes, 2 h, 3 h and 3.5 h after the i.v. cells and albumin, and the animals were killed 30 min later. Cell accumulation is expressed as labelled eosinophils per skin site and oedema as μl of plasma per skin site.

Both CH and CTF produced a dose-dependent eosinophil influx and oedema. Most of the cell accumulation and oedema occurred within 30 minutes of the i.d. injections. The extracts were not specific for eosinophils since neutrophils also accumulated in a dose-dependent manner. Histamine, PAF and prostaglandins do not appear to play a role since pyrilamine ($2.5x10^{-8}$ moles/site), WEB 2086 (10^{-7} moles/site) and indomethacin (10^{-8} moles/site) had very little effect on local oedema or cell influx. The addition of anti-proteases to the extracts prior to the i.d. injections partially inhibited local oedema and to a lesser extent the cell influx. PMSF (10 μg/site) inhibited the oedema caused by CH (68.4 $\pm$ 6.1 μl vs 36.4 $\pm$ 4.2 μl, n= 6) and by CTF (58.1 $\pm$ 6.3 μl vs 25.9 $\pm$ 1.2 μl, n=6). Both PMSF and Trasylol (1 μg/site) inhibited the eosinophil influx caused by CTF (39.5 $\pm$ 4.8% and 36.5 $\pm$ 5.3% inhibition respectively, n= 4-6) but were less effective against CH induced cell accumulation.

Cercaria are known to release several enzymes when they come in contact with the host skin. One of these enzymes is known to be a potent protease which may be important for the parasite penetration into the host (McKerrow *et. al*,1991). The relevance of the rapid influx of these inflammatory cells and local oedema formation has yet to be determined, but it is at least partially due to larvae proteases since anti-proteases inhibit both oedema and cell influx.

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257P A NOVEL POTENT BIS(SUCCINIMIDO)HEXANE PEPTIDE HETERODIMER ANTAGONIST (CP-0364) OF BRADYKININ BK_1 AND BK_2 RECEPTORS: *IN VITRO* AND *IN VIVO* STUDIES

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We have recently described a novel class of bis(succinimido)alkane peptide homodimers one of which,CP-0127 (compound 2, table 1), has potent activity against BK_2 medited responses *in vitro* and *in vivo* (Cheronis et al., 1992; Whalley et al., 1992) but has no antagonist activity at BK_1 receptors. Recent studies indicate that while the BK_2 receptor is constitutively present in most animals the induction and expression of the BK_1 receptor may be an important component of subacute or chronic inflammatory states (Ljunggren & Lerner, 1990; Neppl et al., 1991). We describe here a novel peptide Cys-Cys 1,6-bis(succinimido)hexane peptide heterodimer (compound 4) with potent activity *in vitro* and *in vivo* against both BK_2 and BK_1 receptors. Compounds were assayed for BK_2 antagonist activity on the rat uterus as described previously (Cheronis et al., 1992). BK_1 antagonist activity *in vitro* was assessed on the rabbit aorta. Aortic strips were mounted under 2g resting tension in 4 ml tissue baths containing Krebs solution at 37°C and bubbled with 95%O_2/5%CO_2. At time 5h 10^{-7}M des-Arg^9-BK was added to the bath and when a sustained tension was achieved the antagonist was applied in a cumulative fashion. The concentration producing 50% reversal (IC_{50}) of the sustained response was measured. *In vivo* activity against both BK_2 and BK_1 rceptors was assessed against bradykinin- (0.8 nmole) and des-Arg^9-BK- (8 nmole) induced hypotensive responses in rabbits pretreated with LPS from E Coli (10ug/rabbit i.v.). Two known BK_2-antagonists (compound no's 1&2) and a BK_1-antagonist (compound no 3) were tested and compared with the novel heterodimer (compound 4, CP-0364) and the results are shown in table 1.

COMPOUND		UTERUS BK_2	AORTA BK_1	BLOOD PRESSURE BK_2	BLOOD PRESSURE BK_1
1 Hyp^3-DPhe^7-Leu^8-DArg[BK]	(CP-0088)	7.1±0.2[a]	ia	++	ia
2 Hyp^3-Cys^6-DPhe^7-Leu^8-DArg[BK] Hyp^3-Cys^6-DPhe^7-Leu^8-DArg[BK]	(CP-0127)	8.5±0.2	ia	+++	ia
3 des-Arg^9-Leu^8-Lys[BK]	(CP-0298)	ia	7.9±0.1[b]	ia	+++
4 des-Arg^9-Cys^1-Hyp^3-Leu^8-DArg[BK] Hyp^3-Cys^6-DPhe^7-Leu^8-DArg[BK]	(CP-0364)	8.3±0.2[a]	7.5±0.1[b]	+++	+++

Table 1. a) pA_2 ± s.e. mean; b) -log [IC_{50}] ± s.e. mean; ia = inactive; + - +++ = degree of inhibition. n=3-8

Compounds 1 and 2 inhibited only BK_2- and compound 3 only BK_1-receptor mediated responses. Compound 4 (CP-0364) was found to be a potent inhibitor of both BK_2 and BK_1 receptor mediated responses. Compounds such as CP-0364 may be useful in the treatment of inflammatory disorders wherein both BK_2 and BK_1 receptors appear to contribute to the clinical manifestations of the disease.

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258P RELEASE OF INTERLEUKIN-1 (IL-1), TUMOUR NECROSIS FACTOR-α (TNFα) ACTIVITY AND CORTICOSTERONE (CCS) IN EXPERIMENTAL ENDOTOXAEMIA IN LEWIS AND WISTAR RATS

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Lipopolysaccharide (LPS) releases cytokines into serum and increases corticosteroids which may play a feedback role controlling cytokine release. We have investigated LPS-induced serum IL-1 and TNFα activity and corticosterone (CCS) in Wistar and Lewis rats; the latter has a dysfunctional HPA axis, resulting in lower CCS in response to various stimuli (Sternberg et al., 1989).

Male Lewis (Lew) or Wistar (Wis) rats (200-250g, Harlan-Olac) were injected i.p. with LPS (E. coli 055:B5, Sigma) in sterile saline between 09.00 and 10.30. At the indicated time, animals were killed under stress-free conditions, and blood samples taken. CCS was measured by RIA (ICN-Flow); TNFα measured as cytotoxic activity against mouse fibroblast 'L-M' cells (ICN-Flow) and IL-1 as LAF activity using C3/HeJ mouse thymocytes following precipitation of inhibitory activity with 12% polyethylene glycol (Hopkins & Humphreys, 1990).

CCS, IL-1 and TNFα activity were increased in a dose-dependent manner by LPS (0.01-1 mg/kg), the following data all refers to 1mg/kg. CCS was elevated 1h following LPS (514±45 (n=20) and 565±33 (12) mean ± s.e.m. for (n) rats ng/ml, NS (Student's unpaired t test), Lew and Wis respectively), and maintained for at least 6h; compared to the Wistar, CCS was lower in the Lewis (eg at 3h, 272±26 (n=14, Lew) and 479±27 (16, Wis) $P<0.001$). TNFα activity was measurable at 1h and 2h, but more prolonged in the Lewis (eg at 1h, 5080 ± 774 (9) and 3137 ± 767 (11) NS, and at 2h, 4406 ± 887 (7) and 1212 ± 609 (8) $P<0.05$ TNF 'units' (1/dilution giving 50% cytotoxicity) for Lew and Wis respectively). IL-1 activity was measurable at 3h, and declined at 6h; Lewis serum contained more activity than did Wistar (eg at 3h, 35463 ± 4705 and 3452 ± 287 cpm for 1:200 dilution of sera for Lew and Wist respectively). This 'overproduction' of cytokines and lower CCS release may contribute to the increased susceptibility of the Lewis strain to experimental inflammatory disease.

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259P ANGIOGENESIS DURING THE DEVELOPMENT OF CHRONIC GRANULOMATOUS TISSUE AS ASSESSED BY VASCULAR CASTING *IN VIVO*

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Vascular growth is essential for granuloma, such as pannus, development. The definitive measurement of this process relies upon lengthy and costly blood vessel quantitation. The use of Evans blue (EB) incorporation into tissue and Xenon diffusion from tissue are both susceptible to variations in plasma exudation. We describe a modification of the carmine red (CR) vascular casting technique (Kimura et al., 1986), and report the quantitation of the modulation of angiogenesis during granuloma formation *in vivo*.

Chronic granulomatous air pouches were induced by the s.c. injection of 3 ml air into anæsthetised mice (30±2g, Tuck) and 0.5ml Freund's complete adjuvant with 0.1% crotton oil 24 hours later. Mice were dosed p.o. for 6 days with vehicle, heparin (100-5000U), and cortisone or tetrahydrocortisone (THC) with heparin (1 & 1 mg kg^{-1} and 1000U respectively, and cortisone alone. Vascular content was assessed by the formation of a vascular cast after the i.v. injection of 1ml 10% CR, or 1% EB, in 5% gelatin at 40°C into warmed mice, and chilling the carcasses. The dissected tissue was dried at 56°C, weighed, and papain digested (9ml 0.05M phosphate buffer pH7.0, 0.33g l^{-1} N-acetylcysteine, 12U ml^{-1} papain at 56°C for 72 hours). CR & EB were dissolved by the addition of 1ml 0.05M NaOH, centrifuged at 2500g for 20 min., the supernatants filtered (0.22μm), and read at 490nm using a multiwell plate reader (Biotek). Readings were subtracted from non-injected controls treated as above and the results were expressed as either mg dye content per sample or the vascular index (VI) as μg dye/mg dry weight of tissue.

There is an absence of vasculature into which capillary buds develop and are patent by day 6. EB was exuded into the surrounding tissue and pouch exudate, whilst CR was completely contained within the capillary bed. CR content was unaffected by 75μg histamine injected into the pouch whilst EB content was increased by 24%, and a bolus dose of indomethacin (3mg kg^{-1} p.o.) 24 hours previously reduced EB content by 33% with no effect on CR content.

Table 1. The development of granulation tissue, vascular content (mg dye), and VI (μg mg^{-1} in mice treated with angiogenic or angiostatic stimuli). n=8 per group, ***=p<0.001, **=p<0.01, *=p<0.05 Mann Whitney U test compared to vehicle control.

Angiogenic	Dry mass (mg)	Dye (mg)	VI (μg mg^{-1})	Angiostatic	Dry mass(mg)	Dye (mg)	VI (μg mg^{-1})
p.o. Vehicle	219±28.8	0.77±0.08	3.36±0.53	p.o. vehicle	123.7±18.4	0.89±0.12	8.31±1.46
100U Heparin	219±12.0	0.90±0.05 *	4.24±0.32	cortisone (1mg kg^{-1})	120.3± 6.8	0.87±0.08	8.23±0.62
500	214±15.0	0.96±0.04 ***	4.56±0.48 **	THC (1mg kg^{-1})	86.9± 8.7*	0.46±0.03***	5.29±0.61***
1000	210±16.0	1.03±0.03 ***	4.84±0.42 **	Heparin (1000U)	121.9±17.2	0.84±0.06	7.93±0.83
2000	215± 6.5	1.09±0.05 ***	5.00±0.50 ***	Heparin & cortisone	102.9±13.0	0.69±0.04 *	6.03±1.12**
3000	222± 7.5	1.23±0.06 ***	5.60±0.40 ***	Heparin & THC	78.9± 7.3*	0.48±0.04**	7.11±0.85
4000	236±11.5	1.42±0.13 ***	5.97±0.59 ***				
5000	235±10.6	1.94±0.25 ***	8.53±0.11 ***				

CR in gelatin formed a vascular cast of inflammatory tissue which did not exude into the tissue, unlike EB. Classical angiogenic treatment resulted in a dose related increase in VI, and angiostatic therapy elicited the opposite effect. The otherwise inert steroid THC elicited angiostatic activity in the absence of heparin, and could form the basis for safe angiostatic therapy in disease. We suggest this model is useful as a rapid method for the assessment of drugs for their effects on the vascularity of inflammatory tissue *in vivo*.

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260P THE COMBINED USE OF DEHYDROEPIANDROSTERONE AND DEXAMETHASONE INHIBITS ANGIOGENESIS IN THE RAT WITHOUT CAUSING IMMUNOSUPPRESSION

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We have previously shown, in a rat sponge model, that daily local or systemic administration of 5 or 50 μg dexamethasone (DX) profoundly inhibited the sponge-induced angiogenesis, but there were severe losses in body, spleen and thymus weights (Hori *et al.*, 1992). Using a different model, Blauer *et al.* (1991) reported that thymic atrophy after acute DX treatment was associated with immunosuppression. Furthermore, dehydroepiandrosterone (DHEA) pretreatment of mice significantly protected the animals against DX-induced thymic atrophy. Here we examined if DHEA possesses anti-angiogenic activity and/or protective effects against the DX-induced immune organ weight losses in this model.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (150-200g) and, using a ^{133}Xe clearance technique (Andrade *et al.*, 1987), neovascularisation was assessed as a function of blood flow through the implants every two days over a period of 14 days. The ^{133}Xe clearance technique has been validated by comparison with absolute blood flow measurements by ^{113}Sn microsphere wash-in technique, measurement of neovasculature by carmine dye method and colorimetric measurement of haemoglobin levels in the implants (Hu *et al.*, 1992a & 1992b). The effects of test substances on angiogenesis were confirmed histologically.

Daily administration of 1, 10 or 100 μg DHEA into the sponges produced no apparent effect on sponge-induced neovascularisation. The neovascular response was inhibited by administration of 5 μg DX with or without 100 μg DHEA. The 6 min ^{133}Xe clearance values obtained on Day 8, 10, 12, 14 for control group and treated groups were 21.3±1.0, 30.4±0.9, 35.4±1.2, 40.5±0.9 %; 15.8±1.3, 18.8±1.9, 19.1±1.1, 20.3±0.5 % and 15.3±1.1, 18.3±1.6, 18.1±2.0, 22.4±0.5 % respectively (between the treated and control group, $P < 0.01$, $n = 4$-8). Histological studies of sponge sections stained with haematoxylin & eosin or the endothelial cell marker, *Bandeirea simplicifolia* lectin I, isolectin B_4, showed that both cellular infiltration and neovascularisation were profoundly inhibited in the treated sponges as compared to controls. But the immunosuppressive effect of DX was antagonised by DHEA ($P < 0.01$, $n = 4$-8), e.g. the body, spleen and thymus weights were 172±3 (g), 495±25 (mg), 58±6 (mg) in DX group and 263±3 (g), 823±10 (mg), 309±9 (mg) in DX + DHEA group. If the animals were treated with daily doses of 50 μg DX, the co-administration of 100 μg DHEA produced a partial protection. Furthermore, systemic administration of DHEA was also effective, e.g. daily intraperioneal injection of 100 μg DHEA inhibited the effect of 5 μg DX on body, spleen and thymus weights.

Thus, it seems that DHEA antagonises the immunosuppressive actions of DX without compromising its anti-angiogenic activity in the rat. These results suggest the combined use of DHEA and glucocorticoids could have therapeutic benefit in angiogenic diseases.

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261P ANTI-INFLAMMATORY AND PHARMACOKINETIC PROPERTIES OF A MURINE ANTI-CD 18 MONOCLONAL ANTIBODY AND ITS HUMANIZED COUNTERPART IN THE RABBIT

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The adhesive interaction between leukocyte β2 integrins (CD11/CD18) and their ligands/counter-receptors (e.g. ICAM-1) on vascular endothelium is of critical importance in neutrophil (PMN) emigration to sites of inflammation (Springer, 1990). Although murine monoclonal antibodies (McAb) directed against CD18 can prevent PMN diapedesis and PMN-dependent inflammation in several models (Carlos & Harlan,1990), their therapeutic utility is limited by the immunogenicity of murine immunoglobulin (Ig). To circumvent this problem we humanized the murine anti-CD18 McAb IB4 (mIB4, $IgG_{2a}\kappa$) by grafting its complementarity determining regions into a human IgG_4 framework ($IgG_4\kappa$) whilst retaining the avidity of the murine McAb (Mark et al, 1992). In this study we compared the pharmacokinetics and activity of intravenously administered mIB4 and humanized (h)IB4 in the rabbit. Dermal inflammation was evoked by intradermal injection of recombinant human (rh) C5a (100 pmol/site) or PAF (10 nmol/site), and 6 mm punch biopsies were removed 3 hours later. Plasma extravasation was quantified as local accumulation of [^{125}I]-human serum albumin, and PMN accumulation was quantified as the myeloperoxidase content of the biopsy. Plasma levels of mIB4 and hIB4 were measured by ELISA, and cell-bound mIB4 and hIB4 were estimated by FACS analyses.

When administered at 1 mg/kg 15 mins prior to challenge, mIB4 and hIB4 abolished PMN accumulation elicited by PAF and rhC5a, and suppressed the oedematous response to rhC5a but not PAF, whereas human IgG4 and a murine anti-CD11a (OKM-1) were ineffective. Moreover, mIB4 and hIB4 displayed comparable potency in attenuating rhC5a-induced PMN accumulation and plasma extravasation (ED_{50} values ~0.2 mg/kg). Following i.v administration at 1 mg/kg, the peak plasma level of hIB4 (15.7 μg/ml) exceeded that of mIB4 (5.1 μg/ml), but both were in excess of levels necessary to saturate PMN CD18 (1 μg/ml). Clearance of both McAbs from plasma was rapid (plasma half-life = 4.8 hours for mIB4 and 3.3 hours for hIB4), whereas the clearance time of cell-bound McAb was much longer (half-life between 24 - 48 hours for mIB4, and between 48 - 72 hours for hIB4). The durations of biological action of mIB4 and hIB4, determined by injecting McAbs at different times prior to initiation of dermal inflammation, were prolonged and correlated with extent of saturation of PMN CD18: rhC5a-induced PMN accumulation was 50% inhibited at 44 hours following hIB4 injection, and at 26 hours following mIB4 injection. These data indicate that humanization compromises neither the pharmacokinetic properties nor the anti-inflammatory profile of mIB4, and suggests that hIB4 may be effective in modulating CD18-dependent, leukocyte-mediated inflammatory processes in man.

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262P METHOTREXATE/INDOMETHACIN INTERACTION IN NORMAL AND TUMOUR CELL LINES: EFFECTS OF FOLATE CONCENTRATION

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Indomethacin (INDO) potentiates the cytotoxicity of the folate analogue methotrexate (MTX) to the murine NC carcinoma *in vivo* and *in vitro* (Gaffen *et al.*, 1985; Bennett *et al.*, 1987). We set out to determine whether the interaction also occurs in human bone marrow cells, as these are particularly at risk from cytotoxic damage. Furthermore, since standard culture media contain supraphysiological levels of folate we have examined the MTX/INDO interaction at a physiological folate concentration in NC and bone marrow cells. The findings are encouraging with regard to the possible clinical use of MTX/INDO combinations.

NC carcinoma cells were cultured in Eagle's minimal essential medium, and lymphoblast-like cells of the normal haemopoietic line RPMI 1788 in Iscove's modified Dulbecco's medium, formulated to contain either standard (respectively 2 μM and 8 μM) or physiological (20 nM) folate concentrations. Cell growth was determined by microturbidimetry (Gaffen *et al.*, 1985).

INDO (1 and 2 μg ml^{-1} for NC cells and 0.1 - 5 μg ml^{-1} for lymphoblasts) had no significant effect on cell growth at either standard or physiological folate concentrations. The mean growth reductions of NC cells by MTX 4 and 8 ng ml^{-1} in physiological folate were 55.1 ± 4.1 and 68.8 ± 3.4 % compared to 24.0 ± 5.1 and 56.7 ± 2.5 % in the standard folate concentration ($P<0.0001$, Student's t-test, n=12). INDO 1 μg ml^{-1} potentiated the cytotoxicity of MTX 4 and 8 ng ml^{-1} to NC cells at both folate concentrations (2 μM folate by 33.3 ± 4.0 and 15.2 ± 5.6 % respectively; physiological (20 nM) folate by 25.8 ± 4.8 and 22.6 ± 5.5 %; $P<0.03$, n=12).

With the lymphoblasts, in contrast, the concentration-dependent reduction of growth by MTX 20 - 80 ng ml^{-1} was *less* at the physiological than at the high folate concentration (physiological folate, 6.4 ± 3.4 to 16.1 ± 4.8 %; 8 μM folate, 31.2 ± 2.3 to 39.3 ± 3.0 %; all $P<0.04$, n=18 to 42). Also contrary to the NC cells, with physiological folate INDO 0.1 and 1 μg ml^{-1} *reduced* the cytotoxicity of MTX 40 ng ml^{-1} (by 10.3 ± 4.7 and 11.9 ± 3.3 %, both $P<0.04$, n=18) and a similar trend occurred with MTX 20 ng ml^{-1} (7.9 ± 4.2 and 6.4 ± 3.4 % less cytotoxicity with INDO, $P<0.08$ and $P<0.06$, n=18). However, in 8 μM folate INDO 1 μg ml^{-1} potentiated MTX 20 and 40 ng ml^{-1} by 12.7 ± 5.8 and 9.4 ± 4.5 % (both $P<0.04$, n=18) whereas the lower INDO concentration (0.1 μg ml^{-1}) had no significant effect.

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263P OXIDISED LOW DENSITY LIPOPROTEIN SUPPRESSED BRADYKININ-INDUCED PROSTACYCLIN RELEASE BY CULTURED ENDOTHELIAL CELLS

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Oxidatively modified low density lipoprotein (Ox-LDL) is thought to be implicated in primary events of atherosclerosis by decreasing endothelial function (Flavahan, 1992). We have therefore investigated the effect of chronic Ox-LDL exposure (3 days) on prostacyclin release from bovine aorta endothelial cells.

Cells, prepared by enzymatic digestion, were plated in 1 cm diameter multiwells at a density of 10^5 cells per well and grown to confluence (24 hr) in the presence of LDL or Ox-LDL (20 μg/ml), or vehicle. Growth medium was then replaced by medium 199 for 48 hours. The endothelial cells were washed with 2 x 1 ml Krebs solution and then incubated 30 min at 37°C with fresh Krebs solution (1 ml), with or without bradykinin (Bk). The prostacyclin content of the Krebs solution was determined by radioimmunoassay of 6-keto-prostagladin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$). Results are expressed as pg 6-keto-$PGF_{1\alpha}$.μg^{-1} protein. LDL was prepared from human plasma by sequential ultra centrifugation. Oxidation of LDL was carried out in the presence of $CuCl_2$ (4 μM).

Chronic exposure (3 days) of endothelial cells to Ox-LDL decreased the amount of protein per well by 25 % versus control (table). Basal formation of 6-keto-$PGF_{1\alpha}$ was decreased after Ox-LDL incubation versus LDL and control groups. Bk-induced 6-keto-$PGF_{1\alpha}$ formation was decreased by 48 % for the highest concentration tested after exposure to LDL. Exposure to Ox-LDL completely abolished the formation of Bk-stimulated 6-keto-$PGF_{1\alpha}$, and attenuated basal formation of 6-keto-$PGF_{1\alpha}$.

Table : Effects of LDL and Ox-LDL (20 $\mu g.ml^{-1}$) on proteins (μg per well) and basal or Bk-induced 6-keto-$PGF_{1\alpha}$ formation (pg.μg $prot^{-1}$).

Groups	Proteins	n	6-keto-$PGF_{1\alpha}$ Basal	1 nM	10 nM	100 nM	n
control	99.7±1.8	40	3.6±0.6	11.1±0.9	19.7±2.7	62.2±5.6	12
LDL	92.4±1.7	24	2.6±0.7	12.8±1.5	18.6±4.1	34.3±6.7*	12
Ox-LDL	74.9±4.0¶	24	1.8±0.9*+	00.2±0.1*+	00.4±0.2*+	00.6±0.3*+	12

Values are means ± sem. (¶) significantly different to all groups (ANOVA), and (*) to control or (+) to LDL (Kruskal-Wallis test).

These results suggest that chronic exposure to Ox-LDL alters growth of endothelial cells in culture and completely inhibits bradykinin-induced prostacyclin release. Moreover, the fact that basal and stimulated release of prostacyclin are differentially altered by Ox-LDL might indicate two different pathways for the eicosanoid release. These effects could contribute to the local formation of the fatty streaks by suppression of a major cytoprotective agent.

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264P PREVENTION OF CORONARY THROMBOSIS IN THE DOG BY INHIBITORS OF PLATELET AGGREGATION

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A thromboxane (TXA_2) receptor antagonist (ICI192605), a TXA_2 synthase inhibitor (dazoxiben), a combined TXA_2 antagonist/inhibitor (D1542), aspirin and a $5HT_2$ receptor antagonist (ICI170809) all inhibit platelet aggregation. Adrenaline enhances platelet aggregation and increases the rate of arterial thrombus formation. The maximum effectiveness of these drugs has been measured as an increase in the infusion of adrenaline required to restore the rate of thrombus formation to that before drug treatment, in a stenosed/damaged coronary artery of an anaesthetised dog (pentobarbitone 30mg/kg i.v.).

The maximum effects of aspirin, ICI192605 and ICI170809 were similar and caused dose ratio increases in adrenaline of: 24(10-57); 40(11-150); 39(12-126) [mean,95%CI] respectively. Dazoxiben and D1542 were more effective requiring increases in adrenaline of 151(62-363) and 186(30-1190) [$P<0.05$]. In platelets activated ex-vivo by collagen: aspirin, dazoxiben, and D1542 inhibited TXA_2 by (>95%), also dazoxiben and D1542 caused a 15 fold increase in PGD_2 an inhibitor of aggregation, probably accounting for their greater effectiveness.

The addition of TXA_2 receptor blockade (ICI192605) to TXA_2 synthase inhibition (dazoxiben) is known to further reduce the rate of thrombus formation (McAuliffe et al 1992). D1542 has the advantage that these two properties are combined within the same drug and was the most effective at preventing thrombus formation.

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265P TRIMETAZIDINE REDUCES MYOCARDIAL INFARCT SIZE, RELATIVE TO AREA AT RISK, AFTER TEMPORARY CORONARY ARTERY OCCLUSION IN THE RABBIT

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Trimetazidine has been shown to have anti-ischaemic effects in isolated tissue preparations, possibly by membrane stabilising effects (Kiyosue et al., 1986). We attempted to demonstrate these effects, in vivo, by studying the influence of trimetazidine on myocardial infarct size in the rabbit.

Fourteen New Zealand White rabbits weighing from 2.5 - 4.2kg were pretreated 3 hours before they were anaesthetised. Seven received trimetazidine $3mg.kg^{-1}$ i.v., and seven its vehicle. Induction was by pentobarbitone $20mg.kg^{-1}$ and maintenance by positive pressure ventilation using 50% N_2O/O_2 and 1.5% halothane gas mixture. The heart was exposed by lateral thoracotomy, and the obtuse marginal coronary artery was occluded by an encircling ligature, applied for 45 minutes. The ligature was then released and the chest closed with the suture in situ. The animals thus received 24 hours of normothermic blood reperfusion to the infarcted area, before being heparinised and killed by barbiturate overdose. The heart was removed and placed on a Langendorff column and perfused with heparinised normal saline; the vessel was re-ligated and 10-15μm zinc/cadmium particles were infused into the coronary tree to define the area at risk. The heart was then stained with triphenyltetrazolium, after slicing into 2mm sections. The areas of infarction, defined by absence of triphenyltetrazolium staining, and areas at risk were delineated by planimetry. A p value of <0.05 was taken to represent statistical significance.

Mean infarct size expressed as a percentage of the left ventricle (LV) in the vehicle only group was 19.4 ± 7.7 vs 22.8 ± 21.0 (means ± s.d.) in the trimetazidine treated group (NS); the area at risk was also comparable between the two groups (vehicle: 28.3 ± 7.4 vs 29.8 ± 10.4 (% LV) NS). Because of the range of sizes, the ratio of infarct size to area at risk was compared for each individual animal (Torr et al., 1989). A significant reduction in infarct size, in relation to area at risk, became apparent in the trimetazidine pretreated group (0.420 ± 0.123 vs 0.687 ± 0.204, $p< 0.025$, Mann-Whitney). Plotting infarct size against area at risk for the two groups showed two linear relations with similar slopes for both; however, the mean point of the regression for the trimetazidine pretreated group was significantly lower ($p< 0.025$, ancovar).

In this model, 45 minutes of coronary occlusion was followed by a 24 hour recovery period to allow full development of the infarct. Pretreatment with trimetazidine $3mg.kg^{-1}$ i.v. was effective in reducing infarct size relative to area at risk.

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266P SUPPRESSION OF INTRACORONARY THROMBOSIS BY TRIMETAZIDINE WITHOUT ADVERSE HAEMATOLOGICAL EFFECTS

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Trimetazidine is a member of the piperazine group of drugs known to inhibit calcium entry to cells (Renaud J.F., 1988). In vitro, there is evidence that platelet aggregation can be decreased by this agent (Higuchi et al., 1981). An in vivo investigation of intracoronary thrombosis was performed using the Folts' model (critical stenosis with endothelial damage) in eight open-chest anaesthetised beagles (pentobarbitone 30mg.kg^{-1} induction plus 3mg.kg^{-1} half hourly) as previously described (Belcher et al., 1992). The rate of thrombosis was assessed from the rate of flow reduction in the circumflex coronary artery which was 8.8 ± 3.2ml.min^{-2} (mean ± s.d.) during a control period. Trimetazidine, given intravenously at a dose of 1mg.kg^{-1}, completely abolished the blood flow reduction due to accumulating platelet thrombus in 4 animals, and attenuated it in the remainder [slope 3.1 ± 4.8ml.min^{-2} (mean ± s.d.); $p < 0.01$ Wilcoxon]. Further increments of trimetazidine (up to 5mg.kg^{-1}) were given to these four dogs and abolished platelet thrombosis in two, and further attenuated it in the remaining two. There were no systemic haemodynamic effects observed. Adrenaline was then infused to stimulate platelet activation at a rate of 0.4μg.kg^{-1}.min^{-1}, which is sufficient to overcome the anti-aggregatory effects of aspirin in the model (Keller & Folts, 1990); thrombosis was restarted in one dog only. Adrenaline given at 1.6μg.kg^{-1}.min^{-1} resulted in restoration or increase in the thrombosis rate in all animals ($p < 0.01$).

A further six non-operated dogs were anaesthetised and given intravenous trimetazidine 3mg.kg^{-1}. Prothrombin, thrombin and partial thromboplastin times, and fibrinogen levels were not altered. However, aspirin 5mg.kg^{-1} was then given which significantly increased bleeding time, without altering coagulation variables.

These findings suggest that trimetazidine is effective at preventing intracoronary platelet aggregation in this model. Since coagulation factors are spared and bleeding time is unchanged, the cause is unlikely to be inhibition of arachidonic acid metabolism or interference with the fibrinogen or thrombin receptors which might be potentially advantageous.

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267P THE ANTI-ISCHAEMIC PROPERTIES OF CLORICROMENE IN THE RABBIT ARE NOT MEDIATED BY AN ANTI-PLATELET ACTIVITY

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Cloricromene (CLO) is a non-anticoagulant coumarine derivative which inhibits human platelet aggregation (Prosdocimi *et al.*, 1986) and *in vitro* polymorphonuclear cell adhesion to endothelium (Bertocchi *et al.*, 1989). The drug causes coronary vasodilation in dog (Aporti *et al.*, 1978) and exerts a protective effect in several types of experimental shock (Squadrito *et al.*, 1992). We investigated the effect of CLO in a rabbit model of myocardial ischaemia/reperfusion, monitoring *ex vivo* platelet aggregation and haemodynamics.

Adult rabbits (2.5-3.2 kg) were anaesthetized with sodium pentobarbitone (30 mg/kg i.v.) and subjected, at time 0, to 1 h occlusion of the first antero-lateral branch of the left circumflex coronary artery (LAL), followed by 2 h of reperfusion. Left ventricular systolic pressure (LVSP), mean arterial pressure (MAP), heart rate (HR) and lead II limb ECG were continuously recorded. Rabbits were infused with CLO (2 or 18 mg/kg/h i.v.), ibuprofen (IBU, 5 mg/kg/h i.v.) or saline (VEH) starting 15 min before occlusion and continuing throughout the experiment. Aggregation of platelet-rich plasma was examined *in vitro* using ADP (2 μg/ml) or collagen (8 μg/ml) at -15, 0, 60, 120 and 180 min. At the end of the experiment, the LAL was reoccluded and Evans blue dye solution was injected into the left ventricle to determine the area at risk. To distinguish between the ischaemic and the infarcted area, the area at risk was sliced, chopped and incubated for 20 min at 37 °C in nitro-blue tetrazolium (0.5 mg/ml).

Infusion of CLO or IBU caused minimal changes in LVSP, MAP and HR. The pressure-rate index remained unchanged. In the VEH group, LAL-occlusion caused an ST-segment elevation from 0.03±0.01 mV to 0.14±0.02 mV after 20 min which remained elevated for the 1 h occlusion period and, during reperfusion, gradually returned to basal values. CLO at 18 mg/kg/h significantly reduced the occlusion-induced ST-elevation to 0.08±0.02 mV ($p<0.01$) after 20 min and to 0.03±0.01 mV ($p<0.001$) after 1 h occlusion. One hour of occlusion followed by 2 h of reperfusion did not cause any changes in platelet aggregation in the control ischaemic group. Furthermore, 18 mg/kg/h of CLO induced a significant inhibition of the *ex vivo* platelet aggregation, with the effect being more evident against collagen than against ADP. Neither CLO at 2 mg/kg/h nor IBU had any effect on ischaemia-induced ST-elevation, or *ex vivo* platelet aggregation. In all groups the "area at risk" was approximately 30 %. In the VEH group the infarct size was 67.1±2.4 %. The administration of CLO resulted in a dose-dependent reduction in infarct size, to 40.8±7.9 % at 2 mg/kg/h ($p<0.01$) and 32.4±6.6 % at 18 mg/kg/h ($p<0.001$). Ibuprofen infusion decreased infarct size to 45.5±11.7 % ($p<0.05$).

Thus, cloricromene protected the reperfused ischaemic myocardium both in the presence and absence of an *ex vivo* anti-platelet effect. The mechanism of action of CLO on infarction may therefore be via other cell types or cellular mediators present within the myocardium. *(* Visiting Scientist from Fidia Research Laboratories, Abano Terme, Italy)*

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268P EFFECTS OF SUBSTRATE DEPRIVATION AND HYPOXIA ON THE MEMBRANE INTEGRITY IN CULTURED NEONATAL RAT VENTRICULAR MYOCYTES

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Ischemia is a complex event consisting of hypoxia, acidosis, substrate deprivation and accumulation of metabolic wastes. The precise mechanisms that lead to myocardial irreversible cell injury are unknown, but evidence suggests that calcium overload is involved in this pathological process (Chien and Engler, 1990). The purpose of this study was to test an experimental in vitro model of ischemia in isolated rat ventricular myocytes in monolayer culture. Ventricular cells were isolated from neonatal rats and cultured according to Schanne (1972). Cells were either submitted to glucose deprivation, to glycolysis blockade with 2-deoxy-D-glucose (2DG) or to glucose depletion followed by hypoxia. Lactate de hydrogenase (LDH) activity measured according to Wroblewski and LaDue (1955) was taken as an index of irreversible cell damage.

A 24 hour-depletion of glucose only moderately increased LDH leakage into the extracellular medium raising the concentration to 151 ± 12 IU LDH/mg prot (n=7) (vs basal value of 26 ± 5 IU/mg prot, n=4) which averaged 15% of the total cellular LDH. In contrast, a 24 hour period of glycolysis blockade by 2DG (5.6 mM) dramatically increased LDH release in the extracellular medium (1569 ± 124 IU/mg prot, n=5) corresponding to 80% of the total cellular LDH in this set of experiments. Hypoxic conditions were achieved by incubating the cells in an atmosphere saturated with 95% N_2, 5% CO_2 in order to decrease the pO_2 (25.6 ± 2.0 mmHg versus 135.7 ± 4.0 mmHg in controls, $p < 0.001$). Hypoxia on its own had no effect on basal LDH release. However, a 6-hour hypoxic treatment applied to cells exposed to glucose-free medium for 18 hours caused an increase in LDH release from 176 ± 36 IU/mg prot (normoxia) to 602 ± 82 IU/mg prot (hypoxia), $p< 0.05$. This increase in LDH leakage was significantly antagonized by the dihydropyridine derivative elgodipine (IC_{50} = 4.2 µM).

In neonate rat cultured ventricular myocytes on normoxia, glucose deprivation on its own does not cause significant cell damage, suggesting that conversion of glycogen to glucose is efficient enough to maintain cellular energy production. The significant LDH leakage induced by glycolysis blockade with 2DG supports the view that the maintenance of the membrane integrity and the survival of cardiac cells is highly dependent upon energy production via the glycolytic pathway. In contrast, during hypoxia and under glucose removal, glycogen breakdown is not sufficient to preserve cell viability, suggesting that glucose is the major energy-yielding substrate under hypoxic conditions. The ability of elgodipine to reduce anoxia-induced LDH leakage supports the view that part of the cellular damage may involve Ca^{2+} overload through the dihydropyridine sensitive L-type calcium channel.

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269P PHENTOLAMINE ATTENUATES HYPOXIA-INDUCED ABBREVIATION OF THE CARDIAC ACTION POTENTIAL

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Phentolamine protects the heart against ischaemia-induced arrhythmias (Bralet et al., 1985) but the mechanism of this action has not been elucidated. In view of the finding that phentolamine blocks K_{ATP} channels in pancreatic β cells (Plant & Henequin, 1990) we compared the effects of phentolamine and glibenclamide (a K_{ATP} channel blocker) on hypoxia and levocromakalim (a K_{ATP} channel opener) induced effects on the cardiac action potential.

Guinea pig papillary muscles were superfused *in vitro* with Krebs Henseleit solution at 36°C and paced at 1Hz. Action potentials were measured before and after 30 min exposure to vehicle (DMSO) or drug (glibenclamide 10µM or phentolamine 10 & 30 µM) and over a 30 min exposure period to either hypoxia (PO_2 in organ bath reduced from 636±14 to 63±4 mmHg) and removal of the glucose from the salt solution or levocromakalim (20µM). Phentolamine and glibenclamide significantly attenuated the hypoxic abbreviation of the action potential (Fig 1) and phentolamine (30µM) also reduced the upstroke velocity from 187±11 to 137±10 Vs^{-1} (n=5). Levocromakalim shortened the action potential duration from 178±15 to 55±9 ms (n=3); this shortening was prevented by glibenclamide and attenuated by phentolamine (30µM) (APD_{90} was reduced to 91±7 ms; n=5).

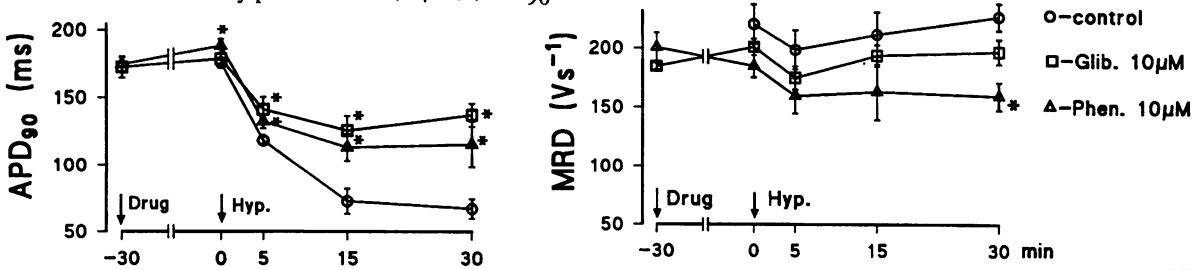

Figure 1. The effects of hypoxia and removal of glucose over a 30 min period on action potential duration measured at 90% of repolarisation (APD_{90}) and maximum rate of depolarisation (MRD) in muscles exposed to vehicle (n=5), glibenclamide (n=4) or phentolamine (n=5). Values are mean ± s.e. mean; *P < 0.05 significantly different from the vehicle treated group.

These results suggest that phentolamine in antiarrhythmic concentrations (Bralet et al., 1985) attenuates the abbreviation of the action potential induced by hypoxia or levocromakalim similarly to glibenclamide. Phentolamine's block of Na^+ channels, as evidenced by the drug's reduction in upstroke velocity, may also contribute to its antiarrhythmic effect.

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270P SR 33589: A NEW AMIODARONE-LIKE ANTIARRHYTHMIC AGENT

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Amiodarone is established to be one of the most effective antiarrhythmic agents currently available for clinical use (Cairns et al., 1991, Nalos et al., 1991) and is thought to be active by prolonging action potential duration (APD) and by its non-competitive inhibition of alpha and beta adrenoceptor stimulation. SR 33589 (2-n-butyl 3-[4-(3-di-n-butylamino-propoxy)benzoyl] 5-methylsulphonamido benzofuran hydrochloride) is a recently synthetised compound, structurally related to amiodarone that has been shown to reduce the incidence of reperfusion-induced arrhythmias in rats (Bruyninckx et al., 1992). In this study we have compared to amiodarone the ability of SR 33589 to prolong APD and inhibit the effects of adrenoceptor stimulation. Male rats were anaesthetized with Nembutal, the chest opened and a suction electrode placed directly on the ventricular tissue. Monophasic action potentials were recorded and heart rate and APD at 90% repolarization were calculated. Amiodarone, SR 33589 or vehicle (PEG/H_2O) were administered intravenously via the femoral vein. Table 1 shows that both compounds prolonged APD_{90} at doses that did not change heart rate by more than 10%. In anaesthetized, atropinized dogs , both amiodarone and SR 33589 inhibited isoprenaline-induced increases in heart rate (0.5 to 2 µg/kg iv; sufficient to produce a 50% increase in heart rate) and adrenaline-induced increases in blood pressure (2.5 to 10 µg/kg i.v.; sufficient to produce a 50 % increase in mean blood pressure) (Table 2).

Table 1 Effect on APD and heart rate in anaesthetized rats

Agent (mg/kg)		ADP_{90} (% change)	Heart rate (% change)
Amiodarone	1	+21 ± 9	-5 ± 3
	10	+23 ± 7*	-9 ± 4
SR 33589	1	+33 ± 6**	-7 ± 3
	3	+29 ± 11*	-7 ± 3
	10	+52 ± 16*	-15 ± 3*

Table 2 Effect on adrenaline-induced increases in blood pressure and isoprenaline-induced increases in heart rate in anaesthetized dogs

Agent (mg/kg)		Increase in mean blood pressure (mmHg)		Increase in heart rate (beats/min)	
		Before drug	After 5 min	Before drug	After 5 min
Amiodarone	1	103 ± 10	86 ± 7**	79 ± 6	74 ± 5*
	5	106 ± 9	68 ± 19*	70 ± 7	51 ± 7*
	10	97 ± 6	33 ± 3***	77 ± 9	37 ± 8**
SR 33589	1	119 ± 10	98 ± 10*	65 ± 9	64 ± 8
	5	100 ± 9	46 ± 7***	71 ± 7	53 ± 9
	10	103 ± 10	46 ± 6***	67 ± 4	41 ± 3***

Values are shown as mean ± s.e.mean of 5-6 determinations, * $P < 0.05$, ** $P < 0.01$, *** P 0.001.
Thus SR 33589 significantly prolongs APD_{90} in anaesthetized rats at lower doses than amiodarone and inhibits adrenoceptor stimulation in anaesthetized dogs. Thus SR 33589 exhibits two potentially antiarrhythmic properties also shown by amiodarone. These actions may well be responsible for the antiarrhythmic actions of SR 33589 (Bruyninckx et al., 1992).

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271P INTERACTION BETWEEN THE ADENYLYL CYCLASE/CYCLIC AMP AND THE PHOSPHOLIPASE C-INOSITOL TRISPHOSPHATE-DIACYLGLYCEROL SYSTEMS IN THE HUMAN ISOLATED RIGHT ATRIUM

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The human right atrium is endowed with receptor systems that mediate their effects via the adenylyl cyclase/cyclic AMP pathway (e.g. β-adrenergic-, H_2-histamine- or 5-HT_4-serotonin-receptors) or cyclic AMP independently - possibly via the phopholipase C (PLC)-inositol trisphosphate (IP_3)-diacylglycerol (DAG) pathway (e.g. angiotensin II- or endothelin-receptors, Brodde et al., 1992). Evidence has accumulated that in many cell systems the cyclic AMP and the PLC-IP_3-DAG-systems can antagonize each other (Hill & Kendall, 1989). The aim of this study was to find out whether such an interaction between the cyclic AMP and the PLC-IP_3-DAG system may also occur in the human right atrium. For this purpose we studied in human isolated right atrial slices the effects of isoprenaline (ISO) and forskolin (FOR) on endothelin-induced inositol phosphate (IP) accumulation as well as the effects of endothelin on ISO- and FOR-induced cyclic AMP accumulation.

Right atrial appendages were obtained from patients without apparent heart failure undergoing coronary artery bypass grafting. None of the patients had been treated with β-adrenocpetor antagonists or -agonists for at least 6 weeks before surgery. IP-generation was assessed as accumulation of total [^{3}H]IP in [^{3}H]myo-inositol labelled atrial slices during a 45 min incubation at 37°C in Krebs-Henseleit-solution that contained 10 mM LiCl. The cyclic AMP content of the slices after 10 min incubation at 37°C with the agonists was assessed by a commercially available radioimmunoassay.

Endothelin (0.001-1 µM) caused concentration-dependent IP-accumulation in the atrial slices (maximum at 1 µM about 75 % above basal IP-accumulation). ISO (1-100 µM), added to the incubation medium simultaneously with endothelin (1 µM), concentration-dependently inhibited IP-accumulation. This ISO-effect was abolished by 10 µM propranolol. Similar to ISO, FOR (1 and 10 µM) significantly attenuated endothelin (1 µM)-evoked IP-accumulation. On the other hand, endothelin (0.01-1 µM) concentration-dependently reduced ISO (10 µM)- and FOR (1µM)-induced cyclic AMP accumulation in the human right atrial slices.

We conclude that in the human right atrium the adenylyl cyclase/cyclic AMP system can inhibit the PLC-IP_3-DAG system; moreover, endothelin inhibits ISO- and FOR-induced cyclic AMP accumulation. Whether this is due to activation of the PLC-IP_3-DAG system or to direct inhibition of adenylyl cyclase remains to be elucidated.

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272P EFFECTS OF TERBUTALINE IN THE HUMAN HEART: *IN VITRO* AND *IN VIVO* STUDIES

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The aim of this study was to characterize the β-adrenoceptor (AR) subtype(s) mediating cardiac effects of the β_2-AR agonist terbutaline (TER). For this purpose we compared the effects of TER and the non-selective β-AR agonist isoprenaline (ISO) in vitro on contractile force and adenylyl cyclase (AC) in human isolated right atria and left ventricles and in vivo on heart rate and contractility in 8 healthy volunteers.

Human right atria were obtained from patients without apparent heart failure undergoing coronary artery bypass grafting, left ventricles from would-be cardiac transplant donors whose hearts could not be transplanted. AC-activity was assessed in cardiac membranes as conversion of [^{32}P]ATP into [^{32}P]cAMP; cumulative concentration-effect curves for the positive inotropic effects of TER and ISO were assessed on isolated electrically driven (1 Hz, 37°C) right atrial strips and left ventricular trabeculae in Krebs-Henseleit solution. In vivo heart rate and pre-ejection period (PEP as a measure of inotropy) were determined in 8 healthy male volunteers during a stepwise incremental intravenous infusion of TER (dose range 25 - 300 ng/kg/min for 15 min) or ISO (dose range 5 - 70 ng/kg/min for 10 min) before (**PRE**) and after (**POST**) the volunteers had been pretreated with the β_1-AR selective antagonist bisoprolol (10 mg orally). Linear regression analysis of the individual dose-response curves was performed to obtain $ED_{50\%}$-values for increases in heart rate and $ED_{25\%}$-values for shortening PEP.

In vitro TER concentration-dependently increased right atrial and left ventricular contractile force (pD_2-values 6.33 and 5.76, respectively) and AC-activity (pEC_{50}-values 6.04 and 5.88, respectively). Compared to the effects of saturating ISO-concentrations maximal increases in contractile force were 85 % (atria) and 49 % (ventricles); maximal AC-activation was 66 % (atria) and 57 % (ventricles). In contrast to ISO, TER-effects on contractile force and AC-activity were in both tissues only antagonized by the β_2-AR antagonist ICI 118,551 (30 nM) but not by the β_1-AR antagonist CGP 20712A (300 nM). In vivo TER and ISO dose-dependently increased heart rate and shortened PEP; however, bisoprolol caused a larger shift to the right for the dose-response curve to ISO than for TER ($ED_{50\ POST}/ED_{50\ PRE}$ for heart rate: ISO 2.01 ± 0.3, n=8; TER 1.09 ± 0.2, n=7, P=0.03; $ED_{25\ POST}/ED_{25\ PRE}$ for PEP: ISO 2.21 ± 0.4, n=8; TER: 1.49 ± 0.2, n=7, P=0.07).

We conclude that in humans TER causes in vitro and in vivo positive ino- and/or chronotropic effects predominantly through activation of cardiac β_2-AR. However, a small β_1-AR component (possibly due to released endogenous noradrenaline via activation of presynaptic β_2-AR) can not completely be ruled out.

273P REGULATION OF β_1- AND β_2-ADRENOCEPTORS IN GUINEA-PIG SINO-ATRIAL NODE AFTER CHRONIC (-)-ISOPRENALINE INFUSION

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Chronic infusion of (−)−isoprenaline (iso) into guinea−pigs produces a marked loss of $\beta 2$−adrenoceptors from the atrio−ventricular conducting system, atrial and ventricular myocardium, cardiac valves and smooth muscle of the aorta with little change in $\beta 1$−adrenoceptors (Molenaar *et al.,* 1990). In this study we have examined the effects of iso infusion on the density of $\beta 1$− and $\beta 2$−adrenoceptors in the sino−atrial node (SAN) and atrial myocardium (RA). ALZET osmotic minipumps were implanted sc under pentobarbitone sodium (30mg kg^{-1}) anaesthesia and (−)−iso (400μg kg^{-1} h^{-1}) or vehicle (1mM HCl) infused for 7 days. Guinea−pigs were then killed, the SA node and surrounding tissues dissected, snap frozen in isopentane cooled in liquid N2 and sections (10μm) cut for staining or receptor autoradiography. Receptors were labelled with (−)[^{125}I] cyanopindolol and the $\beta 1$− and $\beta 2$−adrenoceptors delineated with CGP 20712A (100nM) and ICI 118,551 (70nM) (Molenaar *et al.,* 1990). Labelled sections were apposed to film for 14 days and the images were quantitated using the MCID system and SIMUL (Williams & Summers, 1990). Protein levels were measured in each tissue region using the Coomassie blue staining method (Molenaar *et al.,* 1990). The SAN was identified histologically in the superior vena cava at its point of insertion into the right atrium (RA). The SAN and RA contained both $\beta 1$− and $\beta 2$−adrenoceptors with the SAN having a higher proportion of $\beta 2$−adrenoceptors than RA. Iso treatment reduced $\beta 1$−adrenoceptor density in the SAN (control 49.4 ± 6.2, iso 32.9 ± 1.2 fmol mg^{-1} protein, P = 0.027) but not in RA (control 35.9 ± 4.5, iso 36.4 ± 1.7, P = 0.867, n = 4). Marked reductions in $\beta 2$−adrenoceptors were seen in both SAN (control 8.10 ± 0.50, iso 1.05 ± 0.27, P = 0.0001) and RA (control 3.69 ± 0.58, iso 0.30 ± 0.16, P = 0.002, n = 4). The results demonstrate the high susceptibility of $\beta 2$−adrenoceptors to down−regulation as predicted from structural analysis of the β−adrenoceptor subtypes (Emorine *et al.,* 1991), but also indicate that the $\beta 1$−adrenoceptors in the SAN are more liable to down−regulation than those in the RA.

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274P ARE K_{ATP} CHANNELS INVOLVED IN THE PROFOUND ANTIARRHYTHMIC EFFECTS OF PRECONDITIONING IN ANAESTHETISED DOGS?

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Short periods of coronary artery occlusion profoundly reduce the severity of the ventricular arrhythmias that occur during a subsequent longer period of ischaemia (Vegh et al., 1992). This phenomenon is known as preconditioning. The precise mechanisms are unknown but recently Gross and Auchampach (1992) reported that the K_{ATP} channel antagonist glibenclamide (0.3 mg kg^{-1} iv) prevented the beneficial effects of preconditioning in reducing myocardial infarct size. We used their protocol to examine whether glibenclamide (GLIB) modifies the antiarrhythmic effect of preconditioning.

Dogs were anaesthetised with a mixture of chloralose (60 mg kg^{-1}) and urethane (200 mg kg^{-1}) and the left anterior descending (LAD) coronary artery was occluded for two 5 min periods (preconditioning) with a 20 min reperfusion period between; glibenclamide was given after the first preconditioning occlusion. Twenty min after the second preconditioning occlusion the artery was re-occluded for 25 min and the severity of the resultant ventricular arrhythmias assessed both during ischaemia and subsequent reperfusion. In control dogs the LAD was occluded for 25 min and the myocardium was then reperfused. Blood flow was measured on the left circumflex coronary artery using an electromagnetic flow probe. Glibenclamide had no significant on arterial blood pressure or heart rate, slightly increased LVEDP (from 9.5±1.6 to 11.1±1.4 mmHg; $p<0.05$) and markedly reduced mean coronary blood flow (from 50±7 to 40±7 ml min^{-1}; $p<0.01$).

Table 1. Effect of preconditioning with and without glibenclamide, on the number of ventricular premature beats (VPBs), the incidence and number of episodes of ventricular tachycardia (VT) during ischaemia, the incidence of ventricular fibrillation (VF) both during ischaemia and reperfusion, and on survival in dogs subjected to a 25 min coronary artery occlusion followed by reperfusion.

	n	VPBs	VT % incidence	VT no. of episodes	VF % incidence	VF reperfusion % incidence	survival (%)
Controls	15	528±140	100	5.1 ± 1.6	47	100%	0%
Preconditioning	10	78 ± 27**	20*	0.7 ± 0.3**	0*	60%	40%*
Preconditioning + Glib	10	165 ± 56*	40	3.1 ± 1.8	0*	50%	50%*

*P < 0.05; **P < 0.01 compared to controls.

We conclude that, in the doses used, glibeclamide attenuates but does not abolish the pronounced antiarrhythmic effects of preconditioning. The mechanisms of this protection may thus differ from those involved in infarct size limitation.

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275P MODULATION OF ATP-SENSITIVE K^+ CHANNELS IN INSULIN-SECRETING CELLS BY POLYMYXIN B

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ATP-sensitive potassium (K^+_{ATP}) channels play a major role in coupling stimulation of pancreatic β-cells to the secretion of insulin. Closure of K^+_{ATP} channels by glucose or the sulphonylureas (glibenclamide, tolbutamide, etc) initiates a depolarization of the membrane, the opening of voltage-gated calcium channels and an increase in $[Ca^{2+}]_i$ (Dunne & Petersen, 1991). Conversely activation of the K^+_{ATP} channels hyperpolarizes the cell, lowers $[Ca^{2+}]_i$ and inhibits secretion (Dunne & Petersen, 1991). Polymyxin B is a polycationic antibiotic active against most strains of gram-negative bacteria, but also recognised to have effects on eukaryotic cells, which include the inhibition of protein kinase C and the block of Ca^{2+}-activated K^+ channels in mouse skeletal muscle cells (Storm et al., 1977; Weik & Lonnendonker, 1990).

In the present study we have investigated the effects of interactions between polymyxin B and K^+_{ATP} channels in insulin-secreting cells. The experiments have been carried out using patch-clamp electrophysiology in clonal RINm5F insulinoma β-cells. Single-channel recordings of K^+_{ATP} currents have been made using inside-out, permeabilized ("open") cells, and outside-out patches under quasi-physiological cation gradients; K^+ - rich solution on the inside of the membrane, Na^+ - rich solution on the outside. At either the inside (n = 15 separate patches) or the outside (n = 4) of the cell membrane, polymyxin B (10 - 100 μM) was found to be a highly effective inhibitor of K^+_{ATP} channels. When added directly to the bathing solution, the effects of polymyxin B were rapid in onset and fully reversible (n = 7/8 additions to 7 patches); on average channel open-state probability (p_o) was reduced from 100% to 9 ± 5% of the control value (n = 7, mean ± SEM). Interestingly, when the actions of polymyxin B were studied in the presence of intracellular ATP (0.5 mM) and ADP (0.5 mM), although the inhibitory effects were once again very rapid in onset, channel blockage (p_o = 11 ± 4%) persisted once polymyxin B was removed (n = 20/21 additions to 15 patches). During these experiments channel activity could only be recovered once ATP / ADP was replaced with the nucleotide-free bathing solution. The inhibitory effects of polymyxin B are not dependent upon the availability of Ca^{2+} (all solutions were buffered with 0.5 mM EGTA), and were also seen in patches of membrane excised from cells that had been pre-treated overnight (22 - 24 hours) with 1μM phorbol-myristate acetate (PMA), a procedure used to down-regulate or desensitise protein kinase C (n = 5).

In summary, we have shown that polymyxin B is a highly effective modulator of K^+_{ATP} channels in insulin-secreting cells. Channel inhibition is rapid in onset, and does not depend upon the involvement of protein kinase C. The mechanisms that underlie channel blockade are, as yet, incompletely understood and complicated by the availability of ATP/ADP.

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276P PROSTACYCLIN RELEASE IN EXPERIMENTAL DIABETES: EFFECTS OF EVENING PRIMROSE OIL

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Altered production of endothelium-derived mediators may contribute to diabetic microvasculopathy. We examined prostacyclin release, measured as immunoreactive 6-keto prostaglandin $F_{1\alpha}$, from rat lung, kidney and peripheral nerve. Tissues were taken from control and streptozotocin-diabetic rats which had been treated for 8 weeks with evening primrose oil (EPO) or, as a control for lipid intake, coconut oil (CO), both mixed with powdered diet to 5% w:w which was provided *ad libitum*. Lung and kidney slices were incubated for a 30 min equilibration period (Krebs buffer, 1%BSA, pH 7.4, 37°C, gassed with 95%O_2/5%CO_2), after which the bathing fluid was replaced with fresh buffer and incubation continued for a futher 10 min either in the presence of 10^{-6}M acetyl choline, 10^{-5}M of the calcium ionophore 4-bromo-A23187, 10^{-4}M arachidonic acid, or without agonist (basal). Sciatic nerve segments, with their epi- and perineurial sheaths fenestrated, were also incubated for 30 min equilibration prior to 10 min incubation with or without 10^{-5}M 4-bromo-A23187. Incubation medium was collected and stored at -20°C until assay. Basal prostacyclin release (mean ± SD; pg/mg protein) from lung was significantly higher in EPO- rats irrespective of diabetic state (452.6 ± 190.2 vs 275.4 ± 119.8 in controls, $P<0.01$; 377.8 ± 122.2 vs 212.8 ± 103.0 in diabetics, $P<0.05$). Levels were reduced in CO- diabetics compared to EPO- ($P<0.01$) and, although not statistically significant, CO- controls. Basal prostacyclin release was also significantly reduced in kidney from CO- diabetics (209.2 ± 91.5 vs 384.3 ± 57.5 CO- controls, 334.2 ± 89.4 EPO- controls, $P<0.01$) and was attenuated by EPO treatment (239.5 ± 118.5). In the presence of arachidonic acid, lung prostacyclin release was significantly lower in CO- diabetic rats compared to all other groups (1664.6 ± 437.6 vs 4421.4 ± 1180.6 EPO- controls, 4310.6 ± 1411.8 CO- controls, $P<0.01$; 3004.0 ± 823.6 EPO- diabetics, $P<0.05$) but there were no differences in renal release between any group. Basal prostacyclin release by nerves from CO- diabetic rats was significantly reduced compared to all other groups (8.9 ± 10.27 vs 98.5 ± 44.3 CO- controls, 126.1 ± 80.5 EPO- controls, 98.3 ± 72.6 EPO- diabetics, $P<0.01$). Under acetyl choline- and 4-bromo-A23187- stimulated conditions release from any tissue did not differ significantly from basal. These data thus indicate reduced prostacyclin release from diabetic lung, kidney and nerve, which may be partly due to reduced cyclooxygenase activity. Deficits in release were attenuated, if not prevented, by treatment with EPO. This would therefore also suggest a decreased availability of arachidonic acid for prostacyclin production in diabetes.

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277P ENDOTHELIN RECEPTORS IN HUMAN KIDNEY CHARACTERISED USING NOVEL LIGANDS BQ3020 AND BQ123

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The endothelins (ET), a group of potent vasoconstrictor peptides, are expressed in a wide range of species and tissues (Davenport, A.P., Nunez, D.J. *et.al.* 1990). Three isoforms, ET-1, ET-2 and ET-3, and two receptor subtypes, ET_A and ET_B, have thus far been described in humans. Animal studies have shown the kidney to be one of the most sensitive tissues to the effects of ET, which include reductions in plasma flow and GFR, mesangial contraction and cell proliferation in culture. Our aim was to characterise ET receptors in human kidney using new ligands BQ3020 ([$Ala^{11,15}$]Ac-ET-$1_{(6-21)}$) and the antagonist, BQ123 (cyclo[D-asp-L-pro-D-val-L-leu-D-trp-]), which are ET_B- and ET_A-selective respectively.

Initial quantitative autoradiography of human kidney sections, using these ligands to block receptor subtypes, showed the majority of ET binding to be in the medulla compared with cortex. ET_B binding predominated over ET_A, the latter appearing to localise to medullary rays.

Serial 10μm medulla sections from three fresh frozen normal nephrectomy specimens were incubated with concentrations of [^{125}I]ET-1 in the range 0.01-8 nM. Non-specific binding was determined using alternate sections in the presence of 10^{-6}M ET-1. After washing, the sections were wiped onto filter paper and counted. The data were analysed using iterative curve fitting (LIGAND) and yielded a dissociation constant (K_D) of 0.17 ± 0.04 nM, (mean ± s.e.mean) and receptor density (B_{max}) of 57.7 ± 15.4 fmol/mg protein. This is in the range previously described in other mammalian kidneys, and in a variety of other tissues. Hill coefficients of 0.86 ± 0.03 were obtained; however in this concentration range a two-site fit was not preferred. Further saturation experiments on the same kidneys using [^{125}I]BQ3020 (Amersham International plc) gave a mean K_D for ET_B of 0.36 ± 0.06 nM and B_{max} of 30.0 ± 5.0 fmol/mg protein. The Hill coefficients were again less than unity.

BQ3020 and BQ123 were also employed in competition binding assays with 0.1nM [^{125}I]ET-1, in doses ranging from 10^{-5} to 10^{-12} M. These confirmed each compound's selectivity (BQ3020 giving K_D for ET_B 3.0 ± 1.4 nM versus K_D for ET_A 5.0 ± 1.3μM; BQ123 giving K_D for ET_B 32.8 ± 8.0 μM versus K_D for ET_A 11.8 ± 4.0 nM) and showed the ratio of ET_B to ET_A to be at least 65:35.

This study confirms the presence of at least two ET receptors in human kidney, and shows a thousand-fold selectivity of BQ3020 for ET_B and of BQ123 for ET_A.

Davenport, A.P., Nunez, D.J. & Brown, M.J. (1990) *Clin.Sci.* 77,129-131
F.E.K. is a N.K.R.F. Training Fellow.

278P THE VASOCONSTRICTOR EFFECTS OF ENDOTHELINS AND SARAFOTOXINS IN THE SYSTEMIC CIRCULATION AND RENAL VASCULATURE ARE MEDIATED VIA DIFFERENT RECEPTORS

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Peptides of the endothelin/sarafotoxin (ET/SX) family are potent vasoconstrictors in a variety of vascular beds including the kidney. Their effects are mediated via at least 2 different receptor subtypes (ET_A and ET_B). The selective ET_A-receptor antagonist BQ-123 (cyclo(D-Asp-L-Pro-D-Val-L-Leu-D-Trp)) inhibits the systemic increase in blood pressure induced by ET/SX peptides (McMurdo et al., 1992). Here we compare the antagonistic effects of BQ-123 against constrictions induced by the ET/SX peptides in the systemic and renal circulations.

Wistar-Kyoto rats (male, 300-400 g) were anaesthetized with sodium thiopental (120 mg/kg i.p.). The right femoral and left jugular veins were cannulated for drug administration and infusion of saline (1.5 ml/h), and the right carotid artery for measurement of mean arterial blood pressure (MAP). The left kidney was exposed and an ultrasonic flow probe placed around the left renal artery for measurement of renal blood flow (RBF). All animals were given bolus injections of 0.1, 0.25 and 0.50 nmol/kg of ET1, ET3, SX6b or SX6c. The time between each injection was 1 hour. BQ-123 (1 mg/kg, i.v. bolus) or vehicle was injected 5 min prior to each dose of peptide.

The basal RBF, MAP and renal vascular resistance were 9.4±0.3 ml/min, 120±2 mmHg and 13.2±0.5 mmHg·min/ml, respectively (n=32, for each). Bolus injections of ET1, ET3, SX6b or SX6c produced initial transient depressor responses followed by sustained and dose-dependent increases in MAP with the following rank order of potency: SX6b>ET1>>SX6c>ET3. In the renal vasculature they caused dose-dependent falls in RBF (ET1=ET3=SX6b=SX6c). BQ-123 significantly reduced the systemic pressor effects of all the peptides but was ineffective against the renal vasoconstrictions induced by the ET/SX peptides.

	Vehicle						BQ123					
	rise in MAP (mmHg)			fall in RBF (ml/min)			rise in MAP (mmHg)			fall in RBF (ml/min)		
nmol/kg	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50
ET1	4±1	14±1	38±6	1.5±0.5	2.7±0.6	5.7±1.4	1±1	4±1*	10±1*	0.5±0.2	2.5±0.3	6.2±0.5
SX6b	9±2	30±3	56±3	1.1±0.3	3.9±0.7	5.8±0.5	3±1*	4±1*	4±2*	0.3±0.2	2.3±0.3	6.6±0.6
ET3	1±1	4±2	10±3	0.2±0.1	1.8±0.5	6.8±0.9	0	0*	2±1*	0.6±0.1*	2.0±0.4	5.4±0.9
SX6c	4±1	13±3	14±6	0.9±0.2	2.8±0.3	5.8±0.7	1±1	4±2*	0*	0.8±0.3	2.6±0.4	5.7±0.2

*Table 1: Data are expressed as mean±s.e. mean. n=4 for each group; * p<0.05, unpaired, two-tailed t-test.*

Thus, the pressor effects of the ET/SX peptides in the systemic circulation are inhibited by BQ123 but the vasoconstriction in the kidney is not. ET_A receptors mediate the general pressor effects of the ET/SX peptides, but the receptor mediating the renal vasoconstrictor responses is ET_B-like.

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McMurdo, L. et al. (1992). Br. J. Pharmacol., Dublin meeting, C105.

279P IDENTIFICATION OF ENDOTHELIN ISOFORMS AND PROENDOTHELIN-1 IN THE HUMAN HEART BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND RADIOIMMUNOASSAY

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In humans, three isoforms of endothelin (ET) have been predicted to exist. In the human heart, ET-1 has powerful positive inotropic effects and is a potent constrictor of coronary arteries (Davenport *et al.*, 1989; Howard *et al.*, in press). Our aims were to identify the isoforms of ET in human cardiovascular tissue that, if released, might mediate these actions.

Samples of human atrium and ventricle were collected from beating hearts removed from adult male patients undergoing transplantation for ischaemic heart disease. Samples of each tissue (0.5 to 2g) were homogenised and solid phase extracted using Amprep C_2 minicolumns. The resulting extracts were then either assayed by parallel radioimmunoassay (RIA) of mature ETs and proET-1. The identity of ET isoforms in the extracts was determined using reverse-phase high performance liquid chromatography (RP-HPLC) followed by RIA. The mature ET RIA antisera were raised against the common C-terminus of ET-1 and showed 100% cross-reactivity with ET-2 and ET-3, and < 1% cross-reactivity with the proETs. The proET-1 RIA antisera were raised against the C-terminus of proET-1 and showed < 0.007% cross-reactivity with the mature molecules and proET-2 and proET-3. The sensitivity of detection of both assays was < 1.25 fmol/tube. The inter- and intra-assay variations were < 13% in all cases. Dilution curves of a reconstituted ventricular extract were parallel to the synthetic ET-1 standard curve. The RP-HPLC technique used resolved ET-1, ET-2, ET-3 and proET-1 with baseline separation, and has been validated using both UV (214nm) and RIA detection (10^{-5}M and 10^{-9}M ET mixtures, respectively) on separate, but batch matched, C_{18} columns. Retention times obtained from UV and RIA columns were identical. Aliquots from each of the 1ml fractions collected were assayed by the two RIAs. Standard runs (10^{-9}M mixtures of ETs) were performed with each set of extracts. Blank runs, when assayed, showed no evidence of carryover of immunoreactivity from either standard or sample runs.

Mature ET-1 was detected in all samples of left ventricle (n=3) studied and one sample also contained mature ET-2, ET-3 and proET-1. In the left atrium, all samples studied (n=3) contained mature ET-1 and ET-2. Mature ET-3 and proET-1 were also detected in two of the patients studied. Total immunoreactive mature ET and proET-1 levels were: Right atrium 124.7 ± 4.3, 24.2 ± 3.6; Right ventricle 157.8 ± 41.1, 76.4 ± 6.3; Left atrium 208.1 ± 113, 11.7 ± 1.8; Left ventricle 134 ± 23.2, 41.6 ± 24.7; (fmol/g wet weight, mean ± s.e.mean, for total immunoreactive mature ET and proET-1 respectively).

This study identifies the presence of ET-1 and proET-1 in human cardiac tissue and is consistent with the localisation of these peptides to the endothelial cells by immunocytochemistry. The results also show that ET-2 and ET-3 are expressed in these tissues, as has been demonstrated in the human lung (Marciniak *et al.*, 1992).

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280P EXTRAVASCULAR BIG ENDOTHELIN-1 AND BIG ENDOTHELIN-3 CONVERSION IN THE HAMSTER CHEEK POUCH MICROVASCULATURE

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Endothelin-1 (ET-1) induces potent arteriolar constriction in the cheek pouch (Brain 1988). Intravenous administration of the precursor, human big ET-1 [1-38] (big ET-1), to rats induces a potent pressor response which is sensitive to phosphoramidon (phos, Fukuroda *et al.*, 1990, Matsumura *et al.*, 1990). We have now used intravital microscopy to investigate local effects of both big ET-1 and human big ET-3 [1-42] (big ET-3) applied topically to the microvasculature. Local effects on microvessel calibre of the precursors, in the presence and absence of phos, were studied in the cheek pouch of anaesthetised male Golden Syrian hamsters (80-100g). The pouch with intact blood supply was constantly superfused with Krebs solution (6 ml/min at 35°C). The diameters of individual microvessels (20-40 μm initial diameter) were monitored every min for 15 min following topical application of test agents (10 μl aliquots).

Big ET-1 selectively constricted arterioles in a dose dependent manner and was 10 times less potent than ET-1 (Table 1). Big ET-3 similarly reduced arteriolar diameter and was 30 times less potent than ET-3. The onset of constrictor activity was slower after application of big ET-3 than big ET-1.

Table 1. Effect of big ET-1 and ET-1 on arteriole diameter in the presence and absence of phos, 100 nmol, (% maximal decrease in initial diameter, mean ± s.e.mean, n=5-7 hamsters, N.D. = not determined, * indicates a significant effect of phos, $p<0.05$).

ET-1 (pmol)	alone	+ phos	big ET-1 (pmol)	alone	+ phos
0.0	-	4.0±3.6	0.0	-	2.0±3.4
1.0	28.6±12.1	N.D.	10.0	4.8±5.8	N.D.
10.0	42.0±9.2	44.1±11.1	100.0	62.4±8.1	10.0±14.3*

Phos while not effecting arteriole diameter itself or the constriction observed 5 min after ET-1 (10 pmol) did reduce constriction observed after big ET-1 (100 pmol, Table 1). A similar protocol was carried out using big ET-3. 10 min after big ET-3 (300 pmol) a decrease in arteriolar diameter was observed which was not inhibited by phos (100 nmol): big ET-3 26.2±11.4% decrease; big ET-3 + phos 23.8±8.2% decrease, n=5. A higher dose of phos (300 nmol) induced a slight but not significant inhibition of the constriction to big ET-3 (300 pmol).

The results suggest that a phos sensitive endothelin converting enzyme is situated close to microvascular vessels and is important in the conversion of big ET-1 to a constrictor ie. ET-1. Extravascular big ET-3 is also converted to an active vasoconstrictor in the microcirculation.

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281P STUDIES OF ENDOTHELIN CONVERTING ENZYME IN CULTURED BOVINE AORTIC ENDOTHELIAL CELLS

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Endothelin-1 (ET-1) is derived from a precursor protein of approximately 200 amino acids. Initial proteolytic processing of this pro-hormone to big ET-1 (38/39 amino acids) is similar to other peptide hormones, but the final processing step to ET-1 is unusual and requires a novel endopeptidase activity referred to as endothelin converting enzyme (ECE) (Yanagisawa *et al.*, 1988). This releases the 21 amino acid peptide, ET-1, from big ET-1 by selective cleavage of the Trp^{21}-Val^{22} bond. The majority of ECE activity in disrupted cells is membrane bound and inhibited by the peptidase inhibitor phosphoramidon (PHOS) (Okada *et al.*, 1990). However, the role of this PHOS-sensitive enzyme in the intracellular biosynthesis of ET-1 has not been fully established. Proteolytic processing of peptide hormone precursors generally occurs in the secretory vesicles (Orci *et al.*, 1985). The aim of this work has been to identify ET-1 secretory vesicles in subcellular fractions from bovine aortic endothelial cells (BAEC), and to determine the ECE activity of these fractions. In addition, the conversion of exogenous bigET-1 to ET-1 has been studied using intact cultured BAEC.

BAEC (1 x 10^8 cells) grown in culture to confluence were disrupted with a tight-fitting glass homogeniser in 0.25 M sucrose (10 mM Na HEPES and 1 mM EDTA pH 7.4) and then layered onto a sucrose gradient. After centrifugation at 100,000g for 3 h, ten 1 ml fractions were recovered and assayed for ET-1 immunoreactivity using a radioimmunoassay specific for the C-terminal sequence of endothelin, ET_{16-21} (crossreactivity with big ET-1 <0.015%). For the determination of ECE activity, each fraction was subjected to a further centrifugation step (170,000g for 1 h) to precipitate microsomal membranes and organelles. The pelleted material was then lysed with 50 mM Tris/HCl buffer pH 7.0 and incubated with human $bigET\text{-}1_{1\text{-}38}$ (0.5 nmol/0.1ml). Conversion of bigET-1 to ET-1 at 37°C for 1 h was assessed using the specific C-terminal endothelin RIA. Protein content and galactosyl transferase activity (a specific marker for the *trans* Golgi network) were also determined to establish organelle separation on the sucrose gradient. To measure conversion by intact cells, bigET-1 (1 nmol/ml) was incubated at 37°C with cultured BAEC in 35 mm well plates.

Endothelin immunoreactivity was localised as a discrete peak on the sucrose gradient at the 1.0/1.2 M sucrose interface (7.9 fmol/10^6 cells), suggesting that ET-1 is contained in secretory vesicles. Although ECE activity was present in this fraction (7.2 pmol ET-1 /h/10^6 cells), the major peak of ECE activity was located in the 0.8 M sucrose band (28 pmol ET-1/h/10^6 cells). In the presence of 100 μM PHOS, the membrane bound ECE activity in the 0.8 M sucrose fraction was inhibited by 79%, as previously reported by (Okada *et al.*, 1990). Incubation of bigET-1 with intact BAEC generated ET-1 (2.34 $\pm$ 0.56 pmol/h/well, n = 8), and 100 μM PHOS inhibited this conversion by 82 $\pm$ 1% (n = 8) with an IC50 of 6.4 $\pm$ 1.4 μM. Thus, we have found an ET-1 rich subcellular fraction compatible with the existence of ET-1 secretory vesicles, which is in a different fraction to the peak of membrane bound PHOS-sensitive ECE activity in BAEC. Intact BAEC also convert big ET-1 to ET-1 via a PHOS-sensitive ECE. This latter activity is presumably located on the plasma membrane, and indeed may account for the major peak of PHOS-sensitive ECE found here on gradient centrifugation that was separated from the ET-1 containing fraction.

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282P VASOCONSTRICTORS RELEASE NITRIC OXIDE FROM THE ISOLATED PERFUSED GUINEA-PIG MESENTERIC VASCULATURE IN A CONCENTRATION-DEPENDENT MANNER

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In our originial report describing N$^{\omega}$-methyl-L-arginine (NMA) as an inhibitor of endothelium-derived relaxing factor(EDRF)/nitric oxide (NO) synthesis, we noted that NMA potentiated phenylephrine-induced tone in guinea pig pulmonary arterial rings (Sakuma et al., 1988). Potentiation of vasoconstrictor action by NO synthase inhibitors has subsequently been described in vessels from various species and anatomical locations. In the present study we have assessed the relationship between vasoconstrictor tone and NO production in the isolated mesenteric vasculature of guinea pig.

Male Hartley guinea pigs were anaesthetized and the superior mesenteric artery was cannulated. The Mesenteric bed was isolated and continuously perfused at 37°C with 95% O_2:5% CO_2-gassed Krebs'buffer at 5 ml/min. Perfusion pressure was measured via a pressure transducer. Nitrite, a stable oxidation product of NO, was assayed in the effluent by the Griess assay.

Prior to drug treatment, mesenteric perfusion pressure was 8 ± 2 mmHg and nitrite concentration was 0.26 ± .06 μM (n = 6). Infusion with 150 μM NMA did not affect either perfusion pressure (9 ± 2 mmHg) or nitrite release (0.24 ± 0.05 μM), suggesting that the basal nitrite levels do not arise from NO. However, infusion with 50 ng/ml U46619 caused an increase in perfusion pressure (26 ± 2 mmHg) in association with a significant increase in nitrite release (0.54 ± 0.06 μM, $p < 0.05$). NMA (15-150μM) dose-dependently potentiated the increase in tone caused by 50 ng/ml U46619 (from 28 ± 2 to a maximum of 61 ± 2 mmHg) and abolished the increase in nitrite release (0.03 ± 0.01 μM; $p<0.05$). L-Arginine (15 μmole bolus) transiently reversed these effects of NMA. These findings suggest that U46619-induced tone is offset by activation of NO release. A similar conclusion was reached in studies using diphenyleneiodonium (DPI), an irreversible inhibitor of NO synthase. DPI (1μM), abolished U46619-induced nitrite release and potentiated tone to a degree equvalent to that observed with NMA. However, in contrast with DPI, the effects of DPI were not reversed by L-arginine. The generality of the notion that agents which increase vascular tone also trigger NO release was further studied with other contrictory agents (see Table 1).

		Phenylephrine Dose (nmol)			KCl Dose (mmol)			
		0.5	5.0	50	0.25	0.5	1	2
Perfusion Pressure	CONTROL	0.3±0.2	2.1±0.9	7.4±0.7	0.9±0.3	2.7±0.4	5.9±0.5	9.8±0.4
(increase in mmHg)	+ 150 μM NMA	1.6±0.3	5.0±1.4	14.4±2.3	1.8±0.5	4.8±1.6	8.5±1.2	14.2±1.6
Nitrite Release	CONTROL	20±10	80±30	140±20	20±10	20±20	100±10	270±40
(increase in mmHg)	+ 150 μM NMA	0±0	0±0	0±10	0±0	0±0	20±10	50±20

Bolus injection of either phenylephrine (PE) or KCl elicited dose-dependent increases in perfusion pressure in the mesenteric bed which were accompanied by dose-dependent increases in nitrite efflux into the effluent. NMA potentiated PE- and KCl-induced tone and blocked the associated release of nitrite. These findings provide direct biochemical evidence that NO is released in response to agents which increase vascular tone; this process dampens the action of vasoconstrictors. (supported by NIH grants HL46403 and HL34215).

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283P INDUCTION OF CALCIUM-INDEPENDENT NITRIC OXIDE SYNTHASE IN RAT MESENTERY DURING SEPSIS DOES NOT IMPAIR THE VASCULAR RESPONSE TO VASOCONSTRICTOR AGONISTS

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LPS administration *in vivo* produces hyporesponsiveness to vasoconstrictor agents in the aorta (Beasley et al., 1989), the heart (Smith et al., 1991), and an induction of NO synthase in various organs (Salter et al., 1991). We have investigated the effect of administration to rats of LPS (5 mg/kg; ip) on NO synthase activity in the mesentery compared to that in other organs.

For each time-point (3, 6, 15, 24 h), 3 rats were injected with LPS after which time the various organs were removed and placed into liquid nitrogen. NO synthase activity was measured in organ homogenates by conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline (Mitchell et al., 1991). In separate experiments rats were given LPS (5 mg/kg; ip) or sterile saline for 6 h the mesenteric bed was removed and the arterial and venous portions perfused for measurement of vasoconstrictor responses (Warner, 1990). NO synthase activity was 12±1 pmol cit/mg/20 min in mesenteries from control rats. The brains from control animals also had constitutive NO synthase activity (100±10 pmol cit/mg/20 min). NO synthase activity was undetectable in the liver, heart, lung, spleen, kidney or aorta from these animals. After LPS treatment all organs (except for brain) showed induced NO synthase activity which was maximum after 6h and had returned to control levels after 24 h. The total NO synthase activity (pmol cit/mg/20 min) in mesenteries from LPS treated rats (6h) was 50±10 which was lower than that found in lung (100±10) or spleen (107±12), but higher than that in heart (12.5±5) or aorta (17±6) and equivalent to that found in kidney (42±5) or liver (50±7). Induced NO synthase activity was calcium-independent whereas the constitutive activity in the brain and mesentery from control rats was calcium-dependent. Administration of LPS (5 mg/kg; 6 h) did not reduce the vasoconstrictor actions of endothelin 1 (ET1; 10 pmol), 5-HT (3 nmol), phenylepherine (PE; 3 nmol) or U46619 (100 pmol) in either the arterial or the venous portion of the mesenteric bed (Table 1; n=4-6; increases in perfusion pressure in mmHg)

	Control				LPS (5 mg/Kg; 6hr; ip)			
	ET1	5-HT	PE	U46619	ET1	5-HT	PE	U46619
artery	1.8 ±0.3	13.8±3.4	9.7±2.2	6.0±2.5	2.1±0.4	23.6±7.4	14.4±5.4	4.3±1.6
vein	3.0+0.7	6.5+0.6	4.4+1.1	8.2+0.6	2.6+0.4	4.0+1.1	3.2+0.8	9.8+1.2

These results show that although NO synthase activity is induced in the mesentery there is no hyporesponsiveness to vasoconstrictor agents. This suggests that either the NO synthase activity is induced in cells unable to influence vascular responses or that some other mechanism upregulates the vessels in the mesentery to be more sensitive to constriction.

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284P AMINOGUANIDINE, N^G-MONOMETHYL-L-ARGININE AND N^G-NITRO-L-ARGININE METHYL ESTER CAUSES ENDOTHELIUM-DEPENDENT CONTRACTIONS OF THE PORCINE ISOLATED SPLENIC ARTERY

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N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME) have been reported to produce endothelial- dependent contractions in the rat isolated thoracic aorta (Rees et al., 1990). Since these responses were reversed by L-arginine they appear to be due to inhibition of endothelial nitric oxide synthase. In the present study we have examined whether the arginine analogues, L-NAME, L-NMMA, D-NMMA and aminoguanidine (AG) can produce similar contractions in the porcine isolated splenic artery, and assessed the susceptibility of these responses to L-arginine and guanidino succinic acid (GSA), putative endogenous substrates for nitric oxide synthase (Thomas & Ramwell, 1992).

Spleens from male or female pigs were obtained from a local abattoir. The splenic artery was dissected out and refrigerated overnight in modified Krebs-Henseleit solution containing 2% Ficoll. The following day 5mm ring segments of the vessels were mounted under a final resting tension of 2.5 g wt. for the recording of isometric tension, in organ baths containing Krebs solution maintained at 37°C and gassed with 95% O_2 and 5% CO_2. The endothelium was removed from some segments by inserting a pair of fine forceps into the lumen and gently rolling the preparations on saline-moistened paper tissue.

L-NAME (1-100μM), L-NMMA (1-100μM) and AG (10μM-1mM) produced slow, concentration-dependent contractions in endothelium intact (E+) tissues; L-NAME and L-NMMA were equipotent and about 30 times more potent than AG. The highest concentration of each agent produced approximately 35-40% of the maximum response to 5-hydroxytryptamine (5-HT: 16.4±1.1g wt.). No contraction was observed with 100μM D-NMMA (n=6) in E+ tissues, and none of these agents evoked a contraction in endothelium-denuded (E-) tissues. Substance P and bradykinin produced endothelial-dependent relaxations in segments preconstricted with a submaximal concentration of 5-HT. No relaxations were observed in E(+) segments exposed to 100μM L-NAME. In E+ tissues the L-NAME-induced contractions were reversed by 1mM L-arginine (91.9±2.7%, n=6) but not by either 1mM D-arginine or 100μM GSA.

Our data indicate that these arginine analogues produce substantial endothelium-dependent contractions of the porcine splenic artery which are sensitive to L-arginine. This indicates that the contractions are due to inhibition of constitutive nitric oxide synthase. The finding that L-NMMA and L-NAME were significantly more potent than AG agrees with observations against constitutive nitric oxide synthase in the rat (Corbett et al., 1992).

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285P SYNTHESIS OF N^G,N^G DIMETHYLARGININE BY HUMAN ENDOTHELIAL CELLS

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Endothelium-derived NO is a potent vasodilator which contributes to the physiological regulation of vascular tone in animals and humans (Moncada et al. 1991). The synthesis of NO from L-arginine can be inhibited by N^G,N^G dimethylarginine (ADMA), a compound found in human plasma and urine (Vallance et al. 1992). The source of ADMA is unclear and in the present study we have examined whether human umbilical vein endothelial cells in culture synthesise ADMA.

SV40 transfected human umbilical vein endothelial cells (SGHEC-7) cells were cultured as previously described (Fickling et al. 1992). Primary cultures were prepared from fresh umbilical veins by a modification of the method of Jaffe et al. (1973). After 24h cells were washed twice with PBS and fresh medium added. Cells were then maintained in culture for 1, 2, 4 or 7 days. At the end of the culture period, medium was withdrawn and the cell monolayer removed with trypsin/EDTA (0.5g/L trypsin, 0.2g/L EDTA). Cell number was determined using a haemocytometer, and the number of dead cells estimated by the eosin exclusion technique. Samples of culture medium were centrifuged for 5 min at 900g and frozen at -20°C for up to 2 weeks. Dimethylarginines were measured as described previously (Vallance et al. 1992).

Low levels (0.07±0.03μM; n=4) of ADMA were present in the culture medium prior to exposure to endothelial cells. This concentration of ADMA could be accounted for by the ADMA present in the calf serum and is consistent with previous studies showing the presence of ADMA in plasma and serum. A time-dependent increase in ADMA was seen in medium exposed to actively growing SGHEC-7 cells. After 7 days the concentration of ADMA in the medium was 2.84±1.5μM indicating that 5.09±2.79μg ADMA was released by 10^7 cells in 7 days (n=8). Similar results were obtained with primary cultures of HUVECs which released 5.42±4.45μg ADMA/10^7 cells in 7 days (n=4). When culture medium was incubated for 7 days in the absence of endothelial cells no increase in the concentration of ADMA was seen.

Endothelial cells synthesise NO from L-arginine and metabolise methylated arginine analogues to citrulline and back to arginine again (Hecker et al. 1990). The observation that human endothelial cells synthesise ADMA provides a potential mechanism for the physiological and pathophysiological regulation of NO synthesis within the endothelium.

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286P CITRULLINE CAN RESTORE THE ABILITY OF ACTIVATED MACROPHAGES TO GENERATE NITRIC OXIDE IN THE ABSENCE OF EXTRACELLULAR ARGININE

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Biosynthesis and release of nitric oxide (NO) by macrophages is an important cytotoxic mechanism in non-specific immunity (see Moncada et al., 1991). In macrophages, NO is synthesized with L-citrulline from L-arginine, by a Ca^{2+}-insensitive NO synthase which is induced following exposure of the cells to lipopolysaccharide (LPS). Since macrophages appear to metabolize L-citrulline to L-arginine (Wu and Brosnan, 1992), we have now investigated the characteristics of L-citrulline transport and metabolism to determine whether L-citrulline can sustain NO synthesis in the absence of extracellular L-arginine. Experiments were performed using the murine macrophage cell line J774. Transport of L-citrulline and NO production (assayed by measurement of nitrite accumulation using the Griess reagent) was measured as described previously (Bogle et al., 1992). Metabolism of L-[^{14}C]citrulline was determined by thin-layer chromatography.

Uptake of L-citrulline (50 μM) was time-dependent and linear for up to 10 min, and hence subsequent measurements were made after 5-min incubations. Citrulline transport was reduced from 9 $\pm$ 0.3 nmol/10^6 cell/h (n = 3) at 37°C to 1.1 $\pm$ 0.03 nmol/10^6 cell/h (n = 3) at 0°C. L-citrulline uptake was saturable with an apparent K_t = 0.23 $\pm$ 0.03 mM and V_{max} = 23 $\pm$ 1 nmol/10^6 cells/h. Activation of J774 cells with LPS (1 μg/ml; 24 h) did not markedly alter the kinetics of L-citrulline uptake (K_t = 0.33 $\pm$ 0.01 mM and V_{max} = 26 $\pm$ 0.3 nmol/10^6 cell/h).

When J774 cells were incubated with L-[^{14}C]citrulline for 24 h, significant amounts of L-[^{14}C]arginine and L-[^{14}C]ornithine were detected in cell extracts. In control cell extracts 17 $\pm$ 1 % of the [^{14}C] radioactivity was associated with L-[^{14}C]arginine which increased to 40 $\pm$ 4 % in cells treated with LPS (1 μg/ml) and interferon-τ (100 U/ml). Activation of J774 cells with LPS and interferon-τ also resulted in nitrite production (56 $\pm$ 7 nmol/10^6 cell/24 h) which was completely dependent on the presence of L-arginine in the culture medium. Incubation of activated J774 cells with L-citrulline (0.01 - 10 mM) in the absence of extracellular arginine resulted in a concentration-dependent production of nitrite, reaching 21 $\pm$ 1 nmol/10^6 cell/24 h in the presence of 10 mM L-citrulline. These results show that macrophages transport and metabolize L-citrulline to L-arginine which can partially restore their ability to generate NO in the absence of L-arginine.

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287P MODULATORS OF THE L-ARGININE-NITRIC OXIDE PATHWAY: DIFFERENTIAL EFFECTS ON CUTANEOUS PERMEABILITY TO BRADYKININ AND HISTAMINE IN THE GUINEA-PIG

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Vasodilators such as prostacyclin have been shown to enhance mediator induced cutaneous plasma protein extravasation (PPE) by increasing local blood flow (Williams, 1979). Nitric oxide (NO) is a potent vasodilator synthesised by endothelial cells from L-arginine (Palmer et al., 1988). L-N^G-nitro arginine methyl ester (L-NAME), an inhibitor of NO synthase (Moore et al., 1990), reduces skin blood flow and inhibits substance P induced PPE in the rat (Hughes et al., 1990). We have studied the effect of substances which interact with the L-arginine (Arg) : NO system on cutaneous PPE induced by the direct-acting permeability mediators histamine (HA) and bradykinin (BK) in the guinea-pig.

PPE was measured in guinea-pig skin as local leakage of intravenously injected ^{125}I-albumin over a 40 min period. Intradermal injections (0.1 ml) were made into shaved flank skin in a balanced Latin square design. PPE was calculated as μl plasma (^{125}I counts in skin site/^{125}I counts in 1 μl plasma) and mean $\pm$ s.e. mean values are shown. The significance of differences between means was assessed by analysis of variance followed by Tukey's test.

Co-injection of L-NAME (0.01-0.1 μmol/site) inhibited PPE responses to BK (0.1-3.0 μg/site) or HA (0.25-3.0 μg/site), although the shifts of the dose-response curves were more pronounced with BK than with HA (n=6). In a within animals comparison (n=6), the response to BK (0.5 μg/site: 77 $\pm$ 8) was significantly reduced by 0.01 μmol (39 $\pm$ 6; $p < 0.01$) and 0.1 μmol (26 $\pm$ 4; $p < 0.01$) L-NAME whereas HA (0.5 μg/site; 91 $\pm$ 11) was not significantly reduced by 0.01 μmol (72 $\pm$ 14), but was significantly inhibited by 0.1 μmol (40 $\pm$ 8; $p < 0.05$) L-NAME. L-Arg (1-10 μmol/site) increased responses to 0.5 μg BK (BK control: 72 $\pm$ 9; BK+1.0 μmol L-Arg: 81 $\pm$ 8; BK+10 μmol L-Arg: 120 $\pm$ 15*; *$p < 0.05$; n=4) whilst responses to 0.5 μg HA were reduced (HA control: 81 $\pm$8; HA+1.0 μmol L-Arg: 67 $\pm$ 6; HA+ 10 μmol L-Arg: 36 $\pm$ 3*, * $p < 0.01$; n=6). D-Arg (10 μmol/site) produced a small increase in the BK response (97 $\pm$ 8; p=NS; n=4) but reduced the response to HA (51 $\pm$ 8; $p < 0.05$; n=6).

These observations show that PPE responses to BK or HA can be modulated differentially and imply differences in the mechanisms by which these mediators produce increased microvascular permeability in guinea-pig skin.

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288P NITRIC OXIDE MEDIATES THE VASCULAR HYPOREACTIVITY TO NORADRENALINE AND DECOMPENSATION IN HAEMORRHAGIC SHOCK

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The role of nitric oxide (NO) and the importance of the induction of NO synthase (NOS) in the development of vascular hyporeactivity to noradrenaline (NA) and vascular decompensation (the progressive fall in blood pressure following the retransfusion of shed blood) was investigated in haemorrhagic shock (HS) in anaesthetized rats. Rats were anaesthetized with sodium thiopental (120 mg/kg) and instrumented for the measurement of mean arterial blood pressure (MAP) and heart rate. Calcium-dependent and calcium-independent NOS activity was measured by the conversion of ^{3}H-arginine to ^{3}H-citrulline in organ homogenates from control rats and from rats subjected to HS. The changes in noradrenaline-induced contractile responsiveness of the thoracic aorta ex vivo were also investigated.

Haemorrhagic hypotension (to 50 mmHg within 5 min) caused a rapid (within 30 min) increase (from 30±2 to 37±4 mmHg, n=7) and a subsequent continuous decrease in the pressor response to NA (1 μg/kg i.v.) to 12±1 mmHg, n=7, at 120 min. Retransfusion of the shed blood at 120 min did not restore the pressor response to NA (9±1 mmHg, n=7). The hyporeactivity to NA was reversed by N^{G}-nitro-L-arginine methyl ester (L-NAME, 1 mg/kg i.v), a potent inhibitor of NO synthesis, applied 10 min after retransfusion (to 25±1 mmHg, n=7). In contrast, L-NAME did not potentiate the NA-induced pressor responses in control animals (n=6). Dexamethasone (3 mg/kg i.v. 60 min prior haemorrhage), a potent inhibitor of NOS induction, had no effect on either the control pressor responses to NA, or the development of hyporeactivity to NA.

To investigate the role of NO in the vascular decompensation following retransfusion, animals were subjected to HS. After the initial bleeding, 50 mmHg of MAP was maintained by further bleeding (early stage, compensatory phase) or retransfusion (later stage, decompensatory phase). After retransfusion of 25% of the shed blood, all shed blood was retransfused and the haemodynamic parameters were monitored for a further 80 min. In control animals vascular decompensation was revealed as a progressive fall in MAP (-13±3 mmHg at 80 min, n=5). This fall was prevented by L-NAME treatment (1 mg/kg before retransfusion; relative change in MAP at 80 min:+2±5 mmHg, n=6). Similarly, dexamethasone-pretreated animals maintained higher blood pressures following retransfusion than controls (relative change at 80 min: +9±4 mmHg, n=7).

In a separate group of animals, HS (50 mmHg MAP) was maintained for 150 or 330 min. At 150 min after the initiation of haemorrhage, there was no change in the NA-induced contractions of the aorta. No induction of a calcium-independent NOS was detected in brain, kidney, mesentery, aorta or heart. There was, however, a significant NOS induction in the lung, liver and spleen an 150 min. At 330 min of HS, an attenuation of the contractile responsiveness of the aorta to NA was observed. This was associated with an induction of NOS in the lung, liver, spleen, mesentery, kidney and aorta.

Our results demonstrate that an enhanced formation of NO mediates the vascular hyporeactivity to NA and vascular decompensation associated with HS in the anaesthetized rat. The time course of NOS induction and the effects of dexamethasone suggest that the constitutive NOS is responsible for the early hyporeactivity to NA, whereas an induction of NOS may be involved in the vascular decompensation.

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289P MECHANISMS UNDERLYING VASODILATATION INDUCED BY *E. COLI* LIPOPOLYSACCHARIDE (LPS) IN THE RABBIT CUTANEOUS MICROCIRCULATION

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Inflammatory reactions induced by gram negative bacteria are characterised by vasodilatation, oedema formation and the infiltration of inflammatory cells (Cybulsky *et al.*, 1988). LPS is thought to be important in the induction of these features. Oedema formation induced by the intradermal (i.d.) injection of microbes in rabbit skin has been shown to be dependent on both increased venular permeability and arteriolar vasodilatation (Williams & Peck, 1977; Von Uexkull *et al.*, 1992). In this study, we have investigated blood flow changes induced by i.d. injection of E.coli LPS in the rabbit skin, using a laser-Doppler flow meter.

Rabbits were anaesthetized with intravenous (i.v.) pentobarbitone sodium (30 mg kg^{-1}) and the dorsal skin shaved and depilated with a commercial depilatory cream. Basal blood flow readings were recorded and the agents under investigation, all prepared in phosphate buffered saline solution, were injected i.d. in 0.1 ml volumes, each agent having four replicates per animal. Blood flow readings were then taken every hour during the 4h that followed i.d. injections. The results were expressed as mean ± SEM of absolute changes in red blood cell flux (% of a standardized signal) compared with flux at sites prior to injection.

Intradermal injections of 3, 10 and 30 μg/site LPS (E.coli, serotype O55:B5) induced a dose-related increase in blood flow with a maximum response at 2h. For comparison, calcitonin gene-related peptide (CGRP, 10^{-11} mol/site) induced a maximal increase in blood flow at 30min, which was of equivalent magnitude to the response produced by 10 or 30 μg/site LPS. The vasodilator effect of 10 μg LPS at 2h was inhibited by the co-injection of either actinomycin D (5 μg/site) or L-N^{G} nitro-arginine methyl ester (L-NAME, 10^{-7} mol/site) by 100 % ($n=5$, $p<0.001$) and 58 % ($n=6$, $p<0.05$) respectively, whereas the CGRP response at 30min was not significantly affected by either drug. Pretreatment of rabbits with dexamethasone (2 mg kg^{-1} i.v., 3.5h before challenge) suppressed the LPS-induced increase in blood flow by 82 % ($n=5$, $p<0.01$). In contrast, depletion of circulating neutrophils with i.v. mustine hydrochloride (1.75mg kg^{-1}, 3 days before the experiment) had no significant effect on the response induced by 10 μg LPS ($n=6$).

These results demonstrate that LPS causes delayed vasodilatation of the rabbit cutaneous microvasculature. This response is inhibited by dexamethasone and L-NAME and depends on protein synthesis. However, the neutrophil accumulation induced by LPS is not necessary for the vasodilator response. Thus, the vasodilatation induced by LPS in rabbit skin may involve the expression of the inducible form of nitric-oxide synthase in the vessel wall.

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290P REGIONAL HAEMODYNAMIC CHANGES DURING CHRONIC INFUSION OF LIPOPOLYSACCHARIDE (LPS) IN CONSCIOUS RATS

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Acute administration of large bolus doses of LPS causes rapid-onset hypotension in anaesthetised rats (Thiemermann & Vane, 1990). However, in the clinical setting the development of endotoxaemia is likely to be more insidious and accompanied by production of inducible nitric oxide (NO) synthase (see Moncada *et al.*, 1991). We have simulated the clinical picture by giving a continuous, 24 h infusion of a low dose (150 μg kg^{-1} h^{-1}) of LPS (E. coli serotype 0111:B4 (Sigma)) in conscious, Long Evans rats, chronically instrumented with renal, mesenteric and hindquarters pulsed Doppler probes and intravascular catheters. All surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.); catheters were implanted 7-10 days after the flow probes, and at least 24 h before experiments were begun. Some of the results are summarised in Table 1.

Table 1: Cardiovascular changes during infusion of LPS in conscious, Long Evans rats (n = 7). Values are mean ± s. e. mean; * P<0.05 versus baseline (Friedman's test).

Time	1 h	3 h	8 h	24 h
Heart rate (beats min^{-1})	29 ± 9*	-8 ± 5	36 ± 17	97 ± 14*
Mean blood pressure (mmHg)	-3 ± 3	0 ± 3	3 ± 3	-15 ± 3*
Renal flow (%)	1 ± 8	37 ± 6*	37 ± 9*	68 ± 18*
Mesenteric flow (%)	-8 ± 3	-22 ± 6*	-8 ± 8	5 ± 7
Hindquarters flow (%)	-5 ± 7	-10 ± 6	10 ± 9	64 ± 17*
Renal conductance (%)	4 ± 8	38 ± 10*	33 ± 9*	97 ± 22*
Mesenteric conductance (%)	-4 ± 3	-21 ± 7*	-10 ± 9	24 ± 9*
Hindquarters conductance (%)	0 ± 9	-9 ± 8	7 ± 10	94 ± 21*

The only significant change during the first 1 h of LPS infusion was a small tachycardia. Thus, the dose of LPS used was, as intended, without acute haemodynamic effects. Nonetheless, 3 h into the LPS infusion, although mean arterial blood pressure was unchanged, there was a clear increase in renal flow and conductance and decrease in mesenteric flow and conductance. The changes in the renal vascular bed were still present 8 h after onset of LPS infusion, but the mesenteric vasoconstriction was not. After 24 h LPS infusion there was hypotension, tachycardia, and renal, mesenteric and hindquarters vasodilatation, although only the renal and hindquarters vascular beds showed an increase in flow. These complex haemodynamic changes likely reflect an interplay between factors such as sympathoadrenal activity, the renin-angiotensin system, vasopressin and inducible NO synthase, all of which are known to be stimulated by LPS.

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291P MODULATION OF TUMOUR BLOOD FLOW BY INDUCTION OF NITRIC OXIDE SYNTHESIS

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Tumour vasculature is functionally inadequate in that it is often maximally dilated and unresponsive to vasoactive stimuli. We have previously demonstrated that inhibition by L-arginine analogues of the production of nitric oxide (NO) reduced blood flow in a murine adenocarcinoma Colon 26 hosted in sponge implants and this effect was reversed by administration of L-arginine. In marked contrast, the effects of the same inhibitors on blood flow in sponge-induced non-neoplastic granulation tissue was minimal (Andrade et al.1992), suggesting that NO is involved in regulating tumour blood flow. We have now extended this investigation to another tumour cell line, a B16 melanoma and also determined which NO-synthase (NOS) might be responsible for the synthesis of NO in these tumours. Subcutaneously implanted sponges in anaesthetized Balb/c and C57 mice were used to host Colon 26 and B16 tumour cells ($1x10^6$,injected into the sponges two days postimplantation) and the local blood flow in the implants was assessed by washout of ^{133}Xe (expressed in terms of t½ in min±s.e.m). Frozen cryostat sections from the implants removed at days 7,12 and 14 were immunostained with rabbit antisera to constitutive and inducible isoforms of NOS prepared from rat brain and from the lung of endotoxin treated rats respectively. L-NAME (N^G-nitro-L-arginine methyl ester, 3 and 30μg) injected into sponges followed by ^{133}Xe washout measurement in Colon 26-bearing implants, on day 12, increased t½ (reduced blood flow) from 6±0.7 min to 9±1 min ($p<0.05$) or 14±1.3 min ($p<0.01$)(30μg). Further increase in t½ (16±1.5 min, $p<0.01$) was observed in the same tumours 2 days later with L-NAME (30μg). Similar results were obtained in B16-bearing implants so that increases in t½ from 5±1.2 min to 8±0.8 min ($p<0.05$) and to 10±1 min ($p<0.05$) were observed on 12-day old tumours following 3μg and 30μg L-NAME, respectively. Two days later, in these tumours the t½ increased to 12±0.9 min ($p<0.01$) with 30μg L-NAME. In control implants an increase in t½ from 4±0.9 min to 7±1 min ($p<0.05$) was observed only at day 14 with 30μg L-NAME.

Inducible NO synthase-like immunoreactivity was found in the endothelium of tumour capillaries and in the vascular smooth muscle of infiltrating differentiated vessels in well established tumours (12-14 days after cell injection). Only negligible immunoreactivity was found in the endothelium of vessels in granulation tissue throughout the whole 14 day period. Immunoreactivity for constitutive NOS was not evident. These results strongly suggest that NO is involved in regulation of Colon 26 and B16 tumour blood flow and that NO production is mediated by an inducible enzyme.

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292P CONTROL OF SMOOTH MUSCLE FROM THE PIG PROXIMAL URETHRA

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Urethral pressure profilometry performed in anaesthetised pigs has shown that there is a region of high pressure some 2-3 cm below the bladder neck which is thought to have a role in the maintenance of continence and is known to relax prior to micturition. We have studied the control of smooth muscle of this proximal urethral region. Strips of circular smooth muscle were dissected from proximal urethras obtained at the abattoir from female Landrace pigs, and mounted in 0.2 ml organ baths as previously described (Brading & Sibley, 1983). The tissues were perfused with warmed (35-37°C) Krebs solution and tension was measured isometrically using Dynamometer UFI transducers and recorded on a multichannel pen recorder. Application of phenylephrine and carbachol produced concentration-dependent increases in proximal urethral tone whilst isoprenaline caused a reduction. Atropine (10^{-6}M) and guanethidine ($3x10^{-6}$M) had no effect on the high level of spontaneous tone generated in this region, suggesting that although the tissue possesses muscarinic and adrenergic receptors, release of their agonists is not necessary for the maintenance of high tone.

Field stimulation of intrinsic nerves produced complex responses which, depending on the stimulation parameters, varied from pure relaxation to combined relaxation and contraction. These responses were sensitive to tetrodotoxin ($3x10^{-6}$M). Guanethidine and atropine eliminated the contractile components of the response, but not the relaxations, demonstrating a non-adrenergic non-cholinergic (NANC) origin of the latter. Predominantly relaxant responses were initiated by 1 second trains of 0.1 msec 20 volt pulses, 0.5-10Hz. Inhibitory NANC innervation has also been seen in pig and human urethra by Andersson et al. (1983) and Klarskov et al. (1983). Sodium nitroprusside, an exogenous donor of nitric oxide, produced concentration-dependent relaxations of the urethral smooth muscle. Maximal relaxation, expressed as a percentage of the relaxation seen in calcium free solution, was achieved at a concentration of 10^{-5}M (95 ± 3%; mean ± s.e.m., n=24). Nitro-L-arginine (10^{-5}M), a competitive inhibitor of nitric oxide synthase, completely abolished the nerve mediated relaxations. Reversal of this effect could be achieved by addition of a ten fold excess of the L-enantiomer of arginine. Reduction in the nerve mediated relaxations was also observed following addition of 10^{-5}M oxyhaemoglobin, a scavenger of nitric oxide in extracellular media.

Together, these results suggest that spontaneous tone of this smooth muscle can be enhanced by excitatory cholinergic and adrenergic transmission, and inhibited by NANC innervation, the transmitter of which appears to be nitric oxide. Preliminary observations of Persson and Andersson (1992) support this latter conclusion.

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293P SIMULTANEOUS SINGLE UNIT RECORDING AND FAST CYCLIC VOLTAMMETRY IN BRAIN SLICES

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Carbon fibre microelectrodes (CFM) have previously been used to monitor extracellular action potentials (Armstrong James & Millar, 1979) or, using fast cyclic voltammetry (FCV), to measure "real time" stimulated monoamine efflux in the central nervous system (Stamford, 1990). In this study we combined single unit recording and FCV at a single CFM in superfused slices of rat locus coeruleus (LC), dorsal raphe (DR) and substantia nigra (SN). Briefly, a CFM was slowly lowered into the slice until a spontaneously active single unit could be discriminated. Monoamine efflux was then evoked using trains of constant current pulses (50 pulses, 0.2 ms duration, 10 mA, 100 Hz, every 10 mins) applied to an adjacent tungsten stimulating electrode and monitored by FCV. The polarograph (a Millar Voltammetric Analyser) was set to scan every 500 ms and used in a voltage follower mode between scans. The voltage signal was amplified, gated and filtered. The "spikes" were then discriminated and counted for 450 ms during each 500 ms period using Neurolog modules.

Spontaneously active cells could be isolated and recorded in all three nuclei. These showed stable spike characteristics for periods of up to 2 hours (maximum period investigated) and application of the voltammetric input waveform did not alter the pattern of spontaneous activity. In the LC 22 units were recorded. Spikes had a mean duration of 961 ± 65 µs (mean ± s.e.m.) and a mean amplitude of 93 ± 9 µV. Stimulation caused an increase in firing rate in 16 units (73%), inhibited activity in 2 (9%) and produced a biphasic response (excitation followed by inhibition of firing or vice versa) in 4 (18%). Mean peak stimulated monoamine efflux was equivalent to 495 ± 73 nM noradrenaline. In DR and SN units had mean durations of 1256 ± 194 and 1445 ± 319 µs and mean amplitudes of 67 ± 7 and 80 ± 14 µV respectively (both n=4). Cells in the DR were excited by electrical stimulation whilst in the SN the predominant response (3 of 4) was inhibition. Peak stimulated monoamine efflux in DR was equivalent to 45 ± 3 nM 5-hydroxytryptamine. Amine efflux in SN was not calibrated.

This study shows that the techniques of FCV and single unit recording may readily be combined at single CFMs in brain slices. Stable electrochemical and electrophysiological signals could be recorded for periods of at least 2 hours, thereby allowing pharmacological interventions. The simultaneous recording of amine efflux and unit activity at the same locus should facilitate comparison of drug effects at pre- and postsynaptic sites.

This research was supported by grants from the Royal London Hospital Special Trustees, Smithkline Foundation, Royal Society and University of London Central Research Fund.

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294P MODULATION OF STIMULATED 5-HT OVERFLOW IN SLICES OF THE DORSAL RAPHÉ NUCLEUS OF THE GUINEA-PIG STUDIED USING FAST CYCLIC VOLTAMMETRY

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We have previously shown that the technique of fast cyclic voltammetry (FCV) can measure changes in electrically stimulated endogenous 5-HT overflow ([5-HT]ex) in untreated slices of rat brain (O'Connor & Kruk, 1991a). [5-HT]ex has been shown to be frequency dependent (O'Connor & Kruk 1991b) and sensitive to a number of 5-HT1 ligands in somatodendritic and axon terminal regions of the rat brain (O'Connor & Kruk 1992). Here we report, for the first time, the measurement of [5-HT]ex in untreated slices of guinea-pig brain containing the dorsal raphe (DRN) using FCV.

Experiments were carried out as previously described (O'Connor & Kruk 1991a). Briefly slices (350μm) containing the DRN were superfused with oxygenated ACSF at 32°C at a flow rat of 1.1ml min^{-1}. 5-HT release was monitored with a carbon fibre microelectrode placed centrally in the DRN 80μm below the surface. Single pulse stimulation (0.1ms; 20V) or 5 pulses applied at 100Hz were applied every 5 minutes with a bipolar stimulating electrode, tip separation 200μm. Reproducible evoked release of 5-HT could be measured for more than 5h (typically 8-10nM 5-HT for single pulse and 33-35nM 5-HT for 5 pulse stimulation after electrode calibration; n=10).

[5-HT]ex gave rise to a voltammetric signal identical to that for perfused 5-HT (0.1μM). Omission of Ca^{2+} from the perfusion medium reversibly abolished the electrochemical signal (n=4). Fluvoxamine, the 5-HT uptake inhibitor, caused a 185% increase in the height of the signal and a 236% increase in the time for the signal to return to half its maximal height ($t_{½}$; n=4). The 5-HT1 receptor ligands 8-OH-DPAT and 5-carboxamidotyrptamine (5-CT) produced a concentration-dependent inhibition of [5-HT]ex. ED_{50} values were 25.1±2.9nM and 22.3±3.4nM respectively; Emax values were 79.8±2.5% and 73.3±4.9% respectively (n=4). A pKb value of 7.88 (n=4) was obtained for the 5-HT receptor antagonist methiothepin versus 5-CT.

These results show that the potency and efficacy of these 5-HT1 ligands is greater in guinea-pig than rat brain slices containing the DRN. The data shows that FCV may prove a valuable tool in understanding the control mechanisms of 5-HT release in the guinea-pig brain.

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295P REGIONAL DOPAMINERGIC TERMINAL LOSS IN THE STRIATUM OF VITAMIN E DEFICIENT RATS AS ASSESSED BY [^{3}H]-MAZINDOL BINDING

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^{18}F-DOPA PET scans on four adults with a chronic vitamin E deficiency syndrome showed evidence of nigrostriatal degeneration (personal communication Marsden), suggesting a high susceptibility to oxidative stress (Meydani *et al*,1985). We now examine dopaminergic nerve terminal integrity in vitamin E deficient rats using [^{3}H]-mazindol binding.

Male Wistar rats (n=12) were fed a vitamin E deficient diet (Dyets, USA) or the same diet supplemented with α-tocopherol acetate (40mg/Kg diet) for 52 weeks. Brains were removed, snap frozen, coronal sections (20μm) were cut and thaw mounted onto gelatin-coated slides at four rostral to caudal levels in striatum. [^{3}H]-Mazindol (4nM) binding was performed according to Javitch *et al* (1985), then apposed to tritium hyperfilm for 5 weeks at 4°C and subsequently analysed by computer assisted densitometry to assess specific [^{3}H]-mazindol binding (fmol/mg tissue) in total striatum, and in dorsolateral, ventrolateral, dorsomedial and ventromedial areas at each level. Specific [^{3}H]-Mazindol binding in both control and vitamin E deficient rats was >78% and showed a lateral to medial gradient. At all levels of the striatum there was a trend for a reduction in [^{3}H]-mazindol binding in the vitamin E deficient rats. This was most marked at Level 2 with significant reductions in most areas (Table 1). Reduced [^{3}H]-mazindol binding was also observed in the ventrolateral region of vitamin E deficient rats at Level 3 (31-40%) and Level 4 (32-37%).

Table 1 Striatal [^{3}H]-mazindol binding in control and vitamin E deficient rats at Level 2.

	Left hand side		Right hand side	
	CONTROL	-VITAMIN E	CONTROL	-VITAMIN E
Dorsolateral	229 ± 29	180 ± 11	201 ± 28	151 ± 6
Ventrolateral	269 ± 30	160 ± 6*	204 ± 21	137 ± 8*
Dorsomedial	167 ± 10	141 ± 4*	175 ± 12	143 ± 4*
Ventromedial	160 ± 11	129 ± 5*	144 ± 13	124 ± 4
Overall	203 ± 17	151 ± 5*	182 ± 16	139 ± 3*

Values represent the mean ± 1 SEM, *p < 0.05 compared to control (Student's t test).

Levels (Paxinos & Watson)

	Interaural	Bregma
Level 1	10.7	1.7
Level 2	9.7	0.7
Level 3	8.7	-0.3
Level 4	8.2	-0.8

Vitamin E deficiency apparently causes dopamine terminal loss in rats confirming the PET investigations in vitamin E deficient patients. The results support the concept that nigral cell loss in Parkinson's disease may occur as a consequence of oxidative stress.

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296P CONTRASTING EFFECTS OF REPEATED RESERPINATION ON BEHAVIOURAL AND BIOCHEMICAL CORRELATES OF D_1 AND D_2 RECEPTOR INTERACTION

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Dopamine D1 and D2 receptors have been shown to interact at both the behavioural and biochemical level. Thus, cooperative D1:D2 receptor agonism causes stereotyped behaviour which is not seen when either agonist is given alone (Braun & Chase, 1986). Repeated reserpine administration has been shown to break this link between D1 and D2 receptors, such that agonism of either receptor subtype will induce stereotypy (Arnt, 1985). The situation regarding adenylate cyclase is different in that D2 agonism inhibits D1-stimulated cyclic AMP (cAMP) formation (Stoof & Verheijden, 1986). In view of the reported effects of reserpine on behavioural responses we have now examined the effect of repeated reserpine treatment on the biochemical interaction between D1 and D2 receptors.

Male SD rats (180-200g; Olac) received vehicle or reserpine (1mg/kg, sc) once daily for five days. Two hours after the last dose, striatal homogenates were prepared and the effects of SKF 82958 ((±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine, a full D1 agonist; 1.25-40μM) to stimulate and quinpirole (D2 agonist; 2.5-40μM) to attenuate cAMP production were examined. cAMP was measured by scintillation proximity assay (Amersham). Six point saturation binding assays were also performed, using [3H]-SCH 23390 (0.06-2nM defined by 100μM chlorpromazine) for D1 and [3H]-spiperone (0.03-1nM defined by 10μM sulpiride) for D2 receptors. Behavioural independence of D1:D2 receptors was determined by administering SKF 82958 (1mg/kg ip) and quinpirole (3mg/kg sc), alone and in combination, and assessing the stereotyped licking and biting induced, using a simple 0-2 ranking scale.

Table 1. Effect of repeated reserpinisation on D1:D2 receptor interactions

	Stereotypy (max. score = 12)			Bmax (fmol/mg wet wt.)		Effects on cAMP formation: SKF 82958 stimulation	Effects on cAMP formation: Quinpirole inhibition[a]
	SKF	Quinpirole	SKF + Quin	D1	D2	Vmax (pmol/mg/2.5min)	Apparent IC50 (μM)
Vehicle	0	0	10	75.7 ± 1.91	22.6 ± 0.68	51.2 ± 1.03	47.9 ± 9.81
Reserpine	10	12	12	84.0 ± 2.05••	24.5 ± 0.56•	75.7 ± 5.63•••	44.8 ± 9.88

Biochemical data are mean ± s.e. of mean; •p<0.05, ••p<0.01, •••p<0.001 (Student's t-test); n=6-12. [a]response to 2.5μM SKF 82958

Reserpine treatment significantly increased the number of D1 and D2 receptors (11% and 8% respectively), with no change in Kd. The Vmax for cAMP production in response to SKF 82958 was also significantly increased with no change in Km. However, this treatment had differential effects on the biochemical and behavioural consequences of D1:D2 stimulation. Hence, both SKF 82958 and quinpirole could independently induce stereotypy following reserpinisation, whereas quinpirole was still able to inhibit cAMP production in tissue from reserpinised rats; the apparent IC50 was unchanged. Overall the results indicate that although repeated reserpinisation uncouples the D1:D2 behavioural responses, it does not alter the interaction associated with cAMP formation.

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297P PHARMACOLOGICAL STUDIES ON THE HUMAN DOPAMINE D_3 RECEPTOR EXPRESSED IN STABLE CELL LINES

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A novel dopamine receptor (D3) has been recently cloned which has a distinctive CNS localisation and pharmacological profile (Sokoloff *et al.*, 1990; 1992). It is not known how this receptor is functionally coupled. Since cell lines may vary in their G-protein content and the secondary messengers, we have stably expressed the human D3 (hD3) receptor in CHO, Rat 1 fibroblast and NG108-15 cells. Binding studies were performed using [^{125}I] iodosulpiride as previously described (Sokoloff *et al.*, 1990). Results for each cell line demonstrated high affinity saturable binding to a single population of receptors (Table 1).

Cell Line	Kd (nM)	Bmax (fmol/mg)	Dopamine Ki (nM)	Quinpirole Ki (nM)	Apomorphine Ki (nM)	Bromocryptine Ki (nM)	S-(-)Sulpiride Ki (nM)
hD2 CHO	0.26	700	700	580	29	0.46	4.5
hD3 CHO	0.42	290	25	16	14	2.2	8.0
hD3 NG108-15	0.23	45	35	9.9	16	1.6	6.3
hD3 Rat 1 Fibroblast	0.37	21	57	24	68	1.8	6.2

n=3-8, values are geometric mean. Errors were typically less than 15%.

Displacement studies were performed using 0.2nM radioligand. The hD3 receptor retained high affinity for dopamine and quinpirole in all three cell lines. Functional coupling was estimated by measuring cAMP levels (presence and absence of 1μM forskolin) and arachidonic acid release (enhancement in presence of 1μM A23187). In contrast to the hD2 CHO cell line, dopamine and quinpirole (10-10,000 nM) failed to demonstrate functional coupling in the three hD3 cell lines.

These results suggest that the hD3 receptor possessed similar properties in each of the three cell lines. These cell lines either lack the essential components for functional coupling of the hD3 receptor or this receptor couples in, as yet, an undetermined manner.

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298P CHLORMETHIAZOLE, BUT NOT DIZOCILPINE, INHIBITS DOPAMINE RELEASE IN RAT STRIATUM INDUCED BY HIGH DOSE METHAMPHETAMINE

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We recently reported that administration of chlormethiazole (CMZ) or dizocilpine 15 min before each of a series of high dose methamphetamine (METH) injections protects against the neurotoxic degeneration of striatal dopamine (Green et al, 1992). The possible mechanisms have now been investigated by measuring striatal dopamine (DA) release with *in vivo* microdialysis.

Rats were implanted with microdialysis probes (see Baldwin et al, 1991). On the following day dialysis samples were collected for 8h. After collection of 3 x 20 min baseline samples, one group was injected with saline followed 15 min later by METH (15mgkg^{-1} i.p.). This procedure was repeated 2 further times at 2h intervals. Further groups received CMZ (50mgkg^{-1} i.p.) or dizocilpine (1mgkg^{-1} i.p.) before the METH. DA, HVA and DOPAC were measured in dialysate by h.p.l.c. and electrochemical detection.

Each injection of METH induced a massive release of DA, which attenuated somewhat by the third injection. Dizocilpine did not alter this response (Figure). CMZ, in contrast, markedly inhibited DA release apart from the initial peak (2 way-ANOVA drug x time interaction F(16,96) = 2,73, p<0,005) (Figure). Dialysate HVA and DOPAC concentrations decreased after METH. This response was unaltered by dizocilpine but decreased further by CMZ.

Inhibiting DA function prevents METH-induced neurodegeneration (Schmidt et al, 1985). The reduction of dopamine release by CMZ may therefore be related to its ability to prevent METH neurotoxicity. Dizocilpine, in contrast, presumably protects through other mechanisms, probably involving inhibition of NMDA receptor function.

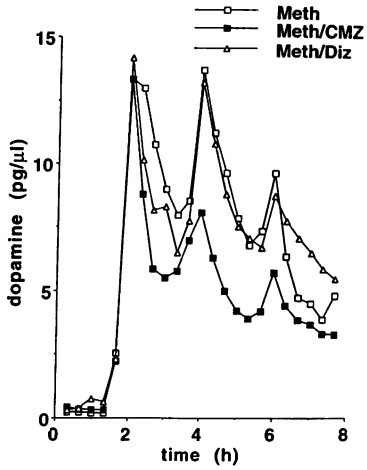

Figure Effect of chlormethiazole (CMZ) and dizocilpine (DIZ) on METH-induced DA release in the striatum. Values are mean dialysate DA concentrations (pg µl^{-1}) Group sizes: METH n=4; METH/CMZ n=5; METH/DIZ n=5.

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299P THE INFLUENCE OF NALOXONE UPON LOCOMOTOR ACTIVITY INDUCED BY A RANGE OF PSYCHOMOTOR STIMULANTS

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The opioid receptor antagonist, naloxone (NX) attenuates the behavioural effects of *d*-amphetamine in a wide range of species and procedures (eg Trujillo et al., 1991). Attenuation of *d*-amphetamine-induced stimulation of locomotor activity by opioid receptor antagonists has been especially well documented (eg Jones and Holtzman, 1992). To investigate the NX/*d*-amphetamine interaction further, the influence of NX upon the actions of psychomotor stimulants with differing mechanisms of actions was assessed in male Sprague-Dawley rats (260-320g, 9-15). Rats received either NX (5mg/kg s.c.) or saline (SAL) as a 30 min pretreatment whereupon cumulative-dose-response curves to each of the compounds were constructed in separate groups of rats (Hooks et al., 1992). Cumulative dosing commenced with injection of the appropriate vehicle (VEH) for each stimulant and was followed at 30 min intervals by increasing doses of the drug. A recording (20 min) of both gross locomotor and fine movements was made commencing 10 min after each injection. The compounds tested included those that release catecholamines from nerve terminals (*d*-amphetamine [0.1 - 6.4 mg/kg s.c.], *l*-amphetamine [0.4 - 24 mg/kg s.c.] and methamphetamine [0.1 - 6.4 mg/kg s.c.]), inhibit catecholamine reuptake (cocaine [3.0 - 56 mg/kg s.c.], methylphenidate [1.0 - 30 mg/kg s.c.], phendimetrazine [3.0 - 100 mg/kg s.c.], mazindol [0.3 - 10 mg/kg s.c.]), or act via other mechanisms (apomorphine [0.03 -1.0 mg/kg s.c.], caffeine [3.0 - 100 mg/kg i.p.] and scopolamine [0.03 - 1.0 mg/kg s.c.]).

Each of the agents caused dose-dependent increases in both the fine and gross counts/20 min. Pretreatment with NX significantly attenuated the gross activity response to both *d*-amphetamine ($F=7.00$, $P=0.014$) and *l*-amphetamine ($F=4.91$, $P=0.035$). For example, the gross counts/20 min were reduced from 1253 ± 130 (SAL) to 812 ± 33 (NX) following 1.6 mg/kg *d*-amphetamine ($P<0.01$). The fine activity response to both of these compounds was unaffected by NX-pretreatment. NX failed to influence the fine or gross activity response to methamphetamine. Of the non-amphetamine-like stimulants, only the actions of cocaine were affected by NX. There was no main effect of NX upon the cocaine-induced increase in gross counts, however, ANOVA revealed a significant interaction ($F=3.11$, $P=0.03$); *post hoc* analysis showed an increase in counts/20 min from 701 ± 135 (SAL) to 1058 ± 138 (NX) following 56 mg/kg cocaine ($P<0.01$). The fine movement response to cocaine was attenuated by NX pretreatment ($F=5.24$, $P=0.03$). For example, the fine counts/20 min were reduced from 848 ± 170 (SAL) to 499 ± 92 (NX) following 56mg/kg cocaine ($P<0.05$). In all cases, NX reduced both the fine and gross counts/20min after the initial VEH injection of the cumulative dose response curve ($P<0.5$-0.01). The findings with *d*-amphetamine confirm our earlier results (Hooks et al., 1992) and extend these findings to *l*-amphetamine. This suggests that for locomotor activity measured under the present conditions only the actions of amphetamine and cocaine are sensitive to NX.

This work was supported in part by Grant DA00541 and Research Scientist Award DA00008 (SGH), both from NIDA, USA.
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300P COMPARED NEUROTOXIC EFFECT OF CHRONIC AND ACUTE MPTP TREATMENT ON NEUROTRANSMITTER SYSTEMS OF THE BASAL GANGLIA OF THE CYNOMOLGOUS MONKEY

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Previous studies from this laboratory (Pérez-Ontaño *et al.*, 1991, 1992) showed that a chronic MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment in the marmoset, spread out over 5-10 months, produced a marked neurotoxicity not only on dopamine (DA) systems by also on serotonin (5-HT) and peptide systems in the basal ganglia. In the present study, two different schedules of MPTP treatment were used in the cynomolgous monkey (Macacca fascicularis) in order to extend the above findings. Acute treatment consisted of 2 MPTP injections (0.75mg/kg, i.v.) spaced one week apart. In chronic treatment, 6-15 injections of MPTP (0.5-1mg/kg, i.v.) spread out over a 3-5 months period, were given until a stable parkinsonian state was achieved.

Acute MPTP treatment decreased DA in the caudate nucleus and putamen by 76.5 and 64.4% respectively. This treatment also decreased the content of the metabolites DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanillic acid) by approximately 80% in both nuclei. Conversely, acute MPTP significantly increased, by 150%, the 5-HT content in the putamen, probably due to the early inhibitory effect of the neurotoxin on type-A MAO. Chronic MPTP produced a much more marked DA depletion, averaging 99.5%, both in caudate and putamen nuclei, along with a striking increment in the quotient HVA + DOPAC/DA, an index of DA turnover. In these chronically MPTP-treated monkeys, there was also a significant noradrenaline depletion in the putamen (mean 73.3%). The 5-HT content in the caudate nucleus was not significantly reduced. Metenkephalin content was increased by 205% in the substantia nigra, no significant changes being observed in the other regions of the basal ganglia.

The present results suggest that the selected schedule of MPTP administration is a major determinant of its neurotoxic features. Moreover, marked differences are present, both at the behavioural and neurochemical level, between the marmoset and a bigger primate such as the cynomolgous monkey. These differences should be taken into consideration at the time of analyzing the results obtained in the MPTP model of parkinsonism.

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301P BASIC FIBROBLAST GROWTH FACTOR (bFGF) INTRAVENTRICULAR INFUSION IN THE MPTP-TREATED MARMOSET

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Basic fibroblast growth factor (bFGF) possesses potent mitogenic activity for mesenchyme derived cells and neurotrophic properties for both central and peripheral neuronal populations, (Gospodarowicz *et al.*, 1986; Unsicker *et al.*, 1987). bFGF enhances the growth parameters of dopaminergic cells, foetal mesencephalic neurons and chromaffin cells *in vitro* and has protective effects in several models of neuronal injury, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridinium (MPP^+) toxicity, (Ferrari *et al.*, 1989; Otto & Unsicker, 1990). We now report on its effect on intraventricular infusion into MPTP-treated common marmosets.

bFGF or vehicle was delivered by infusion from subcutaneously implanted Alzet minipumps into the lateral ventricle of adult common marmosets (*Callithrix jacchus*) rendered chronically parkinsonian by administration of MPTP. Infusion commenced two months after MPTP treatment and the animals received bFGF in low (1.8 µg/L), medium (18 µg/L) or high doses (180 µg/L) over a 28 day period. At weekly intervals automated activity measurements, standardized behavioural disability scoring and blinded videotape analysis were made. Subsequently histological analysis of substantia nigra tyrosine hydroxylase immunoreactivity, Nissl and GFAP staining was performed along with striatal [^{3}H]-mazindol autoradiography.

Two animals in the high dose group developed a neurological syndrome and were killed prior to the end of the study. Both of these animals and all of the other high dose animals revealed hydrocephalus post mortem, which was also observed in one medium dose and two low dose animals. No significant differences were found between control and bFGF-treated animals (employing the Kruskal-Wallis test) with respect to behavioural analysis, nigral TH-cell counts, and subregional [^{3}H]-mazindol binding. Nissl staining revealed proliferation of the choroid plexus and ependyma which was most marked in the high dose animals. GFAP activity was increased about the site of the cannula tip.

Chronic bFGF infusion had no positive effects in modifying the motor disability or histological markers of nigrostriatal destruction induced by MPTP administration in the marmoset. Hydrocephalus and neurological deterioration were observed in some animals, presumably secondary to the stimulation of ependymal and choroid plexus growth by bFGF.

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302P ALTERATIONS IN NEUROPEPTIDES IN BASAL GANGLIA IN PARKINSON'S DISEASE AND INCIDENTAL LEWY BODY DISEASE

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Alterations in neuropeptide levels occur in basal ganglia in Parkinson's disease (PD) but it is not known whether these reflect part of the primary pathology or whether they occur secondary to the loss of dopamine neurones or whether they are produced as a result of drug treatment (Agid & Javoy-Agid, 1985). We now report on peptide changes occurring in incidental Lewy body disease (presymptomatic Parkinson's disease; LB +ve) and end stage PD.

Brain material matched for age and post-mortem delay was dissected to provide dorsal, intermediate and ventral areas of caudate nucleus and putamen, lateral and medial globus pallidus and substantia nigra. Peptides were measured by a combined HPLC/RIA assay as previously described (de Ceballos *et al.*, 1991). Levels of met-enkephalin (met-enk), but not leu-enkephalin (leu-enk) or substance P (SP), were reduced in the intermediate caudate in both PD and LB +ve. The levels of met-enk, leu-enk and SP were reduced in the intermediate putamen in PD. SP, but not met- or leu-enk levels, were increased in PD and incidental LB +ve in the lateral globus pallidus. In the medial globus pallidus there was a trend for met-enk and SP levels to be elevated in both disorders. In substantia nigra met- and leu-enk, but not SP, levels were reduced in PD but there were no changes in LB +ve.

Table 1: Neuropeptide changes (pmol/g tissue) in basal ganglia in PD, LB +ve and control subjects (C).

	Met-enk	Leu-enk	SP
Caudate nucleus			
C	196.7 ± 26.5	13.3 ± 3.0	117.0 ± 38.2
LB +ve	94.4 ± 25.4*	10.8 ± 4.1	85.2 ± 12.9
PD	89.5 ± 20.9*	5.7 ± 3.4	101.9 ± 25.8
Putamen			
C	261.1 ± 79.4	58.9 ± 22.7	195.4 ± 52.6
LB +ve	114.1 ± 18.1	25.6 ± 8.4	199.4 ± 52.6
PD	28.8 ± 4.6*	5.2 ± 3.7*	66.9 ± 15.4*

* $p < 0.05$ vs. controls

The correspondence between some basal ganglia peptide changes in PD and LB +ve disease suggests that these are an integral part of the pathology of the illness.

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303P HAEMOPEXIN IMMUNOREACTIVITY IN NEURONES IN THE HUMAN BRAIN

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Brain iron homeostasis is important for cell metabolism and may play a role in neurodegenerative disorders such as Parkinson's disease. Haemopexin, a 60kDa serum glycoprotein, is involved in the transport of iron containing haem residues in tissues such as liver, by receptor-mediated endocytosis, in an analagous manner to the iron transport protein, transferrin (Smith and Hunt, 1990). Haemopexin therefore has the potential to be a system involved in brain iron homeostasis, in addition to transferrin. The haemopexin system may for example play a role in the transfer of iron from neuronal sites of uptake, mediated by the transferrin receptor, to sites of iron storage in glial cells as ferritin (Morris et al, 1992). As a first stage in the characterization of the haemopexin system in the brain, the immunocytochemical distribution of haemopexin has been studied using a monospecific polyclonal antiserum to haemopexin. A standard ABC immunochemical method has been used in formalin-fixed, paraffin embedded sections of normal human brain (3 males, 1 female, age: 62 ± 10yrs; postmortem delay: 43 ± 21hrs).

Neurones in all the brain regions studied showed immunostaining of the soma, axons and dendrites with some areas showing striking immunoreactivity due to dendritic staining. Glial cells rarely exhibited immunostaining though in some regions, notably those with a high iron content such as the globus pallidus, immunostained astrocytes and microglia were occasionally observed. Oligodendrocytes and choroid plexus epithelial cells lacked haemopexin immunoreactivity.

The presence of hameopexin immunoreactivity in neurones suggests that the haem transport system may play an important role in neuronal iron homeostasis, in addition to that of brain transferrin. Due to its selective role in the transport of haem iron, haemopexin may function by transferring iron in haem residues, predominantly synthesized in neurones, to glial cells for storage in ferritin. The role of haemopexin in brain iron homeostatic mechanisms demands further investigation especially in relation to neurodegenerative diseases such as Parkinson's disease.

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304P BQ123 AND BQ3020 REVEAL ENDOTHELIN ET_A AND ET_B RECEPTOR SUB-TYPES IN HUMAN CARDIAC VENTRICLE AND RAT CEREBELLUM

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Our aim was to use the ET_A and ET_B selective peptides BQ123 (Cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]) (Nakamichi *et al.*, 1992) and BQ3020 ([$Ala^{11,15}$]Ac-ET-$1_{(6-21)}$) to characterise sub-types in human cardiac ventricle and rat cerebellum. In saturation experiments, cryostat sections of human ventricle free wall or adult male Wistar rat cerebellum were incubated at 22 °C with 0.02-10 nM (3-[^{125}Iodotyrosyl^{13}] BQ3020 or (3-[^{125}Iodotyrosyl^{13}] ET-1 (Amersham International plc) for two hours at 22 °C using assay conditions of Davenport *et al.* (1989). Non-specific binding was estimated using 1μM of the corresponding unlabelled peptide.Receptor affinity (K_D) and density (Bmax) were determined using LIGAND. For competition experiments, sections were incubated with a single concentration of [^{125}I] ET-1 (100 pM) and unlabelled BQ123 or BQ3020 (20pM-100μM).

In the cerebellum, the observed association rate constants (mean±s.e.mean for n=3) at 22°C were similar at 0.07±0.01/min for [^{125}I] ET-1 and 0.07± 0.02/min for [^{125}I] BQ3020. The dissociation rate was slow, with 12% of the total specific binding dissociated after 24 h for ET-1 and 30% for [^{125}I] BQ3020. [^{125}I] BQ3020 bound with a similar affinity and density in cerebellum (K_D, 7.9±2.1 nM, Bmax, 4.1±0.7 pmol/mg protein, nH, 1.01±0.01), with Hill slopes close to unity compared to [^{125}I] ET-1 (K_D, 3.4±0.2 nM, Bmax, 4.5±0.6 pmol/mg protein, nH,0.96±0.02). In competition experiments for the non-selective radioligand [^{125}I] ET-1, the affinity of BQ3020 for the ET_A was lower (10.3 μM) than at the ET_B sub-type (41.5 nM) giving a ratio of 22:78%

In the ventricle, observed association rate constants were similar to the cerebellum. Both radioligands had high affinity in ventricle (mean± s.e.mean for n=3): [^{125}I] ET-1, K_D 0.346±0.102nM, Bmax 0.19±0.023 pmol/mg protein), [^{125}I] BQ3020 K_D, 0.107± 0.004 nM; Bmax, 0.019±0.005 pmol/mg protein). In competition for [^{125}I] ET-1, both sub-types were revealed: BQ3020, K_D ET_A, 2.04±0.21 μM; K_D ET_B, 1.38±0.72 nM, (ET_A 67%:ET_B 33%); BQ123, K_D ET_A, 0.73.04±0.22 nM; K_D ET_B, 24.3±2.0 μM, (ET_A 57%:ET_B 43%).
The results demonstrate the presence of both endothelin receptor sub-types, ET_A and ET_B, in human ventricle and rat cerebellum.

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305P CENTRAL ADMINISTRATION OF EXP 3174 OR PD 123319 INHIBITS EFFECTS OF CENTRAL ANGIOTENSIN II IN CONSCIOUS RATS

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All the known effects of angiotensin II (AII) on the cardiovascular system appear to be mediated by stimulation of AT_1-receptors (Wong *et al.*, 1990), in spite of the fact that both AT_1- and AT_2-receptors are widespread in peripheral and central structures (Gehlert *et al.*, 1991). However, there are few studies which have examined the functional effects of central administration of nonpeptide AII receptor antagonists. Therefore, we have measured the haemodynamic effects of intracerebroventricular (i.c.v.) injections of AII, and the influences thereon of the AT_1-receptor antagonist, EXP 3174, or the AT_2-receptor antagonist, PD 123319. Male, Long Evans rats underwent a 3-stage operation under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.). Initially, a guide cannula was implanted in the right lateral cerebral ventricle, and at least 5 days later, pulsed Doppler probes were implanted to monitor renal (R), mesenteric (M) and hindquarters (H) blood flows. After at least a further 7 days, intravascular catheters were implanted, and the following day continuous recordings were made of mean arterial pressure (MAP) and R, M and H blood flow signals; vascular conductances were calculated from the latter divided by MAP. AII (100 ng i.c.v.) caused a prolonged pressor response which was associated with marked M and H vasoconstriction (decreased conductance) and a small, transient R vasoconstriction. The cardiovascular response to AII was quantified by measuring areas under (for MAP) or over (for conductance) curves over 30 min. The effect of either EXP 3174 (1 µg i.c.v., n = 9) or PD 123319 (80 µg i.c.v., n = 9) on responses to AII are shown in Table 1; i.c.v. injection of vehicle had no significant effect on responses to AII.

Table 1: Cardiovascular responses (AUC or AOC) to AII before and after either EXP 3174 or PD 123319.

		Time after EXP 3174 i.c.v.					Time after PD 123319 i.c.v.			
	Control	10 min	3 h	24 h	48 h	Control	15 min	3 h	24 h	48 h
MAP	343 ± 36	78 ± 26*	153 ± 27*	322 ± 23	431 ± 62	458 ± 41	207 ± 40*	88 ± 32*	80 ± 27*	251 ± 48*
R conductance	194 ± 73	170 ± 42	93 ± 25	261 ± 86	232 ± 54	189 ± 55	175 ± 48	43 ± 14	114 ± 37	211 ± 53
M conductance	489 ± 101	210 ± 43*	258 ± 104*	437 ± 53	603 ± 86	836 ± 102	252 ± 55*	202 ± 55*	211 ± 67*	352 ± 90*
H conductance	500 ± 88	131 ± 60*	254 ± 71	393 ± 84	369 ± 84	590 ± 80	342 ± 89	188 ± 44*	261 ± 86*	457 ± 91

Values are mean ± s. e. mean (arbitrary units). * P<0.05 versus respective control (ANOVA).

These data show that the antagonistic profiles of EXP 3174 and PD 123319 are different and indicate that the central effects of AII may involve concurrent, and interdependent, activation of AT_1- and AT_2-receptors, and/or that PD 123319 undergoes a unique biotransformation in the brain to a product(s) with AT_1-receptor antagonist activity.

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306P THE INHIBITION BY LIPOCORTIN 1 OF ACTH SECRETION EVOKED *IN VITRO* BY THE CALCIUM CHANNEL AGONIST, BAY K8644

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Glucocorticoids such as dexamethasone exert potent inhibitory effects on the secretion of corticotrophin (ACTH) by the anterior pituitary gland. Observations that the regulatory effects of dexamethasone on the pharmacologically [corticotrophin releasing factor (CRF-41) and forskolin] evoked release of ACTH from rat anterior pituitary tissue in vitro are mimicked by lipocortin 1 and attenuated by a neutralizing monoclonal anti-lipocortin 1 antibody together with evidence that glucocorticoids promote the externalization of lipocortin 1 by anterior pituitary cells in vitro have led to suggestions that lipocortin 1 mediates some aspects of the regulatory actions of the steroids on ACTH secretion (Taylor *et al* , 1992). The secretory responses of the corticotrophs to CRF-41 and forskolin are dependent on Ca^{++} influx, via L-Ca^{++} channels. Accordingly, In an attempt to elucidate further the mechanism by which lipocortin 1 inhibits the responses to these secretagogues we have now examined the effects of an N-terminal lipocortin 1 fragment ($LC1_{1-188}$, ICI plc), dexamethasone and a monoclonal lipocortin 1 antibody (LC1-Ab) on the release of ACTH in vitro evoked by an L-Ca^{++} channel opener, BAY K8644 (Research Biochemicals Incorporated).

Anterior pituitary tissue was collected post-mortem from adult male CFY rats (≈ 200g body weight) and incubated in conditions described earlier (Hadley *et al*, 1991). ACTH released into the medium was determined by radioimmunoassay and the results were analysed statistically using Duncan's multiple range test.

Using a 60 min contact time, BAY K8644 (0.1nM-1.0nM) caused significant ($p<0.05$, n=5-6) concentration dependent increases in ACTH release. The responses to a submaximal concentration of BAY K8644 (1nM) which produced a significant ($p<0.05$, n=5-6) approximately 2 fold increase in peptide release, were inhibited by nifedipine (1nM). They were inhibited also in a dose dependent manner by $LC1_{1-188}$ (10pg/ml-100ng/ml, $p< 0.01$, n=5-6) which alone, in some instances, (1pg/ml & 1ng/ml), caused a significant ($p<0.05$, n=5-6) increase in the basal release of the peptide. Pre-incubation of the tissue with dexamethasone (0.1μM) did not affect basal ACTH release but reduced by approximately 50% ($p< 0.05$, n=5-6) the response to the BAY K8644 compound. The inhibitory actions of the steroid were overcome by the inclusion of a neutralizing monoclonal anti-lipocortin 1 antibody (Zymed, diluted 1:15000) in the medium. An isotype matched control monoclonal antibody (anti-spectrin α + ß, Sigma Chemical Co, diluted 1:15000) was without effect in this regard.

The results are consistent with the concept that lipocortin 1 contributes to the inhibitory actions of glucocorticoids on ACTH release from rat anterior pituitary tissue and suggest that its actions are effected at the point of, or distal to, the influx of Ca^{++} to the cell.

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307P INHIBITORY EFFECTS OF HUMAN RECOMBINANT LIPOCORTIN 1 AND DEXAMETHASONE ON THE SECRETION OF CORTICOTROPHIN RELEASING FACTOR-41 (CRF-41) IN THE RAT

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We have recently suggested that lipocortin 1 (LC1) acts at the levels of the hypothalamus (Loxley et al., 1991) and the anterior pituitary gland (Taylor et al., 1992) to mediate certain aspects of the powerful inhibitory actions of the glucocorticoids on the functional activity of the hypothalamo-pituitary-adrenocortical (HPA) axis. In the present study we have used in vitro and in vivo models to examine further the influence of human recombinant LC1 (hu-r-LC1, Biogen Inc.) and its stable N-terminal fragment ($LC1_{1-188}$, ICI plc) on the cytokine-induced release of CRF-41 from the rat hypothalamus and to investigate the influence of dexamethasone on the expression of LC1 in this tissue.

In vitro, hypothalami removed from intact male CFY rats (≈200g) and incubated as described previously (Loxley et al., 1991) responded to interleukins (ILs)-1α (50-200pg/ml), -1β (250-1000pg/ml), -6 (5-20ng/ml) and -8 (250-1000pg/ml) with significant ($P<0.05$, n=5-6) increases in the release of immunoreactive (ir)-CRF-41. The maximal responses to IL-1α (200pg/ml), IL-1β (1000pg/ml), IL-6 (20ng/ml) but not IL-8 (500pg/ml) were enhanced significantly ($P<0.05$, n=5-6) by surgical removal of the adrenal glands 7-14 days prior to autopsy. Addition of hu-r-LC1 (10ng/ml), $LC1_{1-188}$ (10ng/ml) or dexamethasone (0.1μM) to the medium had no effect on the spontaneous release of CRF-41 from hypothalami removed from chronically adrenalectomized rats. However, $LC1_{1-188}$ (10ng/ml) reduced significantly the secretory responses to IL-1α (200pg/ml), $P<0.01$, n=6) and IL-1β (500pg/ml, $P<0.05$, n=6) while the full length recombinant molecule (hu-r-LC1, 10ng/ml) caused a significant reduction in IL-6 (5ng/ml, $P<0.01$, n=6) and IL-8 (1000pg/ml, $P<0.05$, n=6) induced CRF-41 secretion. Similar inhibitory effects on the cytokine-induced release of ir-CRF-41 were observed following pre-incubation of the tissue with dexamethasone (0.1μM). Furthermore, western blot analysis revealed that the steroid treatment facilitated the appearance of ir-LC1 on the outer surface of the hypothalamic cells.

In vivo, intracerebroventricular (i.c.v.) administration of IL-1β (2.5-10ng/ml) to conscious rats (via an indwelling cannula placed in the third ventricle under pentobarbitone sodium anaesthesia 7-10 days previously) produced pronounced, dose dependent increases in the serum ir-corticosterone concentration ($P<0.01$, n=4-5) and at the highest dose tested an approximately 6 fold increase in serum corticosterone was apparent. IL-6 (7.5-30ng/ml) was also effective in this regard, ($P<0.01$, n=4-5) although its effects were less marked with the highest dose producing only a 4 fold increase in corticosterone release. Human recombinant-LC1 (0.3 & 0.6μg i.c.v.) had no effect on the serum ir-corticosterone concentration but a higher dose (1.2μg, i.c.v.) produced a small (≈3 fold) increase in steroid release ($P<0.01$, n=4-5). The adrenocortical responses to submaximal doses of IL-1β (10ng, i.c.v.) and IL-6 (30ng, i.c.v.) were reduced significantly ($P<0.05$ and $P<0.01$ respectively, n=4-5) by i.c.v. injection of hu-r-LC1 (0.3-1.2μg) 5 min prior to the cytokine.

The results support the concept that lipocortin 1 may contribute to the regulatory actions of the glucocorticoids on the release of CRF-41 from the hypothalamus.

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308P EFFECT OF CHRONIC ETHANOL TREATMENT ON ENERGY BALANCE IN GOLD THIOGLUCOSE-INDUCED OBESITY

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Gold thioglucose (GTG), after a single injection, causes histological damage focused selectively but not exclusively in the ventromedial hypothalamus (VMH) area (Debons *et. al.*, 1977). As with other VMH lesions, hyperplasia and obesity result. Chronic ethanol treatment has been reported to reverse the obese-diabetic condition in CBA/Ca mice (Connelly & Taberner, 1986) and stimulate brown adipose tissue lipogenesis in these mice (Al-Qatari *et. al.*, 1991). The aim of this study was to compare the effect of ethanol on another obesity model, namely GTG-treated LAC:LCGFCFW (LAC G) mice.

Mice 6-8 weeks old were injected with GTG (400mg/kg, i.p.), body weight and blood glucose monitored for 16 weeks. Mice were housed four to a cage and given 20% w/v ethanol as their sole fluid. Body weight, ethanol consumption and food intake were measured for 5 weeks. *In vivo* lipogenesis rates in brown adipose tissue (BAT) were estimated as described by Mercer & Trayhurn (1983). Data are means ±(n).

The total energy intake of the GTG-treated mice was significantly lower 1134±108(4) KJ/kg/day compared to controls values of 2095±162(4) KJ/kg/day ($p< 0.001$, unpaired ANOVA). Bodyweight of the GTG-treated mice was 65±5.4 (30)g compared to 34±2.3(30)g of age matched controls. Chronic ethanol treatment did not alter total energy balance or body weight of the GTG-treated mice,although there was a slight decrease in body weight of controls. Ethanol represented18% of total energy intake, in both controls and GTG-treated mice. Food intake was slightly reduced in the GTG-treated mice, although total energy was unchanged.
Lipogenic rates in BAT in GTG treated mice were significantly reduced by 70% to 82.9±23.2 (8) µg atoms H incorporated/hr/g fat free tissue weight ($p<0.02$, unpaired t-test). Ethanol-treated age matched controls showed a slight increase in lipogenic rates compared to controls. Chronic ethanol treatment in GTG-treated mice did not restore lipogenic rates back to control values.

This data indicates that in the GTG model of obesity, chronic ethanol treatment does not stimulate thermogenesis. This suggests that ethanol may require the VMH in order to exert its anti-obesity action.

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309P EFFECT OF SUMATRIPTAN ON SYNAPTIC TRANSMISSION IN THE RAT SUPERIOR CERVIAL GANGLION *IN VITRO*

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Sumatriptan has been shown to exert a selective vasoconstrictor action on certain blood vessels (Humphrey et al, 1988). A selective action on cephalic vessels is considered to explain the antimigraine activity of sumatriptan (Parsons et al, 1989). The vasoconstrictor action of sumatriptan contrasts with a depressant action on noradrenergic transmission in isolated saphenous vein (Humphrey et al, 1988).

In the present experiments sumatriptan has been tested on synaptic transmission in the isolated superior cervical ganglion of the rat in vitro.

Ganglia were bathed in a medium containing (mM):- NaCl 118, KCl 3, $CaCl_2$ 1.5, $MgSO_4$ 0.75, $NaHCO_3$ 24, dextrose 12. THe medium was maintained at 25°C and gassed with 95% O_2, 5% CO_2. The postganglionic nerve was placed across a grease seal and maximal synaptic potentials were evoked by cathodal stimulation of the preganglionic nerve with 0.5 msec square pulses at 30 second intervals. The depressant action was measured from the peak amplitude of the postganglionic hexamethonium-sensitive potential.

In six preparations the mean peak amplitude and latency of the ganglionic synaptic potentials (±s.e.mean) were 1.7 ±0.2 mV and 13.6 ±1.1 ms respectively. Concentrations of sumatriptan ranging from 1 nM to 30 µM were applied to four preparations. Threshold depressant actions (7.4% ±2.3 s.e.mean) depression were observed at 0.1 µM concentration. The mean concentrations (µM ± s.e.mean) required to produce 25% and 50% depression from control (100%) values were 3.61 ±0.62 and 10.8 ±1.85 respectively.

Thus it would appear from the present study that the depressant action of sumatriptan is not confined to noradrenergic junctional tissue (Humphrey et al, 1988).

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310P CHARACTERISATION OF A 5-HT RECEPTOR WHICH MEDIATES AN N^G-NITRO-L-ARGININE METHYL ESTER-RESISTANT RELAXATION OF GUINEA-PIG ISOLATED JUGULAR VEIN

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Endothelium-dependent and independent relaxations to 5-hydroxytryptamine (5-HT) have recently been described in a preparation of guinea-pig isolated jugular vein (Gupta, 1992). The endothelium-dependent component involves the activation of a 5-HT_{1D}-like receptor, however, the identity of the 5-HT receptor which mediates the endothelium-independent relaxations to 5-HT is not known. In the present study, the pharmacological properties of the latter receptor have been investigated.
Male Dunkin-Hartley guinea-pigs (300-400g) were killed by cervical dislocation. Isolated rings (2mm in length) were suspended on parallel tungsten wires under a resting load of 0.2-0.5g, in Krebs solution gassed with 95% O_2 and 5% CO_2 at 37°C. Ketanserin (300nM) was present in all experiments to antagonise 5-HT_2 receptor-mediated contractions, and at this concentration, this compound had no effect at the 5-HT receptor under study. Tissues were precontracted with the thromboxane A_2 mimetic, U-46619 (30nM), and after a period of 20min, N^G-nitro-L-arginine methyl ester (100μM) was added directly to the bathing medium to inhibit the endothelium-dependent relaxations to 5-HT receptor agonists. After a further 40min period, a concentration-effect curve to 5-HT receptor agonists was prepared. Following an interval of 15min, the latter procedure was repeated and a second concentration-effect curve to the agonist was prepared in the same tissue. In the agonist studies, the first curve prepared was to 5-HT, and the second curve was either to 5-HT (time control) or test agonist. In antagonist studies, both the first and second curves were to 5-carboxamidotryptamine (5-CT), and the second curve was prepared in either the absence (time control) or presence (test) of antagonist. Antagonists were equilibrated with tissues for at least 60min before preparing the second concentration-effect curve to 5-CT.
Both 5-HT and 5-CT produced concentration-dependent relaxations of the jugular vein (pEC_{50} and maximum effect [expressed as a % relaxation of the maximum effect in curve 1 to 5-HT] values respectively: 5-HT, 6.6±0.1 and 91.3±2.5, n=3; 5-CT, 7.9±0.1 and 112.4±4.8, n=4). The relaxations to 5-HT were not antagonised by tetrodotoxin (100nM), an inhibitor of neuronal function. The relaxations to 5-CT were also resistant to the actions of WAY 100289 (mean concentration-ratio at 1μM, 1.5[0.9-2.4], n=4), a selective 5-HT_3 receptor antagonist (Rhodes *et al.*, 1991). However, 5-CT-evoked relaxations were antagonised by the non-selective 5-HT_1 receptor antagonists, methiothepin (3-100nM), metergoline (0.03-1μM) and mesulergine (0.03-1μM) . Methiothepin produced only a small rightward displacement of the control curve to 5-CT (geometric mean concentration-ratio at 100nM, 4.5[1.7-11.9], n=4), but reduced the maximum effect to 5-CT (pIC_{50} , 7.2[6.8-7.4], n=4). Metergoline and mesulergine behaved as competitive antagonists against the relaxations to 5-CT in this preparation (pA_2 and Schild plot slope values respectively: metergoline 7.6[7.4-8.0], 0.9[0.7-1.2], n=4; mesulergine 7.8[7.6-8.0], 1.0[0.8-1.1], n=4).
In summary, the relaxations to 5-CT, which were 24 fold more potent than 5-HT, were not antagonised by 5-HT_2 and 5-HT_3 receptor antagonists. However, 5-CT-evoked relaxations were sensitive to the actions of the non-selective 5-HT_1 receptor antagonists, methiothepin, metergoline and mesulergine. Therefore, it is proposed that the L-NAME-resistant relaxations to 5-HT and 5-CT observed in preparations of guinea-pig isolated jugular vein could be mediated by a smooth muscle relaxant 5-HT_1-like receptor.

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311P RESPONSES TO 5-HT IN STRIPS OF RABBIT MESENTERIC ARTERY PERMEABILIZED WITH STAPHYLOCOCCUS α–TOXIN

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Noradrenaline induces contraction in part by releasing calcium from an internal IP_3-sensitive store in vascular smooth muscle cells (Kitazawa *et al*, 1989). The activation of 5-HT_2 receptors, which are the predominant 5-HT receptor type present in vascular smooth muscle cells, is associated with the hydrolysis of inositol phospholipids, suggesting that a similar mechanism may be involved in contractile responses to 5-HT. PI-hydrolysis due to 5-HT_2-receptor activation has been demonstrated in rat cerebral cortex and in isolated strips of rat aorta (Conn & Sanders-Bush, 1987; Nakaki *et al*, 1985; Roth *et al*, 1986). In the present study, we investigated the possibility that 5-HT could release calcium from internal stores in the mesenteric artery.

Very small strips of rabbit mesenteric artery (approximately 100μM wide) were cut and attached to a sensitive force transducer. The viability of the strips was tested by inducing contraction to either 10μm noradrenaline or 10μm 5-HT in oxygenated Krebs solution. Viable strips were then placed in a mock intracellular solution (0 Ca^{2+}, 0,2mM EGTA), in which a single, transient contraction could be obtained to either noradrenaline or 10mM caffeine, but not 5-HT. These contractions did not return after subsequent incubation in solution containing 0.08μm calcium. Strips were then permeabilized with staphylococcus α-toxin. After permeabilization, reproducible contractions could be induced in response to either 10μm noradrenaline and 100μm GTP (56 ± 8% of maximal tissue response, n=6) or 10mM caffeine (64 ± 3%, n=8) both in 0 Ca^{2+} solution, but only if the strips had been exposed to mock intracellular solution containing 0.08μm calcium before each stimulus. Prior exposure to caffeine blocked noradrenalines ability to stimulate this contraction. Exogenous IP_3 (100μM) also induced a transient contraction (39 ± 18%, n=4) which was blocked by prior exposure to caffeine. However, measurable contractions were not obtained in response to 5-HT under these conditions, although reproducible contractions to IP_3 could be induced in the same strips. 5-HT did increase the size of contractions induced with 1μM calcium + 100μM GTP, presumably by sensitizing the contractile apparatus.

These observations suggest that contraction to 5-HT in the rabbit mesenteric artery, unlike the contraction to noradrenaline, does not involve the release of calcium from an IP_3-sensitive intracellular store.

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312P A RELATIONSHIP BETWEEN THE STRUCTURES OF SOME N-ALKYL-1,4-DIHYDRO-4-OXOQUINOLINES AND THEIR INSURMOUNTABILITY AS NICOTINIC AND 5-HT_3 RECEPTOR ANTAGONISTS

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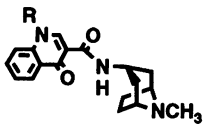
Many 5-HT_3 receptor antagonists are insurmountable in certain functional assays (Ireland & Tyers, 1987). We have observed the 5-HT_3 antagonist effects of 5 closely related N-alkyldihydro-oxoquinolines using 5-HT-evoked depolarization of the rat isolated vagus nerve as the 5-HT_3 test procedure (Ireland & Tyers, 1987). In view of the similarity in structure and function between 5-HT_3 and nicotinic receptors (Maricq et al., 1991) we have also studied antagonism of nicotinic receptor-mediated depolarization. Extracellular recording of depolarization of the desheathed cervical vagus nerve was carried out using a "grease gap" technique (Ireland & Tyers, 1987). Male, Sprague Dawley rats 180-220g were killed by a blow to the head and cervical dislocation. The vagus nerve was placed in a two chamber tissue bath one chamber being perfused (3 ml/min) with Krebs' solution (2.5 mM $CaCl_2$, at 27°C, gassed with 5% CO_2 in O_2) to which drugs were added. Agonist contact time was 3 min (15 min washout) and antagonists (tested at 3 or more concentrations) were equilibrated for 50 min. Two concentration-response curves (CRC's) to either 5-HT, 0.1 to 300 µM or the nicotinic agonist dimethylphenylpiperazinium (DMPP, 1 to 300 µM) were obtained in each tissue.

Table 1

R	pIC_{50} v 5-HT	Apparent pA_2 v 5-HT	pIC_{50} v DMPP	clogP
methyl	<6.5	8.4 (8.3-8.7) ^{a}1.2	4.9 (4.7-5.0)	1.485
ethyl	9.1 (9.0-9.2)	9.1 (8.9-9.7) ^{a}1.2	5.7 (5.5-5.8)	2.014
n-propyl	9.5 (9.2-9.7)	N.S.	6.1 (5.8-6.3)	2.543
n-butyl	9.2 (8.8-9.4)	N.S.	6.4 (6.0-6.6)	3.070
2-methoxyethyl	6.5 (5.8-6.9)	8.8 (8.3-9.8) ^{a}0.8	5.7 (5.6-5.9)	1.260



N.S; CRC's not displaced to the right. pIC_{50}; negative log_{10} of the antagonist concentration (M) which depresses the maximum response by 50%. clogP; calculated octanol:water partition coefficient, 95% limits in brackets, ^{a}Schild plot slope

In control preparations mean maximum depolarizations to 5-HT (n=8) and DMPP (n=18) were 562 and 1080 µV and geometric mean ED_{50} values of 0.7 and 33.8 µM respectively. All compounds were insurmountable antagonists of DMPP-evoked depolarizations with no rightward displacement of CRC's, potency was related to the size of the alkyl group. The compounds antagonized 5-HT_3 receptor-mediated responses at concentrations much lower than those blocking DMPP. Increase in alkyl group size of the antagonist from methyl to propyl was associated with a reduction in the maximum response to 5-HT. With the methyl analogue (10 nM, n=4) the maximum response to 5-HT was reduced to 69.7% of control with little further reduction at higher antagonist concentrations (63.5% at 300 nM, n=6). With the ethyl analogue (1nM, n=7) the maximum 5-HT response was 42.3% of control and 39% at 10 nM (n=4). With these antagonists it was possible to calculate pA_2 values from rightward displacements in CRC's . In contrast the propyl and butyl analogues depressed maximum responses to 5-HT with no shift in CRC's. The 2-methoxyethyl compound, expected to have similar steric properties to its butyl analogue but with reduced lipid solubility, resembled the methyl compound in producing a reduced maximum response to 5-HT (54.0% at 30 nM, n=4) with concentration related rightward shifts in CRC's. A possible explanation of these observations is that the compounds act to block nicotinic responses by a mechanism related to lipid solubility (possibly ion channel block) which does not have the characteristics of competitive receptor antagonism. 5-HT_3 receptor-mediated responses may be antagonized both at the receptor and at a second site, access to which is related to lipid solubility.

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313P FACILITATION OF NONCHOLINERGIC NEUROTRANSMISSION BY RENZAPRIDE (BRL 24924) IN CIRCULAR MUSCLE OF GUINEA-PIG ILEUM

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The substituted benzamide, renzapride (BRL24924), is a gastroprokinetic agent which potentiates twitch contractions to electrical field stimulation of motor nerves in the GI tract and is thought to facilitate the evoked release of acetylcholine by a mechanism involving a 5-hydroxytryptamine (5-HT_4) receptor located prejunctionally on cholinergic nerve terminals. The possibility that renzapride also facilitates the release of other noncholinergic gut transmitters was investigated using intracellular microelectrodes, in electrophysiological studies of junction potentials in the circular smooth muscle of guinea-pig ileum, *in vitro*. Segments (5cm long) of the terminal ileum were opened along the mesenteric border and mucosal/submucosal layers were removed by fine dissection. Ileal muscle strips (5mm by 10mm) were cut in the circular axis and placed in an electrophysiological chamber superfused with oxygenated Krebs solution (5ml/min; 37°C) with the circular muscle left uppermost and the myenteric plexus and longitudinal muscle beneath. With atropine (10^{-6}M) and guanethidine (10^{-6}M) present in the superfusate, renzapride (10^{-12}-10^{-5}M) increased (by up to 36%•4%, mean • s.e.mean; n=9) the amplitude of fast inhibitory and slow excitatory junction potentials (IJPs and EJPs) evoked by electrical field stimulation. Potentiation of noncholinergic IJPs and EJPs occurred without evoking spontaneous junction potentials nor causing significant changes in the membrane potential of circular muscle. Therefore, the possibility was excluded that renzapride caused *either* the spontaneous release of neurotransmitters *or* any appreciable change in electromotive driving force to alter the amplitude of junction potentials. For fast (purinergic) IJPs evoked by single electrical shocks, renzapride (10^{-5}M) caused a leftwards displacement in the stimulus/response relationship and also increased the maximum response to electrical stimulation. These results suggested both an enhanced sensitivity to nerve stimulation and a facilitation of transmitter release. Inhibitory responses by circular muscle to micro-ejections of ATP were not potentiated by renzapride, suggesting the potentiating action of the drug was not due to a postjunctional enhancement of smooth muscle excitability. The potentiating effect of renzapride on fast IJPs was abolished by ICS 205-930 (10^{-6}M) at a concentration known to antagonize 5-HT_4-receptors, without decreasing the amplitude of control fast IJPs. On the other hand, the potentiating effect of renzapride on fast IJPs was reduced but not abolished by spiperone (10^{-6}M), methysergide (10^{-6}M) and granisetron (10^{-7}M), antagonists of 5-HT_1, 5-HT_2 and 5-HT_3 receptor subtypes, respectively; each of these antagonists decreased the amplitude of control fast IJPs and their actions were considered nonspecific. The ability of renzapride to potentiate fast IJPs was either abolished or reduced respectively, when hexamethonium (10^{-4}M) was applied either before or after exposure to renzapride. Thus, potentiation of IJPs by renzapride appeared to involve the indirect activation of nicotinic receptors. Nicotine (10^{-5}M and 10^{-4}M, respectively) was found to enhance temporarily, then reduce to normal, the sensitivity of inhibitory nerves to electrical field stimulation as judged by displacements of the stimulus/response curves for fast IJPs.

It is proposed that renzapride activates 5-HT_4 receptors to facilitate the evoked release of noncholinergic inhibitory and excitatory transmitters. Additionally, renzapride indirectly increases the sensitivity of enteric motor nerves by a mechanism involving the release of acetylcholine and activation of postganglionic nicotinic receptors. In conclusion, the prokinetic activity of renzapride may involve facilitation of all neurotransmission processes between motor nerves and their smooth muscle target.

314P CONTRACTILE RESPONSES TO 5-HT IN ATRIAL BUT NOT IN VENTRICULAR TRABECULAE: A STUDY IN PORCINE AND HUMAN HEARTS

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Positive chronotropic as well as inotropic effects of 5-hydroxytryptamine (5-HT) have been observed in porcine and human atrial tissue (Kaumann, 1990; Villalòn *et al.*, 1990; Kaumann *et al.*, 1991), but no data are available about the direct effects of 5-HT on ventricular tissue. In the present study we investigated inotropic effects of 5-HT on atrial and ventricular trabeculae obtained from hearts of 3 months old pigs, and human donors (Eurotransplant, non-cardiac cause of death). Cardiac tissue was placed in ice-chilled Krebs' buffer. Trabeculae (1 mm thickness) were carefully dissected free and mounted in oxygenated organ baths (37 °C) to measure isometrically developed tension. Tissue was paced at about the resting heart rate *in vivo*; 1.5 Hz for pigs and 1 Hz for humans. Data are presented as mean ± s.e.mean.

Baseline isometric contractile force was significantly higher in ventricular (pig: 422±128 mg, n=13; human: 391±76 mg, n=10) than in atrial tissue (pig: 48±11 mg, n=8; human: 189±55 mg, n=12). A noradrenaline concentration-response curve was used to check the contractile responsiveness of the tissue and responses to 5-HT were expressed as percentage of the response to 10 μM noradrenaline. Noradrenaline (0.01 to 10 μM) caused a concentration-dependent increase in contractile force in both atrial, up to 94±20 mg and 383±79 mg, and ventricular trabeculae, up to 293±71 mg and 719±126 mg at 10 μM noradrenaline, for porcine and human tissue, respectively. In contrast, 5-HT (0.01 to 100 μM) increased force of contraction in atrial tissue (maximum pig: 72±20 % and human: 83±11 % of the response to 10 μM noradrenaline), but not significantly in ventricular trabeculae (12±6 % and 1±1 %, respectively). The absence of a significant ventricular response could not be attributed to a time related deterioration of the tissue, since the noradrenaline concentration-response curve obtained after 5-HT showed similar or even higher contractile effects as the one obtained before 5-HT.

The data show that, in contrast to atrial tissue, contractile force of porcine and human ventricular tissue could not be significantly enhanced by 5-HT. This report demonstrates, to our knowledge for the first time, that in the mammalian heart an agent can elicit a positive inotropic response in the atrium without having a corresponding effect on the ventricle. Besides a contribution to a better insight in the physiology of atrial and ventricular contractility, the present findings have important pharmacological implications, including the fact that drug therapy for heart failure based on 5-HT receptor agonism does not appear feasible.

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315P CARDIOVASCULAR AND METABOLIC EFFECTS OF CGS 21680, A SELECTIVE ADENOSINE A_2 RECEPTOR AGONIST, IN THE RHESUS MONKEY

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A variety of discriminatory ligands with selectivity for adenosine A_1 and A_2 receptors have recently become available (Jacobson et al., 1992). Among these is 2-[p-(2-carboxyethyl)phenylethylamino]-5-N-ethylcarboxamidoadenosine (CGS 21680) which in radioligand binding studies and several functional models behaves as a potent and selective agonist at the A_2 receptor subtype (Hutchison et al., 1989; Balwierczak et al., 1991). In conscious spontaneously hypertensive rats (SHR), CGS 21680 lowers mean arterial blood pressure, a consequence of peripheral vasodilatation, and causes reflex tachycardia (Hutchison et al., 1989). Since important differences in adenosine receptors appear to exist between species (Jacobson et al., 1992), it was considered of interest to evaluate the cardiovascular and metabolic effects of CGS 21680 in the conscious Rhesus monkey.

Ten Rhesus monkeys (7 male) weighing 6.2-11.1 kg were trained to sit in restraining chairs and systolic blood pressure (SBP) and heart rate were measured plethysmographically from the arm. Blood was withdrawn from a cannulated saphenous vein for measurement of plasma renin activity (PRA) and the plasma concentrations of immunoreactive insulin (IRI), glucagon (IRG), glucose, free fatty acids (FFA) and triglycerides by conventional techniques. CGS 21680 or vehicle (n=6) was administered by slow intravenous (i.v.) injection during 30 s.

Doses of 30 (n=4) and 100 (n=10) μg/kg of CGS 21680 produced falls in SBP which were dose-related with respect both to magnitude (-8 ± 2; -18 ± 2 mm Hg, mean ± s.e.mean) and duration (2-3 h; 3-4 h). Hypotension was accompanied by tachycardia and increases in PRA. Plasma glucose concentrations increased markedly during the 30-60 min following drug administration from 86 ± 11 to 142 ± 23 and from 87 ± 5 to 171 ± 14 mg/dl after the 30 and 100 μg/kg doses, respectively. Plasma IRI did not change significantly with time relative to vehicle-treated control values but IRG increased dose-dependently from 154 ± 78 to 767 ± 284 and from 173 ± 44 to 1251 ± 213 pg/ml 30 min following the lower and higher doses of CGS 21680, respectively. Plasma FFA and triglyceride concentrations stayed close to baseline values during the course of the experiment (5 h) in control animals; CGS 21680 had no consistent effects on either parameter.

Thus, low doses of CGS 21680 produce cardiovascular effects in the Rhesus monkey broadly similar to those observed in SHR and consistent with selective adenosine A_2 receptor stimulation. The prominent hyperglycemia can also be attributed to A_2 receptor activation since it is associated with an increase in plasma glucagon, a hormone whose release from the α cells of the pancreas can be triggered by activation of A_2 receptors (Loubatières-Mariani & Chapal, 1988).

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316P REGULATION OF EXTRACELLULAR ADENOSINE PRODUCTION BY ECTONUCLEOTIDES OF RAT CORONARY MICROVASCULAR ENDOTHELIAL CELLS

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Ecto-ATPase, ADPase and 5'-nucleotidase activities capable of hydrolysing ATP sequentially to adenosine are present in many cell types and serve to terminate the actions of extracellular ATP and ADP. The opposing effects of adenosine acting on P_1-purinoceptors and ATP acting on P_2-purinoceptors on the same cell types have suggested a modulatory role for adenosine on the actions of ATP and that the rates of hydrolysis of nucleotides might be regulated. Consistent with this it has been found that the balance between feedforward inhibition of 5'-nucleotidase by ADP and/or ATP and preferential delivery of AMP from ADPase to 5'-nucleotidase determines the rate of adenosine production and this differs in different cell types (Gordon et al., 1986;1989; Meghji et al., 1992).

Coronary microvascular cells were prepared as decribed by Piper et al. (1982). Confluent monolayers of passage 1 cells were preincubated for 30 min with dipyridamole (10 μM) to minimize adenosine uptake and intracellular metabolism. Then they were rinsed three times with Krebs buffer and placed in 0.9 ml of the same buffer on an orbital shaker in a hot box at 37°C. Incubations were started by adding buffer (100 μl) containing unlabelled nucleotide, [^{3}H]-nucleotide (0.22 MBq) and dipyridamole. Subsamples (40 μl) were removed at timed intervals and analysed by thin layer chromatography. After 14 min, the concentration of added ATP (300 μM; n=3) had decreased by 50%. ADP accounted for 53±5%, AMP 8±1% and adenosine 39±6% of this decrease. Similarly after 30 min, 50% of added ADP (300 μM; n=3) was catabolised to form AMP(12±1%), adenosine (85±1%) and inosine (3±1%). However when 5'-nucleotidase was inhibited by the non-hydrolysable ADP analogue, α,ß-methylene ADP or by specific antibodies, AMP accumulated and adenosine formation was virtually abolished. Thus, extracellular nucleotide catabolism to adenosine involves 5'-nucleotidase, although when ATP or ADP is the initial substrate , feed-forward inhibition is not apparent. These observations contrast markedly with findings in macrovascular endothelial cells from porcine aorta (Gordon et al., 1986), where feed-forward inhibition of 5'-nucleotidase by ATP and ADP causes accumulation of AMP and a profound lag in adenosine production from adenine nucleotides. This suggests that ectonucleotidases are present in a complex on microvascular endothelial cells but not on macrovascular endothelial cells. This would result in the local concentrations of substrate supplied from the preceding reactions being much higher than those in the bulk phase on microvascular endothelial cells, thus overcoming the effect of feedforward inhibition. Alternatively, the relative proportion of 5'-nucleotidase activity may be much higher on these cells.

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317P INCREASED UPTAKE OF FIBRINOGEN INTO ARTERIAL WALLS OF UNRESTRAINED CONSCIOUS RATS CAUSED BY ADRENALINE OR ANGIOTENSIN II

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In anaesthetized rabbits the atherogenic uptake of low-density lipoprotein (LDL) by arterial walls is accelerated by adrenaline at its pathophysiological blood concentrations (Cardona-Sanclemente & Born, 1992). Recently this atherogenic effect of adrenaline has been confirmed in another mammalian species, i.e. the rat, and by a different technique in which the animals were conscious and unrestrained (Cardona-Sanclemente, Gorog & Born, 1992). In this communication we extend this results with adrenaline and angiotensin II to the uptake of fibrinogen by arterial walls in the rat.

Adrenaline (40 nM, containing 10 mg/ml ascorbic acid), angiotensin II (8 nM) or saline were infused by osmotic minipump implanted subcutaneously at a delivery rate of 0.5 μl/h for 6 days. Over the whole period, blood pressure and heart rate were determined via a cannula in the carotid artery. Five days after implanting the minipumps, fibrinogen labelled with ^{125}I-tyramine cellobiose (^{125}I-TC-fibrinogen) was injected i.v., allowed to circulate for 24 h and its uptake determined in thoracic aorta. This radioactivity provided a measure of intact and degraded fibrinogen (Pittman et al., 1983).

The results show that in conscious rats the aortic uptake of fibrinogen, like that of LDL, is increased by adrenaline (556 ± 35 vs 667 ± 35 ng fibrinogen/mg dry weight, mean ± s.e.m., n=12, $p<0.025$). Furthermore, this uptake is also increased by angiotensin II (556 ± 35 vs 1162 ± 172, n=11, $p<0.01$). At the concentrations infused, both adrenaline and angiotensin II increased the diastolic blood pressure significantly only after 5 days ($p<0.005$), whereas the blood pressure had decreased progressively in the controls.

These results show that the increased arterial uptake of fibrinogen, like that of LDL caused by adrenaline and angiotensin II, is associated with increased blood pressure .

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318P PROBUCOL INHIBITS MONOCYTE ADHESION TO THE VASCULAR ENDOTHELIUM *IN VITRO* AND *IN VIVO*

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Leucocyte adhesion to vascular endothelium is enhanced within days of starting an atherogenic diet in rabbits. Monocytes subsequently migrate into the vessel wall and are converted into lipid-laden foam-cells within the tunica intima. Macrophage derived foam-cell formation requires low density lipoprotein (LDL) modification into a form that allows its uptake via the scavenger receptor pathway. Susceptibility to LDL oxidation appears to confer an increased risk of coronary heart disease in man (Regnstrom et al. 1992). Agents that inhibit LDL oxidation may also inhibit atherosclerotic lesion development. One such agent is the antioxidant probucol. We have investigated the effects of probucol treatment on mononuclear cell adhesion to endothelial cells *in vivo* and *in vitro*. New Zealand White rabbits were divided into three dietary groups: (i) controls (n=15), (ii) 2% cholesterol (n=11) and (iii) 2% cholesterol with 1% probucol (n=11). Serum cholesterol and probucol were measured before starting the experimental diets and at one and five weeks. Animals were killed at five weeks and perfusion fixed with 4% paraformaldehyde/PBS. The right common carotid artery of each animal was then isolated, opened lengthwise and stained for lipid and cell nuclei with Oil red O and Hoechst 33342 respectively (as described by Rogers and Karnovsky 1988). This method enables identification of adherent monocytes. Blood was taken from the rabbits upon killing and monocytes were isolated by Ficoll-Paque density gradient separation. These monocytes were then labelled with ^{51}-Cr and their adhesion to cultured rabbit endothelial cells *in vitro* was assessed. Serum cholesterol levels were increased significantly within one week in all animals on the 2% cholesterol diet and increased further by 5 weeks. Animals additionally on 1% probucol had lower levels of serum cholesterol at five weeks ($p<0.05$). *In vivo* leucocyte-endothelial adhesion was increased more than six-fold by cholesterol feeding (1.19 ± 0.41 to 7.89 ± 1.24 monocytes/1000 endothelial cells $p<0.001$), and this effect was abrogated by probucol therapy (2.21 ± 0.59 monocytes/1000 endothelial cells $p<0.001$). Monocyte adhesion was inversely related to serum probucol levels ($r=-0.63, p<0.01$). The binding of leucocytes isolated from probucol treated animals to endothelial cells *in vitro* was significantly lower than that of the leucocytes from the animals given cholesterol alone ($12.21\pm1.18\%$ vs $18.78\pm1.23\%, p<0.01; n=5$). These results suggest that part of the antiatherosclerotic action of probucol may be due to the inhibition of monocyte adhesion to the endothelium.

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319P CHARACTERISATION OF FREE RADICAL PRODUCTION BY PORCINE LEUKOCYTES

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Leukocytes are known to produce oxygen derived free radicals following activation by various stimulants but the type of radicals produced by porcine leukocytes has not been well characterized. The aim of this study was to investigate, using free radical scavengers, which types of free radicals are generated by leukocytes from the pig following activation by two nonreceptor mediated stimulants, phorbol myristate acetate (PMA), a protein kinase activator, and ionomycin, a cation ionophore.

Leukocytes were isolated from citrated porcine venous blood, mixed with Histopaque, and allowed to sediment for 45 min at 37°C. The leukocyte rich supernatant was centrifuged at 900 rpm for 7 min and the pellet was resuspended in platelet poor plasma. Cell viability was assessed by trypan blue exclusion and exceeded 98%. A luminometer (Lumi-vette, Chrono-log Corp., Havertown, PA, USA) was used to assay free radical generation as measured by luminol-enhanced chemiluminescence. The reaction mixture contained 4.5 x 10^6 cells/ml, 225 µM luminol plus the drug(s) under investigation at 37°C. The effects of scavengers were examined by addition prior to the stimulant agent. The peak chemiluminescence was measured and data are quoted as mean ± s.e. mean and the effects of scavengers expressed as % change from the response to the stimulant alone.

PMA (0.8 nM-1.6 µM) and ionomycin (0.3 µM-10 µM) caused concentration dependent chemiluminescence. A peak response of 814 ± 112 units (n=17) and 125 ± 61 units (n=4) was produced by 0.8 µM PMA and 3 µM ionomycin respectively. Superoxide dismutase, which eliminates superoxide anion, inhibited PMA (0.8 µM)-induced CL in the concentration range 10-500 U/ml. Inhibitions of 42 ± 3% and 86 ± 1% (n=4, $p<0.05$) were produced by 50 and 500 U/ml superoxide dismutase respectively. Similarly, sodium azide, an inhibitor of myeloperoxidase, caused a marked inhibition of PMA-induced chemiluminescence of 60 ± 5% and 86 ± 2% (n=4, $p<0.05$) with concentrations of 0.01 and 1 mM respectively. Catalase (500 U/ml, n=4) which eliminates hydrogen peroxide, and mannitol (100 mM, n=3), a hydroxyl radical scavenger, had no effect on PMA stimulated chemiluminescence. All of the scavengers studied had a similar profile of activity on ionomycin (3 µM)-induced chemiluminescence.

Porcine leukocytes produce free radicals following protein kinase activation or influx of cations. It is concluded that the free radicals produced by these mechanisms include superoxide anion, but not hydrogen peroxide or hydroxyl radical. The inhibitory effect of sodium azide may suggest a role of myeloperoxidase but a nonspecific effect of this drug cannot be excluded.

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320P IONOPHORE-INDUCED CALCIUM TOXICITY IN RAT PERITONEAL LEUKOCYTES: SUPPRESSION OF SUPEROXIDE GENERATION AND GRANULAR ENZYME SECRETION

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The generation of microbicidal reactive oxygen species such as superoxide during the respiratory burst of phagocytes can be initiated by soluble stimuli whose effects depend upon several intracellular transduction mechanisms. Prominent roles have been assigned to pathways involving protein kinase C and phospholipase D; calcium-dependent and calcium-independent pathways have also been invoked. However, calcium ionophore A23187 is thought to activate the respiratory burst only weakly when compared with chemoattractant FMLP or PKC activators such as phorbol myristate acetate. We have investigated calcium ionophore activation of superoxide generation by rat leukocytes, but find that high concentrations of ionophore induce a calcium-dependent toxic loss of function.

Mixed peritoneal leukocytes (80-90% PMNs) were lavaged from rats given 10 ml 6% glycogen i.p. 16h previously and after differential centrifugation and removal of any erythrocytes by hypotonic lysis were resuspended at 2.5 x 10^6 cells/ml in HBSS containing 1.26 mM Ca^{2+} and 80μM cytochrome c. After incubation at 37°C for 10 min, generation of O_2^- was terminated by pelleting the cells (10 min, 4°C, 400g) and the amount of O_2^- measured at 550nm.

At 10^{-6}M, the stimulants PMA, FMLP (with 10^{-5}M cytochalasin B) and calcium ionophores A23187 and ionomycin caused the generation of 0.52 ± 0.01, 0.10 ± 0.01, 0.11 ± 0.005 and 0.10 ± 0.006 units of O_2^- (where 1 unit is defined as 50 nmoles O_2^-/2.5 x 10^6 cells/10 min incubation at 37°C, results based on at least 15 determinations using rats from 5 or more batches). This shows that calcium ionophores activate the leukocyte respiratory burst to a similar degree as FMLP. Increasing the concentration of stimulant to 10^{-5}M did not significantly alter the yield using PMA or FMLP/cyt B, but there was a large reduction to both ionophores (61.5 ± 1.5%, 63.6 ± 4.5%, $P < 0.001$), suggesting that exposure to very high intracellular calcium (using high ionophore, normal extracellular calcium conditions) damages the cells. This was supported by varying the extracellular calcium: 1.26 mM Ca^{2+} was optimal for O_2^- generation by 10^{-6}M A23187 (0.11 ± 0.01 units), whereas 0 mM was optimal for 10^{-5}M A23187 (0.13 ± 0.02 units). Moreover, calcium chelator EDTA afforded protection against 10^{-5}M A23187 and ionomycin toxicity: in the presence of 0, 0.63 and 1.26 mM EDTA, amount of O_2^- generated was 0.01, 0.03 and 0.09 units for A23187 and 0.02, 0.03 and 0.08 units for ionomycin. Treatment of the leukocytes suspended in 1.26 mM Ca^{2+} with 10^{-6}M and 10^{-5}M A23187 caused leakage of 13.8 ± 0.9% and 32.0 ± 1.5% of the total LDH and secretion of 47.2 ± 1.8% and 13.3 ± 2.6% of lysozyme. As with O_2^- generation, 1.26 mM EDTA protected against the toxic effects of 10^{-5}M A23187 by reducing LDH leakage and enhancing lysozyme secretion.

Thus in rat leukocytes high and sustained levels of free intracellular calcium cause a toxic response which disables the mechanisms for generation of reactive oxygen species and secretion of granular enzymes. This system may be useful for studying the mechanisms and functional effects of calcium overload inside cells.

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321P Mu OPIOIDS INCREASE LIGHT-EVOKED RELEASE OF ACETYLCHOLINE FROM RABBIT RETINA

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In the rabbit retina, acetylcholine (ACh) and γ-aminobutyric acid (GABA) are co-localised to a subpopulation of the amacrine cells in the inner plexiform and ganglion layers (Vaney & Young, 1988). Opioid binding sites in the mammalian retina have a similar distribution (Wamsley et al., 1981). Stimulation of the rabbit retina with light results in an increase in the release of [^{3}H]-ACh but not [^{3}H]-GABA. In the present study, the opioid binding sites in the rabbit retina have been measured and the effects of opioids on light-evoked release of [^{3}H]-ACh have been investigated.

[^{3}H]-Bremazocine (3nM) was used as a universal opioid ligand. The individual sites were labelled with 7.5nM [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (μ), 5nM [^{3}H]-[D-Pen^2, pClPhe^4,D-Pen^5]enkephalin (δ) or 3nM [^{3}H]-CI-977 (k).

The total number of opioid binding sites in homogenates of rabbit retina was 24.9±4.3 fmol mg^{-1} protein (n=7). The numbers of μ-, δ - and k-sites were 24.2±1.61 fmol mg^{-1} protein (n=5), 3.49±0.84 fmol mg^{-1} protein (n=3) and 4.92±0.43 fmol mg^{-1} protein (n=7), respectively.

Stimulation of the dark-adapted rabbit retina with light flashes (3Hz) for 5 min using an eye-cup preparation (Cunningham & Neal, 1983), resulted in a 1.8±0.1-fold increase in [^{3}H]-ACh release (n=9). In the presence of the μ-agonist [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin (10μM), the light-evoked release of [^{3}H]-ACh was significantly greater (n=9;P<.001). This effect was blocked by the opioid antagonist, naloxone (1μM) which itself did not alter [^{3}H]-ACh release (n=3). The effect of [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin was not affected by the glycine antagonist strychnine.

Thus, the endogenous opioid system in the rabbit retina may play a role in regulating the release of ACh in response to physiological stimuli.

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322P AGONIST BINDING AND G-PROTEIN ACTIVATION IN CHO CELLS EXPRESSING M_1, M_2 OR M_3 MUSCARINIC RECEPTORS

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In common with other G-protein linked receptors it is accepted that agonist binding to muscarinic receptor subtypes is guanine-nucleotide sensitive (Hulme et al., 1990). However, although this is clear in the case of the M_2 receptor and its interactions with a pertussis toxin-sensitive G_i-protein, there is little clear evidence for similar interactions with M_1 and M_3 linkage to phosphoinositide signalling through a pertussis-insensitive Gq-like protein. In the present experiments, agonist binding to single subtypes of muscarinic receptors were studied using CHO-K1 cells expressing recombinant M_1, M_2 and M_3 receptors. CHO-M_1, CHO-M_2 and CHO-M_3 cells were characterised with [^{3}H]-N-methyl scopolamine ([^{3}H]NMS) using the selective antagonists pirenzepine, 4-DAMP, AFDX-116 and methoctramine. All agents produced slopes of unity and K_i values characteristic for each subtype as reported elsewhere (Buckley et al., 1989).

Optimal agonist binding conditions were observed with 10mM HEPES/1mM $MgSO_4$/100mM NaCl pH7.4. [^{3}H]NMS binding to membranes under these assay conditions gave binding parameters: K_D=0.128 ± 0.020nM, B_{max}=558 ± 11 fmols/mg protein; K_D=0.462 ± 0.050nM, B_{max}=98 ± 25 fmols/mg protein; K_D=0.286 ± 0.029nM, B_{max}=3045 ± 165 fmols/mg protein in CHO-M_1, CHO-M_2 and CHO-M_3 membranes respectively.

Carbachol competition curves against [^{3}H]NMS in CHO-M_2 membranes were markedly shifted to the right and steepened in the presence of saturating GTP (100μM) or following incubation with pertussis toxin (PTX) (100ng/ml, 20h). In the absence of GTP, carbachol binding curves in CHO-M_3 membranes were steeper than in CHO-M_2 membranes so that the resulting shift to unity in the presence of GTP was less substantial but still significant. PTX increased the slope from control data so that the GTP shift was no longer significant. CHO (VT-1-9) M_3 clones expressing seven-fold fewer receptors than CHO-M_3 membranes (Bmax=430 ± 43 fmols/mg protein) showed less high affinity agonist binding but still shifted significantly to a slope of unity in the presence of GTP. PTX did not significantly affect the data. CHO-M_1 membranes showed no high affinity agonist binding in the absence or presence of GTP.

[^{35}S]GTPγS binding to membranes in an identical buffer containing $3x10^{-6}$M GDP were performed to examine guanine nucleotide exchange by carbachol ($3x10^{-4}$M). 97% of the carbachol-stimulated [^{35}S]GTPγS binding in CHO-M_2 membranes was abolished by PTX. Furthermore, 58% of carbachol stimulation over basal in CHO-M_3 membranes was also PTX-sensitive, as was 35% in CHO (VT-1-9) M_3 membranes.

Together these data suggest that recombinant M_3 muscarinic receptors can associate with pertussis toxin sensitive and insensitive G-proteins, particularly in cells with high levels of receptor expression. Furthermore, the results suggest that high affinity guanine nucleotide-sensitive agonist binding does not reflect agonist activity at phosphoinositide linked muscarinic receptors since CHO-M_1 cells or PTX treated CHO-M_3 cells show little or no high affinity agonist binding but a marked phosphoinositide response (Tobin, Jenkinson & Nahorski, unpublished).

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323P A POSSIBLE LINKAGE OF NON-ADRENOCEPTOR, IMIDAZOLINE BINDING SITES LABELLED BY [^{3}H]-IDAZOXAN TO K^+ CHANNELS AND G-PROTEINS

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The imidazoline derivative idazoxan binds not only to α_2-adrenoceptors but also to non-adrenoceptor, imidazoline binding sites (NAIBS) in various tissues e.g. pig kidney membranes (Vigne *et al.*, 1989). A possible interaction of NAIBS with K^+ channels has been suggested in rabbit kidney cells (Coupry *et al.*, 1990) and human placental cells (Diamant *et al.*, 1992). It has been postulated that non-adrenoceptor imidazoline binding sites labelled by [^{3}H]-clonidine are regulated by GTP (Ernsberger 1992). In this study we have examined whether NAIBS labelled by [^{3}H]-idazoxan in the porcine renal cortex are influenced by agents known to interact with K^+ channels or are affected by GTP.

Porcine renal cortex membranes were incubated with [^{3}H]-idazoxan in 50mM Tris buffer (pH 7.4) for 90 mins at room temperature in a total volume of 0.5ml. Bound radioactivity was separated from free by rapid filtration and non-specific binding was determined in the presence of 10μM cirazoline. Competition curves were constructed using a fixed concentration of [^{3}H]-idazoxan (1-2 nM) and increasing concentrations of the competing ligands cirazoline adrenaline, the monovalent cations NaCl, KCl and CsCl and the K^+ channel blocker 4-aminopyridine (4-AP).

Cirazoline inhibited total [^{3}H]-idazoxan binding with a pKi of 8.34 ± 0.05 and a Hill Slope of -0.96 ± 0.08 (maximum displacement 100%; n=3). Adrenaline (concentration up to 10^{-4}M; n=3) had no effect on [^{3}H]-idazoxan binding suggesting the absence of α_2-adrenoceptors and the existence of NAIBS. 4-AP displaced [^{3}H]-idazoxan from the porcine renal membranes (Table 1). The monovalent cations Cs^+ and K^+ inhibited [^{3}H]-idazoxan binding (Table 1, IC_{50} values are not different at the 5% level of significance; paired t-test). NaCl at 100mM displaced 40.1% ± 5.23% of total specific binding. Saturation curves were then constructed using increasing concentrations of [^{3}H]-idazoxan in the presence and absence of GTP (0.3mM). The B_{max} and the K_d values in the presence of GTP were significantly different ($p < 0.05$; paired t-test) from those without GTP (Table 2).

Table 1.	IC_{50} mM
4-AP	0.108 ± 0.03
CsCl	5.47 ± 1.40
KCl	11.1 ± 3.62
(n=3-5)	

Table 2.	K_d (nM)	B_{max} (fmoles.mg)
[^{3}H]-Idaz.	10.8 ± 1.1	502 ± 60
[^{3}H]-Idaz. + GTP (n=7-11)	14.6 ± 1.7	372 ± 58

Thus, the binding of [^{3}H]-idazoxan at NAIBS in porcine renal cortex membranes is inhibited by Cs^+, K^+ and the potassium channel antagonist 4-AP and by GTP (0.3mM). NAIBS in the porcine renal cortex may therefore represent receptors coupled to K^+ channels possibly by a G-protein linkage.

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324P ALPHA$_1$-ADRENOCEPTORS AND VASOPRESSIN RECEPTORS SHARE A COMMON POOL OF G PROTEINS IN RAT HEPATOCYTES

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High-affinity α_1-adrenergic agonist binding reflects the formation of a ligand-receptor-G protein ternary complex (De Lean *et al.*, 1980) and thus provides a convenient radioligand binding method to study receptor-G protein coupling. If two different types of receptor share a common G protein pool, activation of one of the receptors will allow those receptors to couple to the G proteins and may thereby prevent the other type of receptor from coupling. The possibility that a single G protein pool couples to both α_1-adrenoceptors and vasopressin receptors was investigated in rat liver plasma membranes by constructing [^{3}H]prazosin/adrenaline competition binding curves as previously described (Dasso & Taylor, 1991), and measuring formation of the high-affinity agonist-receptor-G protein ternary complex in the presence and absence of [Arg^8]vasopressin (AVP). AVP evoked a concentration-dependent decrease in the fraction of α_1-adrenoceptors existing in the high-affinity state. In the presence of 1μM AVP, adrenaline bound to a single low-affinity state of the α_1-adrenoceptor. The K_D of this state was indistinguishable from the low affinity state of the control curve. AVP (1μM) and GTP (0.1 mM) together had no further effect on adrenaline binding than had GTP alone. The EC_{50} for the AVP effect on adrenaline binding was 0.49 ± 0.03 nM (n = 3), which is very similar to the K_D of [^{3}H]AVP for its receptor (0.48 ± 0.08 nM, n = 6). This action of AVP was competitively blocked by the V_1-selective AVP antagonist d(CH$_2$)$_5$Tyr(Me)AVP (K_D = 0.27 ± 0.10 nM, n = 3, from Schild analysis, and K_D = 0.26 ± 0.02 nM, n = 3, from competition binding). α_1-adrenergic antagonist binding was unaffected by incubation with AVP. We conclude that in rat hepatocytes α_1-adrenoceptors and V_1-vasopressin receptors share a common pool of G proteins.

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325P FUNCTIONAL IDENTIFICATION OF AN α_{1A}-ADRENOCEPTOR IN RAT EPIDIDYMAL VAS DEFERENS AND AN α_{1B}-ADRENOCEPTOR IN RAT SPLEEN

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Three α_1-adrenoceptor subtypes have been cloned and that the rat vas deferens is rich in the α_{1A}-subtype, the rat spleen in the α_{1B}-subtype while the α_{1C}-subtype has not been found in rat tissues (Lomasney et al, 1991). Binding studies on membranes from COS-7 cells transfected with the cDNA for the 3 subtypes showed that abanoquil had a high affinity for the α_{1A}-subtype, WB4101 for α_{1C} and α_{1A} and chlorethylclonidine (CEC) for α_{1B} and α_{1C} (Marshall et al, 1992). Therefore, the α_1-adrenoceptor subtype(s) mediating contractions to noradrenaline (NA) in the rat vas deferens and in rat spleen were examined using these antagonists.

Non-cumulative contractions to NA (10^{-7}-10^{-4}M) were obtained in the epididymal half of the vas deferens (pD_2 5.6±0.1),(Sprague Dawley rats, 300-400g) and cumulative contractions to NA in splenic strips ($3x10^{-7}$-$3x10^{-4}$M, pD_2 5.2±0.1) in vitro (Krebs solution, 37°C). Prazosin competitively antagonised the NA contractions in both tissues (vas pA_2 8.9, slope 1.1±0.1; spleen pA_2 9.0, slope 1.0±0.1) as did WB4101 (vas pA_2 9.2, slope 1.0±0.1; spleen pA_2 8.2, slope 1.0±0.1). Abanoquil (Greengrass et al, 1991) non-competitively antagonised NA in the vas deferens and at $3x10^{-9}$M produced a 71±2% reduction in the maximum response combined with a 30-fold shift to the right of the concentration-contraction curve. In contrast, in the spleen, abanoquil (same concentration) reduced the maximum by only 8±4% and did not shift the curve to the right. The alkylating agent CEC was incubated with tissues for 30 min then washed out for 30 min before adding NA. In the vas deferens, CEC ($3x10^{-5}$M, effective in the prostate, Marshall et al, 1992) did not alter the contractions to NA whereas in the spleen CEC (10^{-4}M) reduced the maximum by 19±2% and shifted the NA curve 30-fold to the right.

The results with prazosin confirm that NA contractions of the epididymal portion of the rat vas deferens and of the rat spleen are mediated via α_1-adrenoceptors. In the vas deferens, the high potency of WB4101 and abanoquil together with the lack of effectiveness of CEC suggests that the contractions are mediated via the α_{1A}-subtype. On the other hand, in the spleen, the much lower potency of abanoquil and WB4101 combined with sensitivity to CEC is consistent with functional activity via the α_{1B}-subtype. The present evidence suggests that the functional responses to NA in these two tissues are mediated largely through different α_1-adrenoceptor subtypes making the vas deferens and the spleen useful assays for further studies of α_1-adrenoceptor ligands and their mechanisms of action.

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326P THE ROLE OF α_1-ADRENOCEPTOR SUBTYPES IN THE REGULATION OF RENAL HAEMODYNAMICS IN EXPERIMENTAL HYPERTENSION

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The adrenergic nervous system via the α_1-adrenoceptors functions in the regulation of arterial blood pressure and it has been shown that only the α_{1a} subtype plays a major role in the modulation of blood pressure (Piascik et al, 1990). Similarly it has been suggested that the α_{1a} subtype is predominantly involved in the regulation of renal haemodynamics in the normotensive Wistar rats (Munavvar and Johns, 1991) and in the stroke-prone spontaneously hypertensive rats (Munavvar and Johns, 1992). The situation as to which subtypes of the α_1-adrenoceptors are involved in regulating renal haemodynamics in experimental hypertension is unclear. This study was undertaken in anaesthetised (sodium pentobarbital, 60 mgkg^{-1} i.p.) male, 2K1C Goldblatt hypertensive rats, 280-310 g. After tracheostomy, a carotid artery and jugular vein were cannulated for the measurement of blood pressure and infusion of saline. The left kidney was exposed via midline abdominal incision, the renal nerves isolated and dissected for stimulation. An electromagnetic flowmeter probe was fitted to the renal artery for determination of renal blood flow. The iliac artery was cannulated for close arterial administration of all drugs. The renal vasoconstrictor responses to direct renal nerve stimulation (RNS), (1, 2, 4, 6, 8, 10 Hz at 15 V and 0.2 ms), bolus doses of phenylephrine (10, 20, 30 and 40 μg) and methoxamine (20, 40, 60 and 80 μg) were determined before and after the administration of bolus doses of amlodipine (200 and 400 μgkg^{-1}), chloroethylclonidine (CEC) and 5-methylurapidil (5-MeU) at 5 μgkg^{-1} and 10 μgkg^{-1} in three different groups of rats. Throughout the experiment the animals received 25% of the respective bolus doses h^{-1} of amlodipine, CEC and 5-MeU in the form of infusion fixed to deliver 6 ml h^{-1}.

Table 1 — Mean decrease in renal blood flow (ml/min) ± s.e.m.

GROUP	TREATMENT	RNS	PHENYLEPHRINE	METHOXAMINE
A	Control	3.10±0.19	3.51±0.24	1.98±0.14
(n= 11)	200 μgkg^{-1} amlodipine	2.68±0.18*	1.99±0.16*	1.15±0.09*
	400 μgkg^{-1} amlodipine	2.16±0.19*	1.19±0.91*	0.64±0.06*
B	Control	2.88±0.23	2.99±0.10	2.21±0.15
(n=9)	5 μgkg^{-1} 5-MeU	2.32±0.20*	1.61±0.15*	1.07±0.09*
	10 μgkg^{-1} 5-MeU	1.97±0.18*	1.17±0.10*	0.67±0.06*
C	Control	2.49±0.20	3.09±0.24	1.86±0.16
(n=7)	5 μgkg^{-1} CEC	2.16±0.17	3.36±0.23	2.39±0.16*
	10 μgkg^{-1} CEC	2.74±0.22	3.24±0.24	2.49±0.18*

* P<0.05 (ANOVA)

The results obtained in this study indicated that the regulation of renal haemodynamics in the 2K1C Goldblatt hypertensive rats is predominantly modulated by the α_{1a}-adrenoceptors which are sensitive to 5-MeU and dependent on extracellular calcium for activation.

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Human α_{1C}-adrenoceptor: functional characterization in prostate

327P HUMAN α_{1C}-ADRENOCEPTOR: FUNCTIONAL CHARACTERIZATION IN PROSTATE

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Recently three sub-types of the α_1-adrenoceptor, α_{1A}, α_{1B} and α_{1C} have been cloned (see Lomasney *et al*, 1991). However, the functional characterization of the α_1-subtypes in different tissues has not yet been established. The contractile respsonse of the human prostate is mediated by α_1-adrenoceptors (Chapple *et al*, 1989). The present study aimed to define the sub-type of the α_1-adrenoceptor primarily responsible.

Membranes from COS-7 cells/Rat 1 fibroblast cells transfected with rat α_{1A}, hamster α_{1B} and bovine α_{1C} cDNA (Lomasney *et al*, 1991) were incubated with ^{3}H-prazosin (0.2nM) for 30min at 25°C for binding studies. Non-specific binding was determined in the presence of 10^{-5}M phentolamine. Prazosin was found to be non-selective for the three sub-types (K_i±SEM 0.15±0.02, 0.17±0.02 and 0.17±0.01 nM (n=8) for α_{1A}, α_{1B} and α_{1C} respectively). Abanoquil (UK-52,046; Greengrass *et al*, 1991) was selective for the α_{1A} sub-type (K_i±SEM 0.0014±0.0002, 0.13±0.02 and 0.10±0.01 nM (n=10) for α_{1A}, α_{1B} and α_{1C} respectively); WB4101 was selective for α_{1A} and α_{1C} over α_{1B} (1.0±0.08, 16.3±1.4 and 0.35±0.02 (n=16) for α_{1A}, α_{1B} and α_{1C} respectively). Pre-incubation with chlorethylclonidine (CEC) 10^{-5}M for 20min at 37°C (followed by washout) reduced the B_{max} for α_{1B} and α_{1C} by more than 90% unlike the α_{1A} sub-type. Functional *in vitro* studies using strips of human prostate removed at the time of trans-urethral prostatectomy (ages 60-80) were contracted with noradrenaline (NA, 3x10^{-7} - 3x10^{-5}M). These were competitively antagonised by prazosin (pA2 8.5, slope 1.0±0.1) and by WB4101 (pA2 9.0, slope 0.9±0.1). Abanoquil up to 10^{-7}M only shifted the noradrenaline curve less than 3-fold to the right whereas in the rat vas deferens a 100-fold lower concentration markedly antagonised the effect of NA (Burt *et al*, 1992). Pre-incubation with CEC 10^{-5} or 10^{-4}M for 30min at 37°C (washed out for 30 min) reduced the maximum contraction to NA by 35% and 75% respectively.

These results show that the α_1-adrenoceptor mediating contraction of the human prostate has a high affinity for WB4101, a low affinity for abanoquil and is sensitive to inactivation by CEC. This, together with the demonstrated selectivity of these compounds on the different sub-types using cloned cell lines, suggests that the receptor mediating contraction of the human prostate is of the α_{1C} sub-type. This is the first functional demonstration of the α_{1C} subtype in any tissue.

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328P CHARACTERISATION OF CHOLECYSTOKININ RECEPTORS MEDIATING CARDIOVASCULAR EFFECTS IN THE PITHED RAT

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Sulphated cholecystokinin octapeptide (sCCK-8) has been shown to affect heart rate (HR) and mean arterial pressure (MAP) in conscious rats (Janssen et al., 1991). The present study characterises the receptors involved in the cardiovascular effects of sCCK-8 in the pithed rat.

Male AH/A rats (380-450g) were anaesthetised (isoflurane/N_2O mixture), intubated, pithed and respired with room air. The right carotid artery was cannulated for measurement of MAP and HR. The right jugular vein was cannulated for infusion of test agents. Three successive dose response curves (DRC) were obtained to bolus i.v. injection of sCCK-8 (0.1,1 and 10 nmolkg^{-1}), non-sulphated gastrin -17 (G-17) and pentagastrin (PTG, 1,10 and 100nmolkg^{-1}). Antagonists selective at CCK_A receptors (devazepide 1.5-50nmolkg^{-1}) and gastrin/CCK_B receptors (L-365,260, 15μmolkg^{-1} and PD134308, 1μmolkg^{-1}) were added between the 2nd and 3rd DRC. Results were expressed as arithmetic mean ± s.e.mean for changes in MAP and HR and as geometric mean (95% confidence limits) for ED_{50} values.

The MAP and HR under resting conditions were 48.7±1.4 mmHg and 315±11 beats.min^{-1} respectively (n=15). Sulphated CCK-8 caused a dose-dependent increase in MAP and a decrease in HR with ED_{50} values of 1.26 (1.0-1.5)nmolkg^{-1} and 1.20(0.78-1.86)nmolkg^{-1} respectively (n=5). The maximum changes elicited by sCCK-8 (10nmolkg^{-1}) from baseline were 27.6±2.9% (n=15) and 58.1±5.3% (n=15) for HR and MAP respectively. Neither G-17 nor PTG, over the dose range studied, had any effect on HR or MAP (n=4). The sCCK-8 induced changes in MAP and HR were also unaffected by either L-365,260 (15μmolkg^{-1}, n=4) or PD134308 (1μmolkg^{-1}, n=5). Devazepide caused a noncompetitive inhibition of the sCCK-8 induced changes in MAP and HR (Table 1) therefore dose ratios could not be estimated.

Table 1 **Effects of devazepide on changes in MAP and HR induced by sCCK-8 (10nmolkg-1)**

Devazepide dose (nmolkg-1 i.v.)	Control	1.5	5	15	50
% Depression of pressor response	3.5 ± 2.9	11.7 ± 3.3*	33.6 ± 5.4*	39.3 ± 11.7*	43.2 ± 10.1*
% Depression of bradycardia	-2.1 ± 2.4	14.8 ± 3.4*	50.1 ± 6.5*	55.4 ± 8.2*	74.2 ± 5.0*

* represents significant differences from control using Student's t-test for unpaired data, P<0.01

This study has shown that sCCK-8 caused a dose dependent increase of mean arterial pressure and a marked bradycardia in the pithed rat. The ineffectiveness of G-17 and PTG, which have low activity at CCK_A receptors, and the antagonist selectivity were consistent with the view that these responses are elicited, at least in part, through activation of CCK_A receptors.

Janssen, J.J.M., Gardiner, S.M., Compton, A.M. & Bennett, T. (1991) Br. J. Pharmacol. 102, 123-134.

329P DIFFERENTIAL BLOCKADE OF CCK_B/GASTRIN RECEPTOR ANTAGONISTS IN GUINEA-PIG STOMACH AND ILEUM MUSCLE

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Aim: To determine the effects of two CCK-B nonpeptide receptor antagonists (CI-988 and Cam-1028) on muscle contraction evoked by human gastrin I in guinea-pig stomach and ileum.

Methods: Segments of intact ileum (approximately 15 mm long) and strips of stomach body (10-15 mm long, 2-3 mm wide) cut parallel to the circular muscle (mucosa removed) from the same or different guinea-pigs, were suspended in Krebs' solution (O_2/CO_2 95:5; 37°C). Submaximal isotonic contractions were obtained to acetylcholine every 10 min and to gastrin every 30 min. The CCK-B/gastrin antagonists were added separately to different tissues in 10-fold concentration increments (1 pM-100 µM; contact time 30 min) until the contractions to gastrin were greatly reduced or prevented. Vehicle controls (154 mM NaCl) were run in parallel with test strips. Percent inhibition and -log IC_{50} values were calculated from inhibition-response curves.

Results: CI-988 and Cam-1028 (Hughes et al., 1990) had similar antagonist activity, but there was a regional difference. Both drugs inhibited stomach muscle contraction to gastrin at greatly lower concentrations than in the ileum. With stomach circular muscle the -log IC_{50} values for CI-988 and Cam-1028 were: 8.58 ± 0.15 (n=7) and 8.83 ± 0.66 (n=4) respectively, whereas in the ileum longitudinal muscle the compounds were much less potent: CI-988, 4.91 ± 0.27 (n=5); Cam-1028, 5.18 ± 0.24 (n=6). In two of these cases the stomach and ileum from the same animal were compared, with similar results (CI-988 and Cam-1028 stomach -log IC_{50} values 8.48 and 9.38; ileum 5.40 and 5.88 respectively). In the ileum the highest concentration of Cam-1028 and CI-988 (100 µM) inhibited the spontaneous activity by 70-100% and reduced the contractions to acetylcholine by 27-100%, but the lower concentrations usually had no effect. Gastric spontaneous activity and contractions to acetylcholine were not altered by either compound, but the antagonist concentrations were much lower (up to 0.1 µM) than in the ileum.

Conclusion: CI-988 and Cam-1028 potently blocked gastrin-induced contractions of guinea-pig stomach circular muscle, but were about 4000-fold less potent in guinea-pig ileum. The findings indicate that CCK/gastrin receptors differ from each other in these two tissues.

Hughes, J., Boden, P., Costall, B, Domeney, A., Kelly, E., Horwell, D.C., et al. (1990). Proc. Natl. Acad. Sci., 87:6728-6732.

330P EFFECT OF NEUROPEPTIDE Y ON CARDIAC OUTPUT AND ORGAN VASCULAR CONDUCTANCES IN SYROSINGOPINISED RATS

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Neuropeptide Y (NPY) has been shown to increase blood pressure in pithed rats by increasing both cardiac output and vascular resistance in several organs (MacLean & Hiley, 1990). In view of the reported mast cell degranulating activity of NPY (Grundemar & Hakanson, 1991) we have investigated the effects of this peptide on cardiac output and organ vascular conductance in rats pretreated with histamine and 5HT antagonists.

Female, AP strain rats, weighing approximately 350g, were dosed with syrosingopine to deplete catecholamines ($5mgkg^{-1}day^{-1}$ s.c. for 2 days). On the third day they were anaesthetised with sodium pentobarbitone (Sagatal, $60mgkg^{-1}$ i.p.) and ventillated via a tracheal cannula with air supplemented with O_2 ($10mlkg^{-1}$, 50 $cyclesmin^{-1}$). The rats were bilaterally vagotomised, both femoral arteries were cannulated for blood sampling and measurement of blood pressure while the left atrium was cannulated for injection of microspheres. Rats were dosed with tiotidine, mepyramine and methysergide ($5mgkg^{-1}$, $0.5mgkg^{-1}$ and $5mgkg^{-1}$ i.v. respectively). Coloured polystyrene microspheres (E-Z Trac) were injected into the left atrium 1 minute after injection of NPY (1.65nmol) or saline (0.1ml) and a reference blood sample withdrawn ($0.6mlmin^{-1}$). Cardiac output and organ vascular conductance were calculated after extracting and counting the microspheres as described by Hale et al (1988). Results are expressed as mean±s.e.mean, n=4 per group.

Mean arterial pressure was 160±7mmHg and total peripheral resistance (TPR) $1.28\pm0.19mmHgml^{-1}min100g^{-1}$ bodyweight after injection of NPY compared with 89±4mmHg ($p<0.001$) and $0.62\pm0.04mmHgml^{-1}min100g^{-1}$ ($p<0.02$) after saline injection ($p<0.001$). There was no significant difference in heart rate or cardiac index between the two groups (NPY: $291\pm5beatsmin^{-1}$, $10.3\pm1.2mlmin^{-1}100g^{-1}$bodyweight, saline: $308\pm6beatsmin^{-1}$, $11.5\pm1.1mlmin^{-1}100g^{-1}$). After NPY injection, vascular conductance was significantly lower compared with saline injection in mesentery (0.35 ± 0.02 versus $3.54\pm0.57ulmin^{-1}g^{-1}mmHg^{-1}$, $p<0.01$) kidney (8.29 ± 0.93 versus $30.8\pm3.6ulmin^{-1}g^{-1}mmHg^{-1}$, $p<0.001$) and skin (0.19 ± 0.05 versus $0.41\pm0.06ulmin^{-1}g^{-1}mmHg^{-1}$, $p<0.05$) but not in heart, skeletal muscle, brain, stomach, large intestine, small intestine, liver, spleen and lungs.

These results indicate that in syrosingopinised rats treated with antagonists of histamine and 5HT, NPY increases blood pressure by increasing TPR with no effect on cardiac output in contrast with the findings of MacLean & Hiley (1990) in pithed rats. The increased TPR appears to be due mainly to vasoconstriction in mesentery, kidney and skin.

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331P RAPID DESENSITIZATION OF ADRENALINE- AND NEUROPEPTIDE Y-STIMULATED Ca^{2+} MOBILIZATION IN HEL-CELLS

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In the human erythroleukemia cell line HEL neuropeptide Y (NPY) and adrenaline transiently increase intracellular Ca^{2+} by mobilization from intracellular stores via α_{2A}-adrenoceptors and Y_1-like NPY receptors, respectively (Michel et al., 1989; Feth et al. 1992). Since Ca^{2+} levels return towards baseline (≈ 50-70 nM) within few minutes in the continued presence of either agonist, we have now investigated the mechanism underlying this rapid desensitization in more detail using the Ca^{2+} indicator dye Fura-2 at room temperature as previously described (Feth et al. 1992). Formation of 1,4,5-inositol-trisphosphate (IP_3) was determined by a protein binding assay (Amersham, Braunschweig, Germany).

NPY (100 nM) and adrenaline (1 μM) elevated HEL-cell Ca^{2+} by 192 ± 8 and 73 ± 10 nM, respectively (n = 11). A second dose of hormone given 3 min after the first was only 26 ± 7% (adrenaline) or 3 ± 1% (NPY) as effective as the first dose indicating rapid desensitization of the response. If NPY was given first the response to subsequent adrenaline was reduced to 18 ± 4%, but if adrenaline was given first the subsequent response to NPY was not affected (98 ± 6%). Thus, homologous and heterologous desensitization of the Ca^{2+} responses appears to occur. A 30 s pre-treatment with the phorbol ester phorbol-12-myristate-13-acetate concentration-dependently reduced the Ca^{2+} response to both hormones with 100 nM of the phorbol ester inhibiting the adrenaline and NPY response by 75 ± 3% and 60 ± 5%, respectively, indicating that activation of a protein kinase C can desensitize α-adrenoceptor- and NPY-receptor-mediated Ca^{2+} mobilization. The protein kinase C inhibitors staurosporine (1 μM) and calphostin (1 μM) enhanced peak Ca^{2+} increases by adrenaline by only 42 ± 15% ($p < 0.05$) and 10 ± 9% (n.s.) and those by NPY by 22 ± 8% ($p < 0.05$) and 24 ± 7% ($p < 0.05$). The mixed protein kinase A and C inhibitor H8 was without inhibitory effect towards either agonist. A 10 s incubation with adrenaline or NPY enhanced IP_3 formation by only 12 ± 13% (n.s.) and 38 ± 11% ($p < 0.05$).

We conclude that activation of a protein kinase C by phorbol ester can acutely desensitize adrenaline- or NPY-stimulated Ca^{2+} mobilization in HEL-cells but this may play only a minor role in the rapid desensitization of this response following agonist exposure.

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332P THE EFFECT OF cAMP ON HISTAMINE H_1-RECEPTOR-MEDIATED INCREASES IN $[Ca^{2+}]_i$ IN THE HAMSTER VAS DEFERENS CELL LINE DDT_1MF-2

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Histamine (HA) H_1-receptor activation in DDT_1MF-2 cells stimulates the release of Ca^{2+} from intracellular stores and Ca^{2+} influx (Dickenson & Hill, 1992). Since cAMP can alter noradrenaline-induced Ca^{2+} responses in this cell line (Schachter *et al.*, 1992), we have investigated the effect of elevating intracellular cAMP levels ($[cAMP]_i$) on H_1-receptor-mediated increases in $[Ca^{2+}]_i$.

Intracellular Ca^{2+} determinations, ^{3}H-inositol phosphate and ^{3}H-cAMP accumulation in DDT_1MF-2 cells were as described previously (White *et al.*, 1992; Ruck *et al.*, 1990). Forskolin (FK; EC_{50} = 830 ± 131 nM, n=6) and the type IV phosphodiesterase inhibitor rolipram (100 μM) elicited significant increases in the accumulation of ^{3}H-cyclic AMP of 4.0 ± 0.3 fold over basal (n=6; $p<0.05$) and 4.2 ± 0.4 fold (n=11;$p<0.05$) respectively. Pretreatment with FK (10 μM, 15 min), rolipram (100 μM, 45 min) or the membrane-permeant cAMP analogue (Sp)-adenosine 3',5'-monophosphothioate (de Wit *et al.*, 1982; 100 μM, 45 min) attenuated the rise in $[Ca^{2+}]_i$ elicited by 100 μM HA (observed in Ca^{2+}-free buffer containing 0.1 mM EGTA) by 53 ± 3% (n=33; $p<0.05$), 29 ± 7% (n=12; $p<0.05$) and 30 ± 10% (n=4; $p<0.05$) respectively. FK pretreatment (10 μM, 15 min) also attenuated the accumulation of ^{3}H-inositol phosphates elicited by 100 μM HA by 38 ± 6% (n=4). In contrast, FK pretreatment (10 μM, 15 min) failed to inhibit the Ca^{2+} response to 100 μM ATP in Ca^{2+}-free buffer containing 0.1 mM EGTA (96 ± 6% of the maximum response; n=12). Addition of 10 μM FK (in the presence of rolipram; 100 μM; 10 min exposure) 100 sec after the cells had been stimulated with 100 μM HA (in experiments initiated in Ca^{2+}-free buffer containing 0.1 mM EGTA) inhibited the rise in $[Ca^{2+}]_i$ observed when exogenous Ca^{2+} (2 mM) was re-applied by 36 ± 2% (n=16).

To evaluate the effect of FK on intracellular Ca^{2+} store refilling, experiments were performed using ATP as a second Ca^{2+} mobilising agent. After stimulation with HA (100 μM) in Ca^{2+}-free medium, the response to a subsequent addition of ATP (100 μM) was markedly reduced (n=3). However, if 10 μM mepyramine was added after the HA response (to block receptor-activated Ca^{2+} influx) and followed 10 min later by a 5 min exposure to 2 mM Ca^{2+}, a maximal ATP response (measured under calcium-free conditions) was re-established (n=6). FK (10 μM) added at the same time as the mepyramine i.e. 10 min before the re-addition of exogenous Ca^{2+} did not affect the magnitude of the ATP response (n=6).

The results of this study suggest elevating $[cAMP]_i$ attenuates H_1-receptor-activated inositol phospholipid hydrolysis, intracellular Ca^{2+} release and Ca^{2+} influx, but is without effect on intracellular Ca^{2+} store refilling.

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333P MICROINJECTION OF THE NITRIC OXIDE DONOR NITROPRUSSIDE INTO THE HIPPOCAMPUS RESULTS IN A DOSE-DEPENDENT NEURONAL DEGENERATION

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Recent research suggests that excitotoxicity following the activation of NMDA receptors may be nitric oxide (NO)-mediated. NO has been shown to mediate *l*-glutamate neurotoxicity in cortical cultures (Dawson *et al.*, 1991) and NMDA neurotoxicity in hippocampal slices (Izumi *et al.*, 1992). Further, the NO synthase inhibitor *l*-N^G-nitroarginine has been reported to be effective in reducing neuronal damage following focal cerebral ischaemia (Nowicki *et al.*, 1991). This study examined the possibility that NO leads directly to neuronal damage following an intrahippocampal injection of sodium nitroprusside (SNP), a compound that readily dissociates to generate NO (East *et al.*, 1991).

Male Sprague-Dawley rats (250-300g) were deeply anaesthetised with chloral hydrate (400 mg/kg i.p.) and microinjections (1 μl over 5 min, all in 0.9% saline) of either SNP (1,3.3,10 & 33 nmol), potassium ferricyanide (33 nmol), quinolinic acid (100 nmol) or 0.9% saline were made into the rostral hippocampus (coordinates from bregma and dural surface; AP -3.3, L 2.5, V 3.5). Four days after microinjection rats were killed, brains removed and 14 μm frozen coronal sections cut and mounted onto slides. Slide-mounted sections were fixed in 4% paraformaldehyde, rinsed, dehydrated, and stained in 0.01% thionin for histological examination. A dose-dependent neurodegeneration was observed following intrahippocampal microinjections of SNP. SNP (33 nmol) resulted in a virtual absence of pyramidal and dentate granule cells within the ipsilateral hippocampus, with the lesion extending into the ipsilateral thalamus and cerebral cortex. There was little or no damage in the contralateral hippocampus. A lower dose of SNP (1 nmol) produced less neuronal damage, which was limited to the hippocampal formation; dentate granule cells appeared to be more sensitive to the neurotoxic effects of SNP when compared to pyramidal cells. Intermediate doses of SNP produced hippocampal lesions consistent with a dose-dependent mechanism. By comparison, microinjection of quinolinic acid resulted in damage of pyramidal cells within the CA2 and CA3 regions of the ipsilateral hippocampus, similar to the pattern of degeneration produced by the lowest dose of SNP. It is possible that the effects of SNP may be unrelated to the production of NO. East *et al.* (1991) suggested that SNP may have a non-specific action that is mimicked by potassium ferricyanide and attributable to the $Fe(CN_5)^{2-}$ moiety of SNP, produced as SNP generates NO. Microinjection of potassium ferricyanide failed to produce neurotoxicity (as did saline vehicle) excluding such a non-specific action.

This study demonstrates that the microinjection of SNP and subsequent generation of NO within the rat hippocampus results in marked neurodegeneration. Thus, NO, whether endogenously generated via NO synthase following NMDA receptor activation, or directly produced from SNP, appears to be an important signal in the sequence of events leading to excitotoxic cell death.

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332P THE EFFECT OF cAMP ON HISTAMINE H_1-RECEPTOR-MEDIATED INCREASES IN $[Ca^{2+}]_i$ IN THE HAMSTER VAS DEFERENS CELL LINE DDT_1MF-2

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333P MICROINJECTION OF THE NITRIC OXIDE DONOR NITROPRUSSIDE INTO THE HIPPOCAMPUS RESULTS IN A DOSE-DEPENDENT NEURONAL DEGENERATION

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Recent research suggests that excitotoxicity following the activation of NMDA receptors may be nitric oxide (NO)-mediated. NO has been shown to mediate *l*-glutamate neurotoxicity in cortical cultures (Dawson *et al.*, 1991) and NMDA neurotoxicity in hippocampal slices (Izumi *et al.*, 1992). Further, the NO synthase inhibitor *l*-N^G-nitroarginine has been reported to be effective in reducing neuronal damage following focal cerebral ischaemia (Nowicki *et al.*, 1991). This study examined the possibility that NO leads directly to neuronal damage following an intrahippocampal injection of sodium nitroprusside (SNP), a compound that readily dissociates to generate NO (East *et al.*, 1991).

Male Sprague-Dawley rats (250-300g) were deeply anaesthetised with chloral hydrate (400 mg/kg i.p.) and microinjections (1 μl over 5 min, all in 0.9% saline) of either SNP (1,3.3,10 & 33 nmol), potassium ferricyanide (33 nmol), quinolinic acid (100 nmol) or 0.9% saline were made into the rostral hippocampus (coordinates from bregma and dural surface; AP -3.3, L 2.5, V 3.5). Four days after microinjection rats were killed, brains removed and 14 μm frozen coronal sections cut and mounted onto slides. Slide-mounted sections were fixed in 4% paraformaldehyde, rinsed, dehydrated, and stained in 0.01% thionin for histological examination. A dose-dependent neurodegeneration was observed following intrahippocampal microinjections of SNP. SNP (33 nmol) resulted in a virtual absence of pyramidal and dentate granule cells within the ipsilateral hippocampus, with the lesion extending into the ipsilateral thalamus and cerebral cortex. There was little or no damage in the contralateral hippocampus. A lower dose of SNP (1 nmol) produced less neuronal damage, which was limited to the hippocampal formation; dentate granule cells appeared to be more sensitive to the neurotoxic effects of SNP when compared to pyramidal cells. Intermediate doses of SNP produced hippocampal lesions consistent with a dose-dependent mechanism. By comparison, microinjection of quinolinic acid resulted in damage of pyramidal cells within the CA2 and CA3 regions of the ipsilateral hippocampus, similar to the pattern of degeneration produced by the lowest dose of SNP. It is possible that the effects of SNP may be unrelated to the production of NO. East *et al.* (1991) suggested that SNP may have a non-specific action that is mimicked by potassium ferricyanide and attributable to the $Fe(CN_5)^{2-}$ moiety of SNP, produced as SNP generates NO. Microinjection of potassium ferricyanide failed to produce neurotoxicity (as did saline vehicle) excluding such a non-specific action.

This study demonstrates that the microinjection of SNP and subsequent generation of NO within the rat hippocampus results in marked neurodegeneration. Thus, NO, whether endogenously generated via NO synthase following NMDA receptor activation, or directly produced from SNP, appears to be an important signal in the sequence of events leading to excitotoxic cell death.

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334P CYCLIC GMP RESPONSES TO NMDA IN ADULT GUINEA-PIG CEREBELLAR SLICES

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Activation of N-methyl D-aspartate (NMDA) receptors in the rat cerebellum activates soluble guanylate cyclase leading to the formation of the second messenger cyclic GMP. This has now been shown to be mediated by the Ca^{2+}-dependent synthesis of nitric oxide (NO) from L-arginine (Moncada *et al.*, 1991). The stimulation of cyclic GMP formation in the rat brain is age related (Southam *et al.*, 1991) with large accumulations only being evident in neonatal animals. The extrapolation of data gathered in immature animals to the adult condition is hard to justify so we have examined NMDA-stimulated cyclic GMP formation in the adult guinea pig and we report in this communication that the guinea pig cerebellum is an excellent preparation for the evaluation of excitatory amino acid receptor coupling to guanylate cyclase.

Cyclic GMP formation was evaluated by measuring the conversion of [^{3}H]guanine to [^{3}H]cyclic GMP in cerebellar slices (350 x 350 μm) from Dunkin Hartley guinea pigs (300-400 g, either sex). Briefly, slices were preincubated in Krebs Hensleit buffer for 60 min followed by 45 min in the presence of [^{3}H]guanine (9 Ci/mmol, 40 μCi/20 ml). 25 μl aliquots of slices were incubated with agonists for periods up to 10 mins before stopping the reaction with 1 M HCl. [^{3}H]cyclic GMP was then separated from other [^{3}H]-labelled products by sequential Dowex 50/alumina chromatography.

NMDA provoked a concentration-dependent (EC_{50} = 127 ± 3 μM, n = 3) increase in [^{3}H]cyclic GMP accumulation which reached a maximum by 2 min and which was maintained for at least another 8 min. The maximum increase, 2 min in the presence of 1 mM NMDA, varied between 300% and 600% of basal. In the same experiments the maximum responses to NMDA in adult rat and mouse cerebellar slices were only 8% and 5% respectively of the guinea pig responses.

The response to 1 mM NMDA was antagonised by the inhibitor of NO synthesis N^G-nitro arginine (IC_{50} = 0.5 μM) and by oxyhaemoglobin (IC_{50} = 3.7 μM). Methylene blue (10 μM) also abolished the response to NMDA. Pre-incubation of the slices with superoxide dismutase (500 U/ml) enhanced both unstimulated cyclic GMP formation (150% of control, $p<0.01$ students 't' test, n = 3) and, to a lesser extent, that in the presence of 1 mM NMDA (119% of control, $p<0.05$, n = 3). The cyclic GMP response to NMDA was Ca^{2+}-dependent; in nominally Ca^{2+}-free buffer the response to 1 mM NMDA was reduced to 8% of that observed in the presence of 1.2 mM Ca^{2+}. In contrast, cyclic GMP formation in the presence of sodium nitroprusside was unaffected by the omission of Ca^{2+}.

These data are consistent with NMDA stimulating cyclic GMP formation via the Ca^{2+} dependent synthesis of nitric oxide. They demonstrate the advantage of guinea pig cerebellar slices over those from other small laboratory animals for the study of this important signal transduction pathway.

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335P CORTICALLY-EVOKED MONOSYNAPTIC RESPONSES IN THE CAT RED NUCLEUS ARE ANTAGONISED BY AP5 BUT NOT BY AP7

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The magnocellular neurones of the red nucleus (mRN) receive two distinct monosynaptic excitatory afferents, from the interpositus nucleus (IPN) and the sensorimotor cortex (SMC). Evidence suggests that the neurotransmitters in both these pathways are excitatory amino acids (Nieoullon *et al.*,1984; Davies *et al.*, 1986). The interposito-rubral excitatory response is mediated by non-NMDA receptors whereas, the corticorubral input involves both NMDA and non-NMDA receptors (Harris *et al.* ,1991). During the course of our studies to characterise excitatory neurotransmission in the red nucleus we have tested four antagonists considered selective for the NMDA receptor.

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 stimulation of the contralateral IPN and the ipsilateral SMC (<200μA).

Results were obtained from 27 mRN neurones monosynaptically activated from both the IPN and SMC. The NMDA selective antagonists, 2-amino-5-phosphonopentanoate (AP-5) 2-amino-7-phosphonoheptanoate (AP7) 4-(phosphonomethyl) piperazine-2-carboxylic acid (PMPC) and 4-(3-phosphonopropyl) piperazine-2-carboxylic acid (CPP) were tested on synaptic responses and responses to NMDA and α-amino-3-hydroxy-5-methyl isoxazole-4-proprionate (AMPA). All four antagonists depressed or abolished the responses to NMDA whilst having no significant effect on the AMPA responses.. The effect of the antagonists were examined on the monosynaptic responses to cortical stimulation. The results (mean ± SD) are summarised in Table 1.

Table 1. The effect of NMDA antagonists on NMDA , AMPA and SMC evoked synaptic responses

Drug	N	Mean current (nA)	% inhibition of NMDA	% inhibition of AMPA	Mean current (nA)	Number inhibited	% inhibition of SMC
D-AP5	11	13±11	94±10	5±15	45±30	18/20	60±30
RS-PMPC	4	9±3	85±15	0±0	59±59	6/6	65±30
D-AP7	5	9±3	89±10	0±0	62±29	0/9	0±0
R-CPP	7	15±32	92±11	2±5	59±41	3/11	7±23

Contrary to expectation only D-AP5 and RS-PMPC antagonised these excitatory responses whereas D-AP7 and R-CPP had little or no effect. These results provide evidence that two NMDA receptor subtypes exist within the red nucleus.

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336P ACTIVATION OF GLUTAMATE METABOTROPIC RECEPTORS (mGluR) BY SULPHUR-CONTAINING EXCITATORY AMINO ACIDS

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Sulphur-containing amino acids (SAAs), in particular L-cysteine sulphinate (CSA) fulfill a number of CNS neurotransmitter criteria (Cuénod *et al.*, 1990; Griffiths, 1990) and they possess a broad spectrum of activities at ionotropic excitatory amino acid (EAA) receptors (Mewett *et al.*, 1983).

The metabotropic glutamate receptor ($mGluR_1$) which mediates phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis in the CNS is activated by the selective agonists (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3R)-ACPD), (2S,3S,4S)-α-(carboxycyclopropyl)glycine (L-CCG-I) and by quisqualate, ibotenate and glutamate; the latter is widely assumed to be the natural ligand for the mGluR. We have compared several SAAs, glutamate and aspartate with (1S,3R)-ACPD (100μM) in their ability to activate the mGluR. Rat pup cerebral cortical slices (300 x 300μm) were incubated with 75μCi D-*myo*-[2-^{3}H]-inositol for 2 hours, washed and exposed to EAA for 20 min in the presence of 10mM LiCl. The inositol monophosphate formed was separated by ion exchange chromatography and quantified by liquid scintillation counting.

EAA	EC_{50} (μM)	Efficacy (% ACPD Response)
L-glutamate	791 ± 20	82
L-aspartate	584 ± 44	77
L-cysteate	487 ± 2	68
DL-homocysteate	1133 ± 243	30
L-cysteinesulphinate	471 ± 23	70
L-homocysteinesulphinate	401 ± 43	53

Apart from DL-homocysteate, which was virtually inactive, each of the amino acids stimulated the hydrolysis of PIP_2. Although the potencies were relatively low, it is noteable that the other SAAs exhibited greater potency (though lower efficacy) than either glutamate or aspartate. It is thus feasible that the endogenous SAAs may have a significant physiological interaction with mGluRs.

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337P ANALGESIC EFFECTS OF LAMOTRIGINE IN AN EXPERIMENTAL MODEL OF NEUROPATHIC PAIN IN RATS

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The antiepileptic drug, lamotrigine (Lamictal®) has been shown in rats to produce analgesic effects, which suggests that it may be useful in some chronic pain conditions (Nakamura-Craig & Follenfant, 1992). An experimental model of neuropathic pain has been described using diabetic rats (Wuarin-Bierman et al, 1987). We have therefore, now investigated the effects of lamotrigine in this model.

Male Wistar-strain rats (200-250g) were treated with streptozotocin (70 $mgkg^{-1}$ i.p.). Three to four weeks after treatment, blood glucose concentrations were measured by tail clip sampling. Streptozotocin-treated rats with blood glucose levels of >12.0 $mmol\ l^{-1}$ were considered diabetic. Paw withdrawal response (reaction times,s) to paw pressure (40mmHg, constant) was measured using a modification of the Randall-Selitto test (Randall and Selitto, 1975) in both diabetic rats and saline-treated (non-diabetic) controls before, and at various times for up to 24h after drug treatment. Reaction time to paw pressure in diabetic rats prior to treatment was significantly lower than that of non-diabetic controls (mean±s.e. mean 13.6±0.2s, n=102 compared to 26.4±0.3s, n=45; $p<0.001$). Indomethacin (1-10$mgkg^{-1}$ p.o.) had no effect on reaction time whereas morphine, (0.2, 0.5 or 1.0 $mgkg^{-1}$ s.c.) significantly ($p<0.001$) increased reaction times in diabetic rats at 1h after dosing to 17.6±0.7, 22.8±1.2 or 28.2±1.0s (n=8), respectively. Significant analgesia was still present at 5h after morphine, (1.0$mgkg^{-1}$, reaction time, 18.7±1.0s, n=8; $p<0.05$). This dose of morphine also increased reaction times in non-diabetic rats at 1h after dosing (28.8±1.3s, n=4; $p<0.05$). The competitive NMDA-receptor antagonist MK-801, (0.1 $mgkg^{-1}$ s.c.) induced a slight but significant increase in reaction times at 1h after dosing only (16.1±0.8s, n=8; $p<0.05$). Lamotrigine (20 or 40 $mgkg^{-1}$ p.o.) induced significant analgesia at 1h after dosing (reaction times, 18.7±1.4 and 23.9±0.9 s, n=8, respectively; $p<0.01$) which lasted for at least 5h (reaction times; 17.3±1.1 and 23.0±0.8s, n=7, respectively; $p<0.01$) in diabetic rats but had no effect in non-diabetic controls. Paw pressure reaction times had returned to pre-lamotrigine values by 24h after dosing. Blood glucose concentrations were unaffected by any analgesic treatment.

These results support the suggestion that the glutamate release inhibitor, lamotrigine (Leach et al, 1986) may be a useful analgesic in the treatment of diabetic neuropathic pain.

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338P [^{3}H]DIZOCILPINE (MK-801) BINDING PARAMETERS IN RAT HIPPOCAMPUS ARE STRIKINGLY ALTERED BY CHANGES IN BUFFER CONCENTRATION

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N-Methyl-D-aspartate (NMDA) receptors have been widely studied using [3H]MK-801. The methodology is well established, with extensive washing and freezing of membranes routinely performed to remove endogenous amino acids which have activity at this receptor (Wong et al., 1986; Bowery et al., 1988; Williams et al., 1990). We have now critically evaluated this methodology by comparing the effects of freezing, washing and buffer concentration on Kd and Bmax values obtained in rat hippocampal membranes, and the results obtained have important implications for the technique of [3H]MK-801 binding.

Membrane homogenates from rat hippocampus (male Lister hooded, 200-250 g) were incubated with [3H]MK-801 (0.5-48 nM) for 1 h at 25°C in Tris buffer (pH 7.5) containing 50 µM glutamate and 30 µM glycine; non-specific binding was defined with 100 µM MK-801. Membrane preparations were assayed after:- (i) 4 WASHES, (ii) 4 WASHES/FREEZE (-20°C, ≥24 h) or (iii) 4 WASHES/ FREEZE/4 FURTHER WASHES. All preparations were examined using both 5 mM and 50 mM Tris buffer.

Table 1 Effects of freezing, washing and Tris buffer concentration on [3H]MK-801 binding parameters

	4 WASHES		4 WASHES/FREEZE		4 WASHES/FREEZE/4 WASHES	
Parameter	5 mM Tris	50 mM Tris	5 mM Tris	50 mM Tris	5 mM Tris	50 mM Tris
Kd	3.13 ± 0.30	10.07 ± 1.03***	3.42 ± 0.22	12.7 ± 0.94***†	4.34 ± 0.47†	12.4 ± 0.77***†
Bmax	995 ± 114	758 ± 129**	1062 ± 135	752 ± 115**	1045 ± 107	697 ± 50**

Values are mean ± SEM Kd (nM) and Bmax (fmol/mg protein) (n=4-6). *** $p < 0.001$, ** $p < 0.01$ versus 5 mM Tris for same tissue preparation; † $p < 0.05$ versus 4 WASHES (2-way ANOVA followed by Neuman-Keuls multiple comparisons test).

The data in Table 1 showing a 3-fold change in Kd and a 24-33% difference in Bmax clearly demonstrate that buffer concentration has a marked effect on [3H]MK-801 binding, and that the magnitude of this effect is not altered by freezing and extensive washing. Furthermore, the widely used time-consuming protocol of freezing and extensive washing is deleterious compared with the rapid 4 WASHES membrane preparation, as indicated by the increased Kd values. These results indicate that the choice of buffer concentration in [3H]MK-801 binding studies is likely to be a more important determinant of experimental results than the extensive membrane preparation.

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339P ENDOGENOUS ADENOSINE REGULATES THE APPARENT EFFICACY OF 1S,3R-ACPD INHIBITION OF FORSKOLIN-STIMULATED CYCLIC AMP ACCUMULATION IN RAT CEREBRAL CORTICAL SLICES

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We have previously reported that activation of a subclass of "metabotropic" excitatory amino acid receptor inhibits forskolin-stimulated cyclic AMP accumulation in guinea-pig cerebral cortical slices (Cartmell *et al.*, 1992). The 1S,3R- and 1R,3S-stereoisomers of 1-aminocyclopentane-1,3-dicarboxylate (ACPD) appear to be of equal efficacy (approximately 90% inhibition) in producing this response with 1S,3R-ACPD being more potent than 1R,3S-ACPD. In contrast, in rat hippocampus, inhibition of forskolin-stimulated cyclic AMP accumulation was only partially reduced by 1S,3R-ACPD (approximately 50 % inhibition) compared to 1R,3S-ACPD (Schoepp *et al.*, 1992). We have investigated whether these differences in the apparent efficacy are due to species or methodological differences.

Accumulation of [^{3}H]cyclic AMP in [^{3}H]adenine-pre-labelled cross-chopped (350 x 350 µm) cerebral cortical slices from Hooded Lister rats was carried out as previously described (Cartmell *et al.*, 1992).

In the rat cerebral cortex, 30 µM forskolin stimulated [^{3}H]cAMP accumulation (20166 ± 2051 dpm over basal, n=5). 1S,3R-ACPD only partially inhibited forskolin-elicited cyclic AMP accumulation (IC_{50} value 8.8 ± 3.4 µM; maximal inhibition 51 ± 3 %, n=5). In the presence of 1.2 U/mL adenosine deaminase (ADA) in order to reduce the contribution of endogenous adenosine (DeLapp & Eckols, 1992), the response to forskolin was not significantly reduced (14209 ± 3419 dpm over basal, n=4). However, the presence of ADA increased the efficacy of 1S,3R-ACPD (maximal inhibition 88 ± 3 %, $P<0.01$, t-test) without significantly altering the potency (IC_{50} value 2.8 ± 1.2 µM, n = 4).

We conclude that endogenous adenosine plays an important role in regulating the apparent efficacy of 1S,3R-ACPD-elicited inhibition of forskolin-stimulated cyclic AMP accumulation in rat cerebral cortical slices. The likely mechanism of the effect of endogenous adenosine is probably the ACPD enhancement of A_{2b} adenosine receptor-mediated cyclic AMP formation (Alexander & Curtis, 1992). Thus, although in our experiments there appears to be insufficient endogenous extracellular adenosine to enhance the forskolin response (DeLapp & Eckols, 1992), the combination of forskolin and 1S,3R-ACPD with endogenous adenosine may prove sufficient to be significant.

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340P REGIONAL HAEMODYNAMIC RESPONSES TO DIZOCILPINE MALEATE (MK-801) IN CONSCIOUS LONG-EVANS AND BRATTLEBORO RATS

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In conscious, Sprague-Dawley rats, dizocilpine causes a pressor effect accompanied by an increase in renal sympathetic nerve activity (Lewis *et al.*, 1989). Since there is evidence that excitatory amino acids might modulate vasopressin release (Jin & Rockhold, 1989), we have investigated the effects of dizocilipine in conscious Long Evans and Brattleboro (i.e. vasopressin-deficient) rats, chronically instrumented with pulsed Doppler flow probes and intravascular catheters (all surgery was carried out under sodium methohexitone anaesthesia, 40-60 mg kg^{-1} i.p., supplemented as required). There were no significant differences between resting cardiovascular variables in Long Evans and Brattleboro rats. I.v. injection of dizocilipine (0.1 and 1 mg kg^{-1}) caused a dose-dependent pressor effect and renal, mesenteric and hindquarters vasoconstriction in Long Evans rats (Table 1). The lower dose of dizocilipine had no significant haemodynamic effects in Brattleboro rats, but the pattern of response to the higher dose was as seen in Long Evans rats (Table 1), although the pressor effect was greater in Brattleboro rats.

Table 1 Integrated (areas under or over curves (AUC or AOC, 0-10 min), arbitrary units) cardiovascular responses to i.v. bolus injection of dizocilipine (0.1 or 1 mg kg^{-1}). Values are mean ± s. e. mean; n = 7 in both groups.

	Long Evans rats		Brattleboro rats	
	0.1 mg kg^{-1}	1 mg kg^{-1}	0.1 mg kg^{-1}	1 mg kg^{-1}
Mean arterial blood pressure (AUC)	35 ± 6*	55 ± 8*	6 ± 2†	91 ± 12*†
Renal conductance (AOC)	-32 ± 7*	-67 ± 13*	-6 ± 3	-87 ± 10*
Mesenteric conductance (AOC)	-22 ± 7*	-97 ± 9*	-6 ± 3	-96 ± 6*
Hindquarters conductance (AOC)	-38 ± 9*	-70 ± 7*	-15 ± 6	-69 ± 7*

* P<0.05 vs baseline (Wilcoxon test); † P<0.05 vs Long Evans (Mann-Whitney U test).

While the difference in the pressor effect of the low dose of dizocilpine in Long Evans and Brattleboro rats could be taken to indicate an involvement of vasopressin in the former, the accompanying regional haemodynamic profile was not consistent with an action of vasopressin (Gardiner *et al.*, 1988). Moreover, responses to the higher dose of dizocilpine indicate that its pressor and generalised vasoconstrictor actions were most likely due to a widespread increase in efferent vasomotor activity. The difference in the pressor effects of the higher dose of dizocilpine in Long Evans and Brattleboro rats, in spite of similar degrees of vasoconstriction, indicates differential changes in cardiac output.

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341P EFFECT OF NMDA RECEPTOR BLOCKADE ON DOPAMINE OVERFLOW IN RAT CAUDATE PUTAMEN

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Glutamic acid (Glu) acting via NMDA receptors may stimulate the release of dopamine (DA) from rat caudate putamen (CPu) by acting on receptors on nerve terminals of nigrostriatal neurons (Desce et al.,1992 but see Smith & Bolam 1990). NMDA receptor antagonists (e.g. AP-5 and CPP) and an antagonist at the NMDA receptor associated ion channel (e.g. MK-801) fully block this effect. Other investigations however have reported that receptor antagonists can stimulate DA overflow (Moghaddam & Gruen, 1991) or in the case of MK-801 increase DA turnover in the striatum in vivo (Hiramatsu et al., 1989). We have studied the effect of CPP and MK-801 on electrically-evoked DA overflow using fast cyclic voltammetry (Trout & Kruk, 1992). Rats were killed and 350 µm slices of CPu were superfused with artificial CSF containing Mg^{2+} at 32°C. DA overflow was evoked with a single pulse or repeated electrical stimulations (20 pulses at 10, 20 or 50 Hz; all at 0.1 ms, 20 v) applied every 5 min. CPP 1 µM and MK-801 10µM were added to the superfusion medium. In 4 out of 7 experiments CPP 1µM produced a slight inhibition of DA overflow at all stimulation parameters. In the remaining 3 experiments CPP produced potentiation of DA overflow in response to train of pulses whereas it had no effect or even a slight inhibition of the single pulse DA overflow. The changes in the DA overflow in response to CPP treatment were not statistically significant. Treatment with MK-801 10 µM reduced DA overflow (n=6) in response to trains of stimulation, this reduction was statistically significant in responses evoked at 10 and 20 Hz. The results have been summarised in table 1.

Table 1

	(potentiation)		(inhibition)				
	Control	CPP	Control	CPP	Control	Mk-801	
Single Pulse	49±2	36±6	85±31	63±23	43±9	37±8	
20 pulses **10** Hz	90±25	187±83	131±41	98±36	98±5	75±5*	*p<0.05
20 pulses **20** Hz	74±24	89±17	162±56	119±48	112±7	76±9**	**P<0.01
20 pulses **50** Hz	82±29	98±30	194±64	149±57	110±12	85±13	(paired t-test)

All results are expressed as nM DA overflow. Results are mean±s.e.mean

Local stimulation of the CPu is likely to cause depolarisation of a multitude of nerve fibres including glutamatergic nerves. It is therefore feasible that release of Glu after electric stimulation can act via presynaptic receptors on DA nerve endings and release more DA. This argument is supported by the observation that MK-801 reduces the DA overflow evoked by trains of pulses; the results with 1 µM CPP may reflect a threshold effect.

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342P AUTORADIOGRAPHICAL ANALYSIS OF THE BINDING OF [^{3}H]L-689,560, AN ANTAGONIST OF THE GLYCINE SITE ON THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR, TO RAT BRAIN

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Autoradiographical (ARG) experiments have provided evidence for heterogeneity of N-methyl-D-aspartate (NMDA) receptors in rat brain (Monaghan et al, 1988; Maragos et al, 1988). Recently, [^{3}H]glycine binding to rat membrane preparations was reported to be displaced more potently by glycine site antagonists in the hippocampus than in the cerebellum (Yoneda and Ogita, 1991). We have used the glycine site antagonist [^{3}H]L-689,560 (Grimwood et al, 1992) as a tool for ARG studies and to investigate the possible pharmacological heterogeneity of the glycine site on the NMDA receptor in rat brain.

Horizontal sections (10μm) of rat brain were thaw mounted onto gelatinized slides, allowed to dry and stored at -20°C. On the day of use, slides were preincubated for 2 hr at 4°C in assay buffer (50mM Tris-acetate, pH 7.0). Slides were then incubated in fresh assay buffer containing 5nM [^{3}H]L-689,560 (28.4 Ci/mmole; custom synthesis from Amersham) ± test compounds for 1 hr at 4°C. 1mM glycine was used to determine non-specific binding. Sections were washed 4 times for 1 min each in ice-cold assay buffer and then dried in cold air. Slides were apposed to Hyperfilm (Amersham), exposed for 17 days and after development the images were quantified using an image analyser (Imaging Res. Inc.). [^{3}H]L-689,560 binding to a membrane preparation from rat cerebellum was also investigated using methods previously described by Grimwood et al (1992) for cortex/hippocampus membranes.

[^{3}H]L-689,560 showed a high level of specific binding to rat brain sections (Table 1) with the distribution previously reported for the glycine site on the NMDA receptor (McDonald et al, 1988) and no interregional differences were apparent for inhibition by 7-chloro-kynurenate (7-Cl KYNA) and (+)HA-966 (Table 1). In all regions the correct stereoselectivity was apparent for HA-966 and serine. The levels of binding in the cerebellum were too low for reliable analysis of inhibition curves, however, [^{3}H]L-689,560 binding to cerebellum membranes displayed similar pharmacological characteristics to those observed in rat cortex/hippocampus membranes.

Table 1.	Outer Cortex	Inner Cortex	Striatum	CA1	Dentate Gyrus	Cerebellum
Spec.Binding	6.62±0.97 (80%)	4.08±0.57 (71%)	3.37±0.41 (68%)	8.35±0.90 (84%)	7.78±1.29 (83%)	1.20±0.10 (43%)
7-Cl KYNA	0.46(0.29,0.72)	0.57(0.31,1.03)	0.56(0.34,0.91)	0.48(0.29,0.79)	0.52(0.33,0.82)	
(+)HA-966	7.63(4.27,13.6)	10.2(8.90,11.7)	8.25(4.47,15.2)	14.7(9.28,23.1)	10.1(6.83,15.0)	

Levels of specific binding for [^{3}H]L-689,560 (nCi/mg; mean±SEM; % specific binding in parentheses) and inhibition values obtained for 7-Cl KYNA and (+)HA-966 (IC_{50} μM; geometric mean±SEM) (n≥3).

[^{3}H]L-689,560 appears to be an excellent radioligand for ARG studies of the glycine site on the NMDA receptor in rat brain. We found no evidence of pharmacological differences for [^{3}H]L-689,560 binding between the brain regions tested.

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343P MODULATION OF [^{3}H]DIZOCILPINE BINDING *IN VIVO* BY LIGANDS FOR THE NMDA RECEPTOR COMPLEX IN MOUSE BRAIN

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Binding of ^{3}H-dizocilpine to the NMDA receptor complex in mammalian brain tissue is agonist dependent possibly reflecting the use-dependent functional antagonism produced by this non-competitive antagonist (Wong & Kemp, 1991). As a consequence ^{3}H-dizocilpine binding can be regarded as an indicator of the activation state of the NMDA receptor complex (Huettner *et al.*, 1988). In the present study we have examined the influence of modulators of NMDA receptor function on the *in vivo* binding of ^{3}H-dizocilpine in mouse brain. Receptor autoradiography has been used to detect subtle changes in the regional levels of binding.

Male CD1 mice (20-50g) were injected i.v. (tail vein) with saline or test compound (in 100μl). After 15 min 50μCi ^{3}H-dizocilpine (53.1Ci/mmol, 1nCi/ml) was injected via the same route and 10 min later the brains were removed and frozen in isopentane cooled in liquid nitrogen. Non-specific binding was determined by pre-injection of dizocilpine (1mg/kg i.v.). Receptor autoradiograms were prepared as reported by Price *et al.* (1988) with slight modification. Cryostat sections (10μm) were washed twice for 45 s in Tris HCl buffer (pH 7.4, 23°C) and apposed to Amersham Hyperfilm with tritium standards for 4-6 weeks.

The distribution of specifically bound ^{3}H-dizocilpine in vivo was comparable to that obtained *in vitro* in mouse and rat brain. The binding density rank order was: hippocampus > cerebral cortex > caudate putamen > thalamus > cerebellum. Pre-injection of D-serine enhanced ^{3}H-dizocilpine binding in all of these regions with the maximal effect in CA3 (112 ± 9% s.e.m. of basal at 3mg/kg, 123 ± 14% at 10mg/kg and 141 ± 11% at 10 mg/kg, n = 3). L-serine increased binding in the same regions only at 100mg/kg (e.g. CA3, 121 ± 10%). D-cycloserine failed to increase the binding but instead produced a marginal although significant decrease particularly in CA1 and dentate gyrus (15 ± 7% decrease $p < 0.05$ ANOVA). The effect of 7-chlorokynurenic acid was variable with an apparent decrease of 8% at 100mg/kg. Surprisingly the competitive NMDA antagonist, CGP 37849 (DL-[E]-2-amino-4-methyl-5-phosphono-3-pentenoic acid), had no effect (up to 30mg/kg). 1-aminocyclobutane-1-carboxylic acid (100mg/kg) also produced no change in the level of binding.

The comparative potencies of D- and L-serine were in accordance with reported affinities for the glycine receptor binding site *in vitro*. The inhibition produced by D-cycloserine may relate to its partial agonist activity (Emmett *et al.*, 1991) by producing glycine receptor antagonism in regions containing high levels of endogenous agonist. The lack of effect of CGP 37849 was unexpected since antagonism at the NMDA recognition site *in vitro* does reduce ^{3}H-dizocilpine binding.

In conclusion, receptor autoradiography with ^{3}H-dizocilpine can be used to examine regional modulation via the glycine receptor *in vivo*.

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344P INHIBITION OF 1-AMINOCYCLOPENTANE-1S,3R-DICARBOXYLATE-STIMULATED PHOSPHOINOSITIDE TURNOVER BY L-SERINE-*O*-PHOSPHATE

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We have recently shown that phosphoinositide turnover in guinea-pig cerebral cortical slices stimulated by ACPD (1-aminocyclopentane-1,3-dicarboxylate) is resistant to inhibition by quinoxalinedione non-NMDA receptor antagonists (Alexander *et al.*, 1990). Of the isomers of ACPD the 1S,3R- isomer is the most potent with an EC_{50} value of 37 μM (Cartmell *et al.*, 1992). Further investigation of excitatory amino acid-stimulated phosphoinositide turnover has been hampered by the lack of selective antagonists. Serine- *O*-phosphate (2-amino-3-phosphoproprionate, SOP) has been suggested to act as an antagonist of the "metabotropic" excitatory amino acid receptor in rat hippocampal neurones (Nicoletti *et al.*, 1986). We have therefore investigated the isomers of SOP as antagonists of 1S,3R-ACPD-stimulated phosphoinositide turnover in guinea-pig cerebral cortical slices.

Accumulation of [^{3}H]inositol phosphates ($InsP_x$) in slices of guinea-pig cerebral cortex (350 x 350 *m*m) was assessed as previously described (Alexander *et al.*, 1990). SOP was added at least 10 minutes prior to addition of 1S,3R-ACPD. Agonist incubation period was 45 minutes.

D- and L-SOP failed to elicit a significant phosphoinositide response up to 1 mM. 1 mM D-SOP also failed to reduce the response to 100 μM 1S,3R-ACPD (99 ± 5 % control). However, 1 mM L-SOP reduced the phosphoinositide response to 100 μM 1S,3R-ACPD by 59 ± 10 % ($P<0.05$, n=3). Analysis of concentration-response curves to 1S,3R-ACPD indicated a small, non-parallel, rightward shift in the slope (EC_{50} values control 35 ± 3; L-SOP 63 ± 12 μM, $P<0.05$, n=4) with a decrease in the maximal response (E_{max} control 6010 ± 851; L-SOP 4278 ± 715 dpm over basal, $P<0.05$, n=4) in the presence of 1 mM L-SOP.

We conclude that in guinea-pig cerebral cortical slices, L-SOP appears to antagonise phosphoinositide turnover stimulated by the selective "metabotropic" excitatory amino acid receptor agonist, 1S,3R-ACPD. However, the mechanism of antagonism appears to be non-competitive, which will limit its' usefulness as an antagonist. These results further stress the need for more potent, competitive antagonists of the ACPD receptor.

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345P CHARACTERISATION OF 3,4-DIAMINOPYRIDINE-STIMULATED ENDOGENOUS GLUTAMATE RELEASE FROM RAT HIPPOCAMPAL SLICES

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Glutamate is a major excitatory transmitter in the CNS. In addition to its physiological roles, glutamate release is thought to be a key mediator of ischaemia-induced neuronal damage, to which the hippocampus is especially vulnerable. Glutamate release is often studied in vitro from superfused brain slices using sustained depolarisation produced by elevated K^+ as a stimulus. However, it has been suggested that blockade of voltage-dependent K^+ channels by aminopyridines may provide a more appropriate means of mimicking in vivo stimulation by producing "repetitive transient depolarisation" (see McMahon & Nicholls, 1991). Therefore, I have investigated 3,4-diaminopyridine (DAP) stimulation of glutamate release and its modulation by other ion channel blockers.

Glutamate release from superfused hippocampal slices was assessed by a modification of previous methods (Herdon & Nahorski, 1989). Release was stimulated by exposure to DAP or K^+ for two 1 min periods (S_1 & S_2) 40 min apart; other drugs tested were present from 20 min before S_2. All experiments were performed in the presence of the glutamate uptake blocker DL-threo-B-hydroxyaspartic acid (50uM). Glutamate was quantified by dansyl derivatisation followed by HPLC with fluorimetric detection (Okafo & Camilleri, 1991).

DAP (10-100uM) caused a concentration-dependent increase in glutamate release. 100uM DAP produced a 318±22% (S_1) increase over basal levels with an S_2/S_1 ratio of 0.61±0.05 (means±s.e., n=8), whilst 40mM K^+ produced a smaller and less prolonged stimulation (76±14% over basal, S_2/S_1 0.83±0.09, n=4). Both 40mM K^+ and 100uM DAP-stimulated release were abolished by removal of added Ca^{2+} (replacement by 10mM Mg^{2+}); DAP-stimulated release was also virtually abolished by tetrodotoxin (1uM). However, neither the "N-type" Ca^{2+} channel blocker w-conotoxin GVIA (0.1uM) nor the "L-type" blocker nimodipine (10uM) produced any significant reduction in DAP-stimulated release (91±10% and 91±14% of control values respectively, n=4).

The complete tetrodotoxin sensitivity and Ca^{2+}-dependency of DAP-stimulated glutamate release suggest that it involves an exocytotic process mediated by Na^+ channel activation and subsequent Ca^{2+}-channel opening. However, the Ca^{2+} channels involved do not appear to be sensitive to nimodipine or w-conotoxin. Similar results have been reported for release from synaptosomes (eg McMahon & Nicholls, 1991), but the comparative ease of brain slice preparation and use should make this a useful method for further investigation of modulation of release by presynaptic receptors or ion channels.

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346P REACTIVITY OF THE RAT MIDDLE CEREBRAL ARTERY IN CONTROLS AND FOLLOWING ISCHAEMIA

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The development of powerful thrombolytic agents has revolutionised the treatment of myocardial infarction, and their use in acute cerebral arterial occlusion is being widely investigated. Little is known about the pharmacological reactivity of cerebral blood vessels following prolonged periods of acute ischaemia and we have set out to investigate the viability and responses of the the middle cerebral artery (MCA) following 4 hours acute ischaemia in vivo. Information about the effects of ischaemia on pharmacological responses would allow therapeutic strategies to be devised which would maximise blood flow through ischaemic arteries and so achieve optimum reperfusion of ischaemic brain.

We have used an intravascular embolus to occlude the origin of the MCA in anaesthetised rats. Ischaemia is confirmed by measuring cerebral blood flow (CBF) in the MCA territory. Following 4 hours of ischaemia the animal is sacrificed and the ischaemic MCA together with its contralateral normal control harvested. The vessels are mounted on a Mulvany myograph and the in vitro responses recorded in mN active tension. We have measured the contractile responses to 124mM potassium, $PGF_{2\alpha}$ and uridine tri-phosphate (UTP). Using vessels pre-contracted with $PGF_{2\alpha}$ we have investigated the responses to the following vasodilators: noradrenaline (NA), sodium nitroprusside (SNP), histamine (HIST) and papaverine (PAP). Endothelial function has been assessed with Nω-Nitro-L-arginine methyl ester (L-NAME). Results have been analysed using a paired t-test comparing each ischaemic artery with its contralateral control. A p value of <0.05 is considered significant.

Following 4 hours ischaemia the MCA remains viable and contraction in response to potassium is significantly increased 8.0 ±2.4 mN in control and 9.2 ±2.1mN in ischaemic (p=0.003). Maximum responses to $PGF_{2\alpha}$ and UTP are preserved but the sensitivity of the ischaemic vessels is reduced (see table).

EC50 = -log concentration moles/litre SD = standard deviation NS = not significant

Agonist		$PGF_{2\alpha}$	UTP	NA	HIST	SNP	PAP	L-NAME
n		32	19	13	18	13	17	16
EC50 (SD)	Controls	5.07 (0.2)	5.36 (0.2)	6.69 (0.3)	5.54 (0.4)	5.42 (0.6)	5.45 (0.3)	5.43 (0.2)
	Ischaemic	4.96*(0.2)	5.19*(0.3)	6.53*(0.2)	5.46 (0.3)	5.07*(0.5)	5.38 (0.3)	5.29*(0.3)
*p=		0.002	0.004	0.01	NS	0.001	NS	0.01

Following 4 hours ischaemia the MCA remains viable and contractile responses are preserved. Vasodilatation in response to noradrenaline and nitroprusside is impaired as is the response to L-NAME. Our results suggest that treatment with the vasodilators we have studied would tend to divert blood flow from ischaemic to normal vessels. If these findings were applicable in human vessels it would imply that treatment with these vasodilators should be avoided following revascularisation of ischaemic brain.

347P ACE INHIBITORS REVERSE THE COGNITIVE DEFICIT INDUCED BY CAROTID OCCLUSION

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A 45 min period of bilateral common carotid artery occlusion in the rat results in a significant and reproducible deficit in spatial learning ability (Barnes et al 1990). Here we investigate the effects of the putative cognitive enhancing agents captopril and ceranapril (Costall et al 1989) on the deficit in learning associated with carotid occlusion and on blood pressure.

Carotid occlusion (45 min) and assessment of spatial learning ability in the Morris water maze were performed according to the method of Barnes et al (1990) with drug or vehicle (0.9% saline) being administered (i.p.) 40 min prior to testing. Escape latency was assessed as a measure of learning with swim speed being indicative of locomotor activity. Previously naïve rats were implanted with a cannula in the descending aorta which was exteriorised to the back of the neck to allow measurement of blood pressure.

The escape latencies (Lat, s) and swim speeds (Sp, m/s) for sham and occluded (Occ) rats treated with either vehicle, captopril or ceranapril are shown below.

	Vehicle	0.1 ng/kg	1 ng/kg	10 ng/kg	0.1 μg/kg	1 μg/kg	10 μg/kg	100 μg/kg
Captopril:								
Lat Sham	14.5±1.8	16.3±2.3	18.1±1.8	21.9±2.3	17.3±1.9	19.2±1.9	20.3±1.7	22.3±2.2
Lat Occ	55.5±2.7	47.6±3.1	40.6±2.6*	36.5±2.7*	34.3±2.7**	27.6±2.9***	28.8±2.3***	52.2±2.4
Sp Sham	20.6±1.6	20.7±2.1	19.9±0.9	20.5±2.2	19.7±1.3	16.6±1.7*	14.5±2.3**	11.6±1.9***
Sp Occ	21.4±2.2	20.8±1.8	20.3±1.4	21.2±0.9	20.2±2.3	17.2±1.5*	15.0±1.2**	10.1±2.1***
Ceranapril:								
Lat Sham	18.0±1.9	13.2±1.7	10.1±1.3	16.4±1.8	14.1±1.9	15.0±2.7	13.3±2.5	-
Lat Occ	38.9±3.9	28.9±4.2	20.0±3.4*	30.9±4.2	23.9±3.3**	24.3±2.9**	29.3±3.5	-
Sp Sham	20.8±2.4	20.4±2.0	17.9±1.4	20.1±1.9	19.0±1.9	19.5±1.7	19.3±2.1	-
Sp Occ	21.4±2.2	21.2±2.1	20.6±2.0	19.3±1.6	20.9±2.2	20.9±2.1	22.1±2.1	-

*P<0.05, **P<0.01, ***P<0.001. ANOVA plus Dunnett's t test for vehicle v drug, n = 8. Mean ± S.E.M.

Captopril and ceranapril caused reductions (P<0.05, n = 4) of 23.6% and 18.9% respectively in blood pressure of conscious freely moving rats at the highest doses used.

Both captopril and ceranapril caused a reversal of the deficit in spatial learning ability at doses which failed to reduce locomotor activity or blood pressure. These data provide further evidence for the cognitive enhancing properties of these agents.

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348P THE INFLUENCE OF THE NUMBER OF DAILY TRIALS ON THE ACQUISITION OF THE MORRIS WATER MAZE TASK BY RATS

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The Morris water maze is a method for demonstrating spatial learning and its disruption by scopolamine in the rat (Morris 1984). The present experiment investigated the effect of varying the number of training trials on the performance of the task and the response to scopolamine pretreatment.

Male hooded Lister rats were trained in an 8-day paradigm in the water maze. On the first day of training each animal was placed onto the hidden island (located 1-2 cm below the suface of the water) for 30s before testing commenced. For each trial the rat was placed in the pool and the time (s) taken to reach the island (escape latency) was recorded as an indication of learning ability. Groups of rats were trained for 4, 2 or 1 trials per day to a fixed island position for 4 days and to a new fixed island position for another 4 days. Finally, a group of rats was exposed to the maze for 1 trial per day and the island position was randomised (RI) from trial to trial such that it was never in the same position on two consecutive days. Scopolamine (0.5mg/kg i.p.) was administered 20 min before commencing the training trials.

Table 1. The effect of different numbers of trials per day on escape latencies (s) of saline (SAL) and scopolamine (SCOP) treated rats

Trials/day	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8
4 SAL	51.0±6.9	14.0±5.9	5.7±1.3	4.0±1.0	7.7±2.0	6.3±2.3	2.9±0.4	2.0±0.2
4 SCOP	54.9±10.7	30.6±8.3	25.5±7.1***	19.7±11.6	29.0±11.7*	12.7±3.0	9.9±3.3*	6.9±1.4***
2 SAL	63.0±7.9	49.9±14.6	39.0±15.9	18.6±7.7	35.4±7.5	17.1±4.3	16.6±4.9	12.0±4.1
2 SCOP	82.7±11.5	72.7±9.2	58.4±14.1	41.7±11.7	35.4±11.8	24.6±8.9	21.1±7.1	22.9±7.0
1 SAL	82.7±10.6	63.0±13.0	28.5±10.4	13.7±7.1	58.5±11.1	29.0±11.6	22.7±11.6	22.7±11.6
1 RI SAL	80.1±9.9	47.7±10.1	31.5±10.1	32.5±8.8	21.6±7.0	49.6±11.3	30.9±9.2	53.8±8.9
1 RI SCOP	90.5±9.5	90.0±8.3***	66.3±11.5*	43.0±11.5	48.8±10.2	53.7±8.9	59.0±11.2	42.2±9.6

Data are presented as mean ±s.e.m. and were analysed using one-way ANOVA and Dunnett's t-test (n = 6-10) *P<0.05, **P<0.01, ***P<0.005.

These data demonstrate that the Morris water maze task is easily and rapidly learned by rats since, even with 1 trial/day, learning occurred over 8 days. Scopolamine impaired maze learning irrespective of the numbers of trials/day. Impaired learning of the task by saline treated rats was only evident when the position of the island was constantly moved.

These results suggest that the number of trials/day should be considered in the experimental design to avoid a "floor" effect, particularly if a cognitive enhancement is to be considered.

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349P DIFFERENCES IN LEVELS OF ANXIETY IN THREE OUTBRED RAT STRAINS

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Hooded rats have been reported to be less anxious than albino rats in some (Costall *et al*, 1989; Moulton & Morinan, 1989) but not all (Pellow *et al*, 1985) studies using the elevated plus maze (EPM). In the present study, we have investigated inter-strain variability in the EPM, and in three other models of anxiety.

The behaviour of adult male Lister Hooded (LH), Sprague-Dawley (SD) and Wistar (W) rats was observed in the EPM, light/dark box and Open Field over a 5 min period. In addition, the total time spent in social interaction by pairs of unfamiliar rats of the same strain was measured in a 10 min test.

Table 1. Strain Differences in Behavioural Measures of Anxiety

Strain	Open: Total Entries (%)	Open: Total Time (%)	Time in Light(s)	Inner Circle Entries	Social Interaction(s)
SD	15.6 ± 3.8	6.7 ± 2.0	55.2 ± 10.8	0.5 ± 0.3	85.9 ± 6.2
W	20.4 ± 5.5	7.0 ± 2.3	57.9 ± 13.5	0.2 ± 0.2	83.3 ± 10.2
LH	30.9 ± 8.6•	20.5 ± 4.5 #	92.7 ± 9.3 #	3.1 ± 1.3 #	128.9 ± 9.0 #

Each value represents the mean ± s.e.m. of (n=8-10) •P<0.05 compared to SD. # P<0.05 compared to SD and W

LH rats showed a significant increase in exploration of the more aversive areas of the EPM, light/dark box and Open Field; and in the time spent in social interaction, compared to the albino strains (Table 1). In contrast, general locomotor activity as measured by the total numbers of arm entries, light/dark transitions and line crossings, was similar in the three strains. In conclusion, these data suggest that strain difference may contribute to the variability in behavioural responses reported for some anxiolytic drugs.

D.D. is a B.Sc (Hons) Pharmacology Student.

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350P NON-NOXIOUS STRESS AND THE BENZODIAZEPINE INVERSE AGONIST, FG7142, HAVE OPPOSING EFFECTS ON NORADRENALINE RELEASE IN RAT FRONTAL CORTEX

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The benzodiazepine inverse agonist FG7142 induces anxiety in man and other species (File & Baldwin, 1987) and generalises to stress in studies of stimulus discrimination (Leidenheimer & Schechter, 1988). Both anxiety and the response to stress have been linked with increased noradrenaline (NA) release in the CNS. We have used *in vivo* microdialysis to compare the effects of non-noxious stress (novelty) and a single injection of FG7142 on NA release in rat frontal cortex.

Male hooded Lister rats (300-350g) were anaesthetised with 1.5-5.0% halothane and a 5mm long dialysis probe (200 µm diameter Hospal) implanted into the frontal cortex. 3 x 30 min baseline samples were collected 24h later (Baldwin et al., 1991), followed immediately by either injection of FG7142 (10 or 20 mg/kg i.p.), vehicle (1% tween in water i.p.) or exposure to a novel, brightly lit cage. Dialysate was collected for a further 3.5h. Samples were stored at -70°C and NA measured by HPLC-ECD.

TABLE: Effects of FG7142 (10 mg/kg) on dialysate noradrenaline concentrations

Group	Time: 0.5	1.0	1.5	2.0	2.5	3.0	3.5
Vehicle	103 ± 10	82 ± 6	92 ± 8	100 ± 9	103 ± 15	97 ± 11	92 ± 8
FG7142	99 ± 7	91 ± 4	88 ± 6	83 ± 4	81 ± 4*	72 ± 3**	75 ± 6**

Values show mean ± SEM of NA content (pg/30 min) expressed as % basal levels. Samples were collected at 0.5h intervals following injection. **p<0.01, *p<0.05 (cf basal levels: Duncan's test following 1 way ANOVA on raw data. n=11 (vehicle); n=6 (FG7142).

FG7142 (10mg/kg) caused a significant reduction in NA release (ANOVA: $F(9,45) = 5.7$, $p<0.0001$; see TABLE), but FG7142 (20mg/kg) had no effect. Exposure to a brightly lit novel environment apparently increased NA release compared with that in unstressed control rats but over the entire time course this was not statistically significant. The reduction in NA release by FG7142 contrasts with the effects of non-noxious stress, but could be relevant to the dose-dependent ß-adrenoceptor upregulation caused by a single injection of this drug (Stanford et al., 1989).

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351P THE BENZODIAZEPINE RECEPTOR AGONISTS FG 8205 AND DIAZEPAM MAY EXERT THEIR ANTICONVULSANT ACTION VIA DIFFERENT MECHANISMS

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The anticonvulsant activity of the benzodiazepine (BZ) receptor agonist, FG 8205, against pentylenetetrazol (PTZ) has been reported to be relatively less sensitive to the BZ receptor antagonist flumazenil, when compared to diazepam (Tricklebank et al, 1990). In order to further characterise the anticonvulsant mechanisms involved, we carried out a dose-response analysis with FG 8205 and diazepam in the presence and absence of the BZ-receptor antagonists flumazenil, Ro 15-4513, CGS 8216 and propyl-β-carboline (βCCP) in mice treated with PTZ. The potencies of FG 8205 and diazepam against seizures induced by the inverse BZ receptor agonist 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) were also compared.

PTZ (120 mg kg^{-1}, t = 0 min) was injected s.c. and mice then observed for tonic seizures over a 30 min period. Prior to this, they were treated with FG 8205 or diazepam (i.p., t = -30 min) followed by flumazenil (i.p., t = -15 min) CGS 8216 (i.p., t = -30 min) or Ro 15-4513 (i.p., t = -15 min). Dose response curves for FG 8205 and diazepam (n=8 per dose group) either alone or in combination with antagonist, were determined two or three times. ED_{50} values were obtained by probit analysis and dose-ratios then calculated as a measure of antagonism. In experiments where βCCP was used as the antagonist, PTZ (80 mg kg^{-1}) was injected i.v., one min after βCCP (i.v.) and seizures occurring within two min were noted. The inverse agonist DMCM was given i.v., (2.5 mg kg^{-1}) 30 min after treatment with FG 8205 or diazepam, and tonic seizures scored in the two min period following injection of the convulsant.

Against PTZ, FG 8205 (ED_{50} = 0.11 mg kg^{-1}) and diazepam (ED_{50} = 0.18 mg kg^{-1}) were equally protective. FG 8205 was less sensitive to the antagonists flumazenil (20 & 40 mg kg^{-1}) CGS 8216 (3 mg kg^{-1}) and Ro 15-4513 (10 mg kg^{-1}) than was diazepam: respective dose-ratios for FG 8205 were 1.7, 6.4, 15.8 and 8.0, whereas for diazepam they were 6.7, 18.0, 47.0 and 22.5. Use of βCCP (3 mg kg^{-1}) gave dose-ratios of 2.2 and -0.53 when tested against diazepam and FG 8205, respectively, if a full tonic seizure was scored. However, when clonic/tonic seizures were measured, the dose-ratio for diazepam increased to 5.6 whereas that for FG 8205 was 0.12, indicating the insensitivity of FG 8205 to this antagonist. FG 8205 and diazepam also protected mice from seizures induced by DMCM. In contrast to results obtained when using PTZ, FG 8205 was markedly more potent, having an ED_{50} of 0.005 mg kg^{-1} compared to 0.67 mg kg^{-1} for diazepam. Taken together, the pattern of the results suggests a difference in the mechanism of anticonvulsant action of FG 8205 and diazepam.

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352P THE BENZODIAZEPINE RECEPTOR LIGAND RU34347 HAS BIPHASIC ACTIONS IN THE *IN VITRO* RAT CEREBELLAR SLICE

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The soluble benzodiazepine receptor inverse agonist RU34347 has a low affinity for classical benzodiazepine receptor binding (Gardner, 1989). At 10μM, the compound has been shown to reduce the period of stimulus evoked inhibition in the cerebellum, but at 1μM a biphasic effect on the stimulus evoked inhibition occurred with an agonist-like increase in the period of inhibition followed by the expected reduction in inhibition. At even lower concentrations (10nM), only the agonist like component was seen (Bagust *et al.*, 1990) and this was blocked by the benzodiazepine receptor antagonist flumazenil. But, in the same study, 10nM of the benzodiazepine inverse agonist Ro19-4603 only elicited a decrease in the period of inhibition. The aim of this study was to determine the threshold of the agonist-like response to RU34347 and to determine whether very low concentrations of Ro19-4603 have a biphasic effect on the period of inhibition.

Extracellular recordings were obtained from single cells in the Purkinje cell layer of the cerebellum as previously described (Hussain *et al.*, 1990). 1nM RU34347 produced a consistent increase in the period of stimulus evoked inhibition which reversed whilst the compound was still being perfused (mean maximal inhibition 215$\pm$22.6% of control, n=5, $p<0.01$), as did 100pM RU34347 (277.9$\pm$50.9% of control, n=5, $p<0.01$). The effect is still present at 10pM (286.6$\pm$43, n=5, $p<0.01$). 1nM Ro19-4603 however produced a reduction in the period of inhibition (55.1$\pm$13%, n=6, $p<0.01$) but 10pM Ro19-4603 had no effect on the period of stimulus evoked inhibition (101.2$\pm$4.1%, n=6, non-significant).

These data suggest that the biphasic effect of low concentrations of RU34347 on the period of stimulus evoked inhibition in rat cerebellar slices is mediated by a mechanism not activated by to Ro19-4603. Also, the effect is probably mediated through a non-classical benzodiazepine receptor, since the magnitude of response is still large at 10pM and the compound has an IC_{50} at classical benzodiazepine receptors of only 1μM. One possible explanation is that RU34347 acts on the cerebellar BDZ_3 receptor (Luddens *et al.*, 1990).

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353P ASSESSMENT OF THE DEPENDENCE POTENTIAL OF THE IMIDAZOPYRIDINE ANXIOLYTIC, ALPIDEM, IN RATS

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Alpidem is a clinically effective non-benzodiazepine (BZ) imidazopyridine anxiolytic. It has been suggested to be an "anxioselective" drug devoid of adverse side effects, due either to a profile of selective actions at *some* BZ receptor subtypes (Langer et al. 1990), or to the fact that it *may* be a partial BZ agonist (Haefely et al. 1991; Langer et al. 1991). To assess the dependence potential of alpidem, its effects were studied in rats at doses at the top of the range reported to have anxiolytic actions in animals (Zivkovic et al. 1990). Effects of alpidem and its withdrawal on 24 h food and water intake and body weight were compared with vehicle treated controls. Acutely, alpidem (10, 30 and 100 mg/kg, i.p., b.i.d.) decreased body weight and food intake and *enhanced* water intake in a dose-related fashion. Maximal effects of alpidem were seen (at 100 mg/kg) on treatment days 2 or 3; these involved a 3 % loss in body weight, a 60 % reduction in food intake and a 100% increase in fluid intake. Alpidem resembles typical BZs in inducing hyperdipsia. However, at the high doses studied, it differs from BZs, which have well-known hyperphagic actions and stimulant effects on body weight. The suppressant actions of alpidem on food intake and weight may be due to sedative effects of these high doses (Zivkovic et al. 1990), rather than a genuine appetite-suppressant action. During 21 consecutive days of treatment full tolerance developed to *all* effects of alpidem. Withdrawal was *not* associated with any aphagia/hyperphagia, loss/gain in body weight, or hyper/hypodipsia. Thus after b.i.d. treatment with high "anxiolytic" doses of alpidem for a prolonged period of time tolerance developed, but alpidem did not induce the type of appetitive withdrawal signs seen with conventional BZs (Goudie et al. 1991). These data support Perrault et al.'s (1991) suggestion that alpidem has limited dependence potential since it induces no proconvulsant withdrawal effect after chronic treatment.

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354P INHIBITION BY CHLORMETHIAZOLE OF THE HARMALINE-INDUCED INCREASE IN CYCLIC GMP CONTENT IN MOUSE CEREBELLUM

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We have previously shown that chlormethiazole (CMZ) can inhibit seizures induced by injection of N-methyl-DL-aspartate, although it does not interact with the NMDA receptor complex directly (Cross et al, 1992). We have now examined whether CMZ inhibits the stimulation of cyclic GMP (cGMP) content in the cerebellum induced by harmaline injection. This involves release of glutamate and is potently blocked by NMDA antagonists (Wood, 1990).

Male TO mice (23-28g) were injected with harmaline (100mgkg^{-1} s.c.) or saline (control) 15 min prior to killing with focussed microwave irradiation. In other experiments CMZ (10-100mgkg^{-1} i.p.), pentobarbitone (5-50mgkg^{-1} i.p.) or dizocilpine (0.5-3.0mgkg^{-1} i.p.) were given 5 min before harmaline. Tremor severity (0-3) was assessed immediately before death. Cerebellar cGMP was measured using a commercial radioimmunoassay kit.

Harmaline (100mgkg^{-1}) increased cGMP content from 2.1± 0.2 (n=10) to 4.8 ± 1.1 (n=10) pmolmg^{-1} tissue (mean ± s.e.mean). Pre-treatment with dizocilpine, pentobarbitone and CMZ antagonised the harmaline stimulation of cGMP (ED_{50} values in mgkg^{-1}; CMZ: 10.4; pentobarbitone: 6.5; dizocilpine: 0.5 [estimated]).

CMZ (ED_{50} : 50mgkg^{-1}) and pentobarbitone (ED_{50} : 30mgkg^{-1}) inhibited harmaline-induced tremor, while dizocilpine was ineffective up to 3mgkg^{-1} (Figure).

These data indicate that, like other GABA potentiating drugs, CMZ can inhibit glutamate stimulated cerebellar cGMP content as can dizocilpine. The failure of dizocilpine to inhibit tremor suggests that NMDA receptor activation is not involved. This is also perhaps suggested by the lack of correlation of ED_{50} values for inhibiting cGMP content and tremor in the experiments involving CMZ and pentobarbitone.

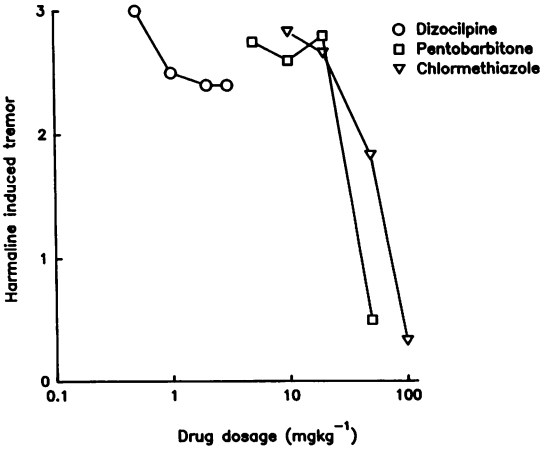

Figure The effect of dizocilpine, chlormethiazole and pentobarbitone on harmaline-induced tremor. Each point is the mean of 4-6 observations.

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355P CARDIAC ACTIONS OF BENZODIAZEPINES

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There is still controversy on the action of benzodiazepines on the cardiovascular system (Gonzalez et al., 1990) and data on the effects of newer derivatives need completion. In this report actions of adinazolam and alprazolam are compared to those of diazepam and midazolam in the Langendorff heart. Moreover, effects of the antagonist flumazenil (Anexate) on inotropic actions of adinazolam and alprazolam have been determined.

Male rats were used. The aorta was cannulated, the heart excised and perfused with Tyrode (37 °C). Throughout an experiment the heart kept its own pace. To measure the contraction force, a balloon connected to a pressure transducer was placed in the left ventricle. After equilibration for 20 min, the experiment was performed using subsequent increasing concentrations diazepam or midazolam in the range from 2 x 10^{-5} to 6 x 10^{-4}M, adinazolam and alprazolam in the range of 3 x 10^{-8} to 1 x 10^{-5}M. Perfusion went on during 10 min for each concentration. Midazolam and adinazolam were soluble in water, diazepam was dissolved in propylene glycol and alprazolam in Krebs, acidified with 0.1 M HCl.

Control experiments (n=12) with the solvents show no effect on the contraction force. Diazepam produces a concentration dependent triphasic effect on inotropy; i.e. at relatively low concentrations (2 x 10^{-5}M) a stimulation is observed, and at higher concentrations (4 x 10^{-5} to 6 x 10^{-4}M) a short lasting inhibition, followed by a transient activation (n=12). Midazolam induces a significant concentration dependent stimulation of the contractile force, although much weaker than that produced by diazepam (n=12). At even higher concentrations of diazepam (higher than 1 x 10^{-4}M) and midazolam (2 x 10^{-4}M) the stimulation decreases. Adinazolam and alprazolam, in concentrations thousands of times lower (3 x 10^{-8} to 1 x 10^{-6}M) induce a dose dependent stimulation of inotropy. Flumazenil (1 x 10^{-5}, 5 x 10^{-5}, 1 x 10^{-4}M) by itself has no effect. The lowest concentration (1 x 10^{-5}M) however, produces a parallel shift of the diazepam curve to the right, indicating antagonism. Flumazenil (1 x 10^{-4}M) induces a shift to the left indicating synergism (n=12). Midazolam effects are not affected by flumazenil (n=6). No significant effects of flumazenil on actions of adinazolam and alprazolam have been found as yet.

In conclusion, benzodiazepines elicit both, concentration dependent positive and negative inotropic actions. The heart appears to be thousands of times more sensitive for the positive inotropic actions of adinazolam or alprazolam as compared to diazepam or midazolam. Effects of different benzodiazepines are affected in different ways by flumazenil, suggesting several types of actions and/or receptors in the heart muscle.

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Adinozalam and Alprazolam were kindly supplied by Upjohn Nederland N.V.

356P LACK OF EFFECT OF DELTA SUBUNIT ANTIBODIES ON $GABA_A$ RECEPTOR FUNCTION IN RAT CEREBELLAR MEMBRANES

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γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. The $GABA_A$ receptor is a multi-subunit GABA-gated chloride channel complex, which can be allosterically modulated by a variety of compounds. A number of subunits and isoforms have recently been identified (α1–6, β1–4, γ1–3, δ and ρ) but the functional role of many of these subunits is at present unknown. We have therefore investigated the effect of an antibody raised against the δ subunit on biochemical indices of $GABA_A$ receptor functional activity as a probe for the role of the δ subunit.

In these studies a peptide (δ_{1-11}) which corresponds to the N-terminal amino acid sequence of the rat δ subunit (Shivers et al. 1989) was synthesised. For antibody production the peptides were coupled via an additional cysteine to bovine serum albumin. Antibodies were raised in New Zealand white rabbits, with the production of the immune response being monitored by enzyme-linked immunosorbent assay with the synthetic peptide as the antigen. The polyclonal antibodies (in serum) were investigated for their effects on modulation of TBPS and flunitrazepam binding. A control rabbit serum was also tested in each assay. The binding of [^{35}S]-TBPS and [^{3}H]-flunitrazepam to rat cerebellar membranes was studied according to the methods of Gee (1988) and Martin & Doble (1983) respectively.

The binding of [^{35}S]-TBPS was allosterically displaced by GABA, the steroid 5α-pregnan-3α-ol-20-one, the barbiturate pentobarbital, the pyrazolopyridine tracazolate and by ethanol with IC_{50}'s of 2.5μM, 0.92μM, 39μM, 2.1μM and 0.26M respectively. The δ_{1-11} antibody (at a concentration of approximately10^{-7}M) had no effect on any of these modulators. The δ_{1-11} antibody also had no apparent effect on the inhibition of [^{3}H]-flunitrazepam binding by diazepam (IC_{50} 4.1nM).

In conclusion, in membranes prepared from rat cerebellum where there is a high level of δ subunit (Benke et al., 1991), there was no evidence that the δ_{1-11} peptide antibody affected $GABA_A$ receptor function.

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357P THE EFFECTS OF SYSTEMIC ADMINISTRATION OF BACLOFEN ON POST-PRANDIAL AND HISTAMINE-INDUCED WATER INTAKE IN RATS

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Drinking occurs in rats after a period of water deprivation, during and after meals, and following the administration of pharmacological agents such as histamine (see Ebenezer et al, 1992). We have previously demonstrated that systemic administration of (±)baclofen inhibits water intake in water-deprived rats by a non-aversive mechanism (Ebenezer et al, 1992). In the present study we investigated the ability of baclofen to inhibit (i) post-prandial drinking, and (ii) drinking induced by systemic injections of histamine. Male Wistar rats (n=16, body wt. 250-360g) were used in this study. They had free access to food and water unless otherwise stated. In the first experiment rats (n=8) were deprived of food for 22h prior to each experimental session. They were allowed to feed ad libitum for 60 min but had no access to water during this period. The food was removed and they were then injected s.c. with either physiological saline (control) or baclofen (1-4 mg kg^{-1}), and 15 min later were presented with water. Water intake was measured over the next 30 min as described previously (Ebenezer et al, 1992). A repeated measures design was used, with each rat receiving all doses of drug and saline in a random fashion. In a second experiment, non-deprived rats were injected s.c. with either saline followed 5 min later by saline, histamine (10 mg kg^{-1}) followed by saline, saline followed by baclofen (2 mg kg^{-1}), or histamine (10mg kg^{-1}) followed by baclofen (2 mg kg^{-1}). Water intake was measured over the next 120 min. Each rat received all treatments in a random fashion. The results of the first experiment showed that baclofen caused a dose-related reduction in post-prandial water intake, with the peak inhibitory effects occurring during the first 15 min after water presentation. Thus, during this period the 1, 2 and 4 mg kg^{-1} doses of baclofen decreased water intake from a control value of 4.3 ± 1.2 ml to 3.1 ± 0.6, 0.86 ± 0.4 and 0.25 ± 0.3 ml ($P<0.01$ in each case) respectively. The results of the second experiment indicated that while histamine (10 mg kg^{-1}) increased water intake from a control value of 0.2 ± 0.1 ml to 4.75 ± 1.7 ml ($P<0.01$) over the 120 min measurement period, pretreatment with baclofen (2 mg kg^{-1}) significantly reversed the dipsogenic effect of histamine ($P<0.01$), with the rats drinking just 0.1 ± 0.04 ml of water. The results of this study extend previous results (Ebenezer et al, 1992) and show that systemic administration of baclofen will inhibit post-prandial and histamine-induced water intake in rats.

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358P PHORBOL ESTER TREATMENT DOES NOT AFFECT ADENOSINE A_2 OR PROSTACYCLIN IP RECEPTOR FUNCTION IN NG108-15 CELLS

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The phorbol ester phorbol 12-myristate, 13-acetate (PMA) has been shown to mediate ß-adrenoreceptor desensitization in 1321N1 astrocytoma cells, by a mechanism that is distinct from agonist mediated desensitization (Toews et al., 1987). The neuroblastoma x glioma hybrid cell line, NG108-15, expresses adenosine A_2 and prostacyclin IP receptors which, like ß-adrenoreceptors, are coupled to stimulation of adenylate cyclase via an interaction with Gs (Kelly et al., 1990). In this study we have investigated the effect of pretreatment with PMA on adenylate cyclase responses in NG108-15 cells.

NG108-15 cells (passage 16-25) were grown to confluency in $80cm^2$ flasks. Cells were pretreated with 1µM PMA or vehicle in Dulbecco's modified Eagle's medium for 1h at 37°C. Cells were then harvested and washed three times in phosphate buffered saline and frozen at -70°C until required. Adenylate cyclase activity was assayed in cell homogenates as previously described (Kelly et al., 1990). Data were compared using paired t-tests, with $P<0.05$ taken to be significant.

Basal adenylate cyclase activity was unaffected by pretreatment with PMA, being 18.2±5.1 and 17.0±4.3 pmol $cAMP.min^{-1}.mg\ protein^{-1}$ in control and treated cells respectively. Similarly the increase in adenylate cyclase activity produced by maximal concentrations of the IP receptor agonist iloprost, the A_2 receptor agonist N-ethylcarboxamido adenosine (NECA) and sodium fluoride were not significantly affected by PMA pretreatment, responses to 1µM iloprost being 42.5±12.7 and 53.3±19.6, to 100µM NECA being 22.9±7.2 and 28.0±13.9 and to 10mM NaF being 10.3±3.4 and 10.8±3.7 pmol $cAMP.min^{-1}.mg\ protein^{-1}$ in control and treated cells respectively. All values are mean±s.e.mean, n=3.

Thus there is no effect of PMA on responses to iloprost and NECA in NG108-15 cells and in this respect the regulation of IP and A_2 receptor responsiveness in these cells appears to differ from ß-adrenoreceptor regulation in 1321N1 cells.

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359P TOLERANCE AND CROSS-TOLERANCE TO THE SEDATIVE EFFECTS OF DIHYDROPYRIDINES: DIFFERENT PATTERNS OF DEVELOPMENT WITH FELODIPINE AND NITRENDIPINE

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We have previously demonstrated that tolerance occurs to the effects of the dihydropyridine calcium channel antagonist, isradipine, on spontaneous locomotor activity (Ripley and Little, 1992). Felodipine appears to differ from other dihydropyridine calcium channel antagonists (including nitrendipine, nimodipine and isradipine) in that it did not protect against the ethanol withdrawal syndrome (Watson *et al.*, 1992). We have now examined the occurrence of cross tolerance between these compounds, in two separate sets of experiments (i and ii).

Male TO mice (30-35g) were used in all experiments. All drugs were suspended in Tween 80, 0.5%. Chronic nitrendipine, 50 mg/kg, or felodipine, 10 mg/kg, i.p., was given every 12h for 13 days; control animals received vehicle injections. At 24h after the last chronic injection, nitrendipine, 50 mg/kg, isradipine, 10 mg/kg, felodipine, 10 mg/kg or Tween were given i.p. Spontaneous locomotor activity of pairs of mice (n values 6 - 8 pairs per treatment group) was measured, by counts of infrared beam crossing, between 5 min and 30 min after the acute injections. $P < 0.05$ (Student's t-test) for the following comparisons: **a** compared with Tween/Tween; **b** compared with Tween/nitrendipine; **c** compared with Tween/isradipine; **d** compared wth Tween/nitrendipine; **e** compared with Tween/nitrendipine

i)

Chronic/acute	Counts
Tween/Tween	4706 ± 553
Tween/nitrendipine	2701 ± 370 **a**
Tween/isradipine	1456 ± 261 **a**
Nitrendipine/nitrendipine	4094 ± 378 **b**
Nitrendipine/isradipine	3090 ± 286 **c**

ii)

Tween/Tween	5444 ± 411
Tween/felodipine	2853 ± 215
Felodipine/felodipine	3025 ± 334
Felodipine/nitrendipine	3093 ± 281 **d**
Nitrendipine/felodipine	3369 ± 258
Tween/nitrendipine	1998 ± 300
Nitrendipine/nitrendipine	3336 ± 282 **e**

Repeated administration of nitrendipine significantly reduced the sedative effects of nitrendipine and isradipine, but did not significantly alter those of felodipine. Chronic felodipine treatment did not cause tolerance to felodipine, although it decreased the effects of nitrendipine. Measurements of whole brain concentrations of nitrendipine showed that the tolerance was not due to changes in metabolism of this compound and that the concentrations at 24h were extremely low. These results support our previous conclusions that the central effects of felodipine differ from those of other dihydropyridine calcium channel antagonists.

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360P THE RELATIONSHIP OF ANALGESIA PRODUCED BY XYLAZINE HYDROCHLORIDE AND PLASMA LEVELS IN NORMAL SHEEP

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Experiments measuring the mechanical nociceptive threshold (Nolan et al, 1987), to evaluate the analgesic effects of xylazine; an alpha2 adrenoceptor agonist, showed a biphasic response in normal sheep after an administration of 50ug/kg IV. This prompted us to investigate a possible correlation between the plasma levels of xylazine and the degree of analgesia experienced by the sheep. To achieve this, a radioreceptor assay for alpha2 agonists was developed using 0.47nM [^{3}H]clonidine as the radioligand binding to alpha2 adrenoceptors in a crude membrane preparation (approx. 4.2mg protein/ml) from rat cerebral cortex. Xylazine hydrochloride, at concentrations ranging from 1 x 10^{-10} to 1 x 10^{-4} M. was used as the displacer in the preparation of the standard curve. After the administration of 75ug/kg IV. xylazine hydrochloride to 6 sheep, blood was recovered via a catheter placed in the jugular vein at time intervals 1, 2, 4, 6, 8, 10, 15, 20, 30, 45, 60 and 90 minutes after injection. The plasma obtained was treated with alumina at a concentration of 100mg/ml to extract any catecholamines that were present, since they have been found to interfere with the assay. Chloroform extraction was performed followed by evaporation and reconstitution in 50mM Tris buffer pH 7.5, this enabled levels down to 0.14ng/ml to be detected from blood samples of 8.0ml.

The assay of plasma from the sheep showed 2 peaks; the first appearing one minute after the administration of xylazine, and the second lower peak appeared 15 minutes later with an edge trail of between 10 and 20 minutes. By use of a more refined mechanical pressure test (Chambers et al, 1990) on the same sheep, a biphasic response to xylazine was demonstrated, with maximum analgesia being experienced immediately after the administration of 50ug/kg xylazine IV, while a second lower analgesic peak appeared 15 minutes later.

The radioreceptor assay demonstrated the existence of two peaks, which correlated well with the times of analgesia as shown in the mechanical nociceptive test, suggesting that the biphasic analgesic response seen is associated with the plasma levels of alpha2 adrenoceptor agonist activity.

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361P EFFECTS OF AGING AND CHRONIC CLORGYLINE AND DESIPRAMINE ON CNS POSTSYNAPTIC α_2-ADRENOCEPTORS MEDIATING MYDRIASIS IN THE RAT

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Clonidine induces a dose-dependent mydriatic effect in the anaesthetized rat by a CNS action through inhibitory postsynaptic α_2-adrenoceptors (α_2R) (Koss, 1986). Chronic administration of antidepressant drugs has been shown to induce desensitization of this α_2R funtional response in adult rats (Menargues et al., 1990). In this study the effect of aging (1, 3 and 24 months) on clonidine-induced mydriasis was investigated in anaesthetized male Sprague-Dawley rats (sodium pentobarbitone, 60 mg/kg, i.p.). The dissociation constant (K_A) for clonidine was also assessed during aging by means of established methods (James et al., 1989) after partial functional recovery (2 days) from complete irreversible inactivation of α_2R by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 1.6 mg/kg, i.p.). The effects of clorgyline (1 mg/kg/day, i.p.) and desipramine (10 mg/kg/day, i.p.) after 1, 4, 7, 14, 21, 28 and 35 days of treatment on clonidine-induced mydriasis were also assessed to evaluate the degree of desensitization of this α_2R functional response during aging. The pupil diameter was measured using a monocular macroscope (10x) with a reticule (0.1 mm gradation) under a white and soft light. All cumulative dose-response curves for clonidine (1-3000 μg/kg, i.v.) were analyzed by use of nonlinear regression. The comparison of ED_{50} values for clonidine in 1, 3 and 24 months old rats (ED_{50}: 16±1, 22±1 and 8±1 μg/kg, respectively, n=4-10) indicated the existence of supersensitive α_2R in aged rats ($P<0.001$), whereas the observed progressive increase of maximal mydriatic effects with aging (Emax: 3.7±0.1, 4.5±0.1 and 5.9±0.1 mm, respectively) could be normalized by the corresponding eye's weight. EEDQ (6 h) abolished clonidine-induced mydriasis which returned gradually (1-12 days) to control values. In young, adult and aged rats, treatment with EEDQ for 2 days resulted in significant decreases of clonidine sensitivity (ED_{50} increased 2.9 to 10.8-fold) with marked reductions of maximal mydriatic effects (Emax decreased by 22-59%, $P<0.001$). Analysis of the hyperbolic regression between the equieffective doses of clonidine before and after EEDQ administration allowed to estimate the various dissociation constant values for clonidine in young, adult and aged rats (K_A: 62±14, 76±8 and 10±1 μg/kg, respectively, n=4-6) which again indicated the existence of supersensitive α_2R in aged rats ($P<0.05$). Chronic (7-35 days) but not acute (1-4 days) treatment with clorgyline or desipramine (n=4-18) resulted in desensitization of clonidine-induced mydriasis in young (ED_{50} increased 1.5-fold, Emax decreased by 14%), adult (ED_{50} increased 1.5-fold, Emax decreased by 15-29%) and aged (ED_{50} increased 2.2 to 4.3-fold, Emax decreased by 9-22%) rats. These data indicate that aging induces supersensitivity of postsynaptic α_2R mediating mydriasis in the rat and that the down-regulation of these α_2R induced by antidepressant drugs is more marked and long-lasting in the aged animals.

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362P FURTHER EVIDENCE THAT MELATONIN IS MONITORED *IN VIVO* BY VOLTAMMETRY

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Melatonin, an indoleamine hormone synthesised in the pinealocytes, appears to be voltammetrically electroactive at the surface of pretreated carbon fibre micro electrodes (mCFE) in vitro at the specific oxidation potential of +580 mV (Crespi et al., 1992). Preliminary differential pulse voltammetric (DPV) experiments have been performed in vivo with the mCFE stereotactically implanted in the pineal gland or in the suprachiasmatic nucleus (SCH) of anaesthetised (chloral hydrate 400 mg/kg i.p.) male rats (Sprague Dawley, 300 g). They indicated the feasibility of simultaneous measurement of the indolaminergic Peak 3 at +250 mV (mainly due to the oxidation of extracellular 5-hydroxyindoleacetic acid (5HIAA)) and a signal at +580 mV which we have called Peak M. By contrast, the same mCFE was unable to monitor the latter signal when implanted in other brain areas (i.e. striatum, accumbens). Furthermore, intravenous or intracerebral injections of exogenous melatonin (Sigma, 5 mg/kg or 2 μg/μl, n=4 respectively) were followed by selective, significant increase of in vivo Peak M.
We have now performed other in vivo experiments with anaesthetised rats prepared for DPV analysis with the mCFE into the SCH. Animals were treated with tryptophan (TRY, 30 mg/kg i.v. n=3) or with n-acetyl serotonin (nA-5HT, 5 mg/kg i.v., n=3) in order to continue to investigate the chemical nature of in vivo Peak M. The data obtained showed that both of these precursors of melatonin were responsible for a transient increase in the size of Peak M with the maximum effect observed after TRY (approx. 320% of control levels within 10 min) and a smaller rise following nA-5HT (about 126% of control values within 20 min). This second treatment was also responsible for an increase in the size of Peak 3 (216% of control values within 15 min) which may have come from an alternative route of degradation of nA-5HT into the SCH. This possibility is supported by the increased levels of both Peak 3 and Peak M following local injection into the SCH of exogenous 5HT (μM, n=3). N-acetyltransferase (NAT) is one of the key enzymes controlling the synthesis of melatonin and its activity is stimulated by butyrate (Wiechmann, 1990). Treatment of anaesthetised rats with sodium butyrate (100 mg/kg i.v., n=3) produced a transient increase in the size of in vivo Peak M and Peak 3.
In conclusion, these data suggest that DPVoltammetry with pretreated micro-biosensors might be the first in vivo method for analysis of melatonergic function(s) in discrete brain areas.

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363P N-BROMOACETYL 5-METHOXYTRYPTAMINE: AN IRREVERSIBLE MELATONIN AGONIST?

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Radioligand and *in vitro* autoradiographic studies have revealed specific, high affinity, G-protein-coupled binding sites for the pineal hormone, melatonin, in discrete areas of the brain (Morgan & Wiliams, 1989). Recently, a derivative of melatonin, N-bromoacetyl 2-[^{125}I]iodo-5-methoxytryptamine ([^{125}I]BraMT) has been reported to identify melatonin binding proteins in membranes from hamster and rat brain (Laudon & Zisapel, 1991; Anis & Zisapel, 1991). The present investigation examined the effects of BraMT on 2-[^{125}I]iodomelatonin binding in chicken brain membranes, and in a biological model of melatonin action, the *Xenopus laevis* melanophore.

Saturation studies revealed that preincubation of chicken brain membranes with BraMT (10^{-8}M, 25°C, 60 min.) did not significantly alter either the maximal number of binding sites (B_{max}) or equilibrium dissociation constant (K_d): control, K_d 55.4 ± 4.8 pM, B_{max} 24.8 ± 2.9 fmol/mg protein; plus BraMT, K_d 57.3 ± 4.3 pM, B_{max} 21.1 ± 3.4 fmol/mg protein (n=3, p>0.05). Melatonin and various analogues induce a dramatic condensation of pigment granules in cultured *Xenopus laevis* melanophores (Sugden, 1991). This effect is completely reversible within 30 min. upon removal of melatonin from the incubation medium. BraMT also produced a concentration-related aggregation of pigment in these cells but removal of BraMT produced an inconsistent and, at best, partial reversal of aggregation. SDS polyacrylamide gel electrophoresis of chicken brain membrane proteins preincubated (60 min., 25°C) with 2-[^{125}I]BraMT (150 pM) indicated that several proteins, with molecular masses ranging from 41 to 95 KDa, were labelled. The incorporation of radiolabel into these proteins was not blocked by melatonin or 2-iodomelatonin (10 μM), but was reduced by BraMT (10 μM).

These studies provide no evidence that BraMT binds irreversibly to chicken brain melatonin receptors or affinity labels specific melatonin binding proteins. Although *Xenopus* melanophores aggregated by BraMT fail to disperse pigment completely after BraMT is washed out, this may be related to the finding that numerous membrane proteins are alkylated rather than to a specific action on a melatonin receptor protein.

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364P THE EFFECTS OF NIMODIPINE ON SALICYLATE OTOTOXICITY IN THE ANAESTHETIZED RAT

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Administration of salicylates is the most popular animal model of tinnitus. The aim of the present study is to investigate the auditory effects of salicylate administration using the auditory evoked brainstem response (AEBR). In doing so we aim to identify possible neurophysiological correlates of salicylate ototoxicity against which the effectiveness of potential tinnitus therapies may be objectively tested.

AEBRs were recorded from Ag/AgCl surface electrodes, placed at the vertex and right mastoid in male anaesthetized rats (Fluanisone 7.5 $mgkg^{-1}$, Midazolam 3.8 $mgkg^{-1}$ and Fentanyl 0.2 $mgkg^{-1}$). Acoustic stimuli (100 μsec bursts of 10 KHz) were delivered free-field. Sodium salicylate (300 $mgkg^{-1}$ in 0.9% NaCl, i.p.) was administered over a 20 minute period. Four hours post administration hearing threshold had increased by 30.8±1.6dB (Mean±1 SEM, n=5). The AEBR consists of four principal peaks I - IV, each peak representing the summed neural activity in regions of the ascending auditory pathway. At 50dB above threshold, prior to salicylate administration, these occurred at 2.09±0.02 ms, 2.92±0.03 ms, 3.48±0.01 ms and 4.44±0.03 ms respectively. Four hours post administration of salicylate there were significant ($p<0.001$, t-test) reductions in the latencies of all four peaks. The latencies now occurred at 1.85+0.03 ms, 2.63±0.01 ms, 3.28±0.02 ms and 4.21±0.4 ms respectively. Administration of vehicle alone did not affect auditory performance. The calcium antagonist Nimodipine has been indicated to be effective in the treatment of tinnitus (Theopold, 1985). In a further series of experiments Nimodipine (2 $mgkg^{-1}$, dissolved in ethanol, s.c.) was administered to rats immediately following administration of salicylate. Four hours post administration hearing threshold had increased by 31.4±2.4dB (n=5). This was not significantly different ($p>0.05$) from that occuring in rats treated with salicylate alone. However, Nimodipine did attenuate the reductions in latencies of peaks I - IV of the AEBR such that only peak II was significantly different ($p<0.02$) from its pre-administration value. Administration of vehicle or Nimodipine alone had no measurable effect on auditory performance.

We believe that we have identified specific neurophysiological correlates of salicylate ototoxicity as indicated by changes in the AEBR. This may be of value in objectively assessing potential tinnitus therapies.

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365P THE PRODUCTION OF CANNABINOID TOLERANCE IN PREPARATIONS OF MOUSE VAS DEFERENS AND OF MYENTERIC PLEXUS-LONGITUDINAL MUSCLE OF MOUSE SMALL INTESTINE

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Our objective was to find an *in vitro* model for studying the mechanisms underlying cannabinoid tolerance. Male MF1 mice were injected once daily for 3 days with delta-9-tetrahydrocannabinol (THC; 20 mg/kg s.c.) as this treatment is known to induce tolerance to the hypothermic effect of THC (see Pertwee 1991). Control animals received injections of Tween 80 (40 mg/kg s.c.). The mice were killed 24 to 28 h after the third injection and their vasa deferentia and small intestines removed. Tissues were also obtained from untreated mice. Vasa deferentia and strips of myenteric plexus-longitudinal muscle dissected from the small intestine were mounted in 4 ml siliconized organ baths, at an initial tension of 0.5 g. The baths contained Krebs solution kept at 37°C and bubbled with 95% O_2 and 5% CO_2. Vasa deferentia were set up in Mg^{++}-free Krebs solution and stimulated supramaximally with 0.5 s trains of 3 pulses (train frequency 0.1 Hz; pulse duration 0.5 ms). Myenteric plexus preparations were stimulated supramaximally with 0.5 s trains of 11 pulses (pulse duration 0.5 ms), using a train frequency (0.2 Hz) that slightly exceeded the rate of spontaneous contractions (Alyami *et al.*, 1991). Isometric contractions were recorded. All cannabinoids were mixed with two parts of Tween 80 by weight and dispersed in saline.

At a submaximal concentration (0.316 nM), the psychotropic cannabinoid, CP 55,940 (Little *et al.*, 1988) produced significantly less inhibition of the twitch response ($P < 0.01$; Student's t test) in vasa deferentia obtained from mice treated with THC (36.9 ± 6.1%; mean ± se; n = 8) than in those obtained from Tween-treated animals (67.0 ± 6.6%; n = 8). The inhibitory effect of 0.316 nM CP 55,940 in vasa deferentia obtained from untreated mice was 70.5 ± 4.4% (n = 6). Experiments with myenteric plexus preparations showed the inhibitory effect of 100 nM CP 55,940 to be significantly less ($P < 0.01$) in tissue obtained from mice treated with THC (14.3 ± 4.4%; n = 8) than in tissue obtained from Tween-treated animals (39.3 ± 4.3%; n = 9). At this concentration (100 nM), CP 55,940 produced its maximum inhibitory effect on the twitch response of myenteric plexus preparations obtained from untreated mice (48.6 ± 4.5%; n = 6). We conclude that both the vas deferens and the myenteric plexus preparation of the MF1 mouse can be rendered tolerant to cannabinoids and that either preparation could be of use in elucidating the mechanisms responsible for cannabinoid tolerance.

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366P DEVELOPMENT OF A MODEL OF ACUTE HINDLIMB ISCHAEMIA IN THE ANAESTHETISED RABBIT: EFFECT OF PENTOXIFYLLINE

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The xanthine, pentoxifylline (P; Trental^R) is currently the major drug used for the treatment of peripheral vascular diseases, including intermittent claudication, although its mechanism(s) of action is unclear. Recently, P was reported to improve skeletal muscle performance in acute hindlimb fatigue in the ferret (Weselcouch & Demusz, 1990). We have now evaluated the effects of P in a model of hindlimb ischaemia in the anaesthetised New Zealand White male rabbit. Anaesthesia was induced and maintained with pentobarbitone sodium 45mg kg^{-1} i.v. administered twice and 90mg kg^{-1} s.c. every two hours. Ischaemia was produced using a variable aortic occluder device to reduce femoral artery perfusion pressure (FAPP) to the hindlimb. Skeletal muscle performance was evoked by sciatic nerve stimulation (6V, 0.7Hz, 0.3ms) and measured using an isometric tension transducer attached to the Achilles tendon, with a loading tension of 160g. Under normal, unrestricted blood flow conditions, two successive 20 min periods of sustained stimulation (S1 and S2), separated by a period of 1 h, evoked reproducible hindlimb muscle performance as judged by (i) AUC, the ratio of the area under the curve of developed tension against time for S2 expressed as a percentage of that for S1, and (ii) Fatigue Index (FI), the ratio of the final developed tension in S2 as a percentage of that for S1. Aortic stenosis of increasing severity produced graded reductions in FAPP, associated with pressure-related decreases in muscle performance (Table 1). A reduction of the normal FAPP to 26mmHg decreased the AUC and FI *without* reducing the peak tension development in S2. Administration of P (1 and 10mg kg^{-1} i.v.) , 30 min prior to S2, had no significant effect on the impairment of muscle performance at FAPP of 26mmHg (Table 1).

Table 1: Effect of P on hindlimb muscle fatigue caused by a reduction in FAPP to 26mmHg in the anaesthetised rabbit (n=6)

Treatment	Vehicle					P (mg kg^{-1})	
	Normal					1	10
FAPP mmHg	59 ± 6	28	26	25	24	26	26
AUC (%)	98 ± 8	80 ± 5	65 ± 5*	52 ± 7*	19 ± 7*	81 ± 8#	63 ± 11#
FI (%)	100 ± 12	58 ± 7*	40 ± 7*	24 ± 6*	9 ± 5*	55 ± 7#	38 ± 12#

* Significantly different from normal, $p<0.05$. # Not significantly different from vehicle control group at 26mmHg.

In conclusion, P failed to protect against acute, ischaemia-induced hindlimb muscle fatigue in the rabbit. These data contrast with those reported in the ferret and indicate a possible species difference in the mechanism(s) of action of P.

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367P AGONIST-INDUCED INOSITOL PHOSPHOLIPID HYDROLYSIS IN CULTURED SMOOTH MUSCLE CELLS FROM HUMAN UMBILICAL ARTERY

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A range of vasoconstrictors can induce smooth muscle contraction in segments of human umbilical artery and vein (Altura *et al.*, 1972; MacLennan *et al.*, 1989). The use of cell culture techniques has provided important information on the signal transduction pathways operating in human umbilical vein endothelial cells (e.g. Jacob *et al.*, 1988; Hallam *et al.*, 1988). In this communication we report that histamine, 5-hydroxytryptamine and bradykinin can stimulate inositol phospholipid hydrolysis in cultured smooth muscle cells derived from human umbilical artery.

Human umbilical artery smooth muscle (HUASM) cells were grown from explant cultures (Hawley *et al.*, 1992). Briefly, explants of the umbilical artery (circa 1 mm^2) were placed in petri dishes containing D-val MEM, 10% foetal calf serum (FCS) and glutamine (2 mM) supplemented with penicillin G (100 U/ml) and streptomycin (100 µg/ml). The medium was changed every 3-4 days. At confluence, the explants were discarded and smooth muscle cells passaged using trypsin/EDTA (Gibco) solution and grown thereafter in DMEM, 10% FCS and glutamine (2 mM). Monolayer cultures (in 24-well cluster dishes) of HUASM cells were loaded for 24 h with ^{3}H-inositol (1 µCi/well) in inositol-free DMEM containing 10% dialysed FCS and glutamine (2 mM). The accumulation of total ^{3}H-inositol phosphates in response to agonists was then measured as described previously for guinea-pig aortic smooth muscle cells (Hawley *et al.*, 1992).

Histamine (1 mM; 45 min) produced a marked increase in total ^{3}H-inositol phosphate accumulation (17.0 ± 0.9 fold over basal levels, n = 9). The EC_{50} value was 16.0 ± 1.4 µM (n = 5). Bradykinin (EC_{50} = 4.5 ± 1.0 nM, n = 3, 10 min stimulation) and 5-HT (EC_{50} = 0.70 ± 0.04 µM, n = 4, 45 min stimulation) produced smaller maximal responses than that obtained with histamine following the same time of incubation (bradykinin, 42 ± 6%, n = 3, 10 min; 5-HT, 13 ± 2.3%, n = 4, 45 min; histamine = 100%). The response to histamine was antagonised by the H_1-receptor antagonist mepyramine (0.1 µM; apparent K_B = 2.9 ± 0.2 nM, n = 3) and the 5-HT response was sensitive to inhibition by ketanserin (0.1 µM; apparent K_B = 4.5 ± 1.8 nM, n = 3).

These data suggest that cultured HUASM cells represent a useful and novel system in which to investigate intracellular signalling in response to smooth muscle spasmogens in this tissue.

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368P DIFFERENTIAL ABILITY OF VARIOUS VASODILATORS TO RELAX RAT AORTAE CONTRACTED WITH NORADRENALINE, PHORBOL 12,13-DIBUTYRATE AND PHORBOL 12-MYRISTATE 13-ACETATE

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Relaxation of blood vessels by endothelium-dependent mechanisms is impaired by protein kinase C activation with phorbol esters. This effect is possibly due to inhibition of nitric oxide production (Morrison & Pollock, 1990). However, it is not clear if protein kinase C activation affects only the generation of nitric oxide, or the biochemical pathways activated by nitric oxide as well.

The present study examined the effect of protein kinase C activation on the ability of various nitric oxide-dependent vasodilators and 8-bromo-cyclic guanosine monophosphate (8Br-cGMP) to relax pre-contracted rings of rat aorta. Rat aortic rings approximately 4mm in length were mounted in an organ bath containing warmed, oxygenated Krebs-Ringer solution and maintained at a resting tension of 2.5 g. The rings were contracted with noradrenaline (0.1 μM) or either of the protein kinase C activators phorbol 12,13-dibutyrate (PDB, 0.1 μM) or phorbol 12-myristate 13-acetate (PMA, 0.5 μM). In noradrenaline pre-contracted rings both acetylcholine (0.01-10 μM) and sodium nitroprusside (0.001-0.5 μM) caused concentration-dependent and complete relaxation. However acetylcholine relaxed PDB-contracted rings to only 57.8 $\pm$ 4.7 % of the maximum observed in noradrenaline-contracted rings. The acetylcholine-induced relaxation in these rings was markedly reduced by N-nitro-L-arginine (100 μM). Sodium nitroprusside completely relaxed PDB-contracted aortic rings, however nitroprusside was less potent in these aortae, the EC_{50} being shifted from 0.015 μM to 0.42 μM. Acetylcholine and sodium nitroprusside were even less potent relaxants in PMA-contracted aortae. Acetylcholine caused a maximal relaxation of 31.6 $\pm$ 7.3 % and sodium nitroprusside to 45.6 $\pm$ 7.9 % of the possible maximum. There was no significant difference in the concentration-relaxation curves of 8Br-cGMP (10 μM-10 mM) in either noradrenaline or PDB-contracted rings. These results suggest that protein kinase C activation inhibits endothelium-dependent relaxations predominantly by inhibiting the production and actions of nitric oxide, rather than the biochemical pathways activated by nitric oxide. Furthermore, PMA is a more potent inhibitor of acetylcholine and nitroprusside-induced vasorelaxation than PDB.

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369P CHANGES IN REACTIVITY OF ISOLATED AORTA FROM YOUNG SPONTANEOUSLY HYPERTENSIVE RATS (SHRs)

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Changes have been reported in the contractile responses of the aorta from SHRs with developed hypertension (Aidulis *et al.*, 1990). Alterations in vascular reactivity in SHRs may be associated with changes in endothelial function (Luscher & Vanhoutte, 1986). We have compared contractions of the aorta from 4-5 wK. old SHRs with control (WKY) rats, in preparations stripped of endothelium, to see if the differences in reactivity reported in this vessel are present during the development of hypertension and are separate from changes in endothelial function.

Endothelium was removed mechanically from isolated aortic rings (5-7 mm) and the rings suspended between two wire triangles in Krebs' solution containing propranolol (10^{-6}M), ascorbic acid (5×10^{-5}M) and EDTA (10^{-5}M) at 37°C gassed with carbogen under a resting tension of 2g. After 1 h equilibration, responses to noradrenaline (NA) (3×10^{-6}M) or KCl (80mM) were elicited and at the peak of the contractile response acetylcholine (10^{-6}M) applied to test for the release of EDRF. Non-cumulative dose-response curves to NA or KCl were then constructed. In other experiments, tissues were depleted of available stored calcium by immersion in Ca^{2+}-free Krebs containing 10^{-4}M EGTA (Ca-EGTA) and activation with 10^{-5}M NA and then cumulative concentration-response curves to $CaCl_2$ were made in Ca-EGTA plus KCl (145mM).

Contractions of aortic rings from SHRs were less sensitive to NA than those from WKYs. This was reflected in a shift to the right of the log dose-response curve to NA with a significant change in EC_{50} (mean EC_{50} ($\pm$ s.e.m.) = SHR, $5.3\pm1.4\times10^{-8}$M, n=8; WKY, $4.4\pm1.0\times10^{-9}$M, n=8, $P<0.001$) but no significant change in E_{max} (SHR, 0.31 ± 0.05 g mg^{-1}; WKY, 0.30 ± 0.04 g mg^{-1}). In contrast, with KCl, there was a significant reduction in E_{max} in rings from SHRs compared with WKYs (SHR, 0.23 ± 0.02 g mg^{-1}, n=12; WKY, 0.31 ± 0.02 g mg^{-1}, n=18, $P<0.001$) but no significant change in EC_{50} (SHR, $6.9\pm$ 1.2mM; WKY, $6.3\pm$ 0.7mM). The results with $CaCl_2$ in depolarized tissues were similar to those for KCl. As an index of intracellular calcium release, contractions were recorded to either (1) NA (3×10^{-6}M) in Ca^{2+}-free Krebs following a 2 min exposure to Ca^{2+}-free Krebs containing 2×10^{-3}M EGTA (EGTA-resistant responses (ERR)) or (2) caffeine (CA) (2×10^{-2}M). Both CA contractions and ERR were reduced significantly in rings from SHRs. The mean ERR in rings from SHRs was 0.09 ± 0.01 g mg^{-1}, n=9 and mean contractions to CA, 0.09 ± 0.01 g mg^{-1}, n=10, whilst in rings from WKYs the mean ERR was 0.17 ± 0.02 g mg^{-1}, n=8, $P<0.001$ and mean contractions to CA were 0.14 ± 0.01 g mg^{-1}, n=8, $P<0.05$.

The results demonstrate reduced reactivity of aortic rings from young SHRs to NA and KCl which is independent of any change in endothelial function and probably associated with reduced calcium entry through potential-dependent channels and release from intracellular stores. The failure to show any change in E_{max} with NA suggests that maximum contractions to NA in aortic rings from the SHR are less dependent on these sources of calcium.

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370P α_1- AND β_2-ADRENOCEPTORS AND ENDOTHELIN RECEPTOR DENSITY IN RAT HEART AFTER ISCHAEMIA AND PROLONGED REPERFUSION

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Pretreatment of rats, subjected to 1 h of left anterior descending coronary artery ligation, followed by 8 days of reperfusion with the free radical scavenger MDL 73404 (3,4-dihydro-6-hydroxy-N,N,N,2,5,7,8, heptamethyl-2H-1-benzopyran-2-ethanaminium 4-methylbenzene-sulfonate; 3 mg/kg/h) a water soluble analogue of α-tocopherol, effectively decreased infarct size, increased cardiac output measured *in situ*, and contractility in isolated hearts. In the present study the distribution of α_1- and β_2-adrenoceptors and endothelin (ET) receptors in heart tissue of rats subjected to an ischaemia and reperfusion procedure has been measured, since a change in receptor density may be responsible for the MDL 73404-induced improvement of heart performance.

Male Sprague-Dawley rats (250 - 300 g; 4 to7 per group) were sham-operated or subjected to 1h of left anterior descending coronary artery ligation followed by reperfusion. Eight days later the rats were killed and the hearts dissected and frozen by immersion in isopentane at -70°C. Coronal sections were obtained from 8 levels along the longitudinal axis. Frozen, 10 µm sections were cut and α_1- and β_2-adrenoceptors and ET receptors were labeled by incubation with [^{3}H]prazosin, [^{125}I]pindolol or [^{125}I]ET respectively. The densities of receptors were estimated in the right and left ventricles, the septum and in the infarcted tissue by quantitative autoradiography. Significance of comparisons was assessed by a Students't test.

Receptor densities in sham-operated rats were not changed by MDL 73404 treatment. Coronary artery ligation did not produce significant changes in receptor densities except in infarcted tissue. In this region of the heart the density of α_1-adrenoceptors decreased from 10.7 ± 4.1 to 2.1 ± 1.2 fmol/mg protein (means ± SD) and ET receptor density from 512.6 ± 87 to 30.0±51.6 fmol/mg protein. (P value < 0.05). However, the density of β_2-adrenoceptors in the infarcted tissue increased from 4.3 ± 1.4 to 13.9 ± 2.2 fmol/mg protein (P < 0.05). Heart tissue from rats subjected to ischaemia and pretreated with MDL 73404 showed a slightly higher density of β_2-adrenoceptors in all regions of the heart compared to non-pretreated rats. The density of β_2-adrenoceptors in the septum (5.3 ± 0.9 fmol/mg protein) and right ventricle (6 ± 1.1 fmol/mg protein) of rats subjected to ischaemia and treated with MDL 73404 was higher (P < 0.05) compared to saline treated rats (septum 3.9 ± 0.4 fmol/mg protein; right ventricle 3.9 ± 0.8 fmol/mg protein) and to MDL 73404 pretreated sham-operated animals (right ventricle 4.1 ± 1.1 fmol/mg protein). In the same cardiac regions, treatment with MDL 73404 tended to increase α_1-adrenoceptors and ET-receptors although the differences were not significant.

Positive inotropic responses are evoked by stimulation of myocardial α_1- and β_2-adrenoceptors and ET receptors. An increased density of these receptor types in MDL 73404 pretreated rats subjected to ischaemia and reperfusion could increase sensitivity of the tissue to local catecholamines and endothelin and be a factor contributing to the improvement of left ventricular function.

371P ISOPRENALINE INFUSION IN RATS: EFFECT ON DENSITY AND NUMBER OF VENTRICULAR β_1- AND β_2-ADRENOCEPTORS

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Chronic agonist stimulation of β-adrenoceptors leads to reduction of tissue adrenoceptor density. Infusion of isoprenaline (ISO) for 6 days has been shown to reduce both β_1 and β_2-adrenoceptor density in rat heart ventricles (Lu and Barnett, 1988). The reduction in adrenoceptors is assumed to be the result of agonist desensitisation. However, it has also been shown in aortic banding experiments that while adrenoceptor density is reduced, total β-adrenoceptor number remains unchanged because of left ventricular hypertrophy (Chevalier et al, 1989). In this study we have re-examined chronic ISO infusion to determine whether reduction of β-adrenoceptor density is due to desensitisation or changes in the heart muscle induced by the treatment.

ISO (40ugkg^{-1}h^{-1}) was infused from subcutaneous Alzet mini-pumps into AP rats (250-300g) as described previously (Kowalski et al, 1990). After 6 days, the ventricles were dissected free of the atria and ventricular membranes prepared. Binding of [^{3}H]-CGP 12177 to these membranes was carried out in HEPES buffer, pH 7.4. The relative proportions of β_1 and β_2-adrenoceptor subtypes were assessed by displacement with CGP 20712 A and LIGAND analysis.

Following ISO infusion, β_1-adrenoceptor density was lowered by 33% from 44.2±0.7 to 29.7±1.1 fmolmgprotein^{-1} (mean±s.e.mean, n=11, p<0.001). In contrast, β_2-adrenoceptor density was reduced by 64% from 25.4±0.6 to 9.1±1.0fmolmgprotein^{-1} (p<0.001). Total ventricular β_1-adrenoceptors were reduced by only 18% after ISO infusion (955±18 to 780±30fmol, p<0.001) while β_2-adrenoceptors decreased by 60% (550±15 to 222±14fmol, p<0.001). ISO infusion caused changes in the ventricles indicative of hypertrophy and fibrosis. Thus the ventricular wet weight (x10^3)/bodyweight ratio increased from 3.06±0.08 to 3.62±0.08 (p<0.001) and ventricular protein increased from 21.6±0.3 to 26.4±0.9mg (p<0.001). Image analysis (Optimax V) of representative sections stained with sirius red showed that collagen content of the left ventricular wall increased from 1.10±1.01% in controls to 14.8±6.8% after ISO (mean±S.D. of 10 fields).

Thus while β_2-adrenoceptors were reduced by agonist desensitisation, the β_1-adrenoceptor population proved to be much more resilient. Analysis of the data suggests that the reduction of β_1-adrenoceptor density was due to a combination of ventricular hypertrophy and accompanying tissue necrosis and not to adrenoceptor desensitisation.

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372P ATYPICAL β-ADRENOCEPTOR ANTAGONIST PROFILE OF ICI D7114 IN GUINEA-PIG AND RAT ISOLATED ILEUM

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We have previously demonstrated that the lipolytic agent, ICI D7114, is a full agonist at the atypical β-adrenoceptor (β_3) in the guinea-pig ileum (Growcott et al., 1991). In the rat ileum, however, the compound is without agonist effects and behaves as an antagonist (Growcott et al., 1992). We suggested that these differences could possibly be explained by differences in the receptor reserve in the two tissues, rather than multiple receptor subtypes. The current study has extended the previous work by comparing antagonist affinity of ICI D7114 at the β_3-adrenoceptor in guinea-pig and rat ileum.

Inhibition-response curves were constructed to isoprenaline and BRL37344 in histamine (1.0μM) and carbachol (0.5μM) pre-contracted guinea-pig and rat ileum respectively as previously described (Growcott et al., 1991 and 1992). Antagonist effects of ICI D7114 (0.01-10.0μM) were then assessed against isoprenaline and BRL37344 and expressed as pA_2 values derived from concentration ratios.

When 0.5μM histamine was used to pre-contract tissues, ICI D7114 (10μM) produced full agonist efficacy in the guinea-pig ileum. However, when higher concentrations (1-10μM) of histamine were employed, ICI D7114 (1 and 10μM) failed to exhibit full efficacy and reduced the pre-contractile responses to histamine by 21±2 and 33±3 % respectively. Under these latter conditions, in the guinea-pig ileum ICI D7114 behaved as an antagonist of the inhibitory responses to isoprenaline and BRL37344 (pA_2 values 6.7±0.1 and 7.0±0.1 respectively). In the rat ileum, where ICI D7114 is without agonist efficacy, a similar degree of antagonism of isoprenaline and BRL37344 was observed (pA_2 values 6.4±0.1 and 6.7±0.4 respectively). These latter values were not different from those obtained in the guinea-pig ileum with ICI D7114.

Based on the hypothesis of Kenakin et al (1980) the current study has demonstrated that manipulation of the receptor reserve can convert the full agonist effect of ICI D7114 to an antagonist effect. The site of action of ICI D7114 is likely to be at the β_3-adrenoceptor as experiments were carried out in the presence of high concentrations (1μM) of β_1 (CGP 20712A), β_2 (ICI 118551) and α_1 (prazosin) - adrenoceptor antagonists. Moreover, it would appear, from the current data, that a single population of atypical adrenoceptors can account for the observed effects.

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373P REGULATION OF RAT CARDIAC β-ADRENOCEPTOR DENSITY AND COUPLING TO ADENYLATE CYCLASE FOLLOWING TREATMENT WITH XAMOTEROL OR ISOPRENALINE *IN VIVO*

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Chronic therapy with the β_1 selective adrenoceptor (AR) partial agonist xamoterol in heart failure is not associated with the tachyphylaxis seen with full agonists. In a rat model, chronic infusion with xamoterol does not downregulate cardiac β ARs (Kowalski *et al*, 1990). Since β_1 AR coupling to adenylate cyclase (AC) is difficult to demonstrate in human heart, we have used this rat model to examine the subtype changes in receptor coupling seen following chronic xamoterol therapy and compared it to the full agonist isoprenaline.

Male AS rats were infused for 6 days by subcutaneous osmotic minipumps with either xamoterol, 400µg/kg/hr (X, n=6) or isoprenaline 40µg/kg/hr (I, n=6) with sham operated rats as controls (S, n=6). Left ventricular membranes were assayed for β AR density using [^{125}I]-iodopindolol (Bmax, fmols/mg protein) and AC activity (pmols cyclic AMP/mg protein/minute). The relative contributions of β AR subtypes to AC stimulation were determined by the inhibition of 1µM isoprenaline stimulated AC activity by the highly β_1 selective antagonist CGP 20712A. Data are given as means +/- sd. The Mann Whitney test was used to compare data between groups.

Total β AR density was significantly reduced in group I (p=0.005), but not X (I=14.93+/-1.41; X=22.23+/-2.95; S=24.89+/-1.56). The ratio of β_1 to β_2 ARs was higher (p=0.005) in group I, but unaltered in X (β_1 ARs as % of total : I=82.50+/-3.15; X=72.83+/-1.29; S=70.93+/-2.58).

Compared to sham treated animals, maximal (10µM) isoprenaline stimulation was significantly reduced in both group I and X (I=21.94+/-2.67*; X=37.41+/-3.95$^+$; S=44.57+/-3.85; *p=0.005, $^+$p=0.02 compared to S). The β_1 AR contribution to isoprenaline stimulation was less in group X (p=0.013), but not I (β_1 AR contribution % of total : I=69.68+/-2.38; X=59.93+/-2.19; S=67.15+/-4.17).

In conclusion we have confirmed that chronic isoprenaline infusion downregulates both β_1 and β_2 ARs in rat heart and that this is associated with reduced stimulation of adenylate cyclase through both subtypes. Chronic therapy with the β_1 selective partial agonist xamoterol however does not downregulate rat cardiac β ARs but selectively uncouples the β_1 AR subtype.

Kowalski et al (1990) *British Journal of Pharmacology* 99, 27-30

374P ISOPRENALINE INHIBITS THROMBOXANE B_2 RELEASE FROM U937 CELLS

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The U937 human monocytic cell line (monos) and its differentiated macrophage-like form (macs) have been well characterised and have been used to investigate macrophage metabolism and function (Harris and Ralph, 1985). Recently, the U937 cell line has been shown to possess ß-adrenoceptors (Liggett, 1989). However, it has been demonstrated that the ß-adrenoceptors on the human airway macrophages are non-functional (Fuller et al, 1988). In this study the release of thromboxane (TXB_2) by the U937 cell line before and after differentiation was investigated following a particulate challenge with opsonised zymosan (OPZ) in the presence or absence of isoprenaline.

The U937 cells were grown in a humidified atmosphere of 95% air, 5% CO_2 at 37°C. Differentiation of the monocytic cells was achieved by the addition of 20% conditioned medium to the growth media for 48h. Both undifferentiated and differentiated cells were incubated with and without OPZ (2mg/ml) in the absence or the presence of either flurbiprofen (n=5) or isoprenaline (n=6), under the above conditions for 1h. The TXB_2 released was assessed by radioimmunoassay.

OPZ (mg/ml)	monos	macs
0.5	29±11	28±18
1.0	73±23	81±41
2.0	99±31	128±6
3.0	158±35	266±45
5.0	143±27	315±26

[Flurbiprofen]M	monos	macs
0	101±21	179±23
10^{-8}	45±8	56±17
10^{-7}	19±8	31±8
10^{-6}	8±3	27±9
10^{-5}	16±5	29±7
10^{-4}	20±8	19±5

[Isoprenaline]M	monos	macs
0	108±12	394±77
10^{-10}	99±11	384±82
10^{-8}	83±12	321±80
10^{-6}	75±5	221±52
10^{-4}	79±7	202±40

TXB_2 pg/10^5 cells (mean ± s.e. mean)

The monocytic and macrophage-like cells released TXB_2 in a dose-dependent manner following challenge with OPZ with the release greater in the macrophage-like cells. The TXB_2 released was inhibited by flurbiprofen ($> 10^{-8}$ M; $p < 0.01$) and isoprenaline ($> 10^{-6}$ M; $p < 0.01$). The inhibition by isoprenaline was reversed by propranolol (10^{-6} M).

The data demonstrate that the human monocytic cell line and its inducible macrophage-like form have functional ß-adrenoceptors, in contrast to the human airway macrophage.

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375P α_1-ADRENOCEPTOR SUBTYPE(S) IN RAT THORACIC AORTA DEFINED USING ABANOQUIL AND OTHER SELECTIVE ANTAGONISTS

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The α_1-adrenoceptor subtype(s) responsible for the noradrenaline (NA) induced contraction of rat thoracic aorta is unclear. In this tissue the mRNA for the α_{1A}-subtype is present with no α_{1B} mRNA detected (Lomasney et al, 1991) and no α_{1C} mRNA has been reported in rat tissues (Schwinn et al, 1991). Functional studies have disagreed about the α_1-subtype mediating NA contractions. Recently several compounds, including the α_{1A}-subtype selective antagonist abanoquil (UK-52,046, Greengrass *et al.*, 1991), have been used to functionally define receptor sub-types in smooth muscle (Marshall et al, 1992). In this study the effects of abanoquil and other compounds have been used to examine the α_1-adrenoceptor subtype(s) in rat thoracic aorta.

Thoracic aortae were removed from male Sprague Dawley rats (300-400g) and cut into 4-5mm rings. The endothelium was then removed and the rings were mounted in organ baths for the recording of isometric tension. Cumulative dose-response curves were obtained to NA (10^{-10}-10^{-3}M, pD_2 8.0±0.1) before and after 30 min equilibration with antagonists. In the case of the alkylating agent CEC, the tissues were washed for 30 minutes following equilibration. The NA contraction curve was shifted non-competitively by abanoquil (10^{-9} and 10^{-8}M) to the right by 10 and 100-fold with a 28±8% (mean±sem) and 86±4% reductions in maximal response. CEC (10^{-5} and $3x10^{-5}$M) reduced the maximal response to NA (up to $3x10^{-4}$M) by 78±3% and 90±3% respectively. However, the effects of this irreversible antagonist were overcome by an increase in NA concentration to 10^{-3}M, reaching 73-85% of the original NA maximal response. The competitive antagonists WB4101 and prazosin gave pA_2 values of 9.8 and 10.4 (slopes 1.2±0.2 and 1.1±0.3) respectively.

The α_1-adrenoceptor responsible for NA-induced contractions in rat thoracic aorta is in part sensitive to CEC (α_{1B}- and α_{1C}-subtypes), WB4101 has a high potency ($\alpha_{1C} > \alpha_{1A} > \alpha_{1B}$) and abanoquil is effective ($\alpha_{1A} >> \alpha_{1B} = \alpha_{1C}$). One explanation is that another receptor subtype may be present which shares the properties of the three currently identified α_1-adrenoceptors. However, it is also possible that α_{1A}- and α_{1B}-adrenoceptors are present and both are required to obtain maximal contractions.

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376P CHARACTERISATION OF α-ADRENOCEPTORS IN THE HIND LIMB VENOUS SYSTEM OF THE HORSE

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Equine laminitis (EL) is a common vascular problem in veterinary medicine. It is now accepted that decreased capillary blood flow to the sensitive laminae underlies EL. A number of α_2-adrenoceptor agonists are used as sedative drugs in the horse (Short *et al*, 1986), including horses at risk of developing laminitis, but only limited studies have been undertaken to characterise the α-adrenoceptors in equine limb blood vessels.

Rings of equine digital and saphenous veins (EDV & ESV) were isolated from hind limbs of mixed breed adult horses, rubbed to remove the endothelium and suspended under 1 to 2 g tension in Krebs-Henseleit solution. Adrenoceptor antagonists (prazosin, 1 nM to 0.1 μM; RX 821002 [2-[2-(2-methoxy-1,4-benzodioxanyl)] imidazoline] 0.01 μM) were added 30 min prior to the addition of agonists. Cumulative dose response curves (CRCs) were constructed to phenylephrine (PE; 0.01 μM to 0.1 mM), BHT 920 (6-Allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d) azepine; 0.01 μM to 1 mM) and noradrenaline (NA), 1 nM to 0.1 mM) in the presence of propranolol (1 μM) and cocaine (3μM). The effects of prazosin (0.1 to 10 nM), RX 821002 (0.01 to 0.3 nM) and BHT-920 (1 to 100 nM) on electrical field stimulation (EFS; 2 ms pulse duration, 20 V, for 1 s at 4 to 50 Hz) of ESV rings were also studied.

Removal of the endothelium did not alter responses to PE or BHT-920 (n=7). Prazosin (0.03μM) inhibited the responses to PE and NA increasing the EC_{50} values by 9.6 ± 2.0 (n=4) and 11.3 ± 2.9 (n=7) fold respectively. Prazosin (0.03 μM) only inhibited the responses to high doses of BHT 920 producing a biphasic CRC (n = 5). RX 821002 (0.01 μM) caused a small increase in the EC_{50} value of NA (2.06 ±0.59 fold), markedly inhibited responses to low doses of BHT-920 and did not affect responses to PE (n=4). EFS frequency-related contractions were inhibited by prazosin, particularly at high frequencies. RX 821002 (0.01 nM to 0.3 nM) significantly enhanced the response to EFS, particularly at low frequencies and BHT-920 (1 nM to 0.1 μM) significantly reduced the responses to EFS.

EDV have postsynaptic α_1 and α_2-receptors, both mediating vasoconstriction. α_2-receptors play only a small part in the constrictor response to NA. No evidence was found for α-receptor-mediated release of endothelially-derived vasodilators in EDV. Our results in ESV are consistent with the presence of inhibitory presynaptic α_2-receptors.

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377P LONG-TERM TREATMENT WITH CLORGYLINE ACCELERATES THE TURNOVER OF BRAIN α_2-ADRENOCEPTORS IN NORMOTENSIVE (WKY) BUT NOT IN HYPERTENSIVE (SHR) RATS

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Long-term treatment with desipramine down-regulates the density of α_2-adrenoceptors (α_2R) in the rat brain and accelerates the turnover of the receptor with a marked increase in its degradation rate (Barturen & García-Sevilla, 1992). The increased α_2R degradation which appears to be the result of the sustained activation of α_2R by the endogenous agonist noradrenaline (NA) after inhibition of neuronal uptake by desipramine, likely explains the induction of down-regulation of brain α_2R. In spontaneously hypertensive rats (SHR) the specific binding of [^{3}H]clonidine and [^{3}H]UK 14,304 (bromoxidine) to brain α_2R as well as clonidine-induced mydriasis (α_2R functional response in the CNS) are reduced when compared to those in sex- and age-matched normotensive Wistar-Kyoto rats (WKY) (Olmos et al., 1991 and unpublished data). Therefore, the SHR offers a genetic model of α_2R down-regulation in the brain to further assess biochemical events associated with this relevant receptor regulatory mechanism. The aim of this study was to quantitate and compare the turnover of α_2R in the cerebral cortex of WKY and SHR, and its modulation during treatment with clorgyline (1mg/kg, i.p. for 21 days), a MAO inhibitor which increases the availability of NA and induces down-regulation of brain α_2R. The recovery of cortical [^{3}H]bromoxidine specific binding (Bmax, defined with 10 μM adrenaline) after almost complete irreversible inactivation (>95% at 6 h) of α_2R by the peptide coupling agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (1.6 mg/kg) was assessed in control and clorgyline-treated rats (EEDQ was given at day 7 and treatment was continued until day 21). Recovery data were fitted (GraFit) to the equation $[R_t]=r/k\ (1-e^{-kt})$ (Mauger et al., 1982), where $[R_t]$ represents the receptor number (Bmax) at a given discrete time t; r is the appearance ("synthesis") rate constant and k is the disappearance ("degradation") rate constant of the receptor that allows to estimate the apparent half-life of the receptor ($t_{1/2}=\ln 2/k$). The ratio r/k represents the density of α_2R at steady-state towards the system tends after irreversible inactivation of the receptors. The simultaneous analysis of recovery curves revealed that the turnover of α_2R in WKY ($t_{1/2}$=8.1±1.5 days; r/k=54 fmol/mg protein) was significantly different of that in SHR ($t_{1/2}$=4.9±0.9 days; r/k=40 fmol/mg protein) ($F[2,54]=3.76$, P=0.03) and that the reduced density of brain α_2R (Bmax or r/k values) in SHR was probably due to an abnormal higher receptor degradation (Δk=66%, $F[1,54]=3.19$, P=0.08) and not to a decreased receptor synthesis (Δr=24%, $F[1,54]=1.34$, P=0.25). Treatment with clorgyline accelerated the turnover of brain α_2R in WKY ($t_{1/2}$=2.1±0.7 days; r/k=29 fmol/mg protein) and the greater increase in receptor degradation (Δk=285%, $F[1,42]=12.7$, P=0.0009) over receptor synthesis (Δr=109%, $F[1,42]=8.53$, P=0.0055) led to down-regulation of receptor density (r/k: 46% lower). In contrast, treatment with clorgyline did not modify the turnover of brain α_2R in SHR ($t_{1/2}$=3.6±1.0 days; r/k=39 fmol/mg protein) ($F[2,42]=0.78$, P=0.46), indicating that these genetically desensitized α_2R cannot be further down-regulated by clorgyline treatment and that they lack the expected adaptive increase in receptor synthesis.

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378P CHARACTERIZATION OF HUMAN α_2-ADRENERGIC RECEPTOR STABLY EXPRESSED IN TRANSFECTED CHINESE HAMSTER OVARY CELL-LINES

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Based upon ligand binding studies four pharmacologically-distinct subtypes of α_2-adrenergic receptors (α_2-ARs) have been postulated (Simonneaux *et al.*, 1991). α_2-AR heterogeneity has been confirmed by molecular biological techniques in that three cDNAs encoding structurally-distinct human α_2-ARs have been cloned and shown to reside on different chromosomes (α_2-C10, α_2-C4 and α_2-C2; Lomasney *et al.*, 1990). For the present study, we have performed a detailed pharmacological characterization of two of these gene products (α_2-C10 and α_2-C2 AR) expressed in transfected chinese hamster ovary (CHO) cell-lines using ^{3}H-rauwolscine as a probe.

Transfected CHO cells which stably express either α_2-C10 or α_2-C2 ARs were grown in monolayer culture and harvested between passage numbers 1-13. ^{3}H-Rauwolscine binding to cell membrane preparations was performed at 25°C and 10 µM phentolamine was used to define non-specific binding. Equilibrium saturation analyses of ^{3}H-rauwolscine binding to α_2-C10 and α_2-C2 transfected cell membranes indicated the binding of this radioligand to single populations of non-interacting sites (K_d= 0.99±0.11 nM for α_2-C10 and K_d= 1.80±0.11 nM for α_2-C2, n=3). The potencies of a number of drugs to compete with ^{3}H-rauwolscine for these α_2-ARs were determined (Table 1). A highly significant correlation (r=0.925, p<0.05) was obtained upon comparing the pKi values of drugs to inhibit ^{3}H-rauwolscine binding to the α_{2A}-AR cell-line HT29 (Simonneaux *et al.*, 1991) and the α_2-C10 cells. A similar analysis of the pharmacological profile of ^{3}H-rauwolscine binding to the α_{2B}-AR subtype of rat neonatal lung (Simonneaux *et al.*, 1991; Bylund *et al.*, 1992), on the other hand, exhibited a good correlation (r=0.978, p<0.05) with the α_2-C2 cells.

Table 1 Inhibition by selected drugs of ^{3}H-rauwolscine binding to membrane preparations of transfected CHO cells

Compound	pKi	
	α_2-C10 AR	α_2-C2 AR
Yohimbine	9.11 ± 0.04	8.20 ± 0.03
SL 86.0715	9.00 ± 0.03	8.71 ± 0.02
Rauwolscine	8.77 ± 0.02	8.65 ± 0.02
SL 84.0418	8.74 ± 0.03	8.58 ± 0.02
Idazoxan	8.32 ± 0.05	7.51 ± 0.04
Spiroxatrine	7.95 ± 0.04	8.97 ± 0.01
Guanfacine	7.56 ± 0.02	5.88 ± 0.16
ARC 239	6.22 ± 0.29	7.90 ± 0.02

Values represent means ± s.e.m. of at least 3 experiments.

These results therefore provide evidence that the α_2-C10 gene product exhibits the pharmacological profile of an α_{2A}-AR, whereas that of the α_2-C2 corresponds to an α_{2B}-subtype.

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379P ACTIVATION OF β_1- OR β_2-ADRENOCEPTORS OPENS PLASMALEMMAL K^+ CHANNELS IN AIRWAYS SMOOTH MUSCLE: EVIDENCE FROM MECHANICAL AND ELECTROPHYSIOLOGICAL STUDIES IN GUINEA-PIG TRACHEALIS

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Isoprenaline promotes K^+-channel opening in rabbit trachealis muscle (Kume et al., 1989). However, studies of salmeterol, an agonist highly selective for β_2-adrenoceptors, indicate that this agent does not mimick isoprenaline in opening K^+-channels. In contrast to isoprenaline, salmeterol failed to hyperpolarize guinea-pig trachealis (GPT) and failed to promote $^{86}Rb^+$ efflux from bovine trachealis (Cook & Small, 1992). The hypothesis (Cook & Small, 1992) that the activation of β_1- but not β_2-adrenoceptors can provoke K^+-channel opening has been tested in studies of $^{86}Rb^+$ efflux using antagonists selective for the β_1- and β_2-adrenoceptor respectively and does not seem to be valid (Chiu et al., 1992).

In the present study, CGP 20712A (1µM) antagonized noradrenaline (>100 fold) in increasing beat frequency in the guinea-pig atria but did not antagonize isoprenaline, procaterol or salmeterol in suppressing the spontaneous tone of GPT. ICI 118551 (100nM) caused >50-fold antagonism of procaterol and salmeterol in suppressing the spontaneous tone of GPT but did not antagonize noradrenaline in increasing atrial rate. Microelectrode recording of membrane potential changes in GPT (Dixon & Small, 1983) showed that the relaxant effect of isoprenaline (100nM) was accompanied by marked hyperpolarisation (13.6 ± 1.3mV, measured after 4 min drug contact; n=8). CGP 20712A (1µM) reduced the ability of isoprenaline (100nM) to hyperpolarize the tissue (5.8 ± 1.4 mV; n=9) but not its ability to suppress mechanical tone. In contrast, ICI 118551 (100nM) reduced the ability of isoprenaline both to hyperpolarize the tissue (-0.4 ± 0.8mV; n=7) and to suppress mechanical tone. Tested in the absence of antagonists, procaterol (10nM) reduced the spontaneous tone of the trachea and caused cellular hyperpolarisation (6.8 ± 2.4mV, measured after 6 min drug contact; n=9). Our electrophysiological findings and the results of $^{86}Rb^+$ efflux studies (Chiu et al., 1992) collectively suggest that the activation of either the β_1- or the β_2-adrenoceptor can promote K^+-channel opening.The failure of salmeterol to hyperpolarize trachealis muscle or to promote $^{86}Rb^+$ efflux cannot be attributed to its selective activation of β_2-adrenoceptors and indicates that K^+-channel opening is not crucial for the relaxant activity of agonists at β-adrenoceptors. The latter idea also receives support from our finding that CGP 20712A can reduce the hyperpolarization induced by isoprenaline but not its mechano-inhibitory effects.

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380P α_1-, α_2- AND β-ADRENOCEPTOR SUBTYPES IN HUMAN AND RAT KIDNEY

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α- and β-adrenoceptors (AR) play an important role in sympathetic regulation of renal function. In order to evaluate whether findings on renal AR obtained in the rat may be comparable with those in humans in this study we compared the abundance of α_1-, α_2- and β-AR subtypes in rat and human kidney.

Human renal cortex was obtained from patients undergoing nephrectomy because of hypernephroma. Rat kidneys were obtained from male Sprague-Dawley rats (weighing 200-250 g). α_1-AR were assessed by [^{3}H]prazosin binding, α_{1A}- and α_{1B}-AR subtypes from competition curves of (+)-niguldipine and 5-methyl-urapidil with [^{3}H]-prazosin binding (Michel et al., 1992). α_2-AR were determined by [^{3}H]rauwolscine binding, α_{2A}- and α_{2B}-AR subtypes from competition curves of ARC-239, prazosin and oxymetazoline with [^{3}H]rauwolscine binding (Michel et al., 1992). β-AR were determined by (-)-[^{125}I]iodocyanopindolol binding, β_1- and β_2-AR subtypes from competition curves of ICI 118,551 and CGP 20712 A with (-)-[^{125}I]iodocyanopindolol binding (Michel et al., 1987). All competition curves were analyzed by nonlinear regression analysis with the iterative curve-fitting program InPlot (GraphPAD software, San Diego, CA). Maximal renal AR-number was assessed from Scatchard-plots of saturation experiments.

In rat kidney maximal AR number was α_2- (94±7fmol/mg protein, n=9) > α_1- (48±7fmol/mg, n=8) > β-AR (22±5 fmol/mg, n=8). Competition experiments revealed that in rat kidney α_{1A}- and α_{1B}-AR coexist with an α_{1A}/α_{1B}-ratio of 40:60 %; α_2-AR appeared to be mostly of the α_{2B}-subtype but a small amount of α_{2A}-AR (<10 %) could not completely be excluded; the β-AR population consisted of both β_1-and β_2-AR with a β_1/β_2-ratio of 60-70: 30-40 %.

In human kidney maximal AR-number was α_2- (71±11fmol/mg, n=5) > β-AR (10±5fmol/mg, n=6), while we were not able to clearly demonstrate α_1-AR. Competition experiments revealed that the α_2-AR were predominantly of the α_{2A}-subtype although a small amount of α_{2B}-AR was detectable (α_{2A}/α_{2B}-ratio = 80:20 %); the β-AR population consisted of both β_1- and β_2-AR but the β_2-AR subtype predominated (β_1/β_2-ratio = 30-40:60-70 %).

These data clearly show that the relative proportion of α_1-, α_2- and β-AR subtypes in rat kidney is markedly different from that in human kidney.

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381P ACTIONS OF ICI D7288 ON SINOATRIAL NODE CELLULAR ELECTROPHYSIOLOGY: WHOLE-CELL CURRENTS

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ICI D7288 (4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride) is a new bradycardic agent (Hargreaves et al., 1992; Marshall et al., 1992). Its actions have been compared with the effects of the known bradycardic agents alinidine and UL-FS 49 (zatebradine) on the whole-cell currents of single dissociated sinoatrial node (SAN) cells in vitro.
SAN cells were dissociated from guinea pig tissues using the method described by Denyer & Brown (1990). Whole-cell patch clamp was used to record membrane currents evoked by voltage clamp step protocols, and the effects of the drugs were investigated.
The principal effect of ICI D7288 was to inhibit the hyperpolarisation activated cationic current (I_f) of the SAN cells. Concentrations of 0.1, 0.3 and 1.0 μM ICI D7288 inhibited the I_f current by 47 ± 5% [10], 67 ± 4% [11] and 77 ± 4% [9] respectively (data are expressed as mean ± SE [n]). These concentrations of ICI D7288 also affected the rate of diastolic depolarisation in isolated SAN tissue preparations (Briggs & Heapy, 1992). In 3 cells where action potentials were also examined, the effect on I_f was accompanied by a slowing of the spontaneous action potential firing rate and decreased rate of diastolic depolarisation. Effects on other currents tested (delayed outward rectifier (I_k) and inward rectifier (I_{k1}) currents) were absent or relatively minor (e.g. I_k: -12 ± 8% [8] for 0.3 μM; I_{k1}: -9 ± 5% [3] for 1μM) at the concentrations which markedly decreased I_f.
Alinidine (10 μM) and UL-FS 49 (0.3 μM) also decreased I_f (51 ± 5% [8] and 37 ± 4% [11] respectively), but reductions of I_k (45 ± 11% [6] and 33 ± 7% [11] respectively) were also observed at these concentrations, which were significantly greater than the effects of 0.1 μM ICI D7288 (alinidine: $P < 0.01$; UL-FS 49: $P < 0.005$). This would explain the greater prolongation of the action potential duration of SAN cells by alinidine and UL-FS 49 than by ICI D7288 (Briggs & Heapy, 1992).
In inhibiting I_f, ICI D7288 resembles the other selective bradycardic agents examined, but it appears to have a degree of selectivity for I_f which the other agents lack, as they have greater inhibitory effects on the delayed rectifier current I_k in addition to their actions on I_f.

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382P ACTIONS OF ICI D7288 ON SINOATRIAL NODE CELLULAR ELECTROPHYSIOLOGY: ACTION POTENTIALS

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ICI D7288 (4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride) is a new bradycardic agent (Hargreaves et al., 1992; Marshall et al., 1992). Its effects have been compared with the effects of the known bradycardic agents alinidine and UL-FS 49 (zatebradine) on the action potentials of cells in guinea pig isolated sinoatrial node (SAN) tissues.
SAN tissues from young male guinea pigs (200 - 400g) were pinned to the base of a perfusion dish and perfused with oxygenated Tyrode's medium at 5 $ml.min^{-1}$ and 33°C. Intracellular action potentials were recorded continuously from cells in the tissue with initial mean diastolic depolarisation rates greater than 85 $mV.s^{-1}$. Drugs were administered by addition to the medium perfused through the dish, and action potential characteristics were measured at 1 minute intervals. At least 30 minutes equilibration time was allowed for each concentration in cumulative dose-response curves.
ICI D7288 (10nM - 3μM) slowed the rate of spontaneous action potential firing predominantly by slowing diastolic depolarisation (-61 ± 5% [6] at 300 nM), with small but significant effects (+10 ± 2% [6]; $P < 0.01$) on the action potential duration (APD). (Data are shown as mean ± SE [n]).
Both alinidine and UL-FS 49 also decreased diastolic depolarisation rate (-56 ± 3% [4] at 30 μM, and -60 ± 10% [3] at 1 μM respectively) and they both also caused significant prolongations of the APD: alinidine by +17 ± 3% [4] at 30 μM ($P < 0.05$), and UL-FS 49 by +31 ± 6% [3] at 1 μM ($P < 0.001$).
These data suggest that the effects of ICI D7288 on the SAN pacemaker cells might be mediated by an inhibitory action on the so-called "pacemaker current" (I_f) which is activated during diastolic depolarisation. This action would reduce the diastolic depolarisation rate and would account for the slowing of action potential firing of the pacemaker. This possibility has been investigated and the results are shown in the adjacent poster.

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383P HAEMODYNAMIC ACTIONS OF ICI D7288, A NOVEL SINO-ATRIAL NODE MODULATOR

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The haemodynamic profile of ICI D7288, (4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride), a novel heart rate modulating compound was defined in six open-chest, pentobarbitone-anaesthetised beagle dogs of the Alderley Park strain.

Measurements were made of heart rate (HR), aortic blood pressure (P_{Ao}), stroke volume (StV) from ascending aortic arch flow and contractile function as maximal left ventricular dP/dt. Cardiac output and systemic resistance were computed from P_{Ao}, StV and HR. Measurements were made at intrinsic and paced heart rates (180 min-1 in all animals) under control conditions and after ICI D7288, 0.02 to 1.0 $mg.kg^{-1}$ i.v., administered at 10 minute intervals as a cumulative dose:response curve. Mean results (±s.e.mean), with values for paced HR given in brackets, are summarised in the following table (* indicates $P<0.05$ by analysis of variance):

	Before ICI D7288	After ICI D7288 0.1 $mg.kg^{-1}$	After ICI D7288 0.5 $mg.kg^{-1}$
HR min^{-1}	151.7 ±9.1 (180.9 ±1.1)	105.1 ±5.2 * (180.2 ±0.5)	83.9 ±4.8 * (180.2 ±0.5)
P_{Ao} systolic mm Hg	128.5 ±3.5 (130.9 ±4.2)	133.3 ±4.6 (135.7 ±3.8)	131.2 ±5.5 (141.0 ±5.2)
P_{Ao} diastolic mm Hg	85.5 ±4.3 (93.0 ±3.2)	79.5 ±4.0 (93.8 ±2.3)	72.6 ±4.0 (98.5 ±2.7)
dP_{LV}/dt $mm\ Hg.s^{-1}$	2572 ±150 (2915 ±163)	2484 ±103 (3194 ±122)	2084 ±84 * (3089 ±104)
St.V. ml	13.0 ±1.5 (11.3 ±1.0)	16.8 ±1.3 (12.1 ±0.9)	18.4 ±1.4 * (12.1 ±0.7)
Cardiac Output $l.min^{-1}$	1.9 ±0.1 (2.0 ±0.2)	1.7 ±0.1 (2.2 ±0.2)	1.5 ±0.1 * (2.2 ±0.1)
Systemic Resistance $dyne.s.cm^{-5}$	4888 ±365 (4801 ±343)	5380 ±311 (4605 ±331)	5939 ±343 (4730 ±318)

ICI D7288 was found to reduce HR without direct depression of ventricular function; other haemodynamic changes observed appeared to be indirect and secondary to HR changes. The properties shown by D7288 - negative chronotropism without negative inotropism - suggest that this compound could be of therapeutic use in the treatment of ischaemic heart disease.

384P REDUCTION OF MYOCARDIAL INFARCTION IN THE FERRET BY INHIBITION OF PURINE TRANSPORT USING S-(P-NITROBENZYL)-6-THIOINOSINE (NBII)

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Elevation of endogenous adenosine by intracoronary or intravenous infusion of adenosine or infusion of adenosine analogues results in cytoprotective effects in models of myocardial ischaemia (Velassco et al, 1991; Norton et al, 1991). In the present study we have investigated the cytoprotective effect of enhancement of endogenous adenosine, with the purine transport inhibitor NBTI, in a model of ischaemia and reperfusion.

Ferrets (male, 1 - 1.5kg) were anaesthetised with sodium pentobarbitone (40 mg kg^{-1}, i.p.) and prepared for occlusion of the left anterior descending (LAD) coronary artery through a left thoracotomy. The animals were artificially ventilated and maintained under anaesthesia by infusion of sodium pentobarbitone (12 - 24 mg hr^{-1}, i.v.). Ferrets were randomly allocated to one of five groups. Groups I - III underwent 90 min ischaemia followed by 4 hours reperfusion. Groups IV and V underwent ischaemia only. Groups I (n = 11) and IV (n = 5) received vehicle; Groups II (n = 6) and V (n = 5) received NBTI, 0.5 mg kg^{-1} (i.v.), prior to LAD occlusion and group III (n = 6) received NBTI prior to reperfusion. Infarct size (INF) was measured using triphenyltetrazoium chloride staining and related to area at risk (AAR). In separate groups neutrophil infiltration of the myocardium was assessed by measurement of myeloperoxidase activity (Bradley et al, 1982) and regional myocardial blood flow using coloured microspheres. Mean (± se) data for AAR and INF are given in the table. The Students unpaired t-test was used for comparison of the drug treated group with the relevant control group.

	GROUP				
	I	II	III	IV	V
AAR/LV (%)	54.0±3.9	50.0±2.8	52.9±3.8	42.6±5.2	45.8±6.4
INF/AAR (%)	84.0±1.7	71.4±3.7	77.7±3.2	59.2±3.7	60.5±5.9
		P<0.001	ns		ns

A small beneficial effect of NBTI upon INF/AAR was observed. No increase in myocardial blood flow to the AAR during ischaemia (0.09 ± 0.04 vs 0.25 ± 0.08 ml min^{-1} g^{-1}) or reperfusion (0.96 ± 0.19 vs 1.51 ± 0.67 ml min^{-1} g^{-1}) was observed. Additionally, no reduction in neutrophil infiltration was detected in the AAR (MPO activity 328 ± 47 vs 359 ± 142 units g wet weight $tissue^{-1}$). The results of this study show that the cytoprotective effect of purine transport inhibition with NBTI was small and seen only if administered prior to ischaemia. The effects were independent of effects on haemodynamics or neutrophil infiltration.

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385P NITRENDIPINE- AND BEPRIDIL-INDUCED CALCIUM RELEASE FROM HEART MITOCHONDRIA ASSOCIATED WITH DEPOLARISATION OF THE MEMBRANE

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Various workers have shown that various Ca^{2+} modifying compounds can alter mitochondrial Ca^{2+} handling by inhibition or stimulation of uptake or release (Baydoun et al., 1990;Matlib and McFarland, 1991). The aim of this study was to investigate such properties further.

Rat heart mitochondria were prepared by a standard homogenisation and differential centrifugation technique, using Nagarse ® to release interfibrillar mitochondria. Ca^{2+} movements were monitored using a Corning Ca^{2+}-selective electrode in a reaction medium containing 2500 μmol sucrose, 50 μmol succinate, 20 μmol potassium dihydrogen orthophosphate and 50 μmol Tris-HCl (pH 7.4). Mitochondrial membrane potential (ψ) was monitored using a TPP^{+}-selective electrode; the reaction medium was similar to that used for Ca^{2+} transport studies except that 2 μmol $MgCl_2$, 100 nmol EDTA and 10 nmol $TPP^{+}Cl^{-}$ were also present. (Kamo et al., 1979).

Additions of bepridil (10-100 μM) to mitochondria pre-loaded with 184 ± 26.2 nmol Ca^{2+} mg $protein^{-1}$, stimulated an immediate release of the cation from the matrix to the external medium, producing an EC_{50} value of 46.6 ± 0.9 μM. Nitrendipine (55-70 μM) also caused a pre-stimulated release of Ca^{2+}, however in contrast to bepridil this effect was not immediate, although the time for which mitochondrial Ca^{2+} was retained within the matrix, was reduced form 13.2 ± 0.6 to 6.9 ± 1.0 min ($n=4; p<0.05$). Nitrendipine and bepridil (10-100 μM) also produced a concentration-related depolarisation of the mitochondrial membrane. In the presence of 50 μM nitrendipine, ψ fell from -202.8 ± 1.4 to -196.4 ± 1.75 mV ($n=4; p<0.05$) whereas the same concentration of bepridil, produced a reduction in ψ from -201.0 ± 2.8 to -143.0 ± 10.1 mV ($n=6; p<0.001$).

In the presence of 65 μM nitrendpine the addition of $BaCl_2$ (50 μM), a known inhibitor of Na^{+}-independent Ca^{2+} release (Luckacs and Fonyo, 1985), resulted in retention time being increased from from 6.9 ± 1.0 to 11.4 ± 1.5 min ($n=4; p<0.05$).

The virtual collapse of ψ in the presence of 50 μM bepridil, associated with significant Ca^{2+} release suggests that the latter is unlikely to be mediated through a specific extrusion carrier, the same can not be concluded for nitrendipine. However, the inhibition of nitrendipine-induced Ca^{2+} release by $BaCl_2$ suggests that at least part of the release occurs through a Na^{+}-independent mechanism (Luckacs and Fonyo, 1985), for example, the H^{+}/Ca^{2+} antiporter. Bepridil is more likely to cause a non-specific increase in permeability to Ca^{2+} which results in Ca^{2+} release either through a reversed uniporter or by activation of the Ca^{2+}-dependent pore mechanism described by Crompton and Costi (1988).

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386P ACYL COMPOUND-MEDIATED MODULATION OF Ca^{2+} TRANSPORT IN RAT HEART MITOCHONDRIA *IN VITRO*

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Inhibition of mitochondrial β-oxidation as a result of ischaemic insult leads to the accumulation of long chain acyl-compounds in the intracellular compartment (Idell-Wenger *et al.*, 1978). *In-vitro* studies involving acyl compounds (ie. palmitoyl carnitine; PC) have demonstrated that these compounds can modify ATP synthesis and Ca^{2+} transport in rat heart mitochondria (Baydoun *et al.*, 1988). We now report the effects of the synthesised isopropyl acyl carnitine esters: palmitoyl, stearoyl, oleoyl and eicosanoyl (Criddle *et al.*, 1991) and the endogenous compounds: palmitoyl carnitine, palmitic acid (PA) and palmitoyl CoA (PCoA) in this preparation.

Tightly coupled rat heart mitochondria were isolated from Wistar rats (250-350g) according to the method of Vercesi *et al.* (1978). Ca^{2+} -ion movements were monitored at 37°C using a Corning Ca^{2+}-selective electrode coupled to a Jenway PHM6 mV meter and BBC SE120 flat bed recorder. Ca^{2+} uptake was initiated by the addition of 2.5mg of mitochondrial protein to a Tris-sucrose buffer. The acyl compounds were administered prior to the introduction of the mitochondria during influx studies. During efflux studies, the acyl compounds were administered following a 5min Ca^{2+} loading period.

Acyl compounds (5-40μM) elicited an inhibition of Ca^{2+} influx into the mitochondrial matrix and stimulated Ca^{2+} efflux rates

Acyl Compound		IC_{50} Influx Rate (μM)	EC_{50} Efflux Rate (μM)
PA	(n=5, p<0.05)	14.95 ± 0.2	12.35 ± 0.5
PCoA	(n=5, p<0.05)	7.15 ± 0.7	6.05 ± 0.2

This trend was similarly observed with high concentrations of PCoA (3-10μM). However at concentrations of 0.1-2.0μM PCoA a concentration dependent stimulation of Ca^{2+} influx rates, Ca^{2+} uptake, and retention times was observed.

Studies employing cyclosporin A (400pmol mg $protein^{-1}$), a Ca^{2+} pore inhibitor (Crompton *et al.*, 1988), demonstrate inhibition of Ca^{2+} efflux rates induced by PC and PCoA; these results are consistent with the hypothesis that PC and PCoA act by opening a Ca^{2+} pore.

The results reported in this study confirm that long chain acyl compounds have the capacity to modify mitochondrial Ca^{2+} handling.

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387P ETOMOXIR MAINTAINS ENDOTHELIAL FUNCTION, AS MEASURED BY ACETYLCHOLINE RELAXATION, IN RABBIT SAPHENOUS VEIN PRECONSTRICTED WITH KCl AND MAINTAINED AT 0% OXYGEN *IN VITRO*

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Accumulation of long chain lipid intermediates such as acyl carnitines has been shown to occur during hypoxia in cardiac tissue with deleterious consequences which can be alleviated to some degree by palmitoyltransferase 1 inhibitors such as, ethyl 2-[6-(4 - chlorophenoxy)hexyl] oxirane - 2- carboxylate (Etomoxir) (Lopuschuk *et.al.* 1988). In smooth muscle preparations acyl carnitines induce positive inotropic responses in isolated chick embryonic myocytes (Patmore *et al* 1990) and potentiate KCl responses of taenia preparations of guinea pig caecum (Spedding & Mir 1987) leading to the hypothesis that they act as endogenous modulators of calcium mobilisation . Described below are experiments on isolated blood vessels which investigate the response to KCl at different levels of oxygen including anoxia (0%) and the effect of Etomoxir. Standard ring preparations of isolated rabbit saphenous vein and artery were stimulated with KCl (5mM - 100mM) at varying oxygen tensions 16%,4%, 1% & 0%. All preparations were incubated in standard Krebs which included 11.1 mM Glucose and bicarbonate buffer. pH was maintained by inclusion of 5% carbon dioxide in the gas mixture the balance being made up with nitrogen. Oxygen tension within the bath was measured continuously by oxygen electrode and change in ring tension measured under isometric conditions. The KCl responses were potentiated in both sensitivity and maximum. This potentiation was completely unaffected by the presence of 10 μM or 30 μM Etomoxir. On going from 16% to 0% the shape of the response, to half maximal (50mM) KCl, changed from a monophasic and maintained response to a biphasic response in which only the lesser secondary component was maintained. In 16% oxygen efficient endothelial dependant relaxation of the maintained response was observed in response to increasing acetylcholine (Ach) 0.1 nM - 10 μM . This relaxation was absent at 0% oxygen in the control tissue but not in the experimental tissue preincubated with 10 μM or 30 μM Etomoxir. In the presence of Etomoxir Ach relaxation is reduced to a third of that occuring in the paired control responses in 16% oxygen but is still statistically significant ($p< 0.05$) , (n=8) .

These results suggest that the potentiation of the KCl response is not due to accumulation of acyl carnitines in the smooth muscle cells. There may however be accumulation of acyl carnitines at 0% oxygen in the endothelium leading to impaired function which is reduced in the presence of Etomoxir. Impaired function has been reported for exogenously applied acyl carnitine *in vitro* (Dainty *et. al.* 1990). Thus it is possible that 0% oxygen generates endogenous acyl carnitine *in vitro*. Moreover Etomoxir appears to protect a mechanism of endothelium dependent relaxation in 0% oxygen which can cause relaxation of KCl depolarised smooth muscle.

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388P INVESTIGATION INTO THE ROLE OF FREE RADICALS IN PLASMA PROTEIN LEAKAGE IN ISCHAEMIC/REPERFUSED RABBIT MYOCARDIUM *IN VIVO*

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Myocardial tissue which has undergone a period of ischaemia displays characteristic signs of an acute inflammatory response. Among the early events characterising this response is oedema formation. Studies have implicated the involvement of free radicals in causing changes in microvascular permeability following ischaemia/reperfusion (Perry et al, 1986). In this study we have examined the effect of three compounds, superoxide dismutase (SOD), catalase (CAT) and dimethylthiourea (DMTU) on oedema formation in a model of myocardial ischaemia and reperfusion in anaesthetised rabbits (Williams et al, 1990).

Male New Zealand white rabbits (2.5-3.5kg) were anaesthetised with i.v. sodium pentobarbitone. Arterial blood pressure and lead I ECG were recorded. ^{125}I-albumin was administered i.v. as a marker of coronary microvascular plasma protein leakage. The left main coronary artery was occluded for either 15 or 30min followed by 30 min reperfusion. Heart tissue was divided into normal zone (NZ) and area at risk (AR) by dye exclusion and levels of ^{125}I were measured by gamma counting. Results are expressed as μl plasma/g tissue ± s.e.mean.

Animals received the following treatments; 1) bolus i.v. injection of SOD (17850Ukg^{-1}min^{-1}) and CAT (62000Ukg^{-1}min^{-1}) in combination or saline, 2 min prior to coronary artery occlusion (CAO). On occlusion animals were infused (i.v.) with SOD (297.5Ukg^{-1}min^{-1}) and CAT (1033Ukg^{-1}min^{-1}) or saline throughout the ischaemic and reperfusion phase, 2) i.v. infusion DMTU (500mgkg^{-1}) or saline over a 10 min period at 30 min prior to CAO. Results are summarized in the table below.

Treatment		n	Myocardial Plasma Volume (μl/g) NZ	AR	% Increase
15min CAO:	control (saline)	4	65±1	98±12	52±18
	treated (SOD + CAT)	4	68±5	94±12	38±10
30min CAO:	control (saline)	5	102±25	172±32	81±25
	treated (SOD + CAT)	5	100±10	207±36	101±23
30min CAO:	control (saline)	6	96±9	232±25	144±19
	treated (DMTU)	7	104±10	252±27	145±24

There was a significant increase in plasma volume in the AR compared with the NZ in all control animals ($p<0.05$). None of the treatments significantly affected this increase in plasma volume. Thus, in this rabbit heart model no evidence was obtained that free radicals are involved in coronary microvascular plasma protein leakage.

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389P THE EFFECT OF LIPOPOLYSACCHARIDE ON PLATELET ACCUMULATION IN THE PULMONARY VASCULATURE OF THE RAT

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Systemic administration of bacterial endotoxins in experimental animals induces many features of septic shock including thrombocytopenia (Mathison & Ulevitch, 1981; Endo & Nakamura 1992). In the present study we have evaluated the effect of systemic administration of lipopolysaccharide from Escherichia coli (LPS, 0127:B8) on the behaviour of labelled platelets in the pulmonary vasculature of rats. Male Wistar rats (250 - 350g) were anaesthetised with urethane (10 % w/v; 10 ml/kg) and an indwelling cannula placed in the tail vein. Collimated 2.5 cm crystal scintillation detectors, linked to an automated isotope monitoring system, were placed over the thoracic and abdominal regions of the supine rats (Oyekan & Botting, 1986). A submaximal dose of ADP (20 μg/kg) was injected via the tail vein, to check the platelet responsiveness. 30 minutes later, either LPS (1-10 mg/kg) or saline were administered to animals in parallel and the accumulation of ^{111}In labelled platelets, into various vascular beds, was followed for the next 4 hours.

Administration of LPS (1 mg/kg) resulted in a fall in platelet associated radioactivity in the thorax. Higher doses of LPS (5 or 10 mg/kg) caused an initial, prolonged platelet accumulation in the thorax, which steadily decreased and which subsequently fell below the basal levels. Concomitant to the pulmonary platelet accumulation, there was a fall in the platelet associated radioactivity in the abdominal region. There was no significant difference in the response to LPS at 5 or 10 mg/kg. In the saline treated group there was no significant change in the platelet associated radioactivity in the thorax over the 4 hour period. 4 hours after the injection of LPS (5 or 10 mg/kg), the response to ADP was significantly reduced compared to the control response to ADP obtained at the beginning of the experiment (from 34.12 ± 1.7% to 23.5 ± 1.2 % n=10, $p < 0.001$ for 5 mg/kg; from 33.5 ± 5.1 % to 17.1 ± 5.2 % n=6 , $p < 0.05$ for 10 mg/kg). No such changes were observed after saline treatment. At the end of each experiment the spleen and a section of the liver were removed, weighed and counted in a gamma counter together with a sample of blood. The radioactivity was expressed as mls of blood equivalents per gram of tissue. In LPS treated rats there was an increase in radioactivity in the liver and spleen compared to the control group (0.56 ± 0.08 n=5; 1.33 ± 0.308 n=8 and 4.9 ± 0.7 n=6; 7.5 ± 0.71 n=9, respectively). Pretreatment with dexamethasone (1 mg/kg i.v. 10 min before LPS), the PAF antagonist BN 52021 (4 mg/kg i.v. 10 min before LPS) or indomethacin (5 mg/kg i.v. 20 min before LPS) had no effect upon the initial LPS-induced pulmonary accumulation of platelets, or the platelet recruitment to the spleen and liver. Furthermore, none of these drugs inhibited the LPS-induced reduction in response to ADP.

In conclusion, systemic administration of LPS in rats leads to platelet accumulation in the lung and causes a reduction in the platelet responsiveness to the agonist ADP.

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390P EFFECT OF PROTEIN KINASE C STIMULATION ON TENSION AND CALCIUM CONCENTRATION IN THE RAT PULMONARY ARTERY: MODULATION BY FORSKOLIN

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In the rat pulmonary artery, activation of protein kinase C (PKC) induces a sustained contraction which seems to be independent of both calcium influx, and calcium release from intracellular stores (Savineau et al., 1991) but is reduced by forskolin. In these studies we have investigated the mechanism by which forskolin and other cAMP-elevating agents alter PKC-mediated contractions.

Rats were anaethestised with ether, and the heart and lungs removed. Experiments were performed on strips (8-10mm in length, 200-400 μm in diameter) isolated from the main pulmonary artery (MPA) and on rings obtained from small (internal diameter <500μm) intrapulmonary arteries (IPA). Isometric contraction of strips and rings was measured by means of an isometric force tranducer (Akers 801) and an automated myograph (Cambustion AM10), respectively. In the latter case, the myograph was combined with an inverting stage microscope (Olympus IMT-2) and spectrofluorimeter (Cairn Research) for the simultaneous estimation of tension and intracellular Ca^{2+}, using the Ca^{2+}-indicator Fura-2. PKC was stimulated by the phorbol ester 12, 13 dibutyrate (PDB).

In MPA, forskolin (0.1-5μM) inhibited in a concentration dependent manner contractions induced by PDB (50nM-5μM, n=5). Blockade of calcium pumps with vanadate (0.5mM) and Ca^{2+} sequestration with ryanodine (25μM) combined with TMB8 (50μM) reversed this inhibitory effect (n=6). Papaverine (1-20μM), theophylline (0.1-2.5mM) and dibutyryl cAMP (0.1-1mM) mimicked the effect of forskolin. In IPA, PDB induced similar contractions, but these were not associated with variations in fura-2 fluorescence (n=7). In contrast, contractions induced by potassium (80mM) combined with PgF2α (10μM) were associated with a large increase in the fura-2 signal. Forskolin (1-5μM) decreased PDB-induced contractions in a dose dependent manner, without change in resting fura-2 fluorescence.

These results demonstrate that PKC-mediated contraction of pulmonary vascular smooth muscle does not involve variations in cytosolic calcium concentration. PKC may therefore increase the Ca^{2+} sensitivity of the contractile machinery, whereas cAMP-elevating agents may counteract this action.

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391P INHIBITION BY PINACIDIL OF *IN VITRO* HYPOXIC PULMONARY VASOCONSTRICTION IN ISOLATED PERFUSED LUNGS FROM PULMONARY HYPERTENSIVE AND NORMOTENSIVE RATS

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Potassium channel opening drugs, such as pinacidil (PIN), may be useful as pulmonary vasodilators where an increase in pulmonary artery pressure is caused by pulmonary vasoconstriction. PIN relaxes isolated preparations of large pulmonary arteries contracted with spasmogens such as noradrenaline (NA) or $PGF_{2\alpha}$. It is not known whether it inhibits the constriction of pulmonary arterioles induced by alveolar hypoxia, a major cause of pulmonary vasoconstriction *in vivo*. Thus the effects of PIN on vasoconstrictor responses to alveolar hypoxia in isolated perfused lungs from Wistar rats have been examined. Lungs from both control and pulmonary hypertensive (PH) rats were used to test the hypothesis that PH is associated with enhanced responsiveness of pulmonary vessels to potassium channel opening drugs (Wanstall & O'Donnell, 1992). Rats were made PH by housing them in a low O_2 environment (10% O_2) for 1 week.

Lungs were perfused *in vitro* (3.5 ml/100g body wt/min) with physiological salt solution (pH 7.4; 37°C) containing 4% w/v bovine serum albumin, 3μM indomethacin, 10 μM N^G-nitro-L-arginine methyl ester and 2.5 nM angiotensin II, and were ventilated at 60 strokes/min at 9 cm H_2O inspiratory pressure. Vasoconstrictor responses to alveolar hypoxia were elicited by ventilating with a hypoxic gas mixture (0-1% O_2, 5% CO_2, balance N_2) until vasoconstriction had reached a plateau (3-4 min). Between each response lungs were ventilated with normoxic gas (20% O_2, 5% CO_2, balance N_2) for 4 min to restore resting perfusion pressure. Initial resting perfusion pressures were: control rats 9.3± 0.2 mmHg (11); PH rats 13.5±0.4 mmHg (12) ($P<0.001$). Responses to alveolar hypoxia were: control rats 8.1±1.1 mmHg; PH rats 9.8±1.6 mmHg ($P>0.05$). When consecutive vasoconstrictor responses were reproducible, PIN was infused into the perfusate for 2 min before, and during, the next two responses. PIN caused a dose-related inhibition of the hypoxic vasoconstrictor responses. The percentage reductions were: control rats 1μM PIN 23±2.8 (4), 3μM 59±11.6 (4), 10 μM 76±4.8 (3); PH rats 1μM 15±15.5 (4), 3μM 24±5.7 (4), 10μM 62±6.3 (4).

In summary, PIN inhibited vasoconstrictor responses to alveolar hypoxia in perfused lungs from rats but the inhibition was no greater in lungs from PH rats than from controls. This contrasts with previous data on large pulmonary arteries contracted with NA, where PIN was more effective in preparations from PH rats than controls (Wanstall & O'Donnell, 1992). This could indicate that the functional changes associated with PH are different for conduit and resistance arteries. Alternatively it could reflect the different mechanisms whereby NA and hypoxia contract pulmonary vascular smooth muscle.

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392P NEUTRAL ENDOPEPTIDASE INHIBITOR, SCH 42495, ATTENUATES THE DEVELOPMENT OF HYPOXIC PULMONARY HYPERTENSION IN RATS

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Atrial natriuretic peptide (ANP) is a natriuretic, diuretic and vasoactive hormone released into the circulation in response to atrial distension. Chronic elevation of circulating ANP (Winter et al., 1990) has been shown to limit the physiological changes associated with exposure to chronic hypoxia. However, the half-life of ANP in vivo is less than 5 minutes in all species suggesting that ANP alone would be of little therapeutic use. One method of clearance of ANP is by the action of a neutral endopeptidase (NEP) enzyme extensively distributed in the lung and kidney (Ronco et al., 1988). Specific inhibitors of NEP have been developed. The effects of SCH 42495, the orally active prodrug of the neutral endopeptidase inhibitor SCH 42354, on the development of hypoxic pulmonary hypertension (PH) in rats were investigated.

Rats (n=4) were kept at 10 % oxygen in a normobaric, hypoxic chamber for 14 days and dosed orally with SCH 42495 (2ml, 30mg/kg) at 12 hourly intervals. Control animals received 2ml aqueous methyl cellulose vehicle (0.4%). Two similarly treated groups of rats were kept in air for 14 days. At 1, 3, 7, 10 and 14 days after the start of dosing, rats were anaesthetized and exsanguinated via the pulmonary artery for determination of haematocrit and plasma ANP levels. Hearts were dissected and weighed for measurement of ventricular ratios. Lungs were perfused with 10% formol saline for histological examination of the pulmonary vasculature.

Table 1

		Treated Hypoxic	Control Hypoxic	Treated Normoxic	Control Normoxic
Body weight	g	178.8 (4.2)	164.6 (3.2)	197.9 (3.8)	205.8(4.3)
RV/[LV+s]		0.39 (0.04)	0.50 (0.05)	0.27 (0.04)	0.30 (0.04)
Haematocrit	%	34.5 (4.27)	48.9 (2.42)	31.3 (4.15)	32.9 (2.20)
Double elastic laminae	%	14.2 (2.17)	35.1 (1.26)	12.0 (1.02)	11.1 (9.33)

Results as mean (s.d)

Treatment with SCH 42495 caused a marked reduction in the pulmonary vascular remodelling and ventricular hypertrophy associated with the development of PH in rats. Maintaining elevated levels of plasma ANP by inhibiting it's metabolism may be therapeutically useful in the treatment of conditions characterized by PH and pulmonary vascular remodeling.

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393P INHIBITION BY POTASSIUM CHANNEL OPENERS OF CHOLINERGIC BRONCHOCONSTRICTION IN THE GUINEA-PIG

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K^+-channel openers (KCOs) may inhibit release of acetylcholine (ACh) from nerve terminals in the airways by a prejunctional mechanism (Ichinose & Barnes, 1990). The aim of the present study was to compare the effects of two KCOs, Ro 31-6930 (Paciorek et al., 1990) and BRL 38227 (Bowring et al., 1991), on contraction generated by electrical stimulation and exogenous ACh in guinea-pig airways.

Strips of guinea-pig trachea were mounted in Krebs solution at 37°C containing indomethacin (3 μM) and propranolol (1 μM) for recording of isometric tension. The effect of Ro 31-6930 (0.1 - 10 μM) or BRL 38227 (0.1 - 10 μM), added cumulatively, were examined on submaximal responses to either EFS (10 s train of pulses; 10 V, 10 Hz, pulse width 0.2 ms) or size-matched responses to ACh (1 - 3 μM) 15 min after addition of the drug. pD_2 values were calculated from normalised data and expressed as mean values with 95% confidence limits (n = 4 - 6). Urethane-anaesthetised, propranolol (1 mg kg^{-1}, i.v.) treated guinea-pigs were prepared for measurement of pulmonary inflation pressure. Bronchoconstriction was evoked by bilateral vagus nerve stimulation (BVS, 30 s train of pulses; 5 - 10 V, 10 Hz, pulse width 0.5 ms) or ACh (10 - 30 μg kg^{-1}, i.v.). Ro 31-6930 (1 - 80 μg kg^{-1}, i.v.) or BRL 38227 (10 - 800 μg kg^{-1}, i.v.) were administered cumulatively, each administration being made 10 min before challenge. ID_{50} values were calculated and expressed as mean ± s.e.mean (n = 4).

In both in vitro and in vivo experiments, responses to ACh, EFS or BVS were not significantly reduced in vehicle- or time-matched controls. On the guinea-pig trachea, Ro 31-6930 and BRL 38227 evoked concentration dependent inhibition of tone generated by EFS with pD_2 values of 7.03 (6.77 - 7.29) and 6.26 (5.91 - 6.61) respectively and of that by ACh with pD_2 values of 7.38 (6.52 - 8.24) and 6.65 (6.16 - 7.13). In the anaesthetised guinea-pig, Ro 31-6930 inhibited the increase in PIP evoked by BVS and ACh with ID_{50} values of 12.9 ± 3.9 and 3.6 ± 1.3 μg kg^{-1} respectively. The corresponding ID_{50} values for BRL 38227 were 356 ± 157 and 37.9 ± 13.4 μg kg^{-1} respectively.

These results provide no evidence that KCO's inhibit prejunctionally the release of ACh in guinea-pig airway smooth muscle.

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394P INTRAVENOUS PAF CAUSES NEUTROPHIL RECRUITMENT AND AIRWAY DYSFUNCTION IN ALLERGIC HORSES

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Allergen challenge of horses with allergic respiratory disease results in airway obstruction and radiolabelled neutrophil accumulation in the lung (Fairbairn et al, 1991). In an initial study, in normal horses, we reported that PAF i.v. caused a transient neutrophil accumulation in the lung accompanied by airway dysfunction (Fairbairn et al, 1992). In the present study, the effects of i.v PAF have been investigated in a group of 5 allergic horses during a period of disease remission.

Equine neutrophils (10^8) were isolated from whole blood, radiolabelled with ^{111}indium-merc, and reinfused back into the donor horse. Radiolabelled neutrophil distribution was monitored *in vivo* using the automated isotope monitoring system (AIMS) and peripheral blood samples were taken for total and differential leucocyte counts. An oesophageal balloon was used to measure changes in pleural pressure (ΔPplmax) and respiratory rate. In a further experiment, in 5 normal horses, erythrocytes were radiolabelled *in vivo* with ^{99m}technecium in order to determine whether PAF altered blood volume.

PAF (5ng/kg i.v.) caused a rapid increase in radiolabelled neutrophils in the lung, maximal at 4min (33.7±8.6%, mean ± s.e.mean), accompanied by a fall in the radioactive counts detected in the leg (-11.6±2.7%). Correspondingly, a significant ($P<0.05$) decrease in the peripheral neutrophil count was observed at 2min (pre: 6.3±0.81, 2min: 3.6±0.79 x 10^6cells/ml). In addition, both ΔPplmax and respiratory rate were transiently increased (ΔPplmax: 4.5±0.9 and 9.2±3.6 cmH_2O, Resp. rate: 11±2.3 and 28±4.4 breaths/min, pre and 2min after PAF respectively). The response of allergic horses to PAF was not significantly different from that previously reported in normal horses (Fairbairn et al, 1992). Despite similar changes in airway function, in normal horses, no increase in ^{99m}technecium counts in the lung was detected following PAF.

In allergic horses, PAF caused transient airway dysfunction together with neutrophil recruitment to the lungs which could not be accounted for by an increase in blood volume. If released during allergen challenge, PAF may therefore be involved in the pathogenesis of equine allergic respiratory disease.

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395P MECHANISMS OF ANGIOTENSIN II-INDUCED PROSTACYCLIN RELEASE IN ISOLATED PERFUSED RAT LUNG

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An infusion of angiotensin II into lungs isolated from rats and perfused at constant flow results in a transient increase in perfusion pressure and release of the vasodilator prostacyclin. Angiotensin II may, therefore, stimulate prostacyclin release either directly, via second messenger-coupling, or indirectly, as a result of mechanical stimulation of the endothelium. The aim of this work was to test the second hypothesis by relating perfusion pressure to prostacyclin release during angiotensin II infusions. Sodium nitroprusside was perfused to relax vascular smooth muscle and to therefore attenuate the pressor response to angiotensin II; under these conditions the perfusate was examined for reduced prostacyclin release with reference to control preparations. The influence of vasoactive mediators on the perfusion pressure response to angiotensin II was also examined using indomethacin and nitro-L-arginine methyl ester (L-NAME) as inhibitors of prostaglandin and nitric oxide synthesis respectively. Lungs were perfused (Krebs buffer, pH 7.4, 37°C, gassed with 95%O_2/5%CO_2) for a 30 min equilibration period followed by a 3 min basal period. An increase in perfusion pressure and prostacyclin release was then provoked by a 5 min infusion of angiotensin II (0.125 ml/min, final concentration $1.2x10^{-6}$M), after which perfusion was continued for a 15 min recovery period. Perfusate was collected and stored at -20°C until assay of 6-keto prostaglandin $F_{1\alpha}$, the stable metabolite of prostacyclin. Perfusion pressure was monitored throughout. Lungs were perfused with 10^{-5}M sodium nitroprusside, 10^{-5}M indomethacin, 10^{-3}M L-NAME or with buffer alone (control). Results are means ± SD. Perfusion with sodium nitroprusside significantly reduced both the angiotensin II induced peak perfusion pressure (mmHg; 10.14 ± 1.41 vs 15.73 ± 4.09, $P<0.01$) and prostacyclin release measured above basal levels (ng; 66.18 ± 31.19 vs 126.05 ± 57.19, $P<0.01$) compared to perfusion with buffer alone. Indomethacin virtually abolished prostacyclin release (8.99 ± 8.18, $P<0.01$) but had no effect on the perfusion pressure increase seen during angiotensin II infusion (15.65 ±13.50). In contrast, L-NAME did not influence the increase in prostacyclin release (94.56 ± 16.32) but markedly exaggerated the perfusion pressure response to angiotensin II (23.79 ± 3.77, $P<0.01$). These results suggest that, in the lung, prostacyclin release in response to angiotensin II challenge is mediated, at least in part, by vasoconstriction. In addition, the vasoconstrictor response is not offset by a cyclooxygenase product such as prostacyclin but is substantially attenuated by nitric oxide.

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396P IDENTIFICATION OF A BINDING SITE FOR [^{3}H]-CROMAKALIM IN VASCULAR AND BRONCHIAL SMOOTH MUSCLE CELLS

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Cromakalim (CRK - BRL 34915) is thought to exert its smooth muscle relaxant effect by an opening of potassium channels resulting in hyperpolarisation of the smooth muscle cell (SMC) membrane (Hamilton et al, 1986). Substantial evidence suggests that potassium channel activator (PCA) drugs, such as CRK, may interact with an ATP-sensitive K^+ channel (see Longman & Hamilton, 1992). Initial attempts to identify a recognition site using [^{3}H]-CRK were unsuccessful (Coldwell & Howlett, 1987). The current paper describes the stereospecific binding of [^{3}H]-CRK to sites on vascular and airways SMC in culture.

Vascular SMC from the intimal layer of rabbit mesenteric artery were dispersed enzymatically and subsequently grown in culture as previously described (Howlett & Longman, 1989). Airways SMC derived from human bronchus were the generous gift of Dr. J. Ward, Dept of Medicine, St Thomas' Hospital, London. [^{3}H]-CRK was prepared by catalytic tritiation (Amersham International) and Dr K. Willcocks (SB, Harlow) at a specific activity of 25.9 Ci/mmole. Cells (2×10^6/tube) were incubated at room temperature with [^{3}H]-CRK in HEPES buffer. After 60 min, the radioactivity bound to the cells was determined following filtration under reduced pressure. Specific binding was defined as that displaced by 1μM BRL 38227 ((-)-enantiomer of CRK).

The specific binding of [^{3}H]-CRK to vascular and airways SMC was saturable and of high affinity. Scatchard analysis of the data described a single site of B_{MAX} 97±33 fmoles/10^6 cells and K_D of 9.8±2.8 nM (n=6) in vascular SMC. Corresponding values for airways SMC were B_{MAX} 36±10 fmoles/10^6 cells and K_D 13.4±4.6nM (n=5). Non-specific binding comprised 32±5.9% (vascular) and 52±7.9% (airways) of total binding at the respective K_D values. [^{3}H]-CRK binding was displaced by cold CRK with IC_{50} values of 19.5±2nM (vascular, n=3) and 26.2±6.6nM (airways, n=4) and by BRL 38227, with IC_{50} values of 11.5±1.5nM (vascular, n=7) and 14.8±2.6nM (airways, n=6). The (+)-enantiomer of CRK, BRL 38226, was inactive at 10μM on both cell types. Binding was also inhibited by other PCA drugs (see Current Drugs,1992) :-SDZ PCO 400 (IC_{50} of 190±44nM, n=3, vascular; 193±95nM, n=4, airways) and FR119748 ($IC_{50}$of 110±55nM, n=4, vascular; 82±31nM, n=3, airways) but not by the potassium channel blocker glibenclamide (10μM).

The data demonstrates the existence of a stereospecific binding site for CRK in vascular and bronchial SMC. Although the affinity of [^{3}H]-CRK is similar in the two preparations, fewer binding sites appear to exist in airways SMC. The lack of inhibition of [^{3}H]-CRK binding by glibenclamide indicates that the latter does not interact directly with the CRK binding site.

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397P BRONCHODILATOR AND CARDIOVASCULAR EFFECTS OF POTASSIUM CHANNEL OPENERS IN THE ANAESTHETISED DOG

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The potassium channel opener (KCO) cromakalim has been shown to protect healthy volunteers from histamine-induced bronchoconstriction (Baird et al, 1988), and preliminary studies suggested this agent would be beneficial in the treatment of nocturnal asthma (Williams et al, 1988). In addition to this bronchorelaxant action, KCOs have also been shown to be potent antihypertensive agents. In this study we have compared the effects of KCOs cromakalim, levcromakalim (LMK), pinacidil, PCO-400 (Sandoz), and Ro 31-6930 (Roche) on nebulised histamine induced bronchoconstriction and total peripheral resistance (TPR) in the anaesthetised dog. This model allows us to evaluate the bronchodilator efficacy of these compounds compared to their cardiovascular effects.

Male beagle dogs (11.0 - 14.5 kg) were premedicated with Dipidolor (4mg kg^{-1} sc) and anaesthetised with chloralose/urethane (0.08g kg^{-1}/0.8g kg^{-1}). The dogs were artificially ventilated, changes in lung resistance (R_L) and dynamic compliance (C_{dyn}) were monitored according to the method described by Alabaster and Keir (1987) in order to assess the bronchoconstriction produced by nebulised histamine (0.25 to 2mg ml^{-1}). A dose of histamine producing an increase in R_L of approximately 200% was determined and repeated every 30 mins until consistent responses were observed. Other parameters measured were left ventricular pressure (LVP), integrated to measure dp/dt (mmHg s^{-1}), mean arterial pressure (MAP) and heart rate (HR). Cardiac output was determined using a thermodilution method, allowing calculation of TPR. Compounds under investigation were infused iv (30 mins per dose) until the histamine response was abolished, and/or maximum (70%) reduction in TPR was achieved.

The doses of compounds (μg kg^{-1} min^{-1}) which caused a 50% inhibition of histamine responses (IC_{50}), and the corresponding percentage fall in MAP at this dose, a 35% fall in TPR (ED_{35}), and a 50% increase in HR (ED_{50}) in the anaesthetised dog are shown in the table (mean ± s.e.mean).

COMPOUND (n)	R_L (IC_{50})	TPR (ED_{35})	HR (ED_{50})	% MAP (R_L IC_{50})
Cromakalim (6)	1.16 ± 0.41	0.83 ± 0.17	1.04 ± 0.06	-6.0 ± 1.78
LMK (6)	0.92 ± 0.39	0.35 ± 0.07	0.34 ± 0.07	-13.67 ± 4.89
PCO-400 (6)	1.58 ± 0.40	1.67 ± 0.24	1.39 ± 0.08	-3.25 ± 3.18
Ro 31-6930 (3)	0.13 ± 0.03	0.06 ± 0.02	0.07 ± 0.04	-1.33 ± 4.81
Pinacidil (4)	23.75 ± 5.8	2.52 ± 0.53	4.07 ± 0.15	-28.5 ± 11.5

As previously shown KCOs caused a fall in TPR which was accompanied by an increase in HR and CO. The results from the table show that none of the KCOs examined showed any selectivity for the airways over their effects on TPR. However pinacidil shows increased selectivity for TPR over R_L by approximately 10 fold. These results clearly show that doses required to inhibit histamine-induced bronchoconstriction are accompanied by significant cardiovascular effects. If the anaesthetised dog is predictive of KCO activity in man these results would suggest that doses used for effective bronchodilator therapy would be accompanied by significant cardiovascular side-effects.

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398P OLIGAEMIA INDUCED BY DIRECT INJECTION OF N^G-NITRO-L-ARGININE METHYL ESTER INTO THE RAT STRIATUM

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Recent in vivo investigations of the effects of NO synthesis inhibitors upon cerebral blood flow have produced contradictory results (Iadocola, 1992; Sonntag et al., 1991). This could be due in part to the systemic hypertension produced by these compounds or to abnormalities in the cerebrovascular physiology of anaesthetized, artificially ventilated animals. The purpose of these studies was to determine whether NO synthesis inhibition by N^G-nitro-L-arginine methyl ester (L-NAME) could alter cerebral blood flow in conscious rats (where physiological regulatory mechanisms are intact) following intracerebral injection.

Local cerebral blood flow was measured using the fully quantitative iodoantipyrine autoradiographic technique (Sakurada et al., 1976) in conscious rats previously implanted with intrastriatal guide cannulae (n =10). The measurement was initiated 60 min after the unilateral striatal injection of either 2µl of mock CSF alone (n = 5), or containing 20µmol L-NAME (n = 5). Animals were sacrificed at the end of a 45 sec infusion of [^{14}C]-iodoantipyrine, and the brains processed for autoradiography. Striatal blood flow was measured from 3 consecutive coronal sections (20µm thick) at 10 pre-set levels exactly 200 µm apart. Because striatal blood flow is inherently heterogeneous and the diffusion of L-NAME may be unpredictably uneven, the total area of striatum was measured at each of these 10 levels and the proportion in which blood flow was less than 50% or 75% of the mean control value was derived.

The total volume of striatal tissue measured in these animals was 25.24 ± 0.9 mm^3 (all data presented as mean ± s.e. mean), and contralateral to the injection sites, striatal blood flow was120 ± 4 ml/100g/min. One hour after the injection of L-NAME, blood flow was reduced to less than 80 ml/100g/min in 12.81 ± 1.38 mm^3 of striatal tissue (40-60% of total area at each level), and a more profound oligaemia with blood flow less than 60 ml/100g/min was measured in 6.51 ± 0.65 mm3 of striatal tissue (20-30% of total area at each level). In contrast, only 1.57 ± 0.16 mm^3 of striatal tissue ipsilateral to CSF injections had an apparent blood flow of less than 80 ml/100g/min (3-8% of total area at each level) and 0.22 ± 0.02 mm^3 was found to be perfused at less than 60 ml/100g/min, values which probably represent intrastriatal white matter flows. Intracranial injections had no effect upon either mean arterial blood pressure or blood gas tensions when compared to pre-injection values.

These results indicate that intracerebral injections of L-NAME can induce oligaemia in the conscious rat brain and are consistent with the hypothesis that NO exerts a tonic vasodilator influence upon the cerebrovasculature. What remains unclear is whether this reduction in flow represents a direct effect upon the cerebral vessels, or whether it is secondary to decreases in metabolic demand.

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399P AN LPS-INDUCIBLE ACTIVITY IN VASCULAR SMOOTH MUSCLE CYTOSOL WHICH CONVERTS GLYCERYL TRINITRATE TO FREE NITRITE

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Cultured vascular smooth muscle cells (VSM) metabolise 1,2,3-glyceryltrinitrate (GTN) to a molecule having the chemical and biological profile of nitric oxide (NO). Although this metabolism is thought to be essential for the clinical actions of GTN, pathways for GTN metabolism are poorly understood. Recently, GTN metabolism by VSM was shown to be potentiated by pretreatment of cells with bacterial lipopolysaccharide (LPS) and destroyed by boiling (Salvemini et al., 1992) suggesting an inducible enzyme. The present study was performed to assess whether an enzyme which metabolises GTN is present in cytosol of VSM.

Cultured aortic smooth muscle cells (passages 10-15) from the rat were grown to confluence and lysed by freezing and thawing in water containing a cocktail of protease inhibitors. Cytosol was prepared by centrifugation at 100,000 x g for 1 h. Also cytosol was prepared from VSM which were activated by treatment with a combination of LPS (30 µg/ml) and interferon-γ (IFN; 50 ng/ml) for 24 h prior to lysis. LPS/IFN resulted in NO synthase induction as measured by nitrite formation using the Griess assay (351±23nmoles/175 cm^2 flask/24h; Mean ± SEM). VSM cytosol was assessed for its ability to metabolise GTN to NO based on: (1) nitrite production, an NO degradation product, (2) oxidation of Fe^{2+}- myoglobin (Fe^{2+}-Mb) to metmyoglobin, a chemical activity of NO, and, (3) the ability to increase the cGMP content of rat lung fibroblasts (RFL-6 cells), a bioactivity of NO.

Incubation of control VSM cytosol with GTN at 100 µg/ml for 1 h at 37°C, pH 7.6, resulted in a significant production of nitrite (0.22±0.04 nmol hr^{-1} mg^{-1} protein). However, nitrite production was significantly greater with cytosol for LPS/IFN-activated cells (96±4 nmol $hr^{-1}mg^{-1}$ protein). Unexpectedly, nitrite production was not associated with the production of a factor which oxidises Fe^{2+}-Mb or increases cGMP in RFL-6 cells, thus, NO is not an intermediate in this process. The ability of cytosol to convert GTN to nitrite increased with protein concentration (2.4±0.11 and 6.9±0.45 pmol min^{-1} for 100 and 400 µg protein, respectively; n=11) and activity was retained by a 10kD pore size filter. Metabolism was specific for GTN in that neither 1,2- nor 1,3-glyceryldinitrate were substrates. Metabolism of GTN was unrelated to NOS since nitrite production was not reduced after depletion of NOS (by passage over DEAE-sephadex resin) or inhibited by 1 mM N^{ω}-methyl-L-arginine. The thiol-alkylating agent N-ethylmaleimide (1 mM) abolished nitrite production from GTN (>95%, n=3). Nonetheless, GTN metabolism to nitrite by VSM cytosol differed from that known to occur non-enzymatically with low M.W. thiol-compounds such as cysteine (CYS) and dithiothreitol (DTT) in that cytosolic activity was lost upon boiling and began to decay after 60 min at 37°C. In contrast, nitrite production by CYS and DTT was neither abolished by boiling nor diminished over a 4 h assay period.

Thus, VSM cytosol contains a factor which is LPS-inducible, > 10 kD, thermolabile, thiol-dependent and which degrades GTN to nitrite without appearing to generate NO as an intermediate. The biological relevance of this activity is unknown; however, it could serve as a physiological means for GTN inactivation and possibly in the development of tolerance to GTN. Alternatively, this activity may produce NO from GTN when in the presence of other required cellular factors, and thereby contribute to GTN bioactivity.

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400P ENDOGENOUS NITRIC OXIDE REGULATES MOTILITY OF THE RAT ISOLATED DUODENUM

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Nitric oxide (NO), synthesized from L-arginine by a constitutive NO synthase (Moncada et al, 1991), has been proposed as a mediator of the non-cholinergic, non-adrenergic (NANC) relaxation of rat or guinea-pig stomach and duodenum, and the canine ileo-cecal junction and duodenum (Li & Rand, 1990; Desai et al, 1991; Irie et al, 1991; Bult et al, 1990; Toda et al, 1990). We have now investigated the actions of endogenous NO formed by both constitutive and inducible NO synthase (Moncada et al, 1991) in modulating spontaneous tone and contractile activity in the rat isolated duodenum.

Segments (3cm) of proximal duodenum from male rats (250-300g) were suspended in a modified Krebs' solution containing $CaCl_2$ (1.25mM) and contractions determined isometrically. Incubation with the inhibitor of NO synthase, N^G-nitro-L-arginine (L-NOARG; 3, 30 and 100μM) produced a dose-dependent significant ($P<0.01$) increase in resting tone, of Δ 133±50, 725±86 and 876±92mg tension (n=11-29) respectively. Likewise, the amplitude of the spontaneous contractions was elevated by 48±17%, 102±13% and 88±12% respectively ($P<0.01$) with these concentrations of L-NOARG. The effects of L-NOARG (30μM) on both duodenal tone and motility were abolished by concurrent incubation with L-arginine (1mM) but not with D-arginine (1mM). The NANC relaxation induced by nicotine (0.3-100μM) was dose-dependently and surmountably inhibited by L-NOARG (3-100μM), with L-NOARG (30μM) reducing ($P<0.01$) the relaxation induced by nicotine (10μM) by 61±14% (n=8) and by the nicotinic agonist 1,1-dimethylphenyl 4-piperazinium (DMPP, 10μM) by 93±6% (n=5). Pretreatment (6h) of rats with endotoxin (lipopolysaccharide from E.coli; $3mgkg^{-1}$) in a dose sufficient to induce nitric oxide synthesis (Moncada et al, 1991), significantly reduced the amplitude of the duodenal spontaneous contractions under basal conditions (from 854±62 to 553±23mg; n=24 for each, $P<0.01$). Furthermore, the increase in basal tone induced by L-NOARG (100μM) was substantially augmented to Δ 1300±204mg (n=18; $P<0.01$). These effects of endotoxin were abolished by pretreatment of the rat with dexamethasone ($1mgkg^{-1}$ s.c.) 2h before challenge (n=14).

These findings *in vitro* indicate that NO synthesis from L-arginine by a constitutive NO synthase is involved in the regulation of spontaneous tone and motility in the rat duodenum as well as in the duodenal NANC relaxation induced by nicotinic stimulation. The observation that endotoxin reduced the spontaneous duodenal contractions, an effect inhibited by dexamethasone pretreatment which prevents NO synthase induction (Moncada et al, 1991), may suggest a role for excessive NO production in the motility disturbances of the gut associated with endotoxaemia.

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401P EFFECT OF L-NITROARGININE METHYL ESTER ON THE DEPRESSION OF RENAL FUNCTION DUE TO CONTRAST MEDIA IN THE ISOLATED PERFUSED RAT KIDNEY

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The acute depression of renal function induced by radiocontrast media has recently been demonstrated in the isolated perfused rat kidney (IPRK)(El Sayed *et al.*1991). This effect has previously been attributed to stimulation of systemic hormones such as ADH, an increase in vasoconstrictor autacoids such as adenosine or a decrease in vasodilator autacoids such as prostacyclin. In the present experiments we have examined the involvement of nitric oxide in the acute renal response to the high osmolar contrast medium diatrizoate (Urografin 325) and the iso-osmolar contrast medium iotrolan (Isovist 300) in the IPRK using the nitric oxide synthetase inhibitor L-nitroarginine methyl ester (L-NAME).

Male Wistar rats (400-500g) were anaesthetised with thiopentone 125mg/kg and the right ureter and renal artery cannulated. The kidney was removed by a non-ischaemic technique and perfused in closed circuit at constant pressure (100mmHg) with a modified Krebs-Henseleit solution containing 6.7% albumin and 14mM mixed amino acids. Renal perfusate flow (RPF) was continuously recorded while glomerular filtration rate (GFR) was measured from clearance of [^{14}C] inulin. L-NAME was added to the perfusate at a final concentration of 10μM and both contrast media at a final concentration of 20mg iodine/ml. Control kidneys were preconstricted with angiotensin II (5ng/min) to mimic the fall in RPF caused by L-NAME. The effect of contrast media shown below was measured in the 20-30 minute period after their addition to the perfusate system.

In experiments performed in the absence of contrast media, both angiotensin II (5ng/min) (n=4) and L-NAME (10μM) (n=4) produced a fall in basal renal perfusate flow (from 62 ±1.8 ml/min/g to 30 ±2.1 ml/min/g for angiotensin II and from 60 ±2.0 ml/min/g to 28 ±1.7 ml/min/g for L-NAME) which was stable with time. In kidneys preconstricted with angiotensin II, diatrizoate produced a significant fall in both RPF (-32 ±2.77%, $p<0.01$ n=6) and GFR (-41 ±2.64%, $p<0.01$ n=6). However, following L-NAME, the decline in RPF (-11 ±3.12%, $p<0.05$ n=6) and GFR (-22 ±3.84%, $p<0.05$ n=6) produced by diatrizoate was markedly reduced. In contrast, iotrolan produced a significant fall in RPF (-20 ±2.34%, $p<0.05$ n=6) and GFR (-23 ±3.28%, $p<0.05$ n=6) in angiotensin II preconstricted kidneys, but a similar fall was also obtained in the presence of L-NAME (-25% ±2.83%, $p<0.05$ n=6) RPF and GFR (-24 ±2.74%, $p<0.05$ n=6).

The results may indicate that part of the acute depression of renal function induced by diatrizoate but not iotrolan is due to inhibition of a vasodilatory process in the kidney dependent on the synthesis of nitric oxide and raises the possibility that the iso-osmolar contrast medium iotrolan could have a reduced potential to induce nephrotoxicity *in vivo*.

El Sayed,A.A *et al.*(1991) Brit.J.Radiol. **64** 435-439

402P EVIDENCE FOR MODULATION OF MOTOR RESPONSES BY INHIBITORY NEUROTRANSMITTER IN THE RAT ANOCOCCYGEUS MUSCLE

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There is evidence that the non-adrenergic, non-cholinergic inhibitory transmitter in the rat anococcygeus muscle is nitric oxide (Gillespie et al., 1989). This study aimed to determine by the use of the nitric oxide synthesis inhibitors L-N^G-nitro-arginine (L-NOARG) and L-N^G-nitro-arginine-methyl-ester (L-NAME) whether activation of the inhibitory innervation could modulate motor responses.

Anococcygeus muscles were dissected from male Sprague Dawley rats as described by Gillespie (1972) and suspended in Krebs Henseleit solution (37°C, 95% O_2/5% CO_2) for isometric tension recording. Tissues were subjected to electrical field stimulation (2-18Hz, 0.5ms pulse width, 30 volts, applied for 0.5s every 10s), which elicited rapid, consistent, monophasic and frequency-dependent contractions. Frequency-response curves were carried out before and after incubation for 20min with L-NOARG (2, 20 and 200μM) or L-NAME (20 and 200μM). Results were expressed as percentages of the maximum contraction obtained to stimulation at 18Hz during the control frequency response curve, which was 2.2 ± 0.1g (mean ± s.e.mean, n=18).

Both L-NOARG and L-NAME caused significant, concentration-dependent enhancement of the motor response to stimulation. For example, 200μM L-NOARG and 200μM L-NAME caused increases in the contraction elicited by stimulation at 18Hz to 156.3 ± 10.2% (n=5) and 159.4 ± 18.5% (n=6) respectively of the control ($p<0.01$). In order to determine the selectivity of this enhancement, contractions to noradrenaline were examined before and after exposure of tissues to L-NOARG. Concentration-response curves to noradrenaline (0.1-100μM) were slightly enhanced following exposure to 200μM L-NOARG, the effect achieving significance ($p<0.05$) only at 10μM noradrenaline. In tissues precontracted to 1.6 ± 0.2g (n=12) with guanethidine (224μM) and stimulated as before, frequency dependent relaxations to stimulation occurred over the frequency range 2-16Hz, the inhibitory frequency-response curve lying to the left of the motor frequency-response curve. To show that the above concentrations of L-NOARG reduced the inhibitory responses, frequency-response curves were carried out in the absence and presence of L-NOARG. 2, 20 and 200μM L-NOARG reduced the relaxations to stimulation: for instance, at 14Hz, relaxations were reduced by 21.5 ± 15.1%, 84.2 ± 5.5% and 100% ± 0% respectively (n=4).

These results support and extend the proposal (Li & Rand, 1989, Gibson et al., 1991) that nitric oxide released on activation of inhibitory innervation can modulate motor responses of the anococcygeus muscle.

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403P HINDQUARTERS VASODILATOR EFFECT OF $MgSO_4$ IN CONSCIOUS RATS: POSSIBLE INVOLVEMENT OF NITRIC OXIDE

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We have recently shown that $MgSO_4$ can inhibit the carotid vasoconstrictor actions of endothelin-1 (ET1) and angiotensin II (AII) but not N^G-nitro-L-arginine methyl ester (L-NAME) (Kemp *et al.*, 1992). In this study we investigated the effects of $MgSO_4$ alone, or in the presence of ET1, AII or L-NAME on renal, mesenteric and hindquarters haemodynamics in conscious, male, Long Evans rats. Animals were chronically instrumented with pulsed Doppler probes (left renal artery, superior mesenteric artery and distal aorta i.e. hindquarters) and intravascular catheters (right jugular vein; caudal artery). All surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p., supplemented as necessary) with at least 7 days between probe implantation and catheterization. Animals received infusions of $MgSO_4$, given as a loading dose of 220 μmol kg^{-1} min^{-1} for 7 min followed by a maintenance dose of 56 μmol kg^{-1} min^{-1}, alone or 20 min after the onset of infusion of ET1 (12.5 pmol kg^{-1} min^{-1}), or AII (50 pmol kg^{-1} min^{-1}), or 60 min after onset of infusion of L-NAME (183 nmol kg^{-1} min^{-1}). The results are summarised in Table 1.

Table 1: Change from baseline for mean arterial pressure (MAP) and vascular conductances in renal (RVC), mesenteric (MVC) and hindquarters (HVC) beds, in the absence (control) or presence of peptide or L-NAME just before onset of $MgSO_4$ infusion (A) and in the absence (control) or presence of peptide or L-NAME at the end of the loading dose of $MgSO_4$ (B). Results are expressed as mean ± s. e. mean; n = 9.

	Δ MAP (mmHg)		Δ RVC (%)		Δ MVC (%)		Δ HVC (%)	
	A	B	A	B	A	B	A	B
Control	-1 ± 1	-13 ± 1*†	5 ± 4	-5 ± 4	3 ± 3	3 ± 5	0 ± 3	77 ± 8*†
ET1	27 ± 3*	-1 ± 2†	-43 ± 4*	-32 ± 3*†	-51 ± 4*	-20 ± 6*†	-20 ± 4*	53 ± 8*†
AII	48 ± 1*	7 ± 1*†	-69 ± 1*	-49 ± 2*†	-61 ± 2*	-36 ± 5*†	-48 ± 3*	40 ± 13*†
L-NAME	29 ± 2*	15 ± 2*†	-35 ± 3*	-34 ± 2*	-49 ± 3*	-41 ± 3*	-42 ± 3*	-28 ± 5*†

* $P<0.05$ versus baseline; † $P<0.05$ versus pre-$MgSO_4$ value (Friedman's test).

$MgSO_4$ alone caused a fall in MAP and a large increase in HVC with no effect on RVC or MVC. ET1, AII and L-NAME caused a significant rise in MAP and significant falls in RVC, MVC and HVC. $MgSO_4$ abolished the pressor effect of ET1 and inhibited substantially that of AII, but only partly supressed the pressor action of L-NAME. In the renal and mesenteric vascular beds, $MgSO_4$ caused a significant inhibition of the vasoconstrictor effects of ET1 and AII, but not that of L-NAME. $MgSO_4$ produced a large increase in HVC in the presence of ET1 and AII, but in the presence of L-NAME, HVC did not rise above baseline. These results suggest that the hindquarters vasodilator action of $MgSO_4$ is largely mediated by nitric oxide, and the action of $MgSO_4$ in the hindquarters vascular bed contributes importantly to its functional antagonism of the pressor effects of ET1 and AII.

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Kemp, P. A., Gardiner, S. M., Bennett, T. & Rubin, P. C. (1992). Neurosci. Lett., **42** (suppl.), S29.

404P L-N^G-NITRO ARGININE METHYL ESTER (L-NAME) REDUCES PLASMA PROTEIN LEAKAGE IN THE SKIN OF THE GUINEA-PIG AND RABBIT

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Local injection of inhibitors of nitric oxide (NO) synthase, such as L-NAME, reduces skin blood flow (Hughes et al., 1990) and inhibits mediator or phlogogen induced permeability responses in rat skin (Hughes et al., 1990; Ialenti et al., 1992) and hind paw (Ialenti et al., 1992). However, Antunes et al. (1991) have reported that L-NAME enhances oedema induced by a variety of mediators in rat hind paw. We have studied the effects of L-NAME on cutaneous plasma protein extravasation (PPE) in the guinea-pig and rabbit. PPE responses were measured as local accumulation of intravenously injected ^{125}I-albumin. All injections were made in a volume of 0.1 ml per site in the shaved flank skin. Treatments were allocated according to a balanced Latin square design. PPE is expressed as μl plasma (^{125}I counts in skin site/ ^{125}I counts in 1 μl plasma) and mean ± s.e. mean values are shown.

In the guinea-pig, i.d. L-NAME or D-NAME (0.001-0.1 μmol/site) produced no significant PPE over a 40 min period compared to phosphate buffered saline (PBS, n=3; data not shown). When co-injected with bradykinin (BK; 0.5 μg/site), L-NAME (0.01-0.1 μmol/site) reduced PPE whereas D-NAME (0.1 μmol/site) had no significant effect. (BK control:76 ± 7; BK+D-NAME: 80 ± 11; BK+0.1 μmol L-NAME: 22 ± 2*; n=5;* $p<0.01$).Similarly, the PPE response to histamine (HA; 0.5 μg base/site) was significantly reduced by 0.1 μmol L-NAME but not by 0.1 μmol D-NAME. (HA control: 138 ± 15; HA+D-NAME: 118 ± 14; HA+L-NAME: 52 ±12*; n=6; *$p<0.01$). In the rabbit, i.d. L- or D-NAME (0.001-1.0 μmol/site) produced no significant PPE over 40 min or 3h compared to PBS (n=3; data not shown). The PPE response to the polycation poly-L-lysine (PLL; 100 μg/site) measured over a 3h period (102 ± 9; n=3) was not affected by co-injection of 1.0 μmol D-NAME (92 ± 5) whereas significant reductions were produced by 0.1 μmol (57 ± 3; $p<0.01$) or 1.0 μmol (33 ± 2; $p<0.01$) L-NAME. In immunised animals the PPE response to antigen (Alternaria tenuis extract 1:30; 65 ± 6; n=3) measured over 40 min, was reduced by L-NAME (0.01 μmol: 34 ± 1; 0.1 μmol: 15 ± 2; 1.0 μmol: 7 ± 0; all $p<0.01$).

Thus, in two species widely used in studies of inflammatory responses, L-NAME reduced cutaneous PPE induced by direct-acting permeability mediators (BK and HA), by antigen or by a polycation (PLL). This effect was not produced by the stereoisomer D-NAME. There was no evidence of potentiation of any response by L-NAME when administered locally (i.d.).

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405P VITAMIN E PROTECTS AGAINST IMPAIRMENT OF ENDOTHELIUM-MEDIATED RELAXATION IN THE CHOLESTEROL-FED RABBIT CAROTID ARTERY

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Hypercholesterolaemia is known to induce endothelial dysfunction, leading to impairment of endothelium-mediated relaxant responses. This is thought to be due to an attenuation in the production and/ or release of endothelium dependent relaxing factor (EDRF), a nitric oxide-related compound (Harrison et al 1991). It is possible that oxidative stress may contribute to these effects since reactive oxygen species are released from monocytes/ macrophages, smooth muscle cells, endothelial cells and from LDL during its modification in the arterial wall.

We have examined the putative role of reactive oxygen species in the early impairment of endothelium-mediated responses by studying the effect of the naturally-occurring antioxidant, vitamin E, on endothelium-mediated relaxations. Male, New Zealand White rabbits of similar age and weight were fed either: (i) a control chow, (ii) 1% cholesterol, or (iii) 1% cholesterol plus 0.2% vitamin E diet, a non-hypocholesterolemic dose for 4 weeks. Animals were then killed, carotid arteries excised and cut into rings approximately 7mm in length. The rings were mounted horizontally in 10 ml organ baths for isometric tension measurements (Stewart-Lee et al 1991). Following the addition of 10 uM noradrenaline, which produced the equivalent to 60-70% of the maximum contractile response, cumulative concentration-response curves were constructed to the endothelium-dependent agonists; acetylcholine (ACh) (0.1-100 uM) and the calcium ionophore A23187 (0.1-100 uM) and the endothelium-independent agent; sodium nitroprusside (SNP) (0.1-300 uM). Carotid artery relaxant responses to ACh at 0.3, 1 and 3 uM were significantly reduced in the cholesterol-fed rabbits (n=10) (14.3±2.1; 29.9±3.8;45.1±5.1 % relaxation respectively) compared to the control group (n=9) (29.8±4.0;51.6±5.3;66.2±4.7 % relaxation respectively) ($p<0.01$;$p<0.01$and $p<0.05$).Relaxant responses were higher in the rabbits fed cholesterol plus vitamin E (n=6) (37.0±3.7;61.2±2.0;76.9±4.3 % relaxation respectively) ($p<0.01$ in each case) compared to animals receiving cholesterol alone. The relaxant responses to A23187 at 3, 10 and 30 uM did not differ significantly between control and cholesterol-fed animals, nor between controls and cholesterol plus vitamin E fed rabbits, but were significantly different for cholesterol (61.6±6.4; 65.1±5.8; 67.8±5.8 respectively) v cholesterol plus vitamin E groups (83.7±4.1; 87.3±4.1;91.8±4.3respectively) ($p<0.05$in each case). Responses to SNP did not differ significantly between the groups. These results indicate that vitamin E is effective in abrogating the deleterious effects of a cholesterol enriched diet on endothelium-mediated response, and support the notion that antioxidants may be beneficial in preventing atherosclerosis.

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406P THE EFFECT OF L-N-NITRO ARGININE METHYL ESTER AND SODIUM NITROPRUSSIDE ON 5-HT- AND KCl-INDUCED RESPONSES IN THE PERFUSED LUNG AND PULMONARY ARTERIES OF THE RAT

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In the isolated-perfused rat lung endogenous nitric oxide (NO) is a more powerful inhibitor of pressor responses mediated through PI (5HT) than depolarization (KCl). (Shaw et al,1992). One reason for this selective inhibition is that the inhibitory mechanisms that operate through elevated intracellular cGMP may be better able to interact with a receptor-operated transduction system (PI) rather than the mechanisms operating through membrane depolarisation. In addition, the factors that regulate NO levels are unclear. Basal release of NO may account for all of its inhibitory action; although increased shear stress arising from the intraluminal pressure of vasoconstriction also may be a powerful stimulus for NO release. In addition 5HT, unlike KCl, may be capable of stimulating NO release directly via $5HT_1$ receptors on the endothelium.

We sought to establish whether the release of NO is mediated directly by 5-HT or by pressure/shear stress and have therefore examined the effects of the NO synthase inhibitor L-N-nitro arginine methyl ester (L-NAME) on pressor responses in the isolated perfused rat lung and compared them with the contractile response of isolated pulmonary arteries where pressure/shear stress are not factors in NO release. In addition we have examined the effect of sodium nitroprusside (SNP) on 5-HT- & KCl-induced pressor and contractile responses in order to determine if NO is a more effective inhibitor of PI-mediated response rather than responses mediated through depolarisation. Male Wistar rats (300 g) were anaesthetised with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and heparinised (2000 i.u. kg^{-1}. i.p.). The trachea was cannulated and the rat respired artificially (stroke vol. = 2.5 ml, rate = 55 $min.^{-1}$). The pulmonary artery was cannulated and and the lungs perfused (6 ml. min^{-1}) with Krebs buffer (35°C, gassed with 95% O_2/ 5% CO_2). Drugs were administered by perfusion to equilibrium. Artery rings from 1st. branch pulmonary arteries from the rat were mounted in organ baths under 2 g tension in Krebs buffer at 35°C, gassed with 95% O_2/ 5% CO_2.

Responses to 5-HT were expressed as a percentage of the maximum KCl response. The maximum pressor response evoked by 5-HT in the absence of L-NAME was 16.8 ± 3.5 %. Inhibition of NO synthase increased this response to 52.3 ± 5.3.% (means ± S.E, n =8, $p < 0.05$). In isolated arteries the maximum contractile response to 5-HT was 117 ± 2 % and was unaltered by L-NAME. Carbachol (100 mM) but not 5-HT (0.1-1mM) relaxed preconsrticted arteries from 350 ± 32 to 225 ± 44 mg tension (means ± S.E, n = 4. $p < 0.05$). SNP inhibited both the pressor & contractile responses to 5-HT but had little or no effect on KCl-induced responses.

These result suggest that 5-HT is a more powerful agonist than KCl in pulmonary arteries. There may exist therefore, a population of arteries in the lung that are insensitive to 5-HT. NO is not released directly by 5-HT from pulmonary arteries suggesting that intraluminal pressure/shear-induced release may be important. NO is a more effective inhibitor of PI-mediated than depolarisation-mediated responses.

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407P COMPARISON OF THE EFFECTS OF NITRIC OXIDE AND BRL 38227 ON MEMBRANE POTENTIAL AND TONE IN THE RABBIT ISOLATED BASILAR ARTERY

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Endothelium-dependent relaxation of the rabbit isolated basilar artery by acetylcholine is accompanied by transient hyperpolarization of the smooth muscle cell membrane and both events are attenuated by inhibitors of nitric oxide (NO) synthase (Rand & Garland, 1992). However, in this vessel exogenous NO failed to evoke smooth muscle hyperpolarization except at concentrations which elicited maximal smooth muscle relaxation (Rand & Garland, 1992). In the rat mesenteric artery NO-evoked hyperpolarization was abolished by the potassium channel blocker glibenclamide (Garland & McPherson, 1992) which also inhibits responses to potassium channel opening drugs such as BRL 38227 (Buckingham *et al.*, 1989) suggesting a similar mechanism of action.
In this study, the contractile and membrane responses of the rabbit isolated basilar arfery to NO and BRL 38227 were investigated. NO gas in concentrations up to 150 μM (n=6) did not alter the resting membrane potential of the smooth muscle cells. When noradrenaline (10-100 μM) was used to contract and depolarize the smooth muscle cells, the addition of NO (0.5-150 μM) evoked dose-dependent, transient relaxation but failed to elicit membrane hyperpolarization. In contrast, the potassium channel opener BRL 38227 (1-100 μM) evoked dose-dependent hyperpolarization of the resting membrane potential of smooth muscle cells in the rabbit basilar artery, the maximal response being 14 $\pm$ 3.2mV (n=6). Furthermore, when noradrenaline (10-100 μM) was used to contract and depolarize the smooth muscle cells, BRL 38227 (0.01-10 μM) elicited dose-dependent relaxation and hyperpolarization (maximal hyperpolarization 25 $\pm$ 2.3mV; n=4). The sulfonylurea compound glibenclamide (10 μM) inhibited both BRL 38227-evoked relaxation and hyperpolarization. The maximal hyperpolarization was completely abolished with this concentration of glibenclamide and the maximal relaxation was reduced by 80 $\pm$ 5.2% (n=6).
These results demonstrate that NO, even at very high concentrations, does not induce membrane hyperpolarization in the rabbit basilar artery. However, this vessel shows similar sensitivity to the potassium channel opener BRL 38227 as other blood vessels indicating that the apparant lack of effect of NO on membrane potential does not reflect the absence of BRL 38227- and glibenclamide-sensitive potassium channels.

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408P TEMPORAL EXPRESSION AND CELLULAR DISTRIBUTION OF NADPH DIAPHORASE STAINING IN THE CHRONIC GRANULOMATOUS TISSUE OF THE MURINE CROTON OIL-INDUCED AIR POUCH

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In neuronal tissues nitric oxide synthase (NOS) is identical to NADPH diaphorase (Dawson et al., 1991). The production of NOS, NADPH-dependent enzymes has been demonstrated in cytokine-stimulated macrophages, neutrophils and endothelial cells (Forsterman 1991). However, there is little information on NADPH diaphorase activity in these cell types in inflammatory tissues. Therefore the presence of NADPH diaphorase activity was studied histochemically in the chronic granulomatous tissue of the murine croton-oil induced air pouch.

Female TO mice (30±2g) were anaesthetized and 3ml of air injected into the dorsal subcutaneous tissue, followed on day 1 by injection of 0.5ml of 0.1% v/v croton oil in Freund's complete adjuvant. The pouch was left for 3,5,7,14,21, and 28 days, animals were then sacrificed by terminal anaesthesia. Blood smears from control and inflamed animals and 10μm cryostat sections from the air pouch through the skin and granulomatous tissue were taken at all time points. Samples were stained for NADPH diaphorase activity according to a modification of the method of Scherer-Singler et al., 1983.

Up to 14 days NADPH diaphorase selectively stained macrophages associated with blood vessels in the loose connective tissue of the subdermis. Occasionally positively stained monocytes were observed in the lumen of vessels in this area. At all time points NADPH diaphorase positive macrophages were seen in the granuloma. As the granuloma developed morphometric analysis showed the macrophages to be associated with the fibrotic regions of the tissue. Endothelial cells of blood vessels in the subdermal region were heterogeneously stained, with the arterioles staining most intensely. However, the majority of the endothelial cells in the developing vasculature of the granulomatous tissue were unstained. Neutrophils were present in the granulomatous tissue, but were not stained. In blood smears from inflamed animals a minority of monocytes were stained for NADPH diaphorase. The number of positive monocytes appeared constant throughout the time course. Monocytes from control smears were unstained.

The observation of NADPH diaphorase stained monocytes in the vasculature, coupled with the finding that the number of positively stained monocytes in peripheral blood was constant, suggests that these cells were activated *en passant* through the inflamed tissue. If NADPH diaphorase staining in the cells of inflammatory tissues is synonymous with NOS, the lack of staining of endothelial cells in the developing vasculature of the granulomatous tissue, could support the finding that NO is an important modulator of leucocyte adherence and infiltration in the inflammatory process.

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409P L-NAME REVEALS AN INCREASED SENSITIVITY TO THE VASOCONSTRICTOR EFFECT OF U46619 IN THE MESENTERIC ARTERIAL AND VENOUS MUSCLES FROM RATS TREATED WITH LPS

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Incubation of rat aortic rings with interleukin-1 or lipopolysaccharide (LPS) inhibits subsequent contractions in a time dependent manner (Beasley et al., 1989) most probably due to the induction of nitric oxide (NO) synthase, leading to elevated levels of NO and increased stimulation of guanylyl cyclase (Beasley, 1990; Fleming et al., 1990). LPS also depresses the vasoconstrictor responses to U46619 in the isolated perfused heart of the rabbit (Smith et al., 1991). Treatment of rats with LPS results in a large induction of NO synthase in the mesentery (Mitchell et al., this meeting). We have therefore examined the effects of LPS treatment on the responses of the arterial and venous vessels of the mesentery.

Male albino Wistar-Kyoto rats (300-350 g), some pretreated (6 h) with LPS (5 mg/kg) were anaesthetised (sodium barbitone, 100 mg/kg). The superior mesenteric artery and vein were cannulated and the vascular bed perfused (10 min, 2 ml/min) with Krebs' buffer containing heparin (100 U/ml). The intestine was then separated from the mesentery and the arteries and veins were independently perfused (2 ml/min) with warmed (37 °C) and gassed (95% O_2: 5% CO_2) Krebs' buffer (Warner, 1990). Changes in perfusion pressures were measured by means of pressure transducers. Vasoconstrictor agents (endothelin-1, ET-1; 5-hydroxytryptamine, 5HT; phenylephrine, PE; U46619) were administered as close arterial or venous bolus doses (1-3 μl). In experiments to examine the effects of inhibition of NO synthase N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) was given as an infusion to the arterial and venous circulations throughout the *in vitro* experimental period.

Pretreatment of rats with LPS had no effect on the responses of either the arterial or venous portions of the circulation to ET-1 (3-30 pmol), 5HT (1-10 nmol), PE (1-10 nmol) or U46619 (30-300 pmol) (n=4-6). In the presence of L-NAME the responses of the arterial vessels from control animals to ET-1 (30 pmol), 5HT (10 nmol), PE (10 nmol) and U46619 (300 pmol) were all significantly potentiated (by 184%, 220%, 264% and 109%, respectively). In the venous vessels there was no significant potentiation of any contractile responses. In the presence of L-NAME the responses of the mesenteric arterial vessels from LPS-treated rats were potentiated to a similar degree in controls (ET-1, 257%; 5HT, 133%; PE, 230%). The effects of U46619 were potentiated further than in control (425%). In the venous vessels from LPS-treated animals L-NAME also potentiated vasconstrictions to U46619 (113%, $p<0.05$) (but not ET-1, 5-HT, or PE) compared to control tissues exposed to L-NAME.

This data suggests that the induction of NO synthase in the mesenteric vessels may be associated with an increase in the sensitivity to contractile agents which counteracts the elevated levels of NO. This effect is particularly relevant to responses to U46619. In addition, our data with tissues taken from control animals illustrates that the production of NO is more important in regulating the responses of arterial than venous vessels.

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410P THE TIME COURSE OF LIPOPOLYSACCHARIDE-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION IN THE RABBIT

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Systemic bacterial infections can impair any or all of the haemostatic mechanisms including the platelets, the coagulation system and the vessel wall. A consequence of sepsis is often consumptive thrombocytopenia, either alone or in conjunction with disseminated intravascular coagulation (DIC). Fibrin formed during this process may result in microvascular thrombosis and end-organ failure, (McKay & Muller-Berghaus, 1967). The aim of the present study was to elucidate the time course of fibrin deposition and other, cellular, events following lipopolysaccharide (LPS) endotoxin-induced DIC in the rabbit. Male NZW rabbits (2.8 - 4.2 kg, n=36) were anaesthetised and instrumented for blood pressure/heart rate monitoring, i.v. drug administration and arterial blood sampling. Human ^{125}I fibrinogen (1μCi/kg i.v. bolus) was given as a marker of fibrin deposition and after 30 minutes animals received an infusion of either control saline (Group 1; 10ml/hr i.v. for 1 hr) or bacterial LPS (10 mg/kg/hr i.v.). Whole blood samples were analysed periodically throughout the experiments for changes in blood gas status and platelet, RBC, and WBC counts. LPS animals were terminated at: 15 min inf.,Grp2; 30 min inf.,Grp3; 60 min inf.,Grp4; 4 hours post a 60 min inf.,Grp 5. Control animals were terminated at 4 hours post inf. Biopsies of selected organs were analysed for ^{125}I fibrin content and deposition ratios (DR) were calculated compared to final blood ^{125}I levels. Results are summarised in the table below.

Parameter	Time point	Group 1, NaCl +4 hrs	Group 2, LPS 15 min	Group 3, LPS 30 min	Group 4, LPS 60 min	Group 5, LPS +4h
Spleen DR	End expt	0.23 ± 0.02	0.30 ± 0.03	0.32 ± 0.04	0.31 ± 0.03 (a)	0.53 ± 0.09 (a)
Kidney DR	End expt	0.18 ± 0.009	0.11 ± 0.006 (a)	0.12 ± 0.008 (a)	0.13 ± 0.016 (a)	0.33 ± 0.04 (a)
WBC	Time 0	4.20 ± 0.31	4.72 ± 0.46	4.40 ± 0.46	3.42 ± 0.07	3.70 ± 0.23
($x10^3/mm^3$)	End expt	4.86 ± 0.62	1.61 ± 0.16 (a,b)	1.26 ± 0.21 (a,b)	0.92 ± 0.17 (a,b)	0.96 ± 0.17 (a,b)
Platelet Count	Time 0	314 ± 29	362 ± 39	374 ± 36	396 ± 32	380 ± 52
($x10^3/mm^3$)	End expt	259 ± 36 (b)	263 ± 39 (b)	262 ± 42 (b)	345 ± 68	237 ± 15 (b)
Mean arterial	Time 0	73 ± 4	75 ± 3	70 ± 4	73 ± 3	71 ± 5
pressure (mmHg)	End expt	66 ± 3	71 ± 2	69 ± 6	71 ± 7	43 ± 4 (a,b)
Heart rate	Time 0	264 ± 14	255 ± 19	231 ± 14	261 ± 11	269 ± 12
(bpm)	End expt	249 ± 17	226 ± 10	220 ± 16	234 ± 17	283 ± 11 (b)

Data are mean ± sem. (a: P<0.05 compared to Group 1, Mann-Whitney U test; b: P<0.05 compared to time 0 within group, Wilcoxon signed ranks test)

Cellular responses to LPS were rapid in onset. Leukopenia was evident by 15 min infusion (Grp 2), became progressively more severe by 60 min infusion (Grp 4) and was then sustained at that level up to 4h post infusion (Grp 5). In contrast, changes in platelet count were neither profound nor sustained. Significant fibrin deposition was seen only in the kidneys and spleen. The renal deposition occured as a late phase response, seen only after 4h (Grp 5), whilst splenic deposition was evident after 15 min (Grp 2), becoming more profound at 4h (Grp 5). These results indicate that there are two distinct phases in the evolution of responses to LPS-induced DIC in this model, an early, cellular response, and a later fibrin response. Clearly, any pharmacological intervention would have to be initiated prior to LPS challenge in order to modulate the initial cellular responses, later phase intervention would need to be aimed at reversing the leukopenia. However, the fibrin deposition could theoretically be attenuated with suitable agents at a stage prior to full expression of this more delayed response.

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411P THE EFFECTS OF ORALLY ADMINISTERED D-MEPHE-PRO-ARG-H (GYKI-14766) ON THROMBIN-INDUCED THROMBOCYTOPENIA AND DISSEMINATED MICROTHROMBOSIS IN RATS

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Thrombin plays a central role in the coagulation cascade and also stimulates platelet activation/aggregation. For this reason antithrombotic agents, such as heparin, are used to prevent or alleviate certain thrombotic disorders. Limitations associated with heparin therapy are well documented (Knabb et al., 1992) Heparin relies on antithrombin III for efficacy and thrombin bound to fibrin monomers may be resistant to inactivation by the heparin-antithrombin III complex. Also, standard heparin is not orally active, necessitating multiple subcutaneous administrations. D-MePhe-Pro-Arg-H (GYKI-14766) is a synthetic , tripeptide reversible thrombin inhibitor, which is reportedly orally active (Bagdy et al., 1992).

In the present study we evaluated the oral activity of GYKI-14766 in anaesthetised rats, first with respect to activated partial thromboplastin time (APTT) and thrombin time (TT). Male Wistar rats (225-360g, n=24) were starved overnight in grid-bottomed cages and allowed water ad libitum. Rats were subsequently randomised to one of four treatment groups for dosing by oral gavage as follows: 0.9% saline (1.0 ml kg^{-1}); GYKI-14766 1.0 mg kg^{-1}; GYKI-14766 3.0 mg kg^{-1}; GYKI-14766 10.0 mg kg^{-1}. The animals were subsequently anaesthetised and arterial blood samples taken at 30 minute intervals for the determination of APTT and TT. We also evaluated the efficacy of orally administered GYKI-14766 to inhibit thrombin-induced thrombocytopenia/disseminated microthrombosis. Animals (n=24) were starved overnight and dosed randomly as follows: 0.9% saline (1.0 ml kg^{-1}); GYKI-14766 1.0 mg kg-1; GYKI-14766 10.0 mg kg^{-1}. The animals were anaesthetised, instrumented and received 1.0 µCi kg^{-1} ^{125}I-labelled fibrinogen (Amersham) i.v. Thirty minutes later, half of the animals that had been pretreated with saline received an infusion of 0.9% saline (2.45 ml h^{-1}) while the remainder, together with groups that had received GYKI-14766, received thrombin (700 U kg^{-1} h^{-1}) at the same infusion rate. Platelet count was determined (Sebia Hemalaser 2AF cell counter) and fibrinogen deposition was quantified (Minaxi gamma counter) in the lungs, heart, kidneys and liver and related to blood ^{125}I-label.

Oral administration of GYKI-14766 in the dose range 1.0-10.0 mg kg^{-1} did not alter APTT. However, 10.0 mg kg^{-1} GYKI-14766 caused a significant, although minimal, elevation in TT (up to 3 fold, $p < 0.05$) within 30 minutes of administration and the elevation was maintained for at least 120 minutes, confirming that GYKI-14766 has activity following oral administration. Systemic administration of thrombin induced a marked thrombocytopenia with deposition of ^{125}I-labelled fibrinogen in the renal microvasculture (see table). Orally administered GYKI-14766 at 10.0 mg kg^{-1}, but not at 1.0 mg kg^{-1}, inhibited renal microthrombosis significantly ($p < 0.05$) and reduced the extent of thrombocytopenia associated with thrombin administration, with minimal prolongation of TT. This study illustrates the therapeutic potential of direct acting thrombin inhibitors such as GYKI-14766, which may have advantages, in particular oral activity, over current heparin therapy.

Group	Renal deposition ratio	% change platelet count
saline control	0.18 ± 0.03	+3.1 ± 8.1
saline + thrombin	1.54 ± 0.37	-20.9 ± 0.9
GYKI-14766 1.0mgkg^{-1} + thrombin	1.16 ± 0.46	-23.8 ± 7.3
GYKI-14766 10.0mgkg^{-1} + thrombin	0.53 ± 0.18*	-14.1 ± 5.0

* $p < 0.05$ compared to saline + thrombin (unpaired t-test)

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412P LACK OF POTENCY OF THE ENDOTHELIN PRECURSOR, BIG ENDOTHELIN-1, ON THE SMOOTH MUSCLE OF THE ISOLATED PERFUSED PORCINE SPLEEN

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In the pig (Hemsen et al, 1991) the intravenous administration of the endothelin-1 (ET-1) precursor (big ET-1) causes a small rise in mean systemic blood pressure. However, accompanying changes in regional vascular resistances suggest that not all vascular territories contribute to this pressor effect. The purpose of the present experiments was to assess the capacity of the porcine splenic endothelium to convert big ET-1 to the active form, ET-1.

Large white pigs (mean weight 24.5 $\pm$ 1.5kg) were anaesthetized with halothane and N_2O, the spleen (mean weight 48.5 $\pm$ 5.9g) was removed and after cannulation of the splenic artery and vein was perfused at constant flow (108 $\pm$ 2.9ml. min^{-1}) with Krebs solution equilibrated with 95% 0_2 and 5% CO_2 at 37°C. The spleen was enclosed in a perspex plethysmograph with continuous recording of spleen volume and splenic arterial perfusion pressure (SAPP; mean initial basal pressure 57 $\pm$ 8.4mmHg). Porcine ET-1, big ET-1 (Peptide Institute Inc., Osaka) and adrenaline (Ad; BDH) were injected as intra-arterial bolus doses over the range 1.0-100pmol (ET-1), 1.0pmol-3.0nmol (big ET-1) and 0.5-500nmol (Ad) to construct dose response curves. Previous results, using the dog spleen (Withrington et al, 1992), were confirmed in the pig spleen since ET-1 produced a dose dependent increase in splenic arterial vascular resistance of prolonged duration with an accompanying reduction in spleen volume. The mean ED_{80} (mean molar dose to increase SAPP by 80% of the control pressure) was 11.7 $\pm$ 3.0pmol and 28.9 $\pm$ 7.9nmol for ET-1 and Ad respectively ($P < 0.01$). In contrast, there were no significant changes in either spleen volume or splenic arterial vascular resistance with any dose of the precursor big ET-1.

Intra-arterial infusion of big ET-1 (1.0nmol min^{-1}), followed by varying periods (1-5 mins) of both arterial and venous occlusion allowed continued contact between the precursor and the splenic vascular endothelium. Subsequent release of the occlusion did not lead to any changes in splenic vascular resistance. In addition, the dose response curve to ET-1 for both capsular and vascular smooth muscle was not shifted in either direction by the concomitant intra-arterial infusion of big ET-1 (1.0nmol min^{-1}).

The results indicate that big ET-1 has no intrinsic efficacy on splenic smooth muscle and that the spleen is not an active site for its conversion to ET-1. This may represent an absence of converting enzyme within the splenic endothelial cells or a lack of uptake of the circulating precursor into the endothelium.

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413P COMPARISON OF PRESSOR RESPONSES TO ENDOTHELIN-1 AND BIG ENDOTHELIN-1 IN THE PITHED RAT

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The pressor effect of big endothelin-1 (big ET-1) in vivo may be mediated by endothelin-1 (ET-1), produced from cleavage of big ET-1, acting on ET receptors (Kashiwabara et al., 1989). The exact nature of the converting enzyme is unknown but it can be inhibited by the metalloprotease inhibitor phosphoramidon (Matsumura et al., 1990). The present study examines the effect of phosphoramidon and BQ-123, an ET receptor antagonist (Ihara et al., 1991), on pressor responses to big ET-1 and ET-1.

Alderley Park rats (280-330g) were anaesthetised with halothane and artificially respired through a tracheal cannula with 40% O_2, 60% N_2. Rats were pithed and the right jugular vein and carotid artery cannulated for administration of drugs and measurement of mean arterial pressure (MAP) respectively. Phosphoramidon or BQ-123 were administered 1 min prior to administration of either big ET-1 (3.0 nmol kg^{-1}) or ET-1 (1.0 nmol kg^{-1}) and changes in MAP assessed (n=4-10). Results are expressed as % inhibition of the pressor response seen in the absence of phosphoramidon or BQ-123.

Table 1. % Inhibition of pressor response to single dose of endothelins.

	Phosphoramidon ($mgkg^{-1}$)			BQ-123 ($mgkg^{-1}$)			
	1.0	5.0	10.0	0.1	0.3	1.0	3.0
Big ET-1	4.8	37.4†	85.4†	10.4*	54.1†	74.6†	-
ET-1	-	6.0	-	8.3	33.0*	48.5†	73.5†

* $p < 0.05$
† $p < 0.001$
unpaired students t-test

Both big ET-1 and ET-1 induced long-lasting increases in MAP (104.2 $\pm$ 2.7 mmHg and 84.0 $\pm$ 3.6 mmHg respectively). The pressor response to ET-1 was preceded by a transient fall in MAP (12.4 $\pm$ 0.9 mmHg) which was unaffected by phosphoramidon but potentiated by BQ-123 (20.8 $\pm$ 1.8 mmHg ($p < 0.01$) at 3.0 $mgkg^{-1}$).

The ability of phosphoramidon to attenuate the pressor response to big ET-1 without affecting the pressor response to ET-1 provides further evidence to suggest that the action of big ET-1 is dependent on its conversion to ET-1. The ability of BQ-123 to inhibit pressor responses to both endothelins suggests that ET-1 produced from cleavage of big ET-1 exerts its pressor effects via an action on the same receptor as exogenously applied ET-1.

Kashiwabara et al., (1989) FEBS Lett. 247, 73-76.
Matsumura et al., (1990) Eur. J. Pharmac. 185, 103-106.
Ihara et al., (1991) Life Sci. 50, 247-255.

414P REGIONAL HAEMODYNAMIC RESPONSES TO i.a. AND i.v. INJECTION OF ENDOTHELIN-1 AND PROENDOTHELIN-1 (1-38) IN CONSCIOUS RATS

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It has been reported that endothelin-1 (ET-1) has lesser effects on arterial blood pressure when given i.v. than when given i.a. in anaesthetised or pithed rats, consistent with ET-1 being cleared in the pulmonary circulation (De Nucci *et al.*, 1988). However, the regional haemodynamic responses to i.a., compared to i.v., administration of ET-1 in conscious rats are not known. Furthermore, the comparative regional haemodynamic effects of i.a. and i.v. injections of proET-1 in conscious rats have not been described. Therefore, we assessed the cardiovascular effects of ET-1 and proET-1 [1-38] given i.v. and i.a. to conscious, male, Long Evans rats. Under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.) animals were instrumented with pulsed Doppler probes (to monitor renal, mesenteric and hindquarters blood flows) and intravascular catheters; surgery was in 2 stages separated by at least 7 days. One day after catheter implantation experiments were begun and they ran over 2 days with animals receiving randomised i.v. or i.a. injections of ET-1 or proET-1 at 50 pmol kg^{-1}. This dose was chosen to give clearly quantifiable results, and because it is about the middle of the dose range for ET-1 used by De Nucci *et al.* (1988). The results are summarised in Table 1.

Table 1: Cardiovascular responses to i.a. or i.v. injection of ET-1 or proET-1 in the same conscious, Long Evans rats (n = 7). Values are mean ± s. e. mean; * P<0.05 for change (Friedman's test).

	ET-1 i.a.		ET-1 i.v.		proET-1 i.a.	pro-ET-1 i.v.
	15 s	5 min	15 s	5 min	15 min	15 min
Δ Heart rate (beats min^{-1})	87 ± 8*	-41 ± 12*	89 ± 7*	-50 ± 9*	-81 ± 16*	-56 ± 9*
Δ Mean blood pressure (mmHg)	-25 ± 3*	31 ± 5*	-30 ± 3*	33 ± 4*	35 ± 4*	35 ± 3*
Δ Renal flow (%)	-48 ± 16	-55 ± 4*	-53 ± 14	-50 ± 8*	-23 ± 4*	-24 ± 5*
Δ Mesenteric flow (%)	-30 ± 8*	-55 ± 4*	-21 ± 7	-49 ± 5*	-31 ± 5*	-25 ± 7*
Δ Hindquarters flow (%)	78 ± 12*	-20 ± 9	71 ± 13*	-21 ± 8*	-32 ± 4*	-33 ± 6*
Δ Renal conductance (%)	-31 ± 21	-65 ± 3*	-37 ± 20	-62 ± 6*	-41 ± 4*	-42 ± 5*
Δ Mesenteric conductance (%)	-7 ± 11	-65 ± 4*	10 ± 11	-61 ± 3*	-47 ± 5*	-43 ± 6*
Δ Hindquarters conductance (%)	138 ± 24*	-37 ± 8*	138 ± 20*	-38 ± 8*	-49 ± 3*	-50 ± 3*

ET-1 caused an initial hypotension, tachycardia and hindquarters vasodilatation, followed by hypertension, bradycardia and renal, mesenteric and hindquarters vasoconstriction; the effects with i.a. and i.v. injections were the same. The dose of proET-1 caused no initial hypotension, but there was a gradual hypertension, bradycardia, and renal, mesenteric and hindquarters vasoconstriction. ProET-1 given i.a. or i.v. had similar effects. These results provide no evidence for substantial pulmonary clearance of ET-1 or for pulmonary activation of proET-1.

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415P REGIONAL HAEMODYNAMIC RESPONSES TO [ALA^{1,3,11,15}] ENDOTHELIN-1 IN CONSCIOUS RATS

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Current dogma holds that endothelin-1 (ET-1) acts through ET_A receptors on vascular smooth muscle to cause contraction and through ET_B receptors on endothelial cells to cause relaxation (Sakurai *et al.*, 1992). There is evidence that [Ala^{1,3,11,15}]ET-1 is selective for ET_B receptors (Saeki *et al.*, 1991), and so we investigated the haemodynamic effects of this analogue *in vivo*. [Ala^{1,3,11,15}]ET-1 was synthesised using solid phase t-Boc chemistry, purified by gel filtration and the sequence confirmed by amino-acid analysis. Peptide concentration was determined by u.v. spectrophotometry. Aliquots were freeze dried and stored at -20°C until reconstituted in physiological saline. Male, Long Evans rats were instrumented with pulsed Doppler probes and intravascular catheters in 2 stages (7 days apart) under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.). At least 24 h after catheter implantation experiments were begun, with animals receiving bolus i.v. injections of [Ala^{1,3,11,15}]ET-1 ranging from 0.15-10 nmol kg^{-1}, with each dose separated by a minimum of 30 min. Table 1 summarises the results.

Table 1: Changes in heart rate (HR), mean arterial blood pressure (MAP) and vascular conductance (VC) following i.v. bolus injection of [Ala^{1,3,11,15}]ET-1 in the same conscious, Long Evans rats (n = 7). Values are mean ± s. e. mean; * P<0.05 for change.

	0.15 nmol kg^{-1}			0.3 nmol kg^{-1}		
	15 s	1 min	3 min	15 s	1 min	3 min
Δ HR (beats min^{-1})	25 ± 11*	9 ± 10	14 ± 12	20 ± 8	-2 ± 7	0 ± 14
Δ MAP (mmHg)	0 ± 1	1 ± 1	-1 ± 1	1 ± 1	2 ± 1	2 ± 1
Δ Renal VC (%)	1 ± 1	-2 ± 1	0 ± 2	0 ± 1	-5 ± 1*	-4 ± 2
Δ Mesenteric VC (%)	-13 ± 3*	-11 ± 2*	-7 ± 2*	-27 ± 4*	-21 ± 3*	-15 ± 3*
Δ Hindquarters VC (%)	23 ± 8*	13 ± 5*	7 ± 3	18 ± 8*	13 ± 10	13 ± 9
	1 nmol kg^{-1}			10 nmol kg^{-1}		
	15 s	1 min	3 min	15 s	1 min	3 min
Δ HR (beats min^{-1})	21 ± 6*	-17 ± 8	-19 ± 8	42 ± 11*	-18 ± 13	-32 ± 11
Δ MAP (mmHg)	6 ± 2*	7 ± 2*	5 ± 2*	4 ± 1*	24 ± 3*	28 ± 4*
Δ Renal VC (%)	-10 ± 3*	-14 ± 2*	-11 ± 3*	-24 ± 6*	-56 ± 3*	-51 ± 5
Δ Mesenteric (%)	-49 ± 5*	-44 ± 4*	-30 ± 4*	-75 ± 3*	-74 ± 2*	-73 ± 2*
Δ Hindquarters VC (%)	27 ± 9*	11 ± 6	3 ± 4	42 ± 11*	3 ± 6	-19 ± 6*

Although [Ala^{1,3,11,15}]ET-1 did cause a transient hindquarters vasodilatation, it is notable that it did not cause an initial hypotension, possibly because of the marked, dose-dependent mesenteric vasoconstriction. While our results differ in some respects from those of Bigaud (1992) (possibly due to his use of anaesthetised rats), we agree with him in that our results provide no evidence for ET_B receptors being specifically coupled to vasodilator processes.

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416P CHARACTERISATION OF THE PROSTAGLANDIN E (EP) RECEPTOR POPULATION IN ISOLATED MOUSE UTERUS

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Previous studies in this laboratory employing eight different species have failed to reveal an animal model in which the myometrium possesses the inhibitory EP_2-receptor found on human myometrium (Senior *et al.*, 1991). Using the available antagonists and selective agonists the aim of this functional study was to define the EP-receptor population on the mouse uterus.

To synchronise oestrous mature female BKW mice were pretreated with stilboestrol (s.c., 100μg $100g^{-1}$) 18h prior to the study. Uterine horns were superfused (2ml min^{-1}) with oxygenated Krebs solution containing 2.79μM indomethacin (Sangha & Senior, 1989). After equilibration of the tissue agonists were injected directly into the flow of the superfusate, immediately after a spontaneous contraction, as bolus doses. As the profile of spontaneous activity and sensitivity to agonists often changed throughout the course of the experiments, comparisons were made between preparations in a non paired manner. Because of the variations in myogenic activity results have been normalised to take into account the level of background activity, thus the results have been expressed as T/B ratios of 'test' (T) agonist responses as a ratio of backgound (B) contractions (see Senior *et al.*, 1991).

The selective EP-receptor agonists used were as follows: butaprost ($EP_2 >> EP_1$ & $EP_3 > 0$); misoprostol and rioprostil ($EP_3 > EP_2 > EP_1$); sulprostone ($EP_3 \simeq EP_1 >> EP_2 \simeq 0$). The antagonists used were AH6809 (10μM) and GR32191 (1μM) to eliminate EP_1 and TP-receptor activity respectively (Senior *et al.*, 1991).

PGE_2 (0.001-100nmoles) evoked a biphasic response, the excitatory component predominating at lower doses but at higher doses inhibition dominated, thus producing a bell-shaped dose effect curve. AH6809 depressed the excitatory element of the response. Misoprostol (0.001-100nmoles) and rioprostil (0.001-100nmoles) produced responses qualitatively similar to PGE_2. The most marked inhibition of myogenic activity was achieved with butaprost (0.001-100nmoles), however, this was not a purely inhibitory response, the initial contractile element being attenuated by AH6809. Sulprostone elicited a purely contractile response which was antagonised by AH6809.

The results of this preliminary study suggest that there is a heterogeneous EP-receptor population on mouse uterus *in vitro*. Therefore, the mouse may represent a suitable *in vivo* model for further investigation of these compounds on the female reproductive tract. This study is currently being extended to cover the other PG receptors present on mouse uterus.

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417P REGIONAL HAEMODYNAMIC RESPONSES TO INDOMETHACIN IN CONSCIOUS RATS WITH STREPTOZOTOCIN (STZ)-INDUCED DIABETES MELLITUS

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There is evidence for abnormalities of prostanoid production in tissues from STZ-treated rats (see Tomlinson *et al.* (1992) for review). Therefore, in the present work we assessed haemodynamic responses to indomethacin (5 mg kg^{-1} i.v. loading dose, given over 10 min, 5 mg kg^{-1} h^{-1} infusion, given over the subsequent 30 min) in conscious, control (i.e. saline-injected, 1 ml kg^{-1} i.p.) and STZ-treated (70 mg kg^{-1} i.p., 3 weeks previously), Wistar rats, that were chronically instrumented with pulsed Doppler flow probes and intra-vascular catheters (all surgery carried out under sodium methohexitone anaesthesia, 40 mg kg^{-1} i.p., supplemented as required (Kiff *et al.*, 1991). At the time of the experiments, blood glucose was greater than 20 mmol l^{-1} in the STZ-treated rats. There was no difference in mean arterial blood pressure (MAP) in the 2 groups (control, 113 ± 4; STZ, 124 ± 2 mmHg; mean ± s. e. mean, n = 8, both groups), but STZ-treated rats had a resting bradycardia (STZ, 289 ± 12; control, 342 ± 13 beats min^{-1}; $P<0.05$, Mann-Whitney U test), raised mesenteric vascular conductance (MVC) (STZ, 99 ± 9; control, 68 ± 5 ([kHz $mmHg^{-1}$]10^3)) and reduced hindquarters vascular conductance (HVC) (STZ, 23 ± 4; control, 46 ± 7 ([kHz $mmHg^{-1}$]10^3)). Responses to indomethacin were assessed from areas under or over curves (AUC or AOC) from 0-40 min. Administration of indomethacin caused a greater pressor effect and bradycardia in STZ-treated rats (AUC and AOC, 24 ± 5 and 67 ± 17 units, respectively) than in control rats (AUC and AOC, 11 ± 3 and 39 ± 7 units, respectively), and these changes were accompanied by reductions in mesenteric blood flow (MBF) and MVC that were larger in STZ-treated (AUC, MBF 8.0 ± 1.4 units, MVC 79 ± 15 units) than in control rats (AUC, MBF, 3.6 ± 0.7 units; MVC, 36 ± 7 units). However, in the presence of indomethacin there was still a significant difference between MBF and between MVC in the 2 groups (STZ, MBF, 10.8 ± 1.0 kHz; MVC, 85 ± 7 ([kHz $mmHg^{-1}$]10^3); control, MBF, 6.0 ± 0.5 kHz; MVC, 51 ± 4 ([kHz $mmHg^{-1}$]10^3)), and reduced in hindquarters blood flow (HBF) and HVC in the STZ-treated rats (HBF, 2.7 ± 0.3 kHz; HVC, 22 ± 3 ([kHz $mmHg^{-1}$]10^{-1})) compared to control rats (HBF, 4.2 ± 0.5 kHz; HVC, 36 ± 5 ([kHz $mmHg^{-1}$]10^3)) was still apparent. Hence, these differences are not likely to have been due to overproduction of vasodilator or vasoconstrictor prostanoids, respectively.

In addition, i.v. bolus injection of acetylcholine (1 nmol kg^{-1}) elicited similar falls in MAP and increases in renal vascular conductance in control rats (AOC and AUC 312 ± 7 and 78 ± 7 units) and STZ-treated rats (327 ± 13 and 71 ± 4 units) and these were not affected by indomethacin (control 322 ± 9 and 74 ± 9 units; STZ 322 ± 13 and 81 ± 8 units). These results corroborate our earlier findings (Kiff *et al.*, 1991) and, unlike the results of Bucala *et al.* (1991) provide no evidence for vasodilator dysfunction in STZ-treated rats.

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418P ABSTRACT WITHDRAWN

419P EFFECTS OF LIPOCORTIN-RELATED PEPTIDES UPON NEUTROPHIL BEHAVIOUR *IN VITRO*

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The peptides AF-1 (MQMKKVLDS; Miele *et al.*, 1988) and 204-212 (SHLRKVFDK; Perretti *et al.*, 1991) have been generated from the consensus sequences of uteroglobin and lipocortin (annexin) 5, and shown to have various anti-inflammatory properties *in vivo* and *in vitro* (Camussi *et al.*, 1990). These actions include inhibition of neutrophil accumulation, although their mechanism of action is unknown. We have investigated their effects upon neutrophil behaviour *in vitro*.

Glycogen-elicited neutrophils from male Wistar rats were pre-incubated with drugs or peptides at room temperature for 15 min before addition to plasma-coated 96-well plates containing stimuli as indicated at a final concentration of $0.5x10^6$ cells/well (0.2ml) in HBSS containing 1.2mM Ca^{2+} and Mg^{2+}. Plates were incubated at 37° for 30 min, washed to remove non-adherent cells and adherent cells quantified using Rose Bengal dye. Neutrophil aggregation was determined in a Payton aggregometer in HBSS using $20x10^6$ cells/ml at 37°.

f-Met-Leu-Phe (fMLP; 0.1-100nM), platelet-activating factor (PAF; 1.9-190nM) and phorbol ester (PMA; 1-100nM) caused a dose-dependent increase in cell adherence which was dependent upon the presence of Mg^{2+}. The peptides AF-1 and 204-212 caused a dose-dependent (2.5-250μM) inhibition of fMLP-induced adherence with IC_{50}'s of 20 and 80μM respectively. The effects upon PAF-induced adherence were less clear-cut, with 65% inhibition at 2.5μM, but no clear dose-response relationship. Neither peptide affected adherence stimulated with PMA. fMLP, PAF and PMA stimulated neutrophil aggregation over the same dose range as for adherence. Neither peptide affected aggregation in response to any of the stimuli. In conclusion, both these peptides selectively inhibit neutrophil adhesion *in vitro*; such an action may contribute to their anti-inflammatory activity.

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420P POTENTIATION OF ACTIVATED NEUTROPHIL-INDUCED CHEMILUMINESCENCE BY THE GUANIDINE DERIVATIVE EI-130

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Stimulated neutrophils produce proteolytic enzymes and reactive oxygen species (ROS). We investigated the effect of the guanidine derivative, 3-chloro-5-hydroxyphenyl p-guanidinobenzoate EI-130 (a trypsin and thrombin serine protease inhibitor) on neutrophil induced chemiluminescence.

Human neutrophils were separated from blood by using methylcellulose sedimentation, Hypaque-Ficoll centrifugation and hypotonic lysis. Neutrophils were stimulated with phorbol myristate acetate (PMA), formylmethionyl-leucyl-phenylalanine (FMLP) or fresh serum opsonized zymosan. Luminol enhanced chemiluminescence was measured by a luminometer, superoxide (O_2^-) production was measured by the cytochrome C reduction technique. EI-130 was tested, and compared with other serine protease inhibitors.

Luminol enhanced chemiluminescence induced by activated neutrophil was significantly (>80%) inhibited by azide and methionine. This suggests that the chemiluminescence is mainly mediated by HOCl. EI-130 potentiated the chemiluminescence dose dependently (10^{-8}M to 10^{-5}M) induced by PMA (100nM), FMLP (1μM) or opsonized zymosan (2mg·ml^{-1}) stimulated neutrophils whereas guanidine and aminoguanidine did not. The increase of the chemiluminescence was 120% to 530% at 10^{-8}M to 10^{-5}M in PMA stimulated neutrophils. This effect was only observed during the activation. Other protease inhibitors, tosyl lysyl chrolomethyl ketone (TLCK), phenylmethylsulphonyl fluoride (PMSF) and trance-4-(aminomethyl)-cyclohexanecarboxylic acid (AMCHA) inhibited the chemiluminescence. There was no interaction in chemiluminescence between EI-130 and luminol and between EI-130 and HOCl. On the other hand, EI-130 inhibited HOCl/H_2O_2/luminol induced cemiluminescence (65% inhibition) and O_2^- production from PMA stimulated neutrophils (23% inhibition) at 10^{-5}M.

It is suggested that the enhancement of neutrophil induced chemiluminescence by EI-130 is not due to its antiprotease activity as other protease inhibitors did not show any enhancement. Chemical interaction between EI-130 and luminol or HOCL is also excluded. As neutrophil induced luminol enhanced chemiluminescence is dependent on H_2O_2/myeloperoxidase (MPO), we suggest that MPO mediated mechanism is involved in this phenomenon, as is the case for ascorbic acid (Bolscher et al., 1984). Although ROS may cause tissue damage, ROS have an important role to defend the body against infection by killing microorganisms using released proteases and ROS. Therefore, EI-130 could act to enhance the bactericidal activity of neutrophils.

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421P ANTIRHEUMATIC DRUGS AND THE DEVELOPMENT OF VASCULATURE IN MURINE CHRONIC GRANULOMATOUS AIR POUCHES

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Vascular growth is necessary for the development of new tissues, including rheumatoid pannus. It is one of the earliest signs of pannus development, and is associated with its phasic growth pattern (Fassbender & Gay,1988). It is suggested that rheumatoid arthritis can be classified 'angiogenesis dependent'. The gold -containing antirheumatic agents inhibit endothelial cell proliferation *in vitro* (Matsubara & Ziff, 1987) We report the activity of several antirheumatic drugs on the vascular content of chronic inflammatory tissue in the murine air pouch, variations of which are used for the assessment of antiinflammatory activity.

Chronic granulomatous air pouches were induced by the s.c. injection of 3 ml air into anæsthetised mice (30±2g, Tuck) and 0.5ml Freund's complete adjuvant with 0.1% croton oil 24 hours later. Mice were dosed p.o. for 6 days with nonsteroidal (NSAID), steroidal and slow acting anti-rheumatic drugs (SAARDs) (see table 1). Vascular content was assessed by the formation of a vascular cast (Colville-Nash et al., 1992) by the i.v. injection of 1ml 10% carmine red in 5% gelatin at 40°C, and chilling the carcasses. The dissected tissue was dried, papain digested, the dye dissolved at alkaline pH, and read at 490nm after centrifugation and filtration. Results were expressed as either mg dye content per sample or the vascular index (VI) as μg dye/mg dry weight of tissue.

Table 1. The development of granulomatous tissue, vascular content (mg dye), and VI (μg mg^{-1} in mice treated with anti-rheumatic drugs). n=8 per group, **=p<0.01, *=p<0.05 Mann-Whitney U test compared to vehicle control.

Treatment (mg kg^{-1})	Dry mass(mg)	Dye (mg)	VI (μg mg^{-1})	Drug (mg kg^{-1})	Dry mass(mg)	Dye (mg)	VI (μg mg^{-1})
p.o. vehicle	123±11.0	2.48±0.24	20.2±0.6	Chloroquine (50)	124± 7.0	0.84±0.06 **	6.6±0.3 **
i.m. vehicle	122±10.0	2.39±0.22	19.5±0.7	Aurothiomalate (20,i.m.)	108± 8.0 *	0.89±0.13 **	8.4±5.5 **
Indomethacin (1)	149±16.0 **	1.77±0.26 **	11.9±0.9 **				
Ibuprofen (30)	142±16.0	1.33±0.09 **	9.4±0.3 **	Auranofin (20)	125± 7.0	0.66±0.07 **	5.0±2.5 **
Piroxicam (2)	130±12.0	2.04±0.11	15.7±1.2	Cyclophosphamide (10)	118± 9.0	0.81±0.06 **	6.9±0.5 **
Dexamethasone (0.5)	53± 8.0 **	1.16±0.15 **	23.7±1.1 **	Azathioprine (30)	137±10.0 *	1.72±0.08 **	11.7±0.5 **
Prednisolone (10)	102± 8.0 *	1.10±0.15 **	10.3±0.8 **	Methotrexate (0.6)	109± 7.0	0.61±0.09 **	5.8±0.3 **
D-penicillamine (100)	107± 5.0 *	0.74±0.13 **	7.1±0.7 **	Levamisole (50)	117± 6.0	1.52±0.39 *	12.7±0.3 **

Those agents which reduce cell proliferation reduced the VI, as did the NSAIDs, except for piroxicam. The gold containing agents reduced the VI significantly as did d-penilliamine, in keeping with their *in vitro* actions. Prednisolone was also effective, but dexamethasone reduced granuloma formation to such an extent that it resulted in an increase in the VI. The activity of the NSAIDs could reflect the angiogenic activity of PGE_2 (Form & Auerbach, 1983). We suggest this model is useful as a rapid method for the assessment of drugs on the developing vasculature of inflammatory tissue *in vivo*.

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422P IONOPHORE, BUT NOT INFLAMMATORY MEDIATORS, INCREASES PRODUCTION OF PAF-LIKE BIOLOGICAL ACTIVITY BY CULTURED GUINEA-PIG EPIDERMAL CELLS

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Human epidermal cells have been reported to produce platelet activating factor (PAF) after stimulation with the ionophore A23187 (Michel et al., 1990). In contrast, increased PAF production was not observed in guinea pig epidermal cells incubated with A23187 for up to 30min, although acetyltransferase activity was detected (Cunningham and Andrews, 1989). In this study PAF production by cultured guinea pig epidermal cells in response to both A23187 and inflammatory mediators has been examined.

Epidermal cell suspensions were prepared by trypsinisation (0.25% 1:250 trypsin; 30min; 37°C) of 0.3mm thickness shave biopsies obtained from the skin of male Dunkin-Hartley guinea pigs (500-750g). The cells were cultured in Eagle's Minimal Essential Medium (MEM) containing 10% foetal calf serum, antibiotics and fungicide for 2 weeks then trypsinised, washed and replated at 10^6/flask. PAF production by cell monolayers was measured 12 days later, and the amount of PAF-like biological activity in lipid extracts of cell and supernatant fractions quantitated in a platelet aggregation assay.

Total PAF production increased when guinea pig epidermal cell monolayers were incubated with 2μM A23187 for 15min at 37°C in MEM containing 0.25% BSA, 100μM acetylCoA and 100nM lyso-PAF, the proportion in the supernatant varying between 42 and 100% (1300 ± 630 vs 625 ± 450pg PAF/10^6cells; mean ± s.e.mean, n=5; $p<0.05$, paired Student's t test; values not corrected for recovery (43 ± 6%; mean ± s.e.mean, n=4)). Thin layer chromatography was used to show that the PAF-like activity co-migrated with synthetic $C_{16:0}$ PAF. No difference was seen between the amounts of PAF recovered from unstimulated epidermal cell monolayers pretreated with the acetyl hydrolase inhibitor phenylmethylsulphonyl fluoride (PMSF; 2mM) and those incubated with recombinant IL-1 (1-50ng/10^6cells) for 2-8h or histamine (10^{-5}-10^{-4}M), LTB_4 (10^{-7}-10^{-5}M) or LTC_4 (10^{-7}-10^{-5}M) for 10 or 30min. PMSF pretreatment did not increase the amounts of PAF produced by ionophore stimulation in the presence (423 ± 122 vs 137 ± 54pg PAF/10^6 cells; mean ± s.e.mean; n=6) or absence (619 ± 256 vs 163 ± 70 pg PAF/10^6cells; mean ± s.e. mean; n=4) of lyso-PAF and acetylCoA.

These results demonstrate that, whilst PAF production by cultured guinea pig epidermal cells could be increased by A23187, under the conditions used, inflammatory mediators were unable to induce PAF formation.

We are grateful to the Skin Diseases Research Fund for financial support.

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423P CHANGES IN THE RESPONSE TO BRADYKININ IN CHRONIC ARTHRITIS

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Bradykinin (BK) is a nonapeptide which has been implicated in the pathogenesis of rheumatoid arthritis. It is released at sites of tissue damage and influences vascular permeability, vasodilatation and pain. These effects are mediated, at least in part, by a neurogenic mechanism involving neuropeptide-containing afferent nerves. It is noteworthy that these nerves are reduced in the inflamed synovium of patients with rheumatoid arthritis. The aim of this study was to investigate the relationship between bradykinin-induced plasma extravasation and synovial innervation in two models of chronic joint inflammation.

Chronic monoarticular arthritis was induced in two groups of Wistar rats (200-250g). The first group were sensitized to methylated bovine serum albumin (mBSA) in Freunds complete adjuvant on two occasions (days 0 and 7) and then challenged with an intra-articular injection of 0.1ml mBSA (5mg/ml in sterile saline) on day 21. The second group received a single injection (0.1ml) into the right knee of 10um latex spheres which remain localised within the intra-articular cavity. BK-induced synovial plasma extravasation was then measured in control and day 21 arthritic animals using a knee perfusion technique. The animals were injected with Evans blue (100mg/kg,i.v.) and the right knee cannulated and perfused with physiological saline. Once a stable baseline was established the knees were perfused with BK. Perfusate samples were collected every 10 minutes and the BK-induced plasma extravasation determined spectrophotometrically. Results are presented as absolute increase in Evans blue (ug/ml) in the first 10 minute fraction. In a second study synovial frozen sections were taken from control and arthritic animals and the innervation was determined using immunocytochemical techniques. The antibody PGP 9.5 was used as a marker for the overall synovial innervation.

A full dose-response curve was obtained for BK-induced plasma extravasation in normal control animals. The EC_{50} value for the response to bradykinin was 0.3uM. This dose of BK was then perfused through the knees of the two groups of arthritic animals. The response in the latex knees was unaltered (controls 0.70 ± 0.14 vs latex spheres 0.93 ± 0.13) but was significantly reduced in the mBSA knees (0.10 ± 0.06, $p<0.05$). A full dose response in the mBSA group revealed an increased EC_{50} (1.0 uM) and a significantly reduced maximal response (1.76 ± 0.11 vs 0.85 ± 0.14, $p<0.05$). Immunocytochemical studies revealed a dense innervation in normal and latex knees but a significant loss of immuno-reactive nerve fibres in the mBSA joint synovium.

We have shown that plasma extravasation in response to BK is reduced in a model of chronic joint inflammation in which there is loss of the normal innervation. We propose that neurogenically-derived mediators are an important component of the overall response to BK in the normal joint. The loss of nerves in rheumatoid synovium and consequent impairment of the neurogenically-derived response may well have important implications for the long-term outcome of this disease.

424P α-MELANOCYTE STIMULATING HORMONE SELECTIVELY INHIBITS THE ACTIONS OF IL-1 ON IMMUNOCOMPETENT CELLS

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It has been proposed that a regulatory network exists between the immune and neuroendocrine systems. The immune system may have a sensory function, sending signals to the pituitary in response to the presence of somatic stimuli (e.g. viruses, bacteria, tumours) and the central nervous system may send signals to the immune system by means of the pro-opiomelanocortin-derived neuroendocrine polypeptide hormones CRF, adrenocorticotrophic hormone (ACTH) or α-melanocyte stimulating hormone (α-MSH). α-MSH has previously been shown to attenuate the pyrogenic actions of the cytokines tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) (Davidson *et al.*, 1992). In the present study the effects of α-MSH on the release of IL-1-like activity from rabbit peripheral blood monocytes (Mø) incubated with either, lipopolysaccharide (LPS), polyinosinic:polycytidylic acid (poly I:C) a double stranded ribonucleotide thought to be representative of viral immunostimulation or human recombinant TNF-α was investigated. The effects of α-MSH on human recombinant IL-1α–, IL-1β– and IL-2-induced thymocyte proliferation was also examined.

Mø were isolated from rabbit peripheral blood by density gradient centrifugation, followed by adherence to plastic. Mø (1 x 10^6/ml) were incubated with either LPS (1 μg/ml), poly I:C (100 μg/ml) or TNF-α (5 μg/ml) at 37 °C, in 5% CO_2 in air for 20 h and IL-1-like activity from supernatants measured using the lymphocyte activating factor (LAF) assay. Thymocytes were also incubated with phytohaemaglutinin (PHA) and either IL-1α (100 pg/well), IL-1β (100 pg/well) or IL-2 (10 units/well) in the presence or absence of α-MSH (10 pg/well - 1 μg/well). The LAF assay was carried out as follows: thymocytes from C3H/HeJ mice were incubated with test substance in the presence of PHA (0.5 μg/ml), pulsed with [^{3}H] thymidine (0.5 μCi /well) after 48 h, incubations were terminated after 72 h by adding trichloroacetic acid and incorporation of radioactivity into acid insoluble material measured. In further experiments Mø were prelabelled with [^{35}S]-methionine for 4 h then incubated with or without α-MSH (10 pg/ml - 1 μg/ml) in the presence or absence of LPS (0.1 μg/ml). Radiolabelled IL-1 in supernatants was estimated by immunoprecipitation with antisera to IL-1α and IL-1β, followed by counting.

LPS, poly I:C and TNF-α induced the release of LAF activity from isolated rabbit Mø. α-MSH had no direct effect on thymocytes themselves but, appeared to reduce the incorporation of radioactivity into acid insoluble material by thymocytes in response to supernatants from Mø incubated with either LPS, poly I:C or TNF-α, IC_{50} values were 0.55 pg/ml, 12 fg/ml and 12 fg/ml respectively. LPS increased the amount of radiolabelled immunoprecipitable IL-1α and IL-1β recovered from the supernatant of Mø from 5366 ± 123 dpm and 2538 ± 89 dpm for unstimulated controls to 6912 ± 142 dpm and 3433 ± 89 dpm for LPS stimulated Mø respectively (all means ± s.d., $P < 0.01$). α-MSH had no significant effect on the amount of radiolabelled immunoprecipitable IL-1α or IL-1β. Incorporation of [^{3}H] thymidine by thymocytes in response to both IL-1α and IL-1β was significantly reduced in the presence of α-MSH compared to that in the absence of α-MSH. The effect of α-MSH was concentration dependent with IC_{50} values of 6.75 pg/ml and 6.95 pg/ml for IL-1α and IL-1β respectively. IL-1 is thought to act through IL-2 resulting in T cell proliferation. IL-2 produced a concentration-dependent increase in incorporation of [^{3}H] thymidine by thymocytes, however, α-MSH (1 fg - 1 mg/well) had no effect on the IL-2-stimulated (10 U/well) incorporation of [^{3}H] thymidine.

α-MSH did not prevent IL-1 release from activated Mø, but did attenuate IL-1-induced thymocyte proliferation by an IL-2 independent mechanism, possibly by interacting with the IL-1 receptor. Hence α-MSH may be an important regulator of the immune response because of its ability to attenuate IL-1-induced effects.

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425P INTERLEUKIN-1 STIMULATES PHOSPHOLIPASE C IN PERIPHERAL BLOOD MONOCYTES

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Interleukin-1 (IL-1) stimulates a wide variety of different cell types during immune responses. In particular monocytes are stimulated to release prostaglandins, T lymphocytes to proliferate and B lymphocytes to release antibodies. The mechanisms by which IL-1 activates cells is poorly understood. Recently much work has been carried out on cell signal transduction systems in non-immune cells with respect to the receptor and GTP-binding protein-mediated activation of phospholipase C. This results in the hydrolysis of membrane phosphatides such as phosphatidylinositol bis-phosphate (PIP_2) which yields inositol trisphosphate (IP_3) thought to be involved in releasing calcium from intracellular sites. The potential phosphorylation of IP_3 can produce inositol tetrakis-phosphate (IP_4) which is thought to be involved in controlling calcium flux between intracellular membranes and the plasma membrane. In this study the effect of IL-1 on the accumulation of inositol phosphates (IPs) in intact monocytes (Mø) and the activation of phospholipase C in Mø homogenates was investigated.

Mø were prepared from rabbit blood by ficoll density-centrifugation followed by incubation in plastic tissue culture petri-dishes for 2 hours after which the adherent cells (Mø) were scraped from the culture dishes. Mø were incubated for 18 hours with [^{3}H]inositol (10 μCi/ml) in inositol-free RPMI 1640. Mø (1 x 10^6 cells in a volume of 200 μl) were then incubated for 10 minutes with 1 mM LiCl followed by IL-1 or RPMI 1640 for appropriate times after which chloroform/methanol (2:1) was added and the aqueous layer was subfractionated for different IPs on Dowex anion exchange columns. Phospholipase C activity was estimated by incubating homogenates (prepared by homogenising Mø in 0.1 M HEPES buffer pH 6.5) with 8.7 mM [^{3}H] PIP_2, 0.1 mM LiCl, 5 μM $CaCl_2$ and 0.1 mM GTP at 37 °C. Ice-cold chloroform/methanol was added and radioactivity in the aqueous phase was measured by liquid scintillation counting. All incubations were carried out at 37 °C, 5% CO_2, 100% humidity. All values are subsequently expressed as the means of n = 4 ± s.d.

IP_3 and IP_4 levels increased ($P < 0.01$) above the control level within the first minute of stimulation with IL-1α and remained greater than the control level after 2 minutes. Neither the IP_3 nor IP_4 level was, however, significantly different than the control level after 4 minutes. The subsequent falls in IP_3 and IP_4 levels were accompanied by a delayed increase in the levels of IP_{1+2}. IL-1β (1.16 nM) also increased the total IPs level from 100 ± 7.2 to 158 ± 9.6 after 2 minutes incubation ($P < 0.01$). The EC_{50} for the accumulation of total IPs recovered from incubations with IL-1α for 4 minutes was 0.59 nM and a maximal accumulation was observed with an IL-1α concentration of 5.78 nM. EGTA (1 mM) prevented the increase in IPs induced by 5.78 nM IL-1α (167.4 ± 10.5 % vs 94.3 ± 8.2 %, $P < 0.01$) but did not significantly affect the IPs level in control incubations (100 ± 8.6 % vs 87.3 ± 8.1%). A maximal increase in the hydrolysis of [^{3}H]-PIP_2 was observed after 15 minutes in the presence of IL-1α (5.78 nM) and a plateau level was maintained from 15 to 60 minutes. The level of [^{3}H]-PIP_2 hydrolysis in control incubations increased at a much lower rate and by 60 minutes had almost attained the same level as that with IL-1α. A maximal increase in [^{3}H]-PIP_2 hydrolysis was observed between 5.78 nM and 28.9 nM IL-1α with an EC_{50} of 0.58 nM. Heating IL-1α (5.78 nM) at 60 °C for 30 minutes prior to incubating it with homogenates abolished its ability to increase [^{3}H]-PIP_2 hydrolysis. IL-1β (1.16 nM) also increased [^{3}H]-PIP_2 hydrolysis in 15 minute incubations from 100 ± 1.54 % to 109.7 ± 1.32 % ($P < 0.01$). In experiments where incubations were carried out with membrane fractions neither IL-1α nor IL-1β significantly altered the level of hydrolysis. Almost identical data was obtained using the cytosolic fractions obtained from Mø homogenates in that neither IL-1α nor IL-1β altered hydrolysis.

The data obtained indicate that IL-1 can increase the accumulation of IPs in particular the biologically relevant IP_3 and IP_4 species in Mø. This would appear to occur through the activation of a phospholipase C, however, there was no effect of IL-1 on the phospholipase C activity of Mø membranes indicating that IL-1 either stimulates a phospholipase C isoform which translocates to the cytosol or that a soluble factor (not Ca^{2+} or GTP) is required for activity.

426P IDENTIFICATION OF INDAPAMIDE BINDING SITES IN GUINEA PIG RENAL MEMBRANES

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Indapamide is generally classified as a "thiazide-like" diuretic. The mechanism of action of indapamide is not clearly established though it appears to lower blood pressure by a combined diuretic and direct vascular action. Indapamide can interact with the renal binding sites of the thiazide diuretic [^{3}H]metolazone (Beaumont *et al*, 1988) but it is likely that vasodilatory mechanisms independent of diuresis may be important. This study has investigated [^{3}H]indapamide binding in guinea pig renal cortex and its displacement by other drugs.

Cortical tissue from guinea pig kidneys was homogenised with a Polytron (3 times, 5s, position 5) in ice-cold Tris/phosphate buffer (pH 7.4) in the presence of the protease inhibitors leupeptin, phenylmethylsulfonyl fluoride and ethylenediaminetetraacetic acid. The homogenate was spun at 4°C at 1000rpm for 15 minutes. The resulting supernatant was spun at 8000rpm for 15 minutes and the supernatant was again spun at 21000rpm for 45 minutes. The final pellet was resuspended in buffer at a protein concentration of 0.5mg/ml and stored at -70°C. Binding studies were miniaturised and done in triplicate on a 96 well microtitre plate. Each well contained 0.1ml solution comprising the renal membrane preparation, [^{3}H]indapamide (40nM) and drugs in buffer. The plates were left on ice for 2h and then the bound radioligand was isolated by rapid filtration of the incubation mixture through Whatman GF/C filters using a cell harvester. The filters, which had been pretreated with 0.3% polyethyleneimine to minimise non-specific [^{3}H]indapamide binding were left overnight to dry and counts recorded on a scintillation counter.

The effects of different diuretics on [^{3}H]indapamide binding is illustrated in table 1.

Table 1. Effects of drugs on [^{3}H]indapamide binding

Drug :	Indapamide	Acetazolamide	Hydrochlorothiazide	Metolazone	Cicletanine	Nicardipine
IC_{50} (nM) :	200(±1%)	32(±1%)	4000(±2.9%)	1300(±2.8%)	N/D	N/D

N/D means no displacement (up to 30μM). N=4; values are in nM (± % error)

This study suggests that the [^{3}H]indapamide binding site in guinea pig renal membranes has the properties of a carbonic anhydrase, in accordance with the findings of Schaeffer *et al* (1990) in pig renal cortex. The physiological significance of this is unknown, though since acetazolamide has been reported to produce cerebral vasodilatation through inhibition of membrane bound carbonic anhydrase (Allen, 1983), it is possible that such inhibition may participate in the antihypertensive effects of indapamide. We are currently examining the direct vasorelaxant actions of the established carbonic anhydrase inhibitor, acetazolamide, in isolated guinea pig resistance arteries.

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427P SHORT RANGE DIFFERENTIAL PULSE POLAROGRAPHY FOR FAST, SELECTIVE ANALYSIS OF CEREBRAL ELECTROACTIVE COMPOUNDS *IN VIVO*

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Differential Pulse Voltammetry (DPV) with pretreated biosensors allows in vivo selective measurement of basal endogenous levels of dopamine (DA), serotonin (5HT), their metabolites (dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5HIAA)) and neuropeptides in situ, in discrete brain areas without recourse to radiolabelling of transmitters stores, perfusion-based techniques or post-mortem analysis (Crespi, 1990). We have now modified DPV in order to reduce the time of analysis from tenths of sec to 1-2 sec without losing selectivity and called this newly reported method Short Range Differential Pulse Voltammetry (SRDPV). Simply, while with DPV the complete oxidation peak is recorded, with SRDPV only the top of each oxidation peak is measured. For example, to monitor Peak 2, occurring at approx. +90 ±10mV and corresponding to the oxidation of extracellular DOPAC in vivo (Crespi, 1990), the initial (Ei) and final (Ef) potentials applied with DPV were -100/+200mV while they were +75 (Ei) and +95 (Ef) with SRDPV. At the scan range of 2mv/0.2sec the effective time of measurement was 30 sec for DPV and only 2 sec for SRDPV. A similar procedure was performed to analyze Peak 3 (5HIAA, occurring at + 230 ± 15mV) with Ei 0mV and Ef +350mV for DPV, or +215 mV and +240mV for SRDPV.
In vitro measurements of increasing (10, 15, 25, 50 μM) concentrations of DOPAC or 5HIAA (chosen on the basis of the suggested in vivo content of these two compounds) were performed with electrically pretreated carbon fibre micro electrodes (12μm diam., n=5) using both DPV and SRDPV. Data indicated that similar sensitivity and selectivity were obtained with both methods at all concentrations, supporting the applicability of SRDPV for in vitro studies.
In vivo, experiments were performed in anaesthetized (Chloral hydrate 500mg/kg i.p.) adult male rats prepared for voltammetry with the biosensor inserted into the striatum (Crespi, 1990). DPV measurements were performed automatically every 5 min and were alternated every 10-20 min with a sequence of 5-10 SRDPV scans performed every 10-30 sec. In 5 rats pargyline treatment (80 mg/kg i.p.) significantly decreased both Peaks 2 and 3 (DPV, 5 ± 5% and 35 ± 15% of control values respectively), as well as Spikes 2 and 3 (SRDPV, 7 ± 5% and 26 ± 18% of control values respectively) within 90 min. In another 5 rats, haloperidol (1 mg/kg i.p.) was responsible for an increase in both Peak 2 (198 ± 11%) and Spike 2 (195 ± 18%) while 5-hydroxytryptophan (15 mg/kg i.p., n=5) produced a 200 ± 28% rise of the size of Peak 3 and a comparable increase in that of Spike 3. Thus, these results support the capability of SRDPV to measure in vivo electroactive compounds with similar selectivity and sensitivity as DPV with improved time resolution.
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428P GLUTATHIONE-RELATED ENZYMES IN BRAIN IN BASAL GANGLIA DEGENERATIVE DISORDERS

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Decreased levels of reduced glutathione (GSH) occur in the substantia nigra (SN) in Parkinson's disease (PD) but not other basal ganglia degenerative disorders (Perry & Yong, 1986). Glutathione peroxidase (Martila & Lorentz, 1988) and glutathione transferase activity is unchanged and there is no corresponding increase in oxidised glutathione levels (Sian *et al.*,1992). We now report on the activity of a range of enzymes associated with glutathione function in brain areas from control, PD and multiple system atrophy (MSA) patients.

Brain tissue (cerebral cortex; Brodmann area 10, caudate nucleus, putamen, globus pallidus and substantia nigra) was obtained from control patients matched for age and post-mortem delay with MSA subjects (mean age; 63.4 ± 3.0) and parkinsonian patients (mean age; 69.2 ± 2.6). Brain samples were homogenised and the supernatant analysed for; γ-glutamyl cysteine synthetase (γ-GCS), glutathione peroxidase (GSHpx), glutathione transferase (GSHt) and γ-glutamyl transpeptidase (γ-GTP) activity using a dual beam spectrophotometer (Meister, 1985).

There was no difference in the activity of either GSHt or GSHpx in any of the brain areas examined in PD compared to controls. However, the activity of GSHpx in MSA was elevated in the lateral globus pallidus and caudate nucleus but not other brain regions. There was no change in the activity of γ-GCS in any brain areas in either PD or MSA. The activity of γ-GTP was increased in the SN in PD but not MSA (Table 1).

Table 1: Activity of glutathione enzymes (nmol/min/mg protein) in the substantia nigra from controls, MSA and PD patients.

GROUP	γ-GCS	GSHpx	GSHt	γ-GTP
Controls	33.4 ± 4.0	145.6 ± 10.7	58.0 ± 10.5	67.4 ± 5.2
MSA patients	36.3 ± 6.1	149.8 ± 10.6	61.1 ± 7.1	61.6 ± 8.5
Controls	30.8 ± 3.1	140.7 ± 2.3	54.8 ± 3.9	71.9 ± 4.1
PD patients	27.9 ± 2.9	148.4 ± 10.7	66.8 ± 4.9	126.9 ± 10.4*

Values are mean ± SEM. n = 3-10. * $p < 0.05$ compared to control subjects (Student's t-test).

These results confirm previous reports of unchanged activity of glutathione peroxidase and glutathione transferase in SN in PD. There appears to be no decrease in the activity of the rate limiting synthetic enzyme (γ-GCS) for reduced glutathione formation. The elevation of γ-GTP activity may indicate altered degradation or translocation of reduced glutathione.

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429P DIFFERENTIAL EFFECTS OF DOPAMINE ON NEURONES OF THE DEEP AND SUPERFICIAL LAYERS OF THE RAT ENTORHINAL CORTEX *IN VITRO*

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The aetiology of schizophrenia has long been associated with hyperactivity in the mesolimbic dopamine (DA) system. However, a more recent suggestion is that schizophrenia involves a decline in activity of excitatory glutamate pathways which is manifested as an apparent overactivity of an opposing DA inhibitory influence (e.g. see Wachtel and Turski, 1990). A prime candidate for the location of such an imbalance is the entorhinal cortex (EC). This area is pivotal in gating activity between the hippocampus and the rest of the limbic system and it has a moderately strong DA innervation (Hokfelt, *et al*, 1974). Post mortem studies have shown severe pathological disruption of the neuronal organization of the EC of schizophrenics, changes which particularly affect the glutamate output neurones (Arnold *et al*, 1991). We have begun to look at DA-glutamate interactions in the EC using a slice preparation from the rat brain. Binding studies have shown a differential laminar distribution of D1 and D2 receptors in the EC (Richfield *et al*, 1989) so we have compared the effects of DA on neurones in layer II and Layer V.

Slices of the EC were cut from the rat brain and maintained *in vitro* at the interface between artificial CSF (34°C) and carbogen gas. Conventional intracellular recordings were made from neurones in layer II and layer V. DA and other agents were applied by bath perfusion.

DA (0.1-1mM) hyperpolarized the majority (22/38) of neurones in layer II of the EC (3.8 +/- 2.9 mV, mean +/- S.D., n=19). This was accompanied by a variable reduction in input resistance (5-30 %). 10 cells were depolarized (3.9 +/- 1.3 mV), again with a variable decrease in input resistance, and the remainder apparently unaffected. In contrast, most layer V cells (15/21) were depolarized by DA with only 2 slightly hyperpolarized and the remaining 4 unaffected. Responses of both deep and superficial cells persisted in TTX (0.5 μM) indicating that they were postsynaptic in origin. In layer II, the D2 receptor agonist quinpirole (up to 50 μM) consistently hyperpolarized cells, even those depolarized by DA. The D1 agonist SKF 38393 (up to 50 μM) either weakly hyperpolarized cells or was without effect. The hyperpolarizing but not depolarizing responses to DA could be reduced with the D2 antagonist, sulpiride (10-40 μM) but the D1 antagonist (SCH 23390, 50 μM) affected neither. In layer V, the depolarizing responses were not mimicked by SKF 38393 (up to 100 μM) or quinpirole (up to 100 μM) nor were they blocked by SCH 23390 (up to 50 μM) or sulpiride (up to 50 μM). Finally, responses to DA in either layer were unaffected by the α- or β-adrenergic antagonists, prazosin and propranolol (up to 50 μM). At present, we conclude that DA hyperpolarization of layer II cells is primarily mediated by D2 receptors whereas the depolarizing responses in both layers cells are neither D1 nor D2.

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430P DIFFERENTIAL INHIBITION OF DOPAMINE UPTAKE BY BENZTROPINE AND COCAINE IN THE RAT CAUDATE PUTAMEN AND NUCLEUS ACCUMBENS

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Marshall et al. (1990) demonstrated a 2-3 fold greater number of dopamine (DA) uptake sites in the caudate putamen (CPu) relative to the nucleus accumbens (NAc) using [^{3}H] mazindol. Fast cyclic voltammetry was used to investigate whether this difference in the number of uptake sites corresponds to a difference in the sensitivity between the CPu and NAc to cocaine and benztropine. DA overflow was measured in rat brain slices prepared as described by Trout & Kruk (1992), following single pulse (1p; 0.1ms, 20V), 4 pulses at 10Hz (4p/10Hz) or 20 pulses at 20Hz (20p/20Hz) electrical stimulation. Dose response curves were determined in the presence of each drug (range 3.10^{-8} to 3.10^{-6} Molar) at each of the stimulation patterns in both CPu and ACb. Maximum increase in DA overflow occured at 1.10^{-6} Molar for both drugs, and EC_{50} values (concentration needed to enhance overflow to 50% of maximum) were determined.

Table 1 Effect of benztropine (1μM) and cocaine (1μM) on electrically evoked DA overflow in the rat CPu and NAc

		Benztropine			Cocaine	
	1p	4p/10Hz	20p/20Hz	1p	4p/10Hz	20p/20Hz
CPu % of Control	280±16**	364±15**	394±17**	235±17	312±29**	287±20**
EC_{50}(.10^{-7}M)	3.3±0.3*	3.3±0.2*	2.6±0.2*	1.3±0.2	1.6±0.2	1.2±0.2
NAc % of Control	146±17	150±20	149±15	202±13	217±7	186±15
EC_{50}(.10^{-7}M)	1.7±0.3	2.1±0.5	1.6±0.3	1.2±0.2	1.1±0.1	0.9±0.1

All values are a mean ±s.e.mean, (n=4). CPu v NAc ** $p<0.001$, * $p<0.05$
Maximum inhibition of uptake resulted in a greater increase in DA overflow in the CPu than the NAc, at all stimulations. The potency of cocaine was similar in both brain regions; the potency of benztropine was significantly lower in the CPu than in the NAc. The difference in the potency of benztropine may be partly due to its antimuscarinic activity as acetylcholine has been shown to have both direct facilitatory and indirect inhibitory control of DA release in the CPu (Gauchy et al. 1991).

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431P DIFFERENCES IN DOPAMINE REUPTAKE INHIBITION BY GBR 12909 IN RAT ANTERIOR AND POSTERIOR CAUDATE PUTAMEN BRAIN SLICES

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We have previously reported, using fast cyclic voltammetry (FCV) at carbon fibre microelectrodes, that ratios of peak dopamine (DA) efflux evoked by trains of 25 pulses applied at 50 Hz (25P / 50 Hz), compared to a single electrical pulse (1P) varied in the caudate putamen (Patel *et al.*, 1992). The anterior caudate putamen (aCPu) contained predominantly low ratio DA release sites whereas the posterior caudate putamen (pCPu) contained predominantly higher ratio DA release sites. Other studies have described a rostral to caudal variation in [^{3}H] DA uptake (Glynn & Yamamoto, 1989), suggesting that the aCPu may contain a higher number of DA reuptake sites than the pCPu. We have now investigated the effects of the highly selective DA reuptake blocker GBR 12909 on DA efflux evoked by 1P and 20 pulses (20P) at 10, 50 & 100 Hz, in low ratio release sites (< 3:1) in aCPu and in high ratio release sites (> 6:1) in pCPu. Experiments were conducted in rat coronal brain slices (350 μm thick) as described by Patel *et al.* (1992). GBR 12909 (0.3 μM) was added to the perfusion fluid and electrically evoked DA was sampled (pre and post drug) at a rate of 4 Hz. GBR 12909 significantly enhanced peak DA efflux following all patterns of stimulation (Table 1).

Table 1. Changes in DA efflux (expressed as % predrug control) in the presence of GBR 12909 (0.3 μM) in aCPu and pCPu.

	1P	20P / 10 Hz	20P / 50 Hz	20P / 100 Hz
aCPu	458.2 ± 40.8	1625.7 ± 64.9	858.5 ± 160.4	709.3 ± 51.4
pCPu	327.5 ± 88.0	402.8 ± 166.1*	191.6 ± 45.1*	305.8 ± 67.4*

Values represent DA efflux (mean ± s.e.mean; $n = 3$ (aCPu), $n = 4$ (pCPu)). * $P < 0.01$, significantly different from aCPu at the same stimulus parameter.

The half-decay time (t½) measured for 1P was significantly increased from 1000 ± 250 ms to 3500 ± 500 ms in the aCPu ($P < 0.01$; $n = 3$), and from 1500 ± 250 ms to 3750 ± 500 ms in the pCPu ($P < 0.01$; $n = 4$). The effect on t½ in the aCPu was significantly greater than that in the pCPu ($P < 0.05$). The present findings demonstrate marked differences in the reuptake system between low ratio DA release sites in the aCPu and high ratio sites of the pCPu. These differences could be a consequence of a heterogeneous distribution in the number of reuptake sites and/or a variation in their affinity for the reuptake blocker.

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432P EVIDENCE FOR INVOLVEMENT OF THE DOPAMINE D_2 RECEPTOR INTOLERANCE TO THE COCAINE DISCRIMINATIVE STIMULUS (CUE)

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Tolerance to the cocaine cue can be induced (Thabit and Goudie, 1992) by the specific dopamine (DA) reuptake inhibitor GBR 12909 (Andersen 1989), in accord with evidence that such tolerance is mediated by central DA systems (Wood et al. 1987). The question of which DA receptor subtypes are involved in the cocaine cue is unresolved (Witkin et al. 1991), although D2 agonists have been found to substitute for cocaine (e.g. Callahan et al. 1991) The present study assessed whether tolerance to the cocaine cue induced by GBR 12909 was also associated with tolerance to the ability of the specific D2 agonist quinelorane (Foreman et al. 1989) to substitute for cocaine. Rats (n=17) were trained to discriminate cocaine (10 mg/kg, i.p.) in a quantal FR 10 drug discrimination assay. Quinelorane (0.015-0.125 mg/kg) generalized fully to cocaine in a dose-dependent fashion (ED_{50} = 0.02 mg/kg). The quinelorane dose/effect curve (DEC) was redetermined after GBR 12909 treatment (20 mg/kg/day, p.o. for 10 or 20 days). Twenty days of treatment shifted the quinelorane substitution DEC significantly (McNemar Test, alpha = 0.05) 16 fold to the right (ED_{50} = 0.32 mg/kg) and also flattened the DEC substantially. Ten days of GBR 12909 treatment produced only a slight shift in the quinelorane DEC, but this was not significant. GBR 12909 treatment (20 days) also produced tolerance to quinelorane's ability to prolong the latency to the first reward and flattening of the DEC, as seen for the drug cue. This study supports previous reports implicating D2 receptors in the cocaine cue. Since tolerance to the D2 cue developed in the same manner as it did for the cocaine cue after GBR 12909 treatment, the results can also be taken as implicating D2 receptors in development of cocaine tolerance.

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433P COCAINE AND BAROREFLEX DEREGULATION

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Acute cocaine administration is associated in man with increased blood pressure coupled with a decrease in heart rate. It was hypothetized that this cardiovascular effect of cocaine resulted from an alteration of the centrally mediated baroreflex which buffers hypertensive and tachycardic effects of physiological stimuli. In other studies, it was reported that a dihydropyridine, nicardipine, or an angiotensin II converting enzyme inhibitor, enalaprilat, (in association with diazepam) administered as antidotes, restored cardiovascular function of intoxicated animal to normal and prevented lethality (Trouvé and Nahas, 1990). The purpose of the present study was to investigate some of the mechanism of action of cocaine on the baroreflex in the rat. Three groups of experiments were performed.

In the first series, 14 Sprague Dawley rats were fitted under pentobarbital anesthesia with a catheter in the caudal artery and their carotids were exposed. The pressure signal from the caudal artery was treated on line by a micro-computer for continuous display of blood pressure and heart rate measurements. Seven animals were administered I.P. 50 mg/kg of cocaine and seven others an equal volume of saline. Seven minutes later, stimulation of the baroreflex was performed by bilateral clamping of the two carotids for a period of two minutes. Same manoeuver was repeated at 14 and 21 minutes. Analysis of variance for repeated measurements indicated that before carotid clamping, there was no significant difference between blood pressure measurements of the saline and cocaine treated groups. After each clamping, a two factor analysis of variance of the repeated measurements of the maximal variation in systolic pressure showed a significant difference between control and cocaine administered groups ($p<0.001$) the former displaying a much greater increment in blood pressure following carotid clamping. In the second series, 5 minutes following cocaine or saline I.P. administration, the animals were treated with nicardipine (1.5 µg/kg/min for 6') or with an equal volume of saline. Following this treatment, bilateral occlusion of the carotid arteries is performed as previously described. Changes in blood pressure following the three successive arterial clampings were not significantly different in both groups. In a third series, 5 minutes after cocaine or saline administration, the animals were given either diazepam (0.7 mg/kg I.M.) and enalaprilat (0.3 mg/kg I.A.) or a similar volume of saline. After bilateral carotid clamping there was no significant difference in blood pressure increments between the saline or enalaprilat-diazepam treated animals. In other experiments, diazepam or enalaprilat administered separately did not prevent the inhibition of the baroreflex produced by cocaine. The mean increases in blood pressure (±SD) after the three clampings (in mmHg) were :

Saline	Cocaine	Diazepam	Nicardipine	Enal + Diaz	Coc + Diaz	Coc + Enal + Diaz	Coc + Nic
36 ± 2	13 ± 3	36 ± 2	33 ± 2	35 ± 2	17 ± 1	40 ± 3	30 ± 2

These experiments indicate that cocaine significantly inhibits the increase in blood pressure caused by a stimulation of the baroreflex, and that administration of known antidotes to cocaine, either nicardipine or the combination enalaprilat-diazepam, correct this deregulation. The hypertensive effects of cocaine administration have been attributed to stimulation of the sympatho-adrenal and renin angiotensin systems (Trouvé and Nahas). Peripherally, this stimulation results in general vasoconstriction. Centrally, an increase in angiotensin II, which modulates the baroreflex, might account for its deregulation by cocaine.

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434P CARDIOVASCULAR TOLERANCE TO COCAINE AND ITS CORRELATES

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Studies on the human and non human primate have reported a cardiovascular tolerance to the hypertensive effects of increasing iv doses of cocaine : increments in blood pressure are not dose related.Cocaine administration is also associated with increased release of catecholamines. These observations led to the hypothesis that the tolerance to the hypertensive effects of cocaine might be associated with a down regulation of post synaptic receptors similar to that which occur in the presence of increased catecholamines.

To test this hypothesis 48 rats were exposed to cocaine (15-60 mg/kg/day) or saline administration for 1 to 3 weeks by means of intra-peritoneal osmotic pumps. All animals survived this initial phase without apparent ill effects. Following this period, their blood pressure response to cocaine was compared and radioligands studies performed to determine the density of the lymphocyte-ß2-adrenoreceptors. In addition morphological studies of the myocardium were done. Three series of experiments were performed .

In a first series, on day 8, 6 cocaine and 6 saline treated animals were fitted under anesthesia with an intraarterial caudal catheter connected via a three way stopcock to a strain gauge and a microcomputer for on line analysis of blood pressure.They were administered at twenty minute interval 10, 20 and 30 mg/kg of cocaine. Resting mean blood pressure was significantly lower ($p<0.05$) in cocaine treated animals (86.7±1.4 mmHg) than in saline controls (93.6±2.5 mmHg). Percentage changes in mean blood pressure of rats chronically treated with cocaine or saline, computed between resting mean blood pressure and peak pressure following cocaine were significantly lower (11-26% versus 23-48%) after each challenging dose of cocaine ($p < 0.01$) in the group of animals treated subcacutely with the alkaloid. These symptoms are indicative of cardiovascular tolerance to cocaine.

In a second series, 24 rats were administered cocaine 60 mg/kg/day or a similar volume of saline for 7 to 14 days. After this period animals were anesthetized, laparatomized and blood sampled from the renal artery. Lymphocytes were prepared for binding studies to ß2-adrenoreceptors. The radioligand was ^{125}I-iodocyanopindolol (ICYP). Specific and non specific binding of ICYP were defined, and the regression lines in Scatchard analysis calculated in order to determine Kd and Bmax which were not significantly different in cocaine treated from those of saline controls (59.2±8.9 and 400.69±19.11 versus 51.8±29 and 468.14±37.76) (Dingeon *et al.*, 1991)

In a third series, 20 saline or cocaine treated (40 mg/kg for 7 to 21 days) were studied.After this period, the animals were anesthetized and bled. Their hearts injected with glutaraldehyde, were removed and prepared for light and electron microscopy examination. Cardiac morphology of animals administered saline was normal. Cocaine treated animals presented disseminated focal lesions of the myocardium, disruption of myocyte and myofibrils, and areas of atypical contraction and intra cytoplasmic vacuolization. Electron microscopy showed consistent disseminated lesions of the cardiomyocytes with disorganization of myofibrils, hydropic infiltration and Z stria out of alignment. Mitochondria were swollen with destruction of the crests and a cloudy appearance.

Tolerance to the hypertensive effects of cocaine administration in the rat chronically administered this alkaloid is not correlated with a down regulation of ß-adrenoreceptors, but it is associated with a measurable decrement in cardiovascular function and dose related myocardial lesions. Cardiac tolerance to cocaine appears related to myocardial damage.

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435P EFFECTS OF NEUROKININS ON NG 108-15 CELLS

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In view of the importance of neurokinins in nervous tissue we have studied the effects of neurokinin agonists in neuroblastoma x glioma hybrid NG 108-15 cells. Our aim was to discover which, if any, of the major subtypes of neurokinin receptor are present on these cells, and which electrophysiological parameters were affected by receptor activation. The neurokinins used were Substance P and three more selective agonists. GR73632 and GR64349 are selective for the NK_1 and NK_2 receptors respectively (Hagan *et al.* 1991) and senktide is selective for the NK_3 receptor (Wormser *et al.* 1986).

Whole-cell patch-clamp recordings were made from undifferentiated NG 108-15 cells. Drugs were applied via the bathing medium, composed of (in mM): NaCl 135, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, HEPES 10, glucose 10 (pH 7.4, 21-24°C). The patch electrodes contained (in mM): KCl 117, EGTA 11, HEPES 11, $CaCl_2$ 1, $MgSO_4$ 2, NaCl 10, ATP 2 (pH 7.2). The cells were clamped at a holding potential of -70mV. Depolarising steps of 100ms duration to +60 mV (0.1Hz) were used to evoke a prolonged outward potassium current. In some experiments the electrodes were filled with (in mM) CsCl 120, TEA 20, EGTA 10, $MgCl_2$ 2, HEPES 10 (pH 7.4) and the perfusate contained (in mM) Choline 115, $BaCl_2$ 10, $MgCl_2$ 2, glucose 10, TEA 5, HEPES 10, tetrodotoxin 0.0003 (pH 7.4). In these conditions calcium currents could be studied directly by applying potential changes to +10mV from a holding potential of -80mV.

Substance P had no effect on resting membrane current when applied for 90s periods at concentrations ranging from 0.3 to 30μM. However it did cause, in a dose dependent manner, a reduction in the voltage-gated potassium current of up to 21.6 $\pm$ 2.1% (mean $\pm$ s.e. mean, n=4) at 30μM. This effect was also observed in the presence of the selective NK_1 agonist GR73632 at 10nM to 1μM, which caused a reduction of 28.3 $\pm$ 2.8% The NK_2 selective agonist GR64349 and the NK_3 selective agonist senktide were orders of magnitude less potent, inhibiting the potassium current by 13 $\pm$ 1% and 14.4 $\pm$ 3% at of 1mM and 100μM respectively. To investigate which component of the potassium current was affected by the NK_1 agonist, cobalt, which blocks voltage-dependent calcium channels selectively (hence blocking the calcium-activated component of the potassium current; Reeve and Peers 1992), was added to the extracellular fluid at a concentration of 4mM. In the presence of cobalt, GR73632 (10μM) had no effect on the residual, calcium-independent potassium current. In conditions where sodium and potassium currents were blocked neither GR73632 (10μM) nor Substance P (20μM) had any effect on calcium currents.

Thus in NG 108-15 cells, neurokinins act on NK_1 receptors to reduce the calcium-dependent component of voltage-gated potassium currents. This is not a direct effect on the calcium channel.

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436P EFFECTS OF NEUROKININS ON RAT SUBSTANTIA GELATINOSA NEURONES

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Neurokinins are believed to play an important role in somatosensory processing in the dorsal horn of the spinal cord. Substance P and neurokinin A are present in small diameter primary afferent fibres, many of which terminate in the substantia gelatinosa. These endogenous neurokinins have some selectivity for different receptor subtypes. We have studied the effects of more selective neurokinin agonists on the activity of neurones in the rat substantia gelatinosa in a slice preparation. The agonists used were GR73632 and GR64349, which are selective for NK_1 and NK_2 receptors respectively (Hagan *et al.* 1991).

Longitudinal slices of lumbar cord 300 to 500 μm thick were prepared from young adult male Wistar rats. Each slice retained several dorsal roots 8 to 12 mm long. The slices were mounted in a recording chamber at 37°C at the interface between artificial CSF and humidified 95% O_2/5% CO_2. Electrodes for stimulation (0.2 Hz, 0.1 or 0.01 ms) were placed on a dorsal root and glass multibarrel micropipettes were inserted into the substantia gelatinosa which could be visually identified under a low magnification microscope. One micropipette barrel contained 3M NaCl for extracellular recording and the others were filled with approximately 1 mM solutions of the neurokinin agonists at pH 4.5 for microiontophoretic application with positive currents.

The results described here are from 101 units which responded to electrical stimulation of dorsal roots. Latencies to the first spike were 5 to 20 ms; this was consistent with stimulation of C-fibre afferents. The responses to microiontophoretic application of the agonists are shown in the table, which gives the number of cells responding in each way to each drug and the range of ejection currents used.

	Total	Excitation	(nA range)	Inhibition	(nA range)	No effect	(nA range)
GR73632	91	56	(0-10)	9	(2-20)	26	(20-80)
GR64349	55	19	(3-20)	4	(2-15)	32	(20-80)

Forty-seven cells were tested with both GR73632 and GR64349: 17 were excited by GR73632 but did not respond to GR64349; 10 were excited by both and 3 were inhibited by both. One cell was inhibited by the NK_2 agonist but not affected by the NK_1 agonist, and 14 cells were not affected by either.

These results suggest that the majority of neurones in the substantia gelatinosa which respond to afferent stimulation possess NK_1 receptors and some of these possess NK_2 receptors as well. The predominant response to agonists at both receptors is excitation.

Hagan, R.M., Ireland, S.J., Jordan, C.C., Beresford, I.J.M., Deal, M.J. & Ward, P. (1991) Neuropeptides **19**, 127-135.

437P PHARMACOLOGICAL PROFILE OF [^{125}I]-[TYR^4]-BOMBESIN BINDING TO GASTRIN RELEASING PEPTIDE- AND NEUROMEDIN B-PREFERRING BOMBESIN RECEPTORS IN RAT CNS

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The mammalian bombesin-like peptides, gastrin releasing peptide (GRP) and neuromedin B (NMB), interact with two distinct receptor types. The GRP receptor displays high affinity for GRP and GRP receptor antagonists (e.g. [D-Phe^6]-bombesin(6-13)-ethyl ester) and low affinity for NMB. In contrast, the NMB receptor displays high affinity for NMB and low affinity for GRP, while GRP receptor antagonists are virtually inactive (von Schrenck *et al.*, 1990). The recent molecular cloning of two different bombesin receptor types supports the above classification (Wada *et al.*, 1991). In this study, we have attempted to identify and characterise GRP and NMB receptor binding sites in rat CNS membranes using radioligand binding techniques.

Binding assays were performed briefly as follows. For GRP receptor assays, rat cerebral cortex membranes (50µg protein) were incubated for 90 min at room temperature with 0.1nM [^{125}I]-[Tyr^4]-bombesin in the presence of 10nM NMB and peptidase inhibitors. For NMB receptor assays, rat olfactory bulb membranes (30µg protein) were incubated with 0.1nM [^{125}I]-[Tyr^4]-bombesin in the presence of 5µM of the selective GRP receptor antagonist [D-Phe^6]-bombesin(6-13)-ethyl ester, under similar conditions. In both assays non-specific binding was defined with 1µM bombesin. Reactions were terminated by rapid filtration and bound radioactivity determined.

Binding of [^{125}I]-[Tyr^4]-bombesin to cerebral cortex membranes (in the presence of 10nM NMB) was inhibited by bombesin related peptides with the following rank order of affinity (IC_{50} values are geometric means, nM; -sem, + sem; n=3): GRP (0.47; -0.12, +0.15) > bombesin (1.5; -0.23, +0.26) > litorin (3.7; -0.72, +0.90) = neuromedin C (4.8; -0.70, +0.82) = [D-Phe^6]-bombesin(6-13)-ethyl ester (4.4; -0.99, +1.27) > [Leu$^{13}\psi$(CH_2NH)Leu^{14}]-bombesin (25; -2.5, +2.7) > NMB (52; -10.7, +13.5). In contrast, [^{125}I]-[Tyr^4]-bombesin binding to olfactory bulb membranes (in the presence of 5µM [D-Phe^6]-bombesin(6-13)-ethyl ester) was inhibited with the following rank order (nM): litorin (0.7; -0.06, +0.07) = NMB (1.1; -0.17, +0.21) > bombesin (5.7; -0.8, +0.9) > GRP (11.9; -0.7, +0.8) > neuromedin C (47; -1.3, +1.3) > [Leu$^{13}\psi$(CH_2NH) Leu^{14}]-bombesin (5346; -1177, +1509) > [D-Phe^6]-bombesin(6-13)-ethyl ester (>10,000).

These results clearly demonstrate the presence of both GRP- and NMB-preferring bombesin receptors in rat CNS. In rat cerebral cortex membranes GRP and the GRP antagonists [Leu$^{13}\psi$(CH_2NH)Leu^{14}]-bombesin and [D-Phe^6]-bombesin(6-13)-ethyl ester display high affinity, whereas in rat olfactory bulb membranes NMB displays higher affinity than GRP, with GRP antagonists being only weakly active. These data are consistent with the observations of Pinnock & Woodruff (1992) who have recently demonstrated the presence of functional GRP and NMB receptors in different regions of the rat CNS using *in vitro* electrophysiological techniques.

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438P THE EFFECT OF GALANIN ON TETRAHYDROAMINOACRIDINE-INDUCED HYPOTHERMIA IN MICE

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We have previously shown that galanin, a 29 amino acid peptide colocalised with acetylcholine and 5-HT in the central nervous system (Crawley and Wenk, 1989), is able to block the hypothermia induced by the muscarinic receptor agonist RS86 (2-ethyl-8-methyl-2,8-diazespiro[4,5]decan-1,3-diol hydrobromide) and the 5-HT_{1A} receptor agonist 8-OH-DPAT, an effect which may be mediated through ATP-sensitive potassium channels (Patel and Hutson, 1991). The acetylcholinesterase inhibitor tetrahydroaminoacridine (THA) also causes hypothermia in mice, presumably due to enhanced cholinergic function. In this study we have investigated the effect of galanin on THA-induced hypothermia and its interaction with glibenclamide.

Male BKTO mice (20-30g) were housed individually at ambient temperature (21 ± 3°C) for at least 60 mins prior to the experiment. They were restrained, and rectal temperatures determined using a Sensotek BAT-12 temperature probe. Mice were pretreated with the peripheral muscarinic antagonist N-methylscopolamine (NMS 1mg/kg, i.p.) or scopolamine (10mg/kg, i.p.) and then injected, under metofane anaesthesia, with either galanin (3, 1, 0.3nmol/5µl icv) or vehicle (artificial csf, 5µl icv). This was followed 20 mins later by either vehicle (saline csf, 4ml/kg i.p.) or THA (15mg/kg, i.p.). The potassium channel blocker, glibenclamide (10nmol/5µl, icm) or ethanol vehicle (5µl icm) were administered 10 minutes prior to the start of the experiment.

THA (15mg/kg, i.p.) significantly decreased rectal temperature with a maximal change of -3.2 ± 0.30°C ($p < 0.05$, n = 8, mean ± sem) at t = 30-50 mins, an effect which was blocked by 10mg/kg scopolamine. Galanin (3, 1, 0.3nmol) dose-dependently blocked the hypothermia induced by THA (-0.9 ± 0.38, -2.1 ± 0.25, and -3.7 ± 0.50°C respectively, n = 7/group) without affecting rectal temperature *per se*. Administration of glibenclamide (10nmol) prior to galanin (3nmol) significantly prevented this ability to block THA-induced hypothermia (vehicle/galanin/THA -0.1 ± 0.38°C, glibenclamide/galanin/THA -3.7 ± 0.46°C; $p < 0.05$, n = 7/group). Radioligand binding studies utilising 0.3nM [^{3}H]-glibenclamide in mouse whole brain homogenates showed that galanin, only weakly displaced [^{3}H]-glibenclamide binding (36% inhibition at 10µM).

These results show that galanin is able to block the cholinergically mediated hypothermia induced by systemically administered THA in mice, and that this effect can be reversed in the presence of the ATP-sensitive potassium channel antagonist glibenclamide. However, as galanin only weakly displaced [^{3}H]-glibenclamide binding in mouse whole brain homogenates, it seems unlikely that galanin is acting directly at this site.

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439P RS-23597-190: APOTENT AND SELECTIVE 5-HT_4 RECEPTOR ANTAGONIST

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5-HT_4 receptors are selectively stimulated by substituted benzamides (e.g. zacopride), substituted pyrrolizidines (eg. SC-53116) or substituted benzimidazolones (e.g. BIMU-8) and are antagonized by SDZ 205,557, DAU 6285 and tropisetron (see Bockaert et al. 1992 for review). Given the lack of discrimination of SDZ 205,557 between $5HT_3$ and $5HT_4$ receptors, the relatively low affinity of DAU 6285 and preference of tropisetron for the $5HT_3$ receptor (Eglen et al., 1992, Waikar et al., 1992, Bockaert et al., 1992), a selective high affinity antagonist is required, to define the receptor unambiguously. The effect of RS-23597-190 ([3-(piperidin-1-yl)propyl 4-amino-5-chloro-2-methoxybenzoate HCl) at $5HT_4$ and $5HT_3$ receptors has been characterized using methods described previously (Baxter et al., 1991; Eglen et al., 1992; Sharif et al., 1990).

5-HT_4 receptor mediated relaxations of rat oesophageal muscularis mucosae to 5-HT (-log EC_{50}=7.6) were antagonized in a surmountable fashion by RS-23597-190. The pA_2 value was 7.8 ± 0.1 and the slope of the Schild regression was 1.2 ± 0.2. RS-23597-190 (0.1 nM-10µM), lacked intrinsic activity in that no relaxations were seen to the compound alone. In guinea-pig ileum, 5-HT_3 receptor mediated contractions to 5-HT, studied under conditions of 5-HT_4 densitization using 10 µM 5-methoxytryptamine, were unaffected by RS-23597-190 at concentrations up to and including 10 µM, suggesting an affinity greater than 10 µM. In competition binding studies, RS-23597-190, displaced [^{3}H]quipazine binding from 5-HT_3 sites in NG 108-15 cell membranes, with an affinity (-log K_i) of 5.7 ± 0.1, nH=1.1 ± 0.2. RS-23597-190 (30 µM) did not exhibit affinity for dopamine D_1 or D_2 receptors in radioligand binding studies. In vivo, following intravenous infusion (0.01-10 $mg.kg^{-1}$, min^{-1}) RS-23597-190 inhibited the von Bezold Jarisch reflex in the rat, elicited by 2-methyl 5-HT (10 $\mu g.kg^{-1}$; iv), with a potency (ID_{50}) of 0.3 $mg.kg^{-1}$. min^{-1}.

RS-23597-190 is a potent and selective 5-HT_4 antagonist, with a selectivity ratio, relative to the guinea-pig 5-HT_3 receptor of greater than 1000 and relative to the NG 108-15 5-HT_3 binding site of 125 fold. The increased selectivity relative to the guinea-pig receptor reflects the atypical nature of the $5HT_3$ receptor in this species. The potency at blocking the von Bezold Jarisch reflex in the rat is in agreement with a low affinity at 5-HT_3 receptors. The use of a continuous infusion of the compound, in vivo, necessitated by its rapid plasma hydrolysis, may enhance its usefulness in whole animal studies of $5HT_4$ receptor function. RS-23597-190 is a novel and potent 5-HT_4 receptor antagonist with, thus far, the greatest selectivity over the 5-HT_3 receptor. As such, it provides a useful pharmacological tool to further characterize the 5-HT_4 receptor.

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440P 5-HT_2 RECEPTORS ACTIVATE POTASSIUM CHANNELS IN HUMAN NEUROBLASTOMA CELLS (IMR-32)

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Recently, this laboratory has reported that 5-HT_2 receptors are present on differentiated IMR-32 cells where they stimulate phosphoinositide metabolism and mobilize intracellular calcium (Elliott *et al.*, 1992; Cholewinski *et al.*, 1992). Here we report the electrical response to 5-HT of this neurally-derived cell.

IMR-32 cells were cultured as previously described (Cholewinski *et al.*, 1992). Cells were superfused at 22-25°C. Intracellular recordings were made in Hanks BSS with 3M K-acetate-filled electrodes (or occasionally 3M KCl). In whole-cell patch clamp recordings (at -50mV), the extracellular solution contained (mM) NaCl 140, KCl 5, $MgCl_2$ 1, dextrose 5, HEPES 10, $CaCl_2$ 2 and the pipette solution contained KCl 125, $MgCl_2$ 1, HEPES 10, Na_2ATP 2, Na_2GTP 1 (both pH 7.4 with NaOH). Agonists were applied via a rapid perfusion system for 10 seconds at 5-20 min intervals. Values are given as median and range.

Intracellular recordings revealed a membrane potential of -36mV (-10 to -59mV) and an apparent input resistance of 75MΩ (25-350 MΩ). 5-HT (10μM) hyperpolarized 34 of 50 cells by 7mV (1 to 26 mV) with an associated reduction in the apparent cellular input resistance. In 4 cells, the hyperpolarization was followed by a small, slow depolarization. The hyperpolarization was also observed using KCl-filled microelectrodes (n=5). Ketanserin (30nM) antagonized the hyperpolarizing response to 5-HT (n=3). In the patch clamp recordings, 5-HT (1-10μM) induced an outward current of 300pA (20 to 1200pA) in 29 of 31 cells which was reduced by ketanserin (10nM, n=3). 2-Methyl 5-HT (10μM) had no effect (n=4). The reversal potential of the 5-HT induced outward current was -81mV (-78 to -88mV, n=10); the predicted value for K^+ ions in these solutions is -82mV. After adding 10mM EGTA to the pipette solution, the 5-HT-induced outward current was absent in 4 out of 6 cells (0pA; 0 to 150pA).

These results show that 5-HT hyperpolarized IMR-32 cells. The reversal potential of the response and its apparent dependence on internal calcium suggest that the outward current was mediated by calcium-activated potassium channels. The pharmacology of this response suggests mediation by 5-HT_2 receptors. The hyperpolarizing response to 5-HT of IMR-32 cells, however, contrasts with the 5-HT_2 receptor-induced depolarizing response reported in central neurones (North & Uchimura, 1991).

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441P LONG-LASTING DESCENDING INHIBITION MEDIATED BY 5-HT IN NEONATE RAT SPINAL CORD

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Segmental reflexes in the spinal cord may be modulated by descending pathways, including those containing 5-HT. In the rat, tryptaminergic pathways to the ventral horn are present at birth (Rajaofetre et al., 1989). We have investigated the capacity of descending latero-ventral pathways to modulate lumbar segmental reflexes. Using isolated thoraco-lumbar cord from 1-2 day old rats, ipsilateral and contralateral segmental responses were recorded from L_4 or L_5 ventral roots on stimulation of one L_4 or L_5 dorsal root. Cords were superfused (4-5ml min^{-1}) with Krebs solution at 19-21°C and roots stimulated or recorded from using suction electrodes (Wallis & Wu, 1991). A bipolar electrode made from fine plaited Ag wire (0.13mm diameter) stimulated the cord surface adjacent to a ventral root, between segments T_{11} and T_{12}.

A single stimulus (50-60V, 0.5ms) to the thoracic cord elicited an intense, long-lasting inhibition of fast, ipsilateral and contralateral, segmental reflexes elicited from an L_4 or L_5 dorsal root (30V, 0.5ms); segmental slow responses were unaffected. The monosynaptic reflex (MSR) was maximally inhibited by 78±3% (mean ± s.e.mean, n=19). Inhibition gradually increased between 0.2 and 2s, there was a plateau of inhibition from 2-20s and thereafter inhibition waned, disappearing around 100s. The polysynaptic reflex (PSR) and the contralateral reflex were inhibited to a smaller extent.

Inhibition of MSR at intervals of 2s and longer was progressively more sensitive to ketanserin, the longer the interval examined. At an interval of 20s, inhibition was 86% blocked by ketanserin (1μM). Ketanserin (1μM), ritanserin (1μM), LY 53857 (1μM) and yohimbine (1μM) all reduced the later phase of inhibition. LY 53857 was less effective than the other antagonists.

The long time course and the complexity of the pathways suggest the involvement of other transmitters. Involvement of noradrenaline or GABA is unlikely, since idazoxan (1μM), prazosin (0.1μM) and propranolol (0.1μM) did not reduce inhibition, nor did bicuculline (10μM) nor 2-OH-saclofen (30-50μM).

We conclude that the later phase of descending inhibition is mediated by 5-HT acting at 5-HT_2 receptors. Supported by the Wellcome Trust.

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442P INHIBITION OF MONOSYNAPTIC POTENTIALS BY 5-HT RECEPTOR AGONISTS IN NEONATE RAT SPINAL CORD *IN VITRO*

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5-Hydroxytryptamine (5-HT) has been reported to depress electrically evoked synaptic potentials in the in vitro neonate rat hemicord preparation (Crick and Wallis, 1991). We have further investigated this depressant action of 5-HT and compared it to the effects of the 5-HT_1 receptor selective ligands 8-hydroxy-2-(di-n-propylamino)-tetralin (DPAT), eltoprazine, flesinoxan and sumatriptan.

Hemicord preparations from neonate rats were superfused in vitro at 25°C with an artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 118, $NaHCO_3$ 24, KCl 3, $MgSO_4$ 0.75, $CaCl_2$ 1.5 and D-glucose 12, equilibrated to pH 7.4 with 95% O_2/5% CO_2. The monosynaptic segmental dorsal root-ventral root potential (DR-VRP) evoked by supramaximal stimulation of a dorsal root (10x threshold, 0.5 ms, 0.033 Hz) was recorded across a grease seal. Drugs were added directly to the ACSF at known concentrations. Results are presented as mean ±s.e.mean (n= number of experiments) where appropriate.

5-HT (1-50 μM) dose dependently depolarized the preparation by 0.63 mV ±0.10 (n=9) and depressed the DR-VRP to 29% ± 5.55 (n=7) of control (100%) values. These effects were maintained in the presence of 5-HT and were not antagonized by spiperone (1 μM) in 5 preparations. The 5-HT_{1A} selective agonist DPAT also depressed the DR-VRP. In 2 separate preparations IC_{50} values were 365 and 414 nM. In contrast flesinoxan had no effect in 3 preparations when perfused either at 100, 500 nM or 1 μM. Eltoprazine (10-100 nM) depressed the DR-VRP: IC_{50} value 32.4 nM ±10.2 (n=6). 100 nM Eltoprazine depressed the DR-VRP to 35.7% ± 10 (n=4) of control values. Sumatriptan also depressed the DR-VRP with IC_{50} values <30 nM in 7 preparations. The maximum depression in the presence of 1 μM sumatriptan was to 10.5% ±1.25 (n=3) of control values.

The results obtained with 5-HT and DPAT agree with those of Crick and Wallis (1991), who speculated about the involvement of 5-HT_{1A} receptors in this effect. However the failure of flesinoxan to depress the DR-VRP and the inability of spiperone to antagonize 5-HT are not consistent with this suggestion. The high potency of both eltoprazine and sumatriptan (and the relatively weak effect of DPAT) suggest that stimulation of a 5-HT_{1B}/5-HT_{1D} like receptor may mediate these depressant effects. If these drug effects are presynaptic in nature, the relevant receptors may be specifically localised to group I primary afferents

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443P THE EFFECT OF 5-HT_3 RECEPTOR ANTAGONISTS ON STIMULANT-INDUCED HYPERACTIVITY IN THE MOUSE

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The 5-HT_3 receptor antagonists, ICS 205-930 and zacopride have previously been shown to reduce cocaine-induced hyperactivity in C57BL/6ByJ mice (Reith, 1990). In the present study, we have examined the effect of the 5-HT_3 receptor antagonists, ondansetron and ICS 205-930 on cocaine-induced hyperactivity in CRH and C57BL/6ByJ mice and the effect of ondansetron on hyperactivity induced by other stimulants in CRH mice.

Male CRH mice (18-22 g; Glaxo-bred) and male C57BL/6ByJ mice (20-25g; University of Essex) were placed in individual activity boxes to habituate. Thirty min later mice were dosed with a 5-HT_3 receptor antagonist or vehicle i.p. and after a further 30 min the stimulant was administered s.c.. Locomotor activity (LMA) was recorded as the number of photocell beam breaks over a 90 min period. Results are expressed as the mean LMA score(0-90min) ± s.e.m in the text and the table below. In the table, the number of mice is shown in parentheses.

Table 1. Effect of 5-HT_3 antagonists on cocaine-induced hyperactivity

Drug [mg kg^{-1}]	Vehicle	Ondansetron [0.03]	Ondansetron [0.1]	Ondansetron [1.0]	ICS 205-930 [0.1]
CRH mice:					
vehicle	833±51 (45)	661±73 (12)	767±84 (11)	987±106 (12)	740±66 (12)
cocaine [5]	1974±99 (42)	2070±201 (12)	2008±238 (12)	2374±311 (12)	2503±237 (22)
cocaine [10]	3115±128 (29)	2733±197 (12)	2873±228 (11)	-	2603±174 (12)
C57BL/6ByJ mice:					
vehicle	682±149 (6)	-	-	-	-
cocaine [5]	1803±227 (7)	-	1836±175 (8)	-	-

Neither ondansetron nor ICS 205-930 modified cocaine-induced hyperactivity in CRH mice and ondansetron also did not modify cocaine-induced hyperactivity in C57BL/6ByJ mice (Table 1; ANOVA 1-way and Dunnett's t-test). In CRH mice, ondansetron (0.1 mg kg^{-1} i.p.) also did not modify the LMA-induced by the following stimulants (Student's t-test pooled or Mann Whitney U-test; n=8-12): morphine (2 mg kg^{-1} s.c.: 1970±163, morphine + ondansetron: 1600±122); MK801 (0.2 mg kg^{-1} s.c.: 3457±264, MK801 + ondansetron: 3585±81) and GBR12909 (10 mg kg^{-1} s.c.: 2778±236, GBR12909 + ondansetron: 2943±183).

In conclusion, 5-HT_3 receptor antagonism failed to modify hyperactivity induced by cocaine, morphine, MK801 or GBR12909.

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444P EFFECTS OF GR68755, A SELECTIVE 5-HT_3 RECEPTOR ANTAGONIST, ON BEHAVIOURS INDUCED BY RAISED MESOLIMBIC DOPAMINERGIC ACTIVITY

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Preclinical evidence from rodent and primate studies indicates that 5-HT_3 receptor antagonists may attenuate the behavioural consequences of a period of raised mesolimbic dopamine function (Costall *et al.*, 1990). The present studies investigated the effects of GR68755, a novel, selective 5-HT_3 receptor antagonist (Kilpatrick et al 1991) in models of elevated mesolimbic dopamine function in the rat induced by a continuous infusion of dopamine into the nucleus accumbens or acute bilateral intra-accumbens injection of amphetamine.

Sprague-Dawley rats (Bradford strain, n = 5-14) were subjected to standard stereotaxic surgery to implant chronically indwelling guides for infusion (via subcutaneously implanted osmotic minipumps) at the centre of the nucleus accumbens (Costall *et al.*, 1981). Following a minimum recovery period of 14 days from the initial surgical procedure, dopamine (50μg/24h) or its vehicle (0.1% sodium metabisulphite) was infused bilaterally into the nucleus accumbens for 12 days. Measurement of locomotor activity (in individual photocell cages) during the infusion period revealed the development of increased locomotor activity in the dopamine-infused animals which achieved a maximum intensity of 276±39.6 counts/60 min compared to vehicle infused animals, 109±11.4 counts/60 min. This hyperactivity response was significantly ($P<0.05$-$P<0.001$) antagonised by fluphenazine (10-100μg/kg i.p. b.d.), hyperactivity counts being reduced to 128±10.6-80±21.2 counts/60 min and by GR68755 (0.001-100μg/kg i.p. b.d.), activity being reduced to 118±20.3-79±16.5 counts/60 min.

Hyperactivity was also induced in the rat following acute bilateral injection of amphetamine (10μg) into the nucleus accumbens. This response could be antagonised by fluphenazine (10ng) or GR68755 (100ng) given as a 30 min intra accumbens pretreatment. Following amphetamine administration, hyperactivity gained a maximum of 73±8.0 counts/5 min which was reduced (at 40 min after amphetamine injection) to 32±15.6 and 33±10.1 ($P<0.05$) in the presence of fluphenazine and GR68755 respectively.

It is concluded that GR68755, a selective and potent antagonist at the 5-HT_3 receptor, is effective over a wide dose range to inhibit the behavioural consequences of enhanced mesolimbic dopamine activity in the rodent.

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445P RESPONSES MEDIATED BY 5-HT_{1D}-LIKE RECEPTORS IN GUINEA-PIG ISOLATED JUGULAR VEIN, BUT NOT IN RABBIT ISOLATED SAPHENOUS VEIN, ARE SENSITIVE TO N^G-NITRO-L-ARGININE METHYL ESTER

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Recently, an endothelial 5-hydroxytryptamine$_{1D}$-like (5-HT_{1D}) receptor has been shown to mediate the relaxations to 5-HT receptor agonists in a preparation of guinea-pig isolated jugular vein (Gupta, 1992). In the present study, the possibility that the relaxations which develop following the activation of this 5-HT_{1D}-like receptor are mediated by nitric oxide has been investigated.

Male Dunkin-Hartley guinea-pigs (300-500g) were killed by cervical dislocation. Isolated rings (2mm in length) of jugular vein were suspended on parallel tungsten wires under a resting load of 0.5g, in Krebs solution gassed with 95% O_2 and 5% CO_2 at 37°C. Agonist-evoked relaxant responses were measured isometrically in rings precontracted with U-46619 (30nM), in the presence of mesulergine (10μM) to antagonise respectively 5-HT_2 and 5-HT_1-like (sumatriptan-insensitive) receptors located on the smooth muscle (Gupta, 1992). Male New Zealand White rabbits (2.0-3.0kg) were killed by intravenous administration of pentobarbitone sodium (Sagatal, 60mg/kg). Isolated rings (3-4mm in length) of saphenous vein were suspended on parallel tungsten wires under a resting load of 1.0g, in Krebs solution gassed with 95% O_2 and 5% CO_2 at 37°C, and treated with pargyline (50μM) and phenoxybenzamine (300nM) for a 30min period. Agonist-evoked contractions of the saphenous vein were measured isometrically (for detailed methods see Martin & MacLennan, 1990).

As described previously, both sumatriptan (30nM-100μM) and 5-HT (1nM-10μM) evoke endothelium-dependent, concentration-related, relaxations of the jugular vein (Gupta, 1992). In the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 0.1-100μM), an inhibitor of nitric oxide synthase, the endothelium-dependent relaxations to both sumatriptan and 5-HT were antagonised non-competitively, and the maximum effect to both agonists was reduced (-$\log_{10}$ of the concentration of L-NAME which reduced the maximum effect to the agonist by 50% [95% confidence limits]: sumatriptan, 5.8[5.1-6.4], n=15; 5-HT, 5.8[5.5-6.0], n=8). At the highest concentration of L-NAME used, 100μM, the endothelium-dependent relaxations to both sumatriptan and 5-HT were inhibited completely. In the saphenous vein preparation, the 5-HT_{1D}-like receptor-mediated contractions to both sumatriptan (30nM-30μM) and 5-HT (3nM-3μM) were resistant to L-NAME (100μM), which demonstrated that L-NAME had no direct antagonist effect at the 5-HT_{1D}-like receptor (L-NAME 100μM, geometric mean concentration-ratio [95% confidence limits]: saphenous vein, sumatriptan 1.0[0.9-3.0], 5-HT 1.2[0.6-2.4], both n=4). Furthermore, relaxations to papaverine (30nM-30μM) in the jugular vein were also resistant to the actions of L-NAME (L-NAME 100μM, geometric mean concentration-ratio [95% confidence limits]: 0.9[0.3-2.2], n=4), which indicated that L-NAME had not altered the ability of the smooth muscle cells of the jugular vein to relax by a non-selective action.

In summary, the selective inhibition of 5-HT_{1D}-like receptor-mediated relaxations of guinea-pig isolated jugular vein by L-NAME, suggests that the generation of nitric oxide is the main mediator resulting from the activation of this endothelial 5-HT_{1D}-like receptor.

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446P OPPOSITE EFFECTS OF FLUOXETINE IN TWO ANIMAL MODELS OF ANXIETY

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There is little published information regarding the effects of acute serotonin specific uptake inhibitors (SSRIs) in animal models of anxiety despite their known clinical utility in anxiety and depressive disorders (Mauri, 1991). Administration of paroxetine for three weeks produced an anxiolytic profile in the elevated X-maze (Cadogan et al., 1992). We report here the acute effects of fluoxetine in the elevated X-maze and the Vogel conlict tests. Naive Male Wistar rats (180-200 g) were used for all experiments. The elevated X-maze procedure was as described previously (Handley and Mithani, 1984) with light intensity of 785 lux. In the Vogel conflict test, rats were deprived of water for 48 hours. A pretest was conducted approximately 4 hours before the test in which rats that made less than 500 licks within a five minute period in the drinking chamber were excluded from further use. During the test, rats were allowed 20 licks before initiating the punishment session during which every subsequent 20th lick was punished by an electric shock of 0.5s duration and intensity 0.4 mA. For both tests, fluoxetine, or water as a control, was administered 30 minutes prior to the start of the test by ip injection. Results are tabulated below and demonstrate that fluoxetine 1.25 - 5 mg/kg had an "anxiogenic" profile in the X-maze, where it reduced both the open / total entry ratio and the time spent on the open arms at doses that did not influence total entries. Higher doses were also "anxiogenic" but reduced total entries. In contrast, in the Vogel test, fluoxetine had an "anxiolytic" profile causing an increase in the number of licks made. There was some evidence of a bell-shaped dose response curve in that at 20 mg/kg, fluoxetine did not significantly alter "anxiety" in either test.

DOSE OF FLUOXETINE	ELEVATED X - MAZE			VOGEL CONFLICT TEST	
(mg/kg) N	OPEN / TOTAL ENTRIES	% TIME ON OPEN ARMS	TOTAL ENTRIES	N	NO.OF LICKS
0 (control) 13	0.22 ± 0.02	8.0 ± 1.1	21.8 ± 1.9	17	102.6 ± 8.9
1.25 6	0.13 ± 0.02**	4.3 ± 0.5	19.5 ± 1.5	7	95.3 ± 14.7
2.5 6	0.11 ± 0.03**	4.1 ± 1.4*	19.5 ± 1.3	7	116.0 ± 20.5
5.0 6	0.06 ± 0.03**	2.0 ± 1.0 **	20.5 ± 3.1	7	310.0 ±113.0**
10 7	0.01 ± 0.01**	0.4 ± 0.4 **	7.6 ± 2.7**	10	355.8 ± 70.5**
20 7	0.21 ± 0.01	4.8 ± 1.9	7.3 ± 1.9**	9	127.9 ± 21.2

All values are mean ± sem of N observations. * $P < 0.05$; ** $P < 0.01$ are significantly different from control by Dunnett's t test after a significant one way ANOVA.

These results with acutely administered fluoxetine may demonstrate that the two tests assess different components of the emotion of anxiety and are evidence that serotonergic drugs have different effects in different models of anxiety.

We wish to thank Dr. M. Critchley for assistance with the Vogel test. The support of the Wellcome Research Laboratories is gratefully acknowledged.

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447P THE ANTIDEPRESSANT PAROXETINE HAS NO EFFECT ON DOPAMINE SYNTHESIS RATE: A REGIONAL STUDY IN RAT BRAIN

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Interactions between dopamine (DA) and 5-hydroxytryptamine (5-HT) systems in the CNS are well documented. Reports suggest that the antidepressant fluoxetine, a 5-HT reuptake inhibitor, either decreases (Baldessarini & Marsh,1990) or has no effect (Baldessarini *et al.*, 1992) on DA synthesis rate in different rat brain regions. We have examined the acute effects of a potent and highly selective 5-HT reuptake inhibitor, paroxetine, on DA synthesis rate, in various rat brain regions, including: frontal cortex, cingulate cortex, caudate-putamen, nucleus accumbens and hippocampus. As an index of efficacy, 5-HT synthesis rate was also measured.

Male CD rats received a single oral dose of vehicle or paroxetine (0.3, 1, 3, 10 and 30 mg/kg, p.o.), and brain tissue was removed 2 h later. DA and 5-HT synthesis rates were assessed, by measuring the accumulation of 3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP), after decarboxylase inhibition using NSD-1015 (100 mg/kg, i.p.), given 75 min after vehicle or paroxetine. L-DOPA and 5-HTP levels were measured using HPLC with electrochemical detection. The effects of 10 mg/kg (means ± s.e.mean), are shown in Table 1. Levels are expressed in pg/µg of protein.

Table 1. Acute effects of paroxetine at 10 mg/kg p.o. on L-DOPA and 5-HTP levels.

	L-DOPA		5-HTP	
	vehicle	paroxetine	vehicle	paroxetine
frontal cortex	13.1 ± 0.9	13.3 ± 1.0	16.2 ± 1.6	9.0 ± 0.7 **
cingulate cortex	15.6 ± 1.8	18.6 ± 1.4	15.0 ± 1.9	8.8 ± 0.5
n. accumbens	203.0 ± 36.3	230.8 ± 34.4	50.1 ± 6.6	25.8 ± 3.5 *
caudate-putamen	260.8 ± 28.0	273.0 ± 29.4	43.8 ± 3.5	20.3 ± 1.5 ***
hippocampus	56.4 ± 4.3	54.7 ± 3.2	128.8 ± 7.6	69.1 ± 3.5 ***

Data tested for significance using one way ANOVA and t-test. *P<0.05, **P<0.01, ***P<0.001. n = 6-8.

Over the dose range tested, paroxetine had no significant effects on DA synthesis rate but did dose dependently decrease 5-HT synthesis rate in some regions following 3, 10 and 30 mg/kg. This inhibitory effect on 5-HTP accumulation is consistent with selective inhibition of 5-HT reuptake. In conclusion, the results indicate that paroxetine had no effect on DA synthesis rate, highlighting its selective action on the 5-HT system. This neurochemical selectivity of paroxetine accords with its lack of extrapyramidal side effects in clinical studies.

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448P EFFECT OF AMINO ACID LOADS ON HIPPOCAMPAL 5-HT RELEASE *IN VIVO* EVOKED BY ELECTRICAL STIMULATION OF THE DORSAL RAPHÉ NUCLEUS AND D-FENFLURAMINE ADMINISTRATION

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In rodents, systemic administration of large neutral amino acids, or mixtures of amino acids, reduces entry of L-tryptophan into the brain and decreases the rate of 5-HT synthesis centrally (Gessa et al., 1974; Kennett & Joseph, 1981). In man, amino acid mixtures have mood lowering effects which have been ascribed to decreased 5-HT release in the brain (Delgado et al., 1990). We have recently shown that the large neutral amino acid L-valine (VAL) decreases electrically-evoked 5-HT release in rat hippocampus *in vivo* (Gartside et al., 1992). Here we describe the effects of a mixture of amino acids on electrically-evoked 5-HT release. We also report the effects of the amino acid mixture and VAL on d-fenfluramine-induced 5-HT release.

In the first study, male Sprague-Dawley rats (270-300g) were anaesthetized with chloral hydrate, and a microdialysis probe and bipolar stimulating electrode were implanted in ventral hippocampus and dorsal raphe nucleus (DRN), respectively. The probe was perfused at 1.2 µl/min with artificial CSF containing 1 µM citalopram. Dialysate was collected every 20 min and assayed for 5-HT by HPLC-EC. After a 2-3 h baseline period, the DRN was stimulated (1 ms, 300 µA, 3 Hz) for 20 min (S1). Animals then received either a mixture of amino acids (850 mg/kg i.p.) or vehicle, and 120 min following S1, received a second stimulation (S2). In a second study, animals had only the microdialysis probe implanted and artificial CSF without citalopram was used. After a 2-3 h baseline period animals were injected with vehicle, VAL (200 mg/kg i.p.) or the amino acid mixture (850 mg/kg i.p.) and then, 1 h (VAL) or 2 h (vehicle and amino acids) later, received d-fenfluramine (10 mg/kg s.c.).

Electrical stimulation of the DRN approximately doubled 5-HT levels in the sample collected during the period of stimulation. In animals (n=7) which received vehicle between stimulations, S1 and S2 released similar amounts of 5-HT (45±16 vs 50±16 fmol, S1 vs S2). However, in animals (n=5) which received the mixture of amino acids between S1 and S2, the amount of 5-HT released by S2 was consistently less than that released by S1 (41±6 vs 28±5 fmol, S1 vs S2; $P<0.02$, paired t-test). In vehicle treated animals, d-fenfluramine caused an approximately 6-fold increase in 5-HT levels (n=5). VAL and the amino acid mixture reduced the effect of d-fenfluramine by about 45 % (n=5) and 20 % (n=6), respectively. Analysis of variance revealed a highly significant time x treatment interaction in the case of both VAL ($F=7.7$, d.f. 6, 54; $P<0.01$) and the amino acids ($F=2.5$, d.f. 6, 60; $P<0.005$). Basal 5-HT ouput was not altered by either VAL or the amino acid mixture.

In summary, in the anaesthetized rat *in vivo*, administration of VAL or a mixture of amino acids attenuates the release of hippocampal 5-HT evoked by either electrical stimulation of the DRN or administration of d-fenfluramine. These findings are relevant to the interpretation of the mood-lowering effects of amino acid loads in man (Delgado et al., 1990).

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449P DOLASETRON MESILATE (MDL 73147EF), A POTENT ANTI-EMETIC 5-HT$_3$ RECEPTOR ANTAGONIST

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5-HT$_3$ receptor antagonists are effective in the treatment of vomiting elicited by cancer chemo- and radiotherapy and may be of use in a variety of central nervous system disorders including anxiety, schizophrenia and cognitive dysfunction (Costall et al. 1990; Aapro, 1991). The aim of the present study was to characterize the actions of a novel 5-HT$_3$ receptor antagonist, dolasetron mesilate ([2α,6α,8α,9aß]-octahydro-3-oxo-2, 6-methano-2*H*-quinolizin-8-yl 1*H*-indol-3-carboxylate monomethane-sulfonate).

Radioligand binding demonstrated a high affinity of dolasetron at 5-HT$_3$ receptors in NG108-15 cell membranes (20 ± 6.6 nM; mean ± s.e.mean; n=6; Boeijinga et al., 1992) but no significant affinity for 5-HT$_{1A}$ or 5-HT$_2$ receptors, α_2 adrenoceptors, dopamine D_2, muscarinic m_1, m_2, m_3, m_4, m_5 or neurokinin NK_1 receptors, measured by standard techniques (IC_{50}s all > 10 µM). Dolasetron was a potent antagonist at 5-HT$_3$ receptors in the rabbit heart (see Fozard, 1984; pA_2 9.8 ± 0.1, n=4). In anaesthetized rats, the von Bezold-Jarisch reflex (BJR) elicited by i.v. injection of 5-HT (1-2 µg/kg) was inhibited by dolasetron (ED_{50} at 5 min, 3 µg/kg); in conscious rats, 1 mg/kg p.o. suppressed the BJR for > 4h. In conscious ferrets with an indwelling cannula in the jugular vein, dolasteron (0.05-0.5 mg/kg i.v. 30 min before and 45 min after cisplatin, 10 mg/kg i.v.) significantly reduced the frequency of retching and vomiting in a dose-dependent manner. The latency to the first vomiting episode was significantly increased by the same dose range. The compound also prevented cisplatin-induced vomiting after oral administration (2 mg/kg).

It is concluded that dolasetron is a potent and selective 5-HT$_3$ receptor antagonist and is an effective anti-emetic agent by the intravenous and oral routes of administration.

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450P TRH ANALOGUE MK771 INDUCES BLINKING: EVIDENCE FOR 5-HYDROXYTRYPTAMINERGIC MODULATION

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Blink rates in humans are known to be altered in a number of psychiatric and neurological disorders, for example they are increased in schizophrenia, Tourette syndrome and depression and reduced in Parkinson's disease. It is generally considered that blinking is primarily under the control of dopamine (Karson, 1989; Elsworth *et al.*, 1991). However, i.c.v. injection in mice of TRH-amide has been shown to induce blinking which was antagonised by the 5-HT2 / 5-HT1C antagonist ritanserin (Dursun and Handley, 1991). We have therefore investigated the effects of a systemically active TRH analogue MK771 (Veber *et al.*, 1976) on blinking in mice and begun to investigate the role of the serotonergic system in modulating this behaviour.

Pairs of male MF1 mice (20-30g, n=6 per treatment group) were treated with MK771 (5.0 mg/kg i.p.) or saline and their blink rates counted in 5 minute bins for 30 minutes following injection. For the dose response relationship, MK771 (0.25-60.0 mg/kg i.p.) was assessed for 5 minutes, 7.5 minutes after administration. The 5-HT1A agonists 8-OH-DPAT (0.1 mg/kg i.p., n=6 per treatment group) and buspirone (1.0 mg/kg i.p., n=6-7 per treatment group) were administered 7.5 minutes prior to MK771 (2.5 mg/kg i.p.) and behaviour was recorded 7.5 minutes later for 5 minutes. Behaviour was recorded on video and analysed later with the observer unaware of the treatment each mouse had received.

MK771 induced a behavioural profile consisting of: blinking (unilateral and bilateral, which was not differentiated on counting), fore-paw licking, fore-paw tremor, ear-scratch, abdominal contractions with vertical tail movements, tail elevation and tremor and infrequent back muscle contractions. MK771 induced blinking (*eg.* 5.0 mg/kg i.p. +5648% (over 30 minutes), $p<0.0005$ *cf.* saline treated mice (unpaired t-test)) which peaked during the second and third bins (5-9.9 and 10-14.9 minutes) and showed a bell-shaped dose response curve (ED50 for linear portion (0.25-6.4 mg/kg i.p.) = 1.72 [1.38-3.63] mg/kg i.p.). 8-OH-DPAT and buspirone attenuated MK771 (2.5 mg/kg i.p.) induced blinking (-46%, $p<0.05$; -40%, $p<0.005$ respectively (two-way-anova with *post-hoc Dunnet's* t-test)).

Thus systemic administration of the TRH analogue MK771 induced blinking in mice consistent with the action of TRH-amide (Dursun and Handley, 1991), and this effect may be modulated by serotonergic systems.

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451P THE 5-HT$_3$ RECEPTOR ANTAGONIST ACTIONS OF THE ENANTIOMERS OF ZACOPRIDE AND SOME OTHER ANTAGONISTS IN THE MOUSE ISOLATED VAGUS NERVE

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The mouse is a species extensively used in behavioural studies of 5-HT$_3$ receptor antagonists but in which there have been few reports of *in vitro* 5-HT$_3$ effects (Newberry et al., 1991). We have investigated the antagonist actions of the S (-) and R (+) enantiomers of zacopride, reported to differ greatly in potency in some behavioural tests (Barnes et al., 1990), and other 5-HT$_3$ antagonists, against the depolarization response to 5-hydroxytryptamine (5-HT) in the mouse isolated vagus nerve (MVN). A grease-gap extracellular recording technique (Ireland and Tyers, 1987) was used to detect 5-HT (10 nM-1mM)-evoked depolarizations of the mouse (male, T.O. strain, 20-50 g) cervical vagus nerve. The nerve was placed in a two chamber tissue bath, one chamber being perfused (3 ml/min) with Krebs' solution (2.5 mM $CaCl_2$, at 27°C, gassed with 5% CO_2 in O_2) to which drugs were added. Agonist contact time was 3 min (15 min washout) and antagonists were equilibrated for 50 min. Depolarizations were expressed as a percentage of an initial response to 5-HT (10 μM) obtained 50 min before a 5-HT concentration-response curve (CRC). One CRC only was obtained in each tissue.

In control tissues a mean maximum depolarization of 0.27 (0.24-0.30) mV, (n=16) was evoked by 5-HT. The CRC was monophasic with an ED_{50} of 0.39 (0.21-0.57) μM. In the presence of either ondansetron (10 nM-0.1μM) or R (+) zacopride (1-30nM) 5-HT CRC's were displaced to the right and appeared biphasic with 5-HT (10 nM-1μM) evoking a depolarization (first phase) of 10-20% of the 5-HT maximum response which was not displaced by increasing concentrations of antagonists. This first phase was not observed in preparations equilibrated (50 min) with 5-methoxytryptamine (10μM) which itself evoked a mean maximum depolarization of 84.4 μV (n=3) in this preparation. A second phase of the CRC to 5-HT was displaced to the right in the presence of either ondansetron or R (+) zacopride with pA_2 values for the antagonists of 8.3 (8.0-8.9; n=11) and 9.3 (9.1-9.6; n=16) and Schild plot slopes of 1.1 and 1.0 respectively, consistent with a 5-HT$_3$ mechanism. Tropisetron (0.1-10 nM; pIC_{50} 9.4), the S (-) enantiomer of zacopride (0.3 and 3nM; pIC_{50} 9.4) and racemic zacopride (0.3 nM-10 nM; pIC_{50} 9.1) markedly depressed the maximum response to 5-HT with little evidence of a rightward shift in CRC's.

Thus, in the MVN a major component of the depolarization evoked by 5-HT was antagonized by ondansetron, suggesting a 5-HT$_3$ receptor mechanism. This component was also blocked by similar concentrations of both enantiomers of zacopride, only the R (+) enantiomer being a surmountable antagonist. These results are similar to those reported using the same preparation of the rat (Coleman et al., 1991) and do not offer an explanation of results in behavioural tests in which the R (+) enantiomer is more potent than the S (-) enantiomer of zacopride. A small component of 5-HT evoked depolarization of the MVN was resistant to 5-HT$_3$ antagonists but was absent in tissues pre-treated with 5-methoxytryptamine, a compound with 5-HT$_4$ receptor affinity.

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452P 5-HT INHIBITION OF BICARBONATE SECRETION BY RAT CAECUM *IN VITRO* MAY INVOLVE ENDOGENOUS PROSTANOIDS

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Luminal bicarbonate secretion by rat caecum in vitro is inhibited by cholinergic agonists (Canfield & Abdul-Ghaffar, 1991). In view of the many similarities between the actions of cholinergic drugs and 5-HT on intestinal ion transport, we have examined the effects of 5-HT on bicarbonate secretion by this preparation.

The caecum was removed from male Wistar rats (250-300g), opened, washed with saline and then tied over the end of perspex tubes (area 1.13cm^2) with the mucosa facing the tube lumen. 5ml of an unbuffered physiological saline gassed with 100% O_2 was placed on the mucosal side and the tube suspended in 30ml of a similar saline, in which 25mM HCO_3^- replaced Cl^-, gassed with 5%CO_2/95%O_2 (pH 7.4) maintained at 37C. Mucosal saline was replaced every 15 min and HCO_3^- secretion determined by back-titration with 5mM HCl. Secretory rate is expressed as μmol cm^{-2} h^{-1} and data shows the fall in rate following drug application. Values are mean ± s.e.mean with n in brackets and $P < 0.05$ was taken as a significant difference between means (t-test). All drugs were added to the serosal side.

5-HT (0.1-50μM) inhibited secretion with a maximum reduction of basal rate of 1.38 ± 0.15 (12) μmol cm^{-2} h^{-1} at 5μM. The response to 50μM 5-HT was not significantly different from the response to maximum bethanechol (Bet, 50μM) in the same tissues: 5-HT = 1.21 ± 0.15 ; Bet = 1.33 ± 0.16 (10). Response to 5-HT was abolished when mucosal Cl^- was replaced with NO_3^- as found previously with Bet. A number of antagonists were tested after 45 min preincubation in two sets of experiments (see table).

	Control 5-HT (10μM)	**+ Methysergide** (10μM)	**+ Metoclopramide** (10μM)	**Control 5-HT** (10μM)	**+ Indomethacin** (10μM)	**+ Atropine** (10μM)
Reduction in bicarbonate secretion (μmol cm^{-2} h^{-1})	1.24 ± 0.22	0.2 ± 0.14*	0.47 ± 0.13*	0.94 ± 0.19	0.35 ± 0.16*	0.94 ± 0.17
n =	5	5	5	11	11	11

*** indicates a significant difference from control $P < 0.05$**

Indomethacin (Ind) also significantly inhibited the response to 10μM bethanechol; Bet = 1.0 ± 0.1 (4), Bet + Ind = 0.12 ± 0.3 (4) μmol cm^{-2} h^{-1}. In a pilot experiment PGE_2 (5μM) reduced basal bicarbonate secretion by a similar amount.

5-HT inhibited luminal bicarbonate secretion in this preparation in a similar manner to bethanechol and the effects of both drugs were reduced by indomethacin. The action of both 5-HT and Bet may involve the release of endogenous prostanoids from the tissue, possibly PGE_2. Determination of the 5-HT receptor types involved and the relationship between 5-HT, Bet and prostanoid production requires further study.

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453P ELECTRICAL FIELD STIMULATION OF RABBIT ISOLATED CUTANEOUS AND PULMONARY MICROVESSELS DEMONSTRATES THE DIVERSITY OF INNERVATION WITHIN THE MICROCIRCULATION

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Many workers are now studying the microcirculation in relation to its control of peripheral resistance. The adventitia of many small arteries contain nerves which can influence the reactivity of the vessel. We have studied the responses to electrical field stimulation (EFS) of isolated segments of dorsal cutaneous and pulmonary microvessels from the rabbit. It is most likely that these types of cutaneous and pulmonary microvessels contribute to the pathogenesis of systemic and pulmonary hypertension, respectively.
Male New Zealand White rabbits (2.5 - 3 Kg) were killed by stunning followed by exsanguination. The lungs were quickly removed and placed in standard Krebs solution at room temperature. The main pulmonary artery was identified and followed down to its most distal point in each lobe. Branches off the main artery (unstretched diameter ~300μm) were excised and mounted on a wire myograph. Alternatively, a 2cm x 2cm section of skin from the area at the base of the spine was removed and placed in Krebs solution at room temperature. Segments of microvessels (diameter ~300μm) were removed and mounted on a wire myograph. Pulmonary and cutaneous arteries were placed under 100mg and 500mg tension respectively. Cocaine (10μM) and propranolol (1μM) were present to block neuronal uptake and beta-adrenoceptors, respectively. EFS was delivered via platinum plates which were fixed to the (plastic) mounting heads of the myograph. The parameters of stimulation were as follows: 4, 8, 16, 32 & 64 Hz; pulse width 0.1ms; train duration 1 second; 50 volts. Stimuli were delivered at 5 minute intervals. Time between frequency response curves was 30 minutes. An additional low frequency long duration stimulus (4Hz / 10 seconds) was also tested.
EFS delivered to the cutaneous arteries produced monophasic responses which returned quickly to baseline. The responses were frequency dependent and ranged from 270± 81mg at 4Hz to 1909±265mg at 64Hz (n=10). Prazosin (0.1μM) caused a significant reduction of responses at 32Hz (43% reduction), 64Hz (47%) & 4Hz/10sec (83%). The addition of α,β-methylene ATP (3μM) caused a transient contraction which returned to baseline within 2-3 minutes. The combination of prazosin and α,β-methylene ATP caused a significant reduction (>90%) of all responses from control levels. The pD_2 for noradrenaline in this tissue is 6.26±0.1. The response to EFS in the pulmonary microvessels was much more variable. Of the 18 preparations studied, 5 gave little or no response to EFS but contracted to either KCl (50mM) or $PGF_{2\alpha}$ (3μM). Of the 13 preparations which responded to EFS, 9 exhibited a marked biphasic response to 32Hz and above. Only 7 preparations gave frequency dependent biphasic responses over the full range of stimuli. The biphasic response consisted of an initial fast component followed by a much longer, slowly developing phase which often took 5 minutes to return to baseline. Incubation with indomethacin (0.3μM) for 30 minutes significantly inhibited the slowly developing phase leaving only the initial fast component.
We have described two microvessels of similar size which have very different neuroeffector mechanisms. The dorsal cutaneous arteries appear to have a rich adrenergic innervation in which co-transmission of ATP plays an important role. Rabbit pulmonary microvessels, *in-situ*, exhibit indomethacin sensitive responses to vagal stimulation (Shirai *et. al.*, 1992). We have shown that a similar result can be obtained *in-vitro* and that there is an initial, indomethacin resistant, component to the response.

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454P CORRELATION BETWEEN VASODILATING RESPONSE TO SELECTIVE ADENOSINE AGONISTS IN RAT AORTA AND BOVINE CORONARY ARTERY

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Adenosine exerts its vasodilating action in different vascular beds by interacting with the A_2 adenosine receptor (Olsson & Pearson, 1990). However, differences have been found, in various species, in response to adenosine and its analogues (Collis, 1991). In an attempt to understand the complexity of the responses evoked by adenosine, several selective adenosine agonists have been tested for their vasorelaxant effects in rat aorta and bovine coronary artery preparations precontracted with PGF2α (3 μM). The results are reported in table 1.

AGONIST	ED_{50} Mean ± S.E.M. (-log M)		Binding Selectivity* $Ki(A_1)/Ki(A_2)$
	Rat Aorta	Bovine Coronary Artery	
CGS 21680	6.94 ± 0.20	7.18 ± 0.17	54.1
NECA	6.40 ± 0.16	6.77 ± 0.06	1.4
2-hexynyl-NECA	6.22 ± 0.22	6.25 ± 0.28	56.5
CV 1808	5.91 ± 0.13	6.29 ± 0.15	8.1
R-PIA	5.73 ± 0.18	5.96 ± 0.09	0.008
S-PIA	5.30 ± 0.11	5.24 ± 0.12	0.025
CCPA	> -3	5.37 ± 0.17	0.002

* Dionisotti et al. (1992) Naunyn Schmiedebergs Arch. Pharmacol. (in press).

Involvement of the A_2 receptor was indicated by the similar order of potency observed in both vascular preparations (CGS 21680 > NECA > 2-Hexynyl-NECA > CV 1808 > R-PIA > S-PIA > CCPA). Moreover, the A_1 selective antagonist DPCPX (100 nM) did not influence ED_{50} values for vasorelaxation. The presence of functional endothelium was indicated by the ability of acetylcholine (30 μM) to relax vascular preparations. Responses obtained to CGS 21680 and NECA were found to be independent from endothelial cells injury. A significant correlation was found between the vasodilating response to A_2 selective agonists in rat aorta and bovine coronary artery ($r=0.86$; $P<0.05$).
In conclusion, our data provide evidence that A_2 receptor-mediated relaxation is similar in the two vascular preparations. In addition, the general potency with which the adenosine agonists evoked responses was related to their affinity for the A_2 receptor subtype.

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455P ELECTROPHYSIOLOGICAL EVIDENCE FOR NICOTINIC ACETYLCHOLINE RECEPTORS IN ISOLATED TYPE I CELLS OF THE NEONATAL RAT CAROTID BODY

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Catecholamine release from type I cells in response to hypoxia and other stimuli is an essential step in carotid body chemotransduction. In the rat, there is evidence that hypoxia-induced release is influenced by acetylcholine, and indeed carbachol can evoke release in normoxic conditions (Shaw, Montague & Pallot, 1989). In this study we have used the whole cell variant of the patch clamp technique to investigate electrophysiological responses of isolated neonatal rat type I cells to various cholinergic agonists and antagonists. Type I cells were isolated as previously described (Wyatt & Peers, 1992).

Cells were perfused with a solution of composition (in mM): NaCl 135, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, HEPES 5, glucose 10 (pH 7.4, 21-24°C) and whole cell recordings were obtained from single cells using electrodes filled with (in mM): KCl 117, K-EGTA 11, HEPES 11, $CaCl_2$ 0.1, $MgSO_4$ 2, NaCl 10, ATP 2, (pH 7.2) at a holding potential of -70mV. Under these conditions carbachol (CCh, 100μM) evoked an inward current of 17.8 ± 4.7pA (mean ± SEM, n=4 cells). Nicotine evoked inward currents in a concentration-dependent manner: 109.3 ± 26.7pA (n=10) at 30μM, 144.4 ± 34.8pA (n=17) at 100μM and 263.5 ± 33.5pA (n=10) at 300μM. Dimethylphenylpiperazinium iodide (300μM) also evoked inward currents of 89.3 ± 33.7pA (n=9), whereas muscarine (100μM) was without detectable effect (n=10). Mecamylamine attenuated nicotine-induced inward currents: at a concentration of 0.3μM, mecamylamine reduced inward currents evoked by 300μM nicotine to 50.4 ± 14.1pA (n=13). In the presence of 3μM mecamylamine, 300μM nicotine failed to evoke reponses in 9 out of 10 cells studied, but in one cell a current of 23pA was seen. Atropine (1μM) had no significant effect on peak inward currents evoked by 100μM nicotine (n=10). When extracellular NaCl was replaced by 90mM $CaCl_2$, nicotine (100μM) evoked inward currents of 129.3 ± 10.6pA (n=10). Application of 100μM nicotine under "current clamp" conditions (in NaCl-containing solutions, using small hyperpolarising currents to hold cells close to the holding potential used in voltage clamp studies) caused type I cells to depolarise from -71.3 ± 1.1mV to -22.6 ± 3.2mV (n=8).

These findings indicate that isolated neonatal rat type I cells possess nicotinic acetylcholine receptors. Their activation might be expected to raise $[Ca^{2+}]_i$, and this in turn might account, at least in part, for the previously reported Ca^{2+}-dependent stimulation of catecholamine release from the intact rat carotid body caused by carbachol (Shaw et al., 1989).

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456P CHANGE OF NICOTINIC AChR CHANNELS IN B-CELLS OF BULLFROG SYMPATHETIC GANGLION DURING DEVELOPMENT

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The developmental change in muscle nicotinic AChR channel kinetics due to change in subunit composition is well documented. However, few studies have examined the possibility of a developmental changes in channel properties of neuronal nAChR since the large size and extensive size of many dendrites make an accurate measurement of synaptic currents difficult (Moss et. al., 1989). The present experiments examined the kinetics of nAChR of bullfrog sympathetic B-cells, their developmental change and new interpretation of the results which have already been published (Marshall & Kojima, 1986).

Synaptic current recordings were made by whole cell patch clamping neurons of the 9th and 10th ganglia of the sympathetic chain. The suction electrode was applied to the interganglionic nerve trunk above the 7th ganglion in order to stimulate preganglionic B-fiber. 90%-10% of the decay phases of evoked epsc and miniature epsc in B-cell were analyzed. Membrane current noise was produced by iontophretic application of ACh from a micropipette filled with 2M AChCl - 30μm away from the cell surface.

Evoked epscs and miniature epscs decayed exponentially while current noise spectra were fitted by two Lorentzian functions at the holding potential -60mV. These results could be explained by two different models of channel kinetics. One of these was chosen after considering Kuba's finding of two populations (fast and slow) of nAChR channel with similar conductances in cultured B-cells (Kuba et. al., 1989).

In the present experiments, the decay time constants of mepsc, eepsc and noise were measured. Their values obtained from tadpoles were 4.16 ± 1.77ms (n=39), 4.46 ± 0.96ms (n=13) and 1.86 ± 0.39ms (n=7), respectively. These recorded from adults frogs were 5.19 ± 1.53ms (n=44), 6.36 ± 1.42ms (n=30) and 2.70 ± 0.47ms (n=10), respectively, which were consistent with those obtained by Kuba's experiment from cultured B-cells. The quantal content increased from 1.36 ± 0.16 (n=7) to 21.60 ± 11.4 (n=9) and quantal size estimated by the amplitudes of mepscs were unchanged.

It is concluded from the present experiments that the properties of nAChR channel in bullfrog sympathetic B-cell may change during development and this change coincides with the time of synaptic formation.

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457P PIRENZEPINE SELECTIVELY INHIBITS BETHANECHOL-STIMULATED SMALL INTESTINAL (SI) MOTILITY IN THE ANAESTHETISED DOG: A POSSIBLE ROLE FOR M_1 RECEPTORS

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Pirenzepine, a selective M_1 antagonist, inhibits bethanechol stimulated gastric acid secretion in the absence of effects on heart rate in the dog (Hirschowitz et al, 1983). In motility studies, pirenzepine antagonised McN-A343 (M_1 agonist) induced inhibition of SI motility and reduced the contractile effect of acetylcholine in anaesthetised dogs (Fox et al, 1985). This study examines the muscarinic receptor mediating SI motility to bethanechol in the anaesthetised dog.

All in vitro studies were conducted in Krebs maintained at 32°C and gassed with 95% O_2 and 5% CO_2. Cumulative agonist dose response curves to carbachol (on guinea pig ileum (contraction) and the spontaneously beating atria (slowing)), and McN-345 (inhibition of the field stimulated vas deferens (Eltze, 1988)) were obtained in the absence and presence of antagonists to determine pA_2 values (Arunlakshana and Schild, 1959). Male beagle dogs (11-14.5 kg) were prepared as described in a previous study (Quinn et al, 1991). SI motility was stimulated by a bolus injection of bethanecol (75-100 $\mu g\ kg^{-1}$) and the resultant activity was expressed as motility index (MI) (mean amplitude x frequency). The dose ($\mu g\ kg^{-1}\ min^{-1}$) to inhibit bethanechol-induced SI motility by 50% and the dose to increase resting heart rate by 50% (ED_{50} values) were calculated.

In vitro atropine was a non-selective muscarinic antagonist, whilst 4-DAMP showed selectivity for M_1 and M_3 receptors and pirenzepine showed selectivity for M_1 receptors (Table 1) confirming published selectivities. In vivo atropine (n=3) was a non-selective, whilst 4-DAMP (n=4) and pirenzepine (n=3) were gut-selective (Table 1).

Table 1	In vitro (pA_2 value(n=5-8)			In vivo (ED_{50})[$\mu gkg^{-1}min^{-1}$]	
	M_3 (Ileum)	M_2 (Atrium)	M_1 (Vas Deferens)	SI(M1)	Heart Rate
Atropine	9.4±0.1	8.7±0.1	9.6±0.1	0.3	0.5
4-DAMP	9.7±0.1	7.9±0.1	9.5±0.1	0.3	1.7
Pirenzepine	7.2±0.04	6.7±0.1	8.1±0.1	1.8	27.0

In vitro the intestinal muscarinic receptor mediating contraction of the guinea pig ileum is of the M_3 subtype and this may explain the potent inhibition seen with atropine and 4-DAMP in vivo. The result with pirenzepine supports a role for M_1 receptors in mediating response to bethanechol. Further evaluation of the roles of M_1 and M_3 receptors requires more selective antagonists.

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458P THE ACTIONS OF ACETYLCHOLINE (ACh) AND A PHE-MET-ARG-PHE (FMRF)-AMIDE-LIKE PEPTIDE ON A DORSAL MUSCLE STRIP OF THE PARASITIC NEMATODE *ASCARIS SUUM*

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FMRF-amide-like immunoreactivity has been demonstrated in *Ascaris* motorneurones (Davenport *et al.*, 1988; Sithigorngul *et al.*, 1990) where it may be colocalized with either GABA or ACh, the inhibitory and excitatory neuromuscular junction transmitters, respectively. An endogenous peptide, AF1 (KNEFIRFamide), has been identified which paralyses worms after injection into the whole animal. AF1 also inhibits spontaneous activity in the inhibitory motoneurones (Cowden *et al.*, 1989). In order to assess the role of this mechanism in AF1 induced paralysis we have investigated actions of AF1 on *Ascaris* dorsal muscle strip and its susceptibility to the nicotinic receptor antagonist mecamylamine.

1 cm of *Ascaris* dorsal body wall muscle was excised anterior to the genital pore (this preparation contains no motoneurone soma) and secured in an organ bath containing artificial perienteric fluid (APF composition in mM; NaCl 67, NaAcetate 67, $MgCl_2$ 15.7, $CaCl_2$ 3, KCl 3, Tris 5, pH 7.6) at 37°C attached to an isometric transducer. A tension of 1g was applied.

ACh elicited a dose-dependent increase in tension pD_2 55μM (n=12) which was reversibly blocked by mecamylamine (pA_2 6.6, Schild slope 0.89). The block was use-dependent not time-dependent i.e. the response to the 1st application of ACh in the presence of mecamylamine (0.1μM) was 70$\pm$6% of control and after the 6th application the response was 34$\pm$6%. In another set of experiments following exposure of the muscle to mecamylamine (0.1μM) for 1 hr, the response to the 1st application of ACh was 67$\pm$3% (n=4) of control. Mecamylamine did not elicit a change in resting tension suggesting that ACh is not released tonically in this preparation. AF1 also increased muscle tension (1μM AF1 was approximately 80%, n=3 the potency of ACh). However, in contrast to ACh it also increased the amplitude and frequency of the spontaneous activity. The response latency was longer for AF1 compared to ACh, taking 3 min to develop. It also reversed more slowly taking an hour for recovery to basal tension. Mecamylamine (10μM, n=4) completely blocked the response to AF1 (1μM).

AF1 has an excitatory action on the muscle strip. It is possible that AF1 is acting to increase the overall cholinergic tone in the preparation and this is supported by the observation that mecamylamine blocks the response to AF1.

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459P MUSCARINIC ACTIONS ON POTASSIUM CURRENTS IN GUINEA-PIG CŒLIAC GANGLION NEURONES MAINTAINED IN CULTURE

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A prominent action of muscarine in amphibian and many mammalian sympathetic neurones is to decrease a non-inactivating potassium current that is active at rest, the M-current. Muscarine also decreases distinct calcium-activated potassium currents, an apamin-sensitive I_{AHP}, and a slow gK_{Ca}. We are interested in the properties of a distinct population of sympathetic neurones in the coeliac ganglion, those that innervate gastrointestinal blood vessels; these neurones can be grown in culture in isolation from other, non-vasomotor, coeliac neurones. We examined the actions of muscarine on membrane currents recorded with intracellular microelectrodes and single-electrode voltage clamp techniques. In normal solution, hyperpolarizing voltage commands from rest evoked slow inward relaxations whose time course, voltage-dependence and pharmacology resembled the M-current: this non-inactivating current activated and deactivated between -65 mV and 0 mV, tail currents were best fit by two exponentials with time constants of 65 $\pm$ 12 ms and 390 $\pm$ 46 ms (n=8), and the current was insensitive to TEA (10 mM), 4-AP (2 mM) and apamin (1 μM). The current was inhibited to a variable degree (45 - 100%) by the inorganic calcium channel blockers, cadmium (100-300 μM) or lanthanum (1-20 μM). Two types of afterhyperpolarizations followed the generation of action potentials, a fast and a slow AH (half durations 0.25 and 1.8 s respectively). Both AHs were blocked by cadmium or lanthanum; the fast AH was abolished by apamin (30 nM) but the slow AH was unaffected by 100-fold higher concentrations. M-like current was present in all cells examined (n=125) but the apamin-sensitive I_{AHP} and slow gK_{Ca} were observed in less than half of these neurones. There was no positive correlation between M-current magnitude and presence of slow gK_{Ca}.
Muscarine produced an inward current at -60 mV associated with a decrease in the M-like current; EC_{50} for inward current and inhibition of M-current was 3 μM. Muscarine (20 μM) abolished the M-current and reduced the instantaneous leak current by 50%. Muscarine also abolished the slow gK_{Ca} (EC_{50} = 0.65 μM) but was without effect on the apamin-sensitive I_{AHP}. These results indicate that coeliac ganglion neurones exhibit an M-current which is relatively sensitive to inhibition by inorganic calcium channel blockers, a distinct slow gK_{Ca} and an apamin-sensitive fast I_{AHP}. Muscarine blocks the former two, but not the latter, currents.

460P EFFECTS OF MECAMYLAMINE AND CAPTOPRIL ON REGIONAL HAEMODYNAMIC RESPONSES TO BRADYKININ IN CONSCIOUS RATS

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Bradykinin injected i.v. has complex haemodynamic effects in conscious rats (Gardiner *et al.*, 1992). Inhibition of kininase II with an angiotensin-converting enzyme inhibitor, such as captopril, could enhance the depressor effects of bradykinin by inhibiting its degradation and also by suppressing the formation of angiotensin II (Stewart *et al.*, 1971). In the present work we assessed the haemodynamic responses to bradykinin in the absence and presence of mecamylamine, and again in the presence of mecamylamine and captopril in the same, conscious Long Evans rats, in order to assess the effects of ACE inhibition in the presence of ganglionic blockade. Male, Long Evans rats were chronically instrumented with pulsed Doppler probes (coeliac, mesenteric and hindquarters) and intravascular catheters; all surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p., supplemented as required). Animals were challenged with i.v. bolus injections (3 nmol kg^{-1}) and 3 min infusions (36 nmol kg^{-1} min^{-1}) of bradykinin before and 20 min after the onset of mecamylamine administration (50 μmol kg^{-1} h^{-1}) and again about 10 min after i.v. bolus injection of captopril (60 μg kg^{-1}). The results are summarised in Table 1.

Table 1 Cardiovascular changes (areas under or over curves; AUC, AOC) after bradykinin in the same, conscious, Long Evans rats (n = 7) under control conditions, in the presence of mecamylamine (M) and in the presence of mecamylamine plus captopril (M+C). Values are mean ± s. e. mean; * P<0.05 versus baseline; [a] P<0.05 versus control; [b] P<0.05 versus mecamylamine.

	Bradykinin Bolus			Bradykinin Infusion		
	Control	M	M+C	Control	M	M+C
Heart rate (AUC; beats)	180 ± 32*	138 ± 32*	324 ± 59*[ab]	303 ± 25*	165 ± 12*[a]	214 ± 20*[ab]
Mean arterial blood pressure (AOC; mmHg min)	-19 ± 4*	-65 ± 18*[a]	-63 ± 13*[a]	-33 ± 12*	-60 ± 9*[a]	-117 ± 19*[ab]
Coeliac conductance (AUC; ([kHz $mmHg^{-1}$]10^3)min)	88 ± 18*	105 ± 27*	45 ± 12*	106 ± 9*	101 ± 13*	54 ± 11*[ab]
Mesenteric conductance (AUC; ([kHz $mmHg^{-1}$]10^3)min)	48 ± 10*	52 ± 12*	44 ± 14*	103 ± 9*	132 ± 17*	75 ± 26*[ab]
Hindquarters conductance (AUC; ([kHz $mmHg^{-1}$]10^3)min)	52 ± 12*	116 ± 28*[a]	119 ± 17*[a]	71 ± 20*	96 ± 18*	95 ± 20*

In the presence of mecamylamine, the depressor and hindquarters vasodilator responses to bolus injection of bradykinin were enhanced; captopril had no additional effect. In contrast, in the presence of mecamylamine, captopril enhanced the depressor response to bradykinin infusion, but this effect was accompanied by reduced coeliac and mesenteric vasodilatation. Hence, the increased hypotension must have been due to reduced cardiac output.

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461P MODULATION OF THE RELEASE OF VASOACTIVE COMPOUNDS BY HYPERCAPNIC STIMULATION IN THE ISOLATED PERFUSED RABBIT CEREBRAL VASCULATURE

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We have previously described an isolated perfused cerebral preparation in the rabbit which enables the release of vasoactive compounds including ATP, substance P, endothelin and vasopressin to be studied under precise and controlled haemodynamic and physiological conditions by bilateral collection of the jugular effluent (Domer et al, 1992). The present study investigated the influence of hypercapnia, purported to be a potent hyperaemic stimulus, upon the release of these compounds. 5 New Zealand White male rabbits, weighing 2.5 - 3.0 kg, were sedated with 'Hypnorm' (fentanyl-fluanisone) at 0.33 ml kg^{-1} i.p. and after 5 minutes anaesthetised with sodium pentobarbitone i.v. via a cannulated marginal ear vein. Details of the surgery, preparation of perfusate and circuit have been described elsewhere (Domer et al 1992). Briefly, a perfluorocarbon emulsion was infused bilaterally into the internal carotid arteries and collected bilaterally from the jugular veins. Control perfusions were conducted at physiological pressure and blood gas concentrations using 100% oxygen as the gassing medium: the flow rate was 5.5 ± 0.7 ml min^{-1}. Each experiment consisted of 3, 5 minute perfusion periods, two using 95% O_2/ 5% CO_2 and one period using 90% O_2 / 10% CO_2. Each of these 5 minute perfusion periods was preceded by 5 minutes of perfusion with the control medium (100% O_2). The mean quantity of substances released during the initial control perfusion period (pmol min^{-1}) were 17.99 ± 2.21 for endothelin, 4.18 ± 0.9 for substance P, 0.61 ± 0.08 for vasopressin and 18.85 ± 6.01 for ATP. A table of the results expressed as % of initial control period value(mean ± sem) for each gas mixture is shown below. Peptides were measured by enzyme-linked immunoabsorbent assay and ATP by the luciferin-luciferase assay.

	ATP	Substance P	Endothelin	Vasopressin
5%	224.67 ± 7.37**	72.71 ± 19.64	268.58 ± 47.39***	663.59 ± 209.84*
5%	247.06 ± 36.99**	32.51±10.38**	163.67 ± 34.88	262.28 ± 132.20
10%	241.69 ± 37.39	87.89 ± 35.54*	217.00 ± 28.62***	337.89 ± 113.88
Final control period	112.72 ± 27.34	24.92 ± 7.07***	133.99 ± 17.13*	581.44 ± 212.76

* P<0.05, ** P<0.01, *** P<0.001, Wilcoxan & Mann Whitney U-test.

The release of each compound presented a unique profile of significant changes. Endothelin, ATP and vasopressin were increased in the effluent during the initial exposure to 5% CO_2, whilst levels of ATP also increasd following the second exposure to 5% CO_2. Only endothelin had a significant increase in release during exposure to 10%CO_2. Substance P had a delayed decrease following the first exposure which lasted through to the second exposure to 5% CO_2 and remained low during exposure to 10% CO_2. These data suggest that a) ATP and perhaps vasopressin play an immediate but short-lived role in the control of cerebral vascular tone during hypercapnia and b) that the sustained changes in release of endothelin and substance P may exert a more profound influence upon the overall control of cerebral vascular tone during and following hypercapnic episodes. The endothelium may be a source of these vasoactive compounds.

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462P REDUCED NERVE GROWTH FACTOR mRNA IN SKIN OF DIABETIC RATS: EFFECTS OF INSULIN

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Experimental diabetes in rats is associated with reduced sciatic nerve levels of neuropeptides - substance P and calcitonin gene-related peptide - whose gene expression is influenced by nerve growth factor (NGF). This study was designed to determine whether primary dorsal root afferents, of cutaneous origin, might sample reduced trophic support in experimental diabetes mellitus, by measuring cutaneous levels of the mRNA for NGF. Male Wistar rats (starting weight 290-310 g) were allotted at random to 3 groups. Two groups were made diabetic with streptozotocin (50 mg/kg i.p.) and all animals left untreated for 2 weeks. For the subsequent 5 weeks all diabetic rats were given a long-acting insulin (heat-treated Ultralente; Novo) at 2.0 I.U./100 g body weight twice weekly to limit morbidity and for the final 4 weeks one diabetic group was given intensive (twice daily) insulin, at doses adjusted with reference to daily blood glucose (by reflectance photometry on tail prick blood), to control glycaemia. The other diabetic group remained on twice-weekly insulin. After 12 wk diabetes the diabetic rats on background insulin were hyperglycaemic (whole blood glucose, 34.2±3.1 (SEM) mmol/l) and lighter (body weight, 307.3±8.3 g) by comparison with controls (8.5±0.5 mmol/l and 569,2±20.0 g) or diabetic rats given intensive insulin (17.7±1.0 mmol/l and 387.2±13.4 g). At death samples of foot skin (1 cm^2) were extracted for RNA by the method of Chomzynski and Saachi (1987). A 450 base truncated sense mRNA for NGF was added as an internal standard, to control for losses during processing. A full length sense mRNA was processed in parallel as an external standard, permitting quantification of yields. Total RNA was isolated and subjected to either Northern transfer or applied to nitrocellulose via a "slot-blot" manifold. Total RNA and poly A^+ were similar in all 3 groups. Northern blots were hybridised using a mouse (Pst 1 fragment) cDNA for NGF and the hybrids detected by autoradiography. Autoradiograms were scanned by an image analysis system (AI Cambridge) and 'corrected' using the internal standard. In the diabetic rats maintained on background insulin only, the NGF mRNA levels were reduced (9.87 ± 2.76 pg/cm^2 skin; n = 6) compared to either control rats (17.87 ± 1.43 pg/cm^2; n = 6) or the diabetic rats given 1 month intensive insulin (19.40 ± 3.8 pg/cm^2; n = 5). These findings indicate a deficit in the level of NGF mRNA in foot skin which may give rise to reduced trophic support of primary afferent cell bodies.

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463P CHARACTERIZATION OF CALCITONIN GENE-RELATED PEPTIDE RECEPTORS IN CEREBRAL VESSELS FROM MAN: VASOMOTOR RESPONSES AND cAMP ACCUMULATION

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Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide with strong vasodilator effects on cerebral arteries both in vitro and in situ (Edvinsson et al. 1985; McCulloch et al. 1986). The purpose of the study was twofold: firstly to examine the effects of the different CGRP agonists and antagonists on isolated cerebral vessels from man. Secondly to examine possible coupling of the CGRP receptors to second messengers such as cAMP and endothelium derived relaxing factor (EDRF).

Human cerebral artries were obtained in conjunction with neurosurgical tomour resections, immersed in aerated buffer solution and immediately transported to the laboratory. For studying the vasomotor responses small circular segments from branches of the middle cerebral artry were mounted on two L-shaped metal prongs. They were given a passive load of 3 mN and allowed to stabilize at this level of tension for 1.5 hrs (for refs. see Edvinsson et al. 1985). Their contractile capacity were tested by exposure to a potassium rich (60 mM) buffer solution. Only vessels responding with strong (> 1mN) contractions were studied further. For second messenger experiments vessel segments were incubated for 15 min in a tube containing aerated Krebs buffer solution and a certain concentration of CGRP analogue. The incubation was postponed by moving the vessel segment to a tube containing acidic ethanol. After homogenization and centrifugation the content of cAMP was measured by radioimmunoassay.

The CGRP analogues produced concentration-dependent relaxations of the $PGF_{2\alpha}$-precontracted arterial segments. In man the order of potency was β-rCGRP > α-hCGRP > α-rCGRP > β-hCGRP with maximum responses between 71 and 91% of precontraction. The CGRP-1 receptor antagonist, hCGRP(8-37) induced a parallel shift of the concentration-response curves induced by the two α-forms of CGRP (human and rat) without any change in maximum effect. Furthermore, hCGRP(8-37) did not cause any significant change in the response to β-rCGRP or β-hCGRP. There were no changes in potency or in maximum responses in experiments performed in the presence of [Tyr^0]CGRP28-37, the other putative antagonist. α-hCGRP potently increased the formation of cAMP in the human cerebral arteries. The antagonist α-hCGRP(8-37)only induced a slight increase in cAMP formation but blocked the response to α-hCGRP. In contrast the response induced by β-CGRP was not blocked by this antagonist. Endothelium removal did not attenuate the response to CGRP.

The present study suggests that there is a difference in receptors for the α- and β-forms of CGRP in human cerebral arteries. It also seems like the CGRP receptor antagonist hCGRP(8-37) acts selectively on receptors mediating α-CGRP induced relaxation while the relaxation induced by β-CGRP is unaffected. The CGRP receptors are coupled to the generation of cAMP in the same concentrations as those causing relaxation.

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464P CAPSAZEPINE INHIBITS LOW pH- AND CAPSAICIN-INDUCED RELEASE OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) FROM RAT SOLEUS MUSCLE

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Capsazepine (CPZ) has been characterized as the first competitive antagonist of the capsaicin receptor (Bevan et al., 1991). In this study we have investigated the ability of various concentrations of CPZ to inhibit the release of CGRP-like immunoreactivity (CGRP-LI) from the rat isolated soleus muscle, as evoked by application of capsaicin (1 μM), low pH (pH 5) solution or KCl (80 mM). These three stimuli have been shown to produce release of CGRP-LI from peripheral endings of primary afferent neurons in this preparation (Sakaguchi et al.,1991; Santicioli et al, 1992). Physiological phosphate buffer (pH 7.4 and 5) solutions and determination of CGRP-LI outflow were made as described previously (Geppetti et al., 1991). Results in Table 1, expressed as total evoked CGRP-LI release (T.E.R.) over a 20 min perfusion period with the three stimuli, indicate that threshold concentrations for CPZ inhibition of CGRP-LI release was 3, 10 and 30 μM toward low pH, capsaicin and KCl, respectively. Basal CGRP-LI outflow was 16.8±3 fmol/g per fraction.

Table 1 CGRP-LI release (T.E.R., fmol/g of wet weight, mean ± s.e.m; n= 5-20) in the presence of various concentrations of CPZ. (*) = significantly different from controls P< 0.05

	Control	Capsazepine concentration (μM)			
		1	3	10	30
Capsaicin	482 ± 69	331 ± 81	359 ± 63	116 ± 24 *	38 ± 16 *
low pH	169 ± 20	110 ± 29	38 ± 10 *	34 ± 13 *	26 ± 9 *
KCl	219 ± 20	204 ± 68	290 ± 29	194 ± 36	116 ± 14 *

Protons have been shown to excite capsaicin-sensitive primary afferent neurons and open the same type of cation channel which is activated following stimulation of the capsaicin receptor (Bevan and Yeats, 1991). The observation that the competitive capsaicin receptor antagonist, CPZ selectively inhibits low pH- and capsaicin-induced activation of primary afferents, implies a connection between the action of protons and activation of the capsaicin receptor, which needs further evaluation.

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465P PHARMACOLOGICAL PROPERTIES OF BMS 180,560, AN INSURMOUNTABLE ANGIOTENSIN II RECEPTOR ANTAGONIST

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(Introduced by D. R. Howlett)

BMS 180,560 (2-butyl-4-chloro-1-[[1-[2-(2H-tetrazol-5-yl)phenyl]-1H-indol-4-yl]methyl]-1H-imidazole-5-carboxylic acid) is a novel non-peptidic angiotensin (A)II receptor antagonist. It selectively inhibits [^{125}I]Sar^{1}Ile^{8}AII ([^{125}I]SI-AII) binding to rat aortic smooth muscle (RASM) cell and rat adrenal cortical AT_1 receptors (K_i=7.6±1.2 and 18.4±3.9 nM respectively) compared to adrenal cortical AT_2 receptors (K_i=37.6±1.3 μM). The K_i value was dependent on the concentration of BSA used in the assays, indicating BMS 180,560 binds to albumin. BMS 180,560 (3-300 nM) increased the K_D values of SI-AII for RASM cell AT_1 receptors and decreased the B_{max} only at high concentrations (300 nM). In isolated rabbit aorta, BMS 180,560 inhibited AII-stimulated contraction with a calculated K_B=0.068±0.048 nM, produced 80% reduction of maximal AII-stimulated contractions at 1 nM BMS 180,560, and functioned as an insurmountable antagonist. Experiments conducted in the presence of 0.1% BSA generated significantly higher K_B values (K_B=5.2±0.92 nM). In similar experiments, losartan behaved as a competitive antagonist with a K_B=2.6±0.13 nM. Contractions of rabbit aorta stimulated by endothelin-1, noradrenaline, KCl, or the TXA_2 receptor agonist U-46619 were unaffected by BMS 180,560 (1 nM).

BMS 180,560 (0.03-3.0 μmol/kg i.v., & 3.0 μmol/kg p.o.) inhibited the AII pressor response in conscious, normotensive rats (maximal inhibition 45-96% & 49%, respectively) and inhibition was maintained for 7 h after the 3.0 μmol/kg doses. BMS 180,560 (3-30 μmol/kg p.o.) produced dose-dependent, long-lasting decreases in the blood pressure of sodium-depleted SHR (45 mm Hg decrease after 24h using 30 μmol/kg p.o.), which were comparable to losartan.

In [^{3}H]-myoinositol-labelled RASM cells, losartan (30, 200nM), shifted the EC_{50} for AII-stimulated [^{3}H]inositol monophosphate formation to higher values, with no change in the maximal response. In contrast, BMS 180,560 (3,10 nM) increased the EC_{50} for AII and decreased the maximum responses by 30 and 80% respectively. This decrease could be attenuated by inclusion of losartan (200 nM) indicating the inhibition was not irreversible.

BMS 180,560 is a potent, specific, orally active antihypertensive AII receptor antagonist, which displays insurmountable receptor antagonism. At concentrations of BMS 180,560 which have no effect on receptor number, BMS 180,560 produced insurmountable antagonism of AII-stimulated second messenger formation. The site responsible for the insurmountable inhibition may lie between receptor and PLC activation.

466P POSSIBLE INFLUENCE OF LIPOPHILICITY ON ANGIOTENSIN RECEPTOR ANTAGONIST POTENCY ESTIMATES IN RABBIT ISOLATED AORTA

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Rabbit isolated aorta is widely used to estimate the affinity of angiotensin receptor antagonists (e.g. Wong et al., 1990). We have now investigated whether the lipophilic angiotensin receptor antagonists, losartan (cLogP = 4.1), and the acid metabolite of losartan, EXP3174, (2-n-butyl 4-chloro 1-[(2'-(1H-tetrazol-5-yl)biphenyl- 4-yl)methyl]imidazole-5-carboxylic acid; Wong et al., 1990: cLogP = 5.4) are subject to retention in this preparation, relative to the hydrophilic peptide antagonist, saralasin (cLogP = -1.5), by comparing the reversibility of their antagonist effects.

De-endothelialised helical strips (1.5-2cm) of thoracic aorta from male, NZW 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. Two consecutive, concentration-response curves to angiotensin II (AII) were constructed in all preparations which were then incubated with antagonist or vehicle. Subsequently, AII curves were constructed in the presence of antagonist/vehicle or following washing (5 times at 10s intervals) with antagonist-free Krebs. Responses to AII were expressed as a percentage of the maximum obtained in the preceding (pretest) curve. Under these conditions, the maximum response to AII was increased ~40% by losartan, and suppressed ~25% and ~40% by saralasin and EXP3174, respectively. Therefore, antagonist activity was expressed in terms of the ratio of AII concentrations required to elicit responses equal to that produced by the EC_{50} in the corresponding pretest curve. Losartan (100nM; 45min incubation) produced a 9.2-fold (95% C.I. = 5.3-15.7, n=4) rightward displacement of the AII curve (Figure 1A). In other preparations incubated with losartan (100nM) and subsequently washed, a similar degree of rightward displacement (6.1-fold; 95% C.I. = 3.4-10.9, n=4) of the AII curve, and a corresponding increase in the maximum response occurred (Figure 1A). Similar to losartan, the antagonist effects of EXP3174 (1nM; 2h incubation, n=4) were not reversed after washing. Saralasin (30nM; 45min incubation) caused a non-parallel rightward displacement of the AII curve (6.9-fold; 95% C.I. = 3.6-13.3, n=6). However, after washing the tissue pre-incubated with saralasin, no displacement of the AII curve (concentration-ratio <2, n=6) was observed (Figure 1B), suggesting that saralasin had been effectively washed out of the tissue.

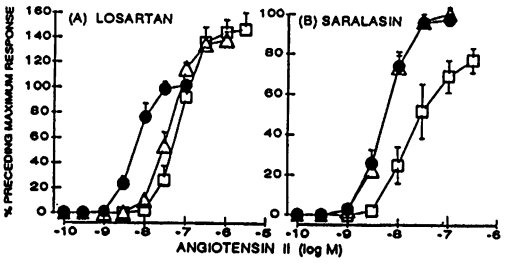

Figure 1: Contractile effect of angiotensin II in the rabbit aorta in the presence of vehicle (●) or subsequent to incubation with A) losartan (100nM,□) or B) saralasin (30nM,□) and then after washing (Δ), in each case.

In conclusion, the AII antagonist effects of the hydrophilic peptide, saralasin, in the rabbit aorta were readily reversed by washing the tissue. In contrast, the non-peptide compounds, losartan and EXP3174, appeared to be subject to tissue retention, probably as a consequence of their lipophilic nature. These results suggest that affinity estimates based on the aqueous concentrations of lipophilic angiotensin receptor antagonists using this preparation should be regarded with caution.

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467P EFFECTS OF THE NON-PEPTIDE ANGIOTENSIN RECEPTOR ANTAGONIST, GR117289, ON BASAL RENAL FUNCTION IN THE ANAESTHETISED RAT

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The kidneys and the renin-angiotensin system play an important role in blood pressure control, and in the pathophysiology of hypertension and heart failure. The recent development of angiotensin receptor antagonists may provide novel agents for the therapy of these disease states. This study has examined the effects of the specific and selective, non-peptide, angiotensin AT_1 receptor antagonist, GR117289 (Marshall et al., 1991; Robertson et al., 1991) on renal function, in the anaesthetised rat.

Male, albino rats (270-375g) were anaesthetised (sodium pentobarbitone 60mg/kg i.p. + 20mg/kg/h i.v.) and the left carotid artery cannulated for measurement of diastolic blood pressure (DBP) and collection of blood samples. The ureters were cannulated for collection of urine and determination of urine output (U_V), and a Doppler flow probe placed around the left renal artery for measurement (in volts) of blood velocity, as an indication of renal blood flow (RBF). Values for glomerular filtration rate (GFR), absolute ($U_{Na}V$) and fractional (FE_{Na}) sodium excretion, and absolute potassium excretion (U_KV) were derived. GR117289 (1mg/kg i.v.) was administered, and cardiovascular and renal function parameters were followed for 90 min after dosing. The renal function data of the left kidney are shown in Table 1. Following administration of GR117289, there was a reduction in DBP, an increase in RBF, and a trend for U_V, $U_{Na}V$ and FE_{Na} to increase. These cardiovascular and renal effects were progressive in onset, achieving maximum response 45-90 min after dosing. U_KV also tended to increase (basal 1.46±0.23 to 1.87±0.22μmol/min at 15 min post dose), although this effect was not maintained, and had returned to basal levels after 75 min. Interestingly, significant ($P<0.05$) increases in basal renal excretory parameters (U_V 0.3±0.1 to 0.6±0.1ml/15 min, $U_{Na}V$ 3.5±1.5 to 8.4±0.9μmol/min, FE_{Na} 1.1±0.4 to 2.5±0.3%), were observed in the right kidney, 90 min after dosing with GR117289.

Table 1 : Effects of GR117289 (1mg/kg i.v.) on cardiovascular and renal function in the anaesthetised rat

	DBP mmHg	RBF volts	GFR ml/min	U_V ml/15 min	$U_{Na}V$ μmol/min	FE_{Na} %
Basal	109±4	3.4±0.3	1.9±0.1	0.27±0.09	3.1±1.3	1.0±0.4
+45 min	95±4*	4.4±0.4*	2.0±0.1	0.41±0.07	5.0±0.8	1.7±0.3
+90 min	88±6*	4.5±0.4*	2.0±0.2	0.44±0.14	5.0±1.5	1.5±0.4

Values shown are arithmetic mean ± s.e.m. (n=6). * $P<0.01$ (Student's paired t-test compared with basal values).

In summary, GR117289 produced a slowly developing vasodepressor effect, accompanied by renal vasodilatation, natriuresis and diuresis in the anaesthetised rat. These data are qualitatively similar to those seen previously in studies where GR117289 was administered to the anaesthetised dog (Clark et al., 1992). The renal vasodilator effect of GR117289 almost certainly reflects antagonism of endogenous angiotensin II at the renal vascular AT_1 receptors and probably accounts for the observed natriuresis. However, the increase in FE_{Na} could indicate a further antagonist effect of GR117289 at AT_1 receptors in the renal tubules.

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468P THE EFFECT OF DEVAZEPIDE ON GASTRIN AND CHOLECYSTOKININ-INDUCED ACID SECRETION IN THE CONSCIOUS GASTRIC FISTULA RAT

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The involvement of cholecystokinin (CCK) in the control of gastric acid secretion is poorly understood. It is reported that CCK can stimulate (Stenning and Grossman, 1969) and also inhibit acid secretion (Lloyd et al, 1992). To further evaluate the involvement of CCK in the regulation of acid secretion we have compared the effects of non-sulphated gastrin-17 (G-17) and sulphated CCK-8 (sCCK-8) in the chronically prepared gastric fistula rat and determined the effect of the selective CCK_A receptor antagonist devazepide on the secretory responses to these peptides.

Female albino rats (approximately 180g) were surgically prepared with a titanium gastric cannula under isoflurane/N_2O anaesthesia and allowed one week to recover. Secretion experiments were carried out on 18h fasted animals, lightly restrained in Bollman type cages. Compounds were administered via a tail vein cannula. Gastric juice was collected from the fistula by drainage every 15 minutes and acid secretion determined by titration. ED_{50} values for the peptides were determined graphically from their respective maxima. Values are expressed as mean ± SEM, (n>5).

In rats receiving a saline infusion (3.4mlh^{-1}), basal acid secretion was 238±38μEq3h^{-1}. Infusion of G-17 (0.01 to 50nmol$kg^{-1}h^{-1}$ produced a dose related increase in acid secretion, with an ED_{50} of 0.75nmol$kg^{-1}h^{-1}$ and a maximum acid output of 958±151μEq3h^{-1}. Infusion of sCCK-8 (0.1 to 10nmol$kg^{-1}h^{-1}$) produced a bell-shaped dose-response curve with an ED_{50} of 0.35nmol$kg^{-1}hr^{-1}$ and a maximum total acid output of 611±83μEq3h^{-1} at 1nmol$kg^{-1}h^{-1}$. Infusion of sCCK-8 at 3nmol$kg^{-1}h^{-1}$ produced a smaller stimulation of acid secretion and at 10nmol$kg^{-1}h^{-1}$, secretion was similar to basal levels. A sub-maximal dose of G-17 (1nmol$kg^{-1}h^{-1}$) was not significantly affected by devazepide (1μmolkg^{-1}), maximum acid secretions were 646±82μEq3h^{-1} and 723±238μEq3h^{-1} respectively. Sulphated CCK-8 at 0.1, 0.3, and 1nmol$kg^{-1}hr^{-1}$ were not significantly affected by devazepide (1μmolkg^{-1}), however devazepide significantly increased acid secretion produced by higher doses of sCCK-8 (3 and 10nmol$kg^{-1}h^{-1}$), altering the profile of the sCCK-8 curve from bell-shaped to monophasic (Table 1).

Table 1 **Effects of devazepide on sCCK-8 induced acid secretion (Total Acid Secreted (μEq3hr^{-1}))**

CCK-8 (nmol$kg^{-1}h^{-1}$)	0	0.1	0.3	1	3	10
Vehicle	238 ± 38	246 ± 64	400 ± 120	611 ± 83	377 ± 113	267 ± 41
Devazepide (1μmolkg^{-1})	346 ± 77	295 ± 51	470 ± 108	718 ± 142	750 ± 78*	657 ± 104*

(* significantly different from vehicle alone by unpaired t test ($p<0.05$))

These studies suggest that, in the conscious gastric fistula rat, sCCK-8 has both stimulatory and inhibitory effects on gastric acid secretion. The apparent inhibitory effects at higher doses of sCCK-8 appear to be mediated via a CCK_A receptor.

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469P MECHANISMS OF ACTION OF THE CARDIOVASCULAR EFFECTS OF CHOLECYSTOKININ OCTAPEPTIDE IN THE PITHED RAT

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Sulphated cholecystokinin octapeptide (sCCK-8) has been shown to have complex effects on heart rate (HR) and arterial blood pressure (MAP) in a pithed rat model which are mediated through activation of CCK_A receptors (Gaw et al., 1992). The objective of the present study was to characterise the mechanisms involved in the pressor responses and bradycardia induced by sCCK-8.

Male albino AH/A rats (380-450g) were anaesthetised with isoflurane/nitrous oxide gas mixture, intubated, pithed and mechanically respired with room air. The right carotid artery was cannulated and connected to a pressure transducer for measurement of MAP and HR. The right jugular vein was then cannulated for infusion of test agents. Three successive dose response curves were obtained to bolus i.v. injection of sCCK-8 (0.1,1 and 10 nmolkg^{-1}). Receptor blocking drugs were added between the 2nd and 3rd dose response curves to sCCK-8. A group of rats was subjected to bilateral adrenalectomy and a curve to sCCK-8 obtained as described above. In addition arterial segments of 3-4mm length were obtained from the aorta, carotid and femoral arteries of the rat and suspended in oxygenated physiological salt solution at 37°C. These segments were attached to isometric force transducers and placed under 1g resting tension. Phenylephrine (1μM) was used to test the viability of the tissues which were washed and later exposed to sCCK-8 (0.1nM-1μM). Values are expressed as mean ± s.e.mean.

Sulphated CCK-8 (0.1-10nmolkg^{-1}) has been shown to produce dose related pressor responses and bradycardia in pithed rats (Gaw et al. 1992). Phentolamine (1μmolkg^{-1}) and guanethidine (2μmolkg^{-1}) depressed the increase in MAP elicited with sCCK-8 (10nmolkg^{-1}) by 59.2±5.2% (n=5) and 60.8±9.6% (n=5) respectively, however these compounds had no effect on HR (changes of -1.9±4.6% and 12.6±3.8% respectively, n=5). Propranolol (3μmolkg^{-1}), atropine (1μmolkg^{-1}) and hexamethonium (2μmolkg^{-1}) had no effect on the pressor response or bradycardia observed with sCCK-8 (n=5). In rats which had been adrenalectomised, sCCK-8 still caused a dose-dependent increase in MAP and bradycardia (changes with 10nmolkg^{-1} sCCK-8 were 48.6±7.7% and 31.8±0.5% of the respective resting values).These results are comparable to those observed in non-adrenalectomised rats (Gaw et al., 1992) In isolated arteries which contracted to phenylephrine, sCCK-8 had no contractile effect at concentrations up to 1μM (n=5).

The *in vitro* data showed that sCCK-8 did not cause direct stimulation of vascular α-adrenoceptors. Since the pressor response to sCCK-8 was not altered by adrenalectomy but was inhibited by either phentolamine or guanethidine it is likely that sCCK-8 stimulates noradrenaline release from sympathetic nerves to produce increases in blood pressure. It is likely that sCCK-8 induced bradycardia is mediated by either a direct action on the heart or activation of a nonadrenergic-noncholinergic neuronal system.

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470P CHOLECYSTOKININ ANTAGONISM IN HUMAN ALIMENTARY MUSCLE BY THE NONPEPTIDE CCK_B/GASTRIN RECEPTOR ANTAGONISTS Cl-988, Cam-1028 AND L-365,260

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Aim: To compare the effects of three nonpeptide CCK-B/gastrin receptor antagonists on contraction of human gastrointestinal muscle to cholecystokinin octapeptide (CCK-OP).

Methods: Macroscopically normal stomach, colon and gallbladder were obtained at surgery from patients with benign or malignant disease. Mesentery and fat were removed and the mucosa/submucosa cut away along the gastric and colonic submucosal plexus. Strips approximately 3 mm wide and 15-20 mm long cut parallel to the circular and/or longitudinal muscle were suspended in Krebs' solution (O_2/CO_2 95:5, 37°C), and submaximal isotonic contractions recorded to acetylcholine (ACh) every 10 min. Submaximally effective amounts of sulphated CCK-OP were added for 2 min every hour (long cycle to avoid tachyphylaxis). L-365,260 (Lotti & Chang, 1989) was dissolved in DMSO, and Cl-988 and Cam-1028 (Hughes et al., 1990) were dissolved in 154 mM NaCl; they were added in 10-fold concentration increments at hourly intervals (0.001-500 μM bath concentrations) until contractions to CCK-OP were greatly reduced or prevented. Parallel vehicle controls enabled the percent changes and -log IC_{50} values to be calculated from plotted antagonist-response curves.

Results: The potencies of the antagonists were relatively low, and although there were quantitative differences between them each compound was equally effective throughout the gastrointestinal tract, both in the circular and longitudinal muscle layers. L-365,260 was the most potent (overall -log IC_{50} 5.84 ± 0.19, n=17). Furthermore, it usually had no significant effect on the contraction to ACh at its -log IC_{50} value (5.84), but in 2/17 experiments 10 μM L-365,260 reduced the response to ACh by 30 and 50%. Cl-988 and Cam-1028 were an order of magnitude less potent (-log IC_{50} values 4.68 ± 0.13, n=14, and 4.94 ± 0.14, n=15 respectively), and the highest drug concentration (100 μM) reduced the contractions to ACh by 20-60% in 5/14 experiments with Cl-988 and by 15-65% in 9/15 experiments with Cam-1028.

Conclusions: L-365,260 was 14- and 8- fold respectively more potent than Cl-988 and Cam-1028 in blocking contractions of human gastrointestinal muscle to CCK-OP. The rather low potency of all three antagonists suggests that CCK-B receptors are less involved in gut muscle contraction than CCK-A receptors (D'Amato et al., 1991).

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471P THE EFFECT OF ATRIAL NATRIURETIC PEPTIDES ON RAT COLONIC MUCOSAL ION TRANSPORT

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The action of atrial natriuretic peptide (ANP) in causing sodium and water diuresis by the kidney, when plasma volume expands has led to the proposal that ANP has a physiological role in the regulation of salt and water balance. As the colon is involved in controlling water and electrolyte movement a number of investigations have been carried out to determine whether ANP can affect transepithelial ion transport in this organ as well as the kidney.

In the rat colon two similar studies have produced different results. Barros et al (1990) found rat atriopeptin III to have no effect on mucosal ion transport. Moriarty et al (1990) found anaritide (fragment 4-28 of human ANP) to cause electrolyte secretion. The present investigation seeks to determine the cause of the discrepancy between the two studies.

Muscle stripped sheets of rat colonic mucosa plus submucosa were set up in Ussing chambers to detect changes in electrogenic ion transport. Values of short-circuit current (s.c.c.) quoted are for an exposed membrane area of 0.64cm^2.

Electrical field stimulation (1ms, 1 and 10Hz, 150 pulses) gave tetrodotoxin (TTX, 3.1μM) sensitive, voltage dependent (2.5 - 40V) increases in s.c.c. Serosal administration of 8-bromocyclic GMP (8-Br cGMP, 22μM-10mM n=4) and sodium nitroprusside (4μM-38mM n=4) produced concentration dependent increases in s.c.c. Anaritide (0.1-10μM, n=4) human ANP (0.1-10μM, n=4) and rat atriopeptin III (0.1-10μM, n=4) did not affect s.c.c. In the presence of bovine serum albumin (4mg/ml) and phosphoramidon (10μM) anaritide (5μM n=4) did not increase s.c.c. Anaritide produced concentration dependent relaxations ($9x10^{-6}$μM - 0.9μM, n=5) of precontracted spirally cut rat aortic preparations.

These results suggest that the atrial natriuretic peptides investigated do not affect electrogenic ion transport in the rat colon.

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472P ENZYME INHIBITORS CAN POTENTIATE HYPERACTIVITY INDUCED BY CENTRAL INFUSION OF NEUROKININ (NK_1) AGONISTS IN GUINEA-PIGS

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Recent work has shown that the NK_1 agonists, substance P (SP), [$Sar^9Met(O_2)^{11}$]-SP, and GR73632 can all increase locomotor activity (LMA) in the guinea-pig following intracerebroventricular (ICV) administration (Brent et al., 1988, Seymour et al., 1991, Mason et al., 1992). As SP and other peptides are rapidly metabolized by peptidase enzymes, inhibitors of this process should enhance their biological activity. The present study investigated the effects on LMA in the guinea pig of three NK_1 agonists SP-methyl ester (SPOMe), [$Sar^9Met(O_2)^{11}$]-SP (Drapeau et al., 1987) and GR73632 (Hagan et al., 1991), both in the absence and the presence of a combination of the endopeptidase 24.11 inhibitor, phosphoramidon (3nmol) (Matsas et al., 1984) and the aminopeptidase inhibitor bestatin (10nmol) (Hooper et al., 1985).

Male guinea-pigs (Interfauna, 200-250g) were cannulated bilaterally with stainless steel cannulae aimed at the lateral ventricles. One week after surgery the animals were habituated to test boxes for 30min prior to a 5μl/side infusion of either drug or vehicle (n=3-12). Motor activity (photocell beam breaks = counts) was subsequently recorded for 30min. After histological verification, data were analysed using an ANOVA and a post-hoc Dunnett's test to assess significance.

GR73632 induced a dose-dependent (100-600pmol) and significant ($p<0.05$) increase in LMA following ICV administration, which was unaltered by the addition of both these enzyme inhibitors {counts±sem: vehicle: 131±26; inhibitor control: 143±48; GR73632 100pmol: 346±109; GR73632 + inhibitors: 256±95}. Both [$Sar^9,Met(O_2)^{11}$]-SP (1-5nmol) and SPOMe (10nmol) significantly increased LMA in the absence of inhibitors {vehicle: 116±19; [$Sar^9,Met(O_2)^{11}$]-SP 1nmol: 347±63; 5nmol: 451±47},{vehicle: 123±33; SPOMe 1nmol: 190±39; 5nmol: 316±52; 10nmol: 821±106}. Indeed, in the presence of both enzyme inhibitors the LMA induced by these relatively metabolically unstable NK_1 agonists (1nmol) was significantly enhanced {[$Sar^9,Met(O_2)^{11}$]-SP + inhibitors: 540±80; SPOMe + inhibitors: 349±102}. Finally, none of the inhibitors had any effect on basal LMA.

Therefore, we have confirmed and extended previous findings demonstrating that NK_1 agonists increase LMA after ICV administration in the guinea-pig. Furthermore, these data suggest that peptidase inhibitors potentiate the LMA induced by compounds susceptible to enzyme degradation {SPOMe and [$Sar^9,Met(O_2)^{11}$]-SP} and also support the proposed stability of GR73632. This implies that endopeptidase 24.11 and/or aminopeptidase are important for the degradation of the NK_1 agonists used in this study. Furthermore, peptidase-resistant NK analogues such as GR73632 are more suitable for elucidating the physiological and pharmacological roles of endogenous NKs in the CNS.

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473P NOVEL CYCLIC PSEUDOPEPTIDES WITH HIGH AFFINITY FOR TACHYKININ NK_2 RECEPTOR SUBTYPES

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The rabbit pulmonary artery (RPA) and hamster trachea (HT) express heterogeneous forms of the NK-2 receptor which are recognized with very different affinities by competitive antagonists (Maggi et al., 1990). We have developed novel cyclic pseudopeptides MEN 10,573 or cyclo(LeuΨ[CH_2NH]Asp(OBzl)-Gln-Trp-Phe-βAla) and MEN 10,612 or cyclo(LeuΨ[CH_2NH]Cha-Gln-Trp-Phe-βAla) which are characterized by high affinity for the NK-2 receptor expressed in the HT. The potency of these novel ligands, expressed as pK_B values (Table 1) has been estimated using bioassays for the NK-1 receptor (guinea-pig ileum, GPI), NK-2 receptor (RPA and HT) and NK-3 receptor (rat portal vein, RPV) using methods described previously (Maggi et al., 1990, 1991a). MEN 10,573 and MEN 10,612 were compared with previously described NK-2 receptor antagonists MEN 10,376, R396 and L659,877 (Maggi et al., 1991b for review). The difference in pK_B values (log units) determined in the HT and RPA assays is also shown in Table 1.

Table 1 pK_B values of MEN 10,573 MEN 10,612 and reference compounds at NK-1, NK-2 and NK-3 receptors

Antagonist	GPI	HT	RPA	Δ HT-RPA	RPV
MEN 10,376	5.66 ± 0.10	5.64±0.09	8.08±0.10	- 2.44	inactive*
R 396	inactive *	7.63±0.11	5.42±0.12	+ 2.21	inactive*
L 659,877	5.60 ± 0.11	7.92±0.03	6.72±0.08	+ 1.20	5.40±0.30
MEN 10,573	6.37 ± 0.16	8.66±0.12	7.26±0.06	+ 1.40	inactive*
MEN 10,612	6.09 ± 0.15	9.06±0.04	7.37±0.05	+ 1.69	inactive*

Each pK_B value is mean ± s.e.m of at least 9 determinations. Peptidase inhibitors (thiorphan, captopril and bestatin, 1 μM each) were added to the bath 15 min before the agonist. (*) inactive at 3 μM.

MEN 10,573 and MEN 10,612 are the most potent antagonists thus far described to block the NK-2 receptor-mediated contraction in the HT. As compared to the cyclic hexapeptide L 659,877 (Leu-Met-Gln-Trp-Phe-Gly) the modifications inserted in MEN 10,573 and MEN 10,612 led to an increase both in absolute potency and selectivity between different forms of the NK-2 receptor.

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474P PP56 DOES NOT INHIBIT NEUROPEPTIDE Y-STIMULATED Ca^{2+} ELEVATIONS IN CULTURED PORCINE AORTIC SMOOTH MUSCLE CELLS

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Neuropeptide Y (NPY) is a potent vasoconstrictor in many vascular beds. Since elevations of intracellular free Ca^{2+} (Ca_i) are an important mediator of smooth muscle contraction we have characterized the effects of NPY on Ca_i in cultured porcine aortic smooth muscle cells (PASMC) and tested possible inhibitory effects of the putative NPY-antagonist PP56 (D-myo-inositol-1,2,6-trisphosphate). PASMC were isolated and cultured as described (Vischer & Buddecke, 1985). Ca^{2+}-measurements were performed in trypsinized cells loaded with 0.5 μM Fura-2 for 1 h at room temperature as described by Motulsky & Michel (1988).

NPY transiently increased Ca_i in a concentration-dependent manner (EC_{50} = 11, 13 nM; n = 2). The increase produced by 100 nM NPY was 177 ± 93 nM (mean ± S.D.; n = 18). To determine the NPY receptor subtype involved, we investigated four NPY analogues selective for the proposed NPY receptor subtypes at 100 nM each. [Pro^{34}]NPY was slightly (80 ± 4% of the NPY response) and PYY considerably less effective (56 ± 9%) than NPY, and NPY_{13-36} was almost inactive (2 ± 3%; n = 3 each) indicating a Y_3-like subtype. Pre-treatment with pertussis toxin (100 ng/ml for 24 h) reduced the NPY-stimulated Ca_i-response by 93 ± 5% compared with untreated controls (n = 4) indicating involvement of a pertussis toxin sensitive G-protein. Chelation of extracellular Ca^{2+} by 5 mM EGTA reduced the NPY-stimulated Ca^{2+} elevation by 52 ± 6% (n = 4) indicating concomitant influx of extracellular Ca^{2+} and mobilization of Ca^{2+} from intracellular stores. The organic Ca^{2+} entry blockers verapamil (10 μM), diltiazem (10 μM) and nifedipine (100 nM) failed to significantly inhibit the increase in Ca_i produced by 100 nM NPY. Thus, voltage-operated Ca^{2+} channels appear not to be involved. PP56 has previously been reported to non-competitively inhibit NPY-stimulated vasoconstriction upon pre-incubation of at least 20 min (Edvinsson et al., 1990). However, pre-incubation of PASMC with PP56 (100 μM) for 45 min did not inhibit the increase in Ca_i produced by 100 nM NPY (259 ± 22 vs. 233 ± 31 nM; n = 3).

We conclude that NPY promotes Ca_i elevations in PASMC via an Y_3-like NPY receptor coupling via a pertussis toxin-sensitive G-protein to mobilization and influx of Ca^{2+} but this response is insensitive to the putative NPY antagonist PP56.

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475P AN *IN VIVO/IN VITRO* MODEL FOR INITIATION, DEVELOPMENT AND PREVENTION OF HEPATOTOXICITY

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Liver slices and isolated hepatocytes have been used as models for the study of cell injury by paracetamol and other drugs *in-vitro*. Using these *in-vitro* systems we and others have found numerous agents which can prevent toxicity even after initiation of injury is complete (McLean & Nuttal, 1978; Mourelle *et al.*, 1990; Shen *et al.*, 1992). Most of these have subsequently been found to be ineffective *in-vivo*. We have developed a system whereby hepatotoxicity is initiated *in-vivo* and subsequent development of injury is observed using an extended *in-vitro* slice technique.

Phenobarbitone pre-treated male wistar rats were dosed with 1g/Kg paracetamol then sacrificed 3 hours later. Slices were cut and incubated in a Ringers solution with gentamicin for 18 hours at 37°C in a shaking waterbath under oxygen. Toxicity was assessed by LDH and K^+ leakage from three slices per experimental condition (McLean & Nuttal, 1978).

Slices from control animals could be maintained with minimal loss of LDH and K^+ over 18 hours. In slices from animals treated with paracetamol there was extensive LDH leakage and potassium loss at 18 hours which was not apparent at 6 hours (Table 1). Several agents which protect against hepatotoxicity in the usual *in-vitro* systems had no protective effect in this new model. These agents also did not protect *in-vivo*. However, animals pretreated with the effective antidote methionine (250mg/Kg), 30 min before paracetamol treatment showed no injury in the slice system at 18 hours.

Table 1.

IN VIVO TREATMENT	% LDH LEAKAGE IN VITRO T6h	T18h	nmoles K^+/mg dry wt. T18h	
CONTROL (n=5)	5.7 ± 3.1	10.8 ± 4.7	245.0 ± 17.3	(means & S.D.
+ PARACETAMOL (n=5)	7.2 ± 3.0	29.3 ± 14.1*	131.5 ± 59.2*	from n expts.
+ METHIONINE + PARACETAMOL (n=2)	3.5 ± 0.6	7.7 ± 2.6	269.5 ± 16.0	* $p<0.05$)

Our new system appears to go some way in bridging the gulf between the *in-vitro* system and *in-vivo* and allows further study of agents which produce hepatotoxicity.

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476P INDOMETHACIN SPECIFICALLY INCREASES THE MAXIMAL VELOCITY OF [^{3}H]-METHOTREXATE UPTAKE BY NC CANCER CELLS

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Indomethacin (INDO) potentiates the anti-cancer effect of methotrexate (MTX) *in vitro* and can extend the survival time of tumour-bearing mice when co-administered with MTX *in vivo* (Bennett *et al.*, 1987). INDO also increased the cellular accumulation of MTX by 60 min (Gaffen *et al.*, 1985) via an unexplained mechanism. We now report that INDO increases MTX accumulation by specifically increasing the Vmax of MTX uptake.

NC cancer cells were grown in Eagle's minimal essential medium containing physiological levels of folic acid (20 nM). Uptake experiments on cells from cultures growing in log phase were performed using a modification of the method previously described (Bennett *et al.*, 1987) using low MTX (30 - 500 nM) and high MTX (1 - 30 μM) concentrations ± INDO 1 μM. Nonspecific MTX binding was determined using identical controls run at 0° C and subtracted from the values obtained at 37° C. All results (mean ± s.e. mean) were analysed using Student's t-test for paired data (2-tailed).

Low MTX concentrations (30 - 500 nM) showed linear uptake of MTX for over 30 min, which indicates inward MTX flux only. Apparent Km [Km(app)] and Vmax [Vmax(app)] values were determined using Lineweaver-Burke plots. Neither value was significantly affected by INDO 1 μM: Km(app) 1.47 ± 0.24 and 1.2 ± 0.10 μM (P = 0.17, n=3); Vmax(app) 10.9 ±1.8 and 9.6 ± 0.5 fmol per sample per 30 min incubation (P = 0.44, n = 3) in the presence and absence of INDO respectively.

At high MTX concentrations (1 - 30 μM), Km and Vmax were determined by the hyperbolic best fit of raw uptake data. INDO enhanced MTX uptake by increasing the Vmax from 65.3 ± 21.1 pmol per sample per 30 min incubation to 88.0 ± 23.5 (P = 0.026, n = 4). There was no significant difference in the Km (26.2 ± 9.2 and 22.1 ± 8.0 μM; P = 0.18, n = 4) with or without INDO respectively.

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477P DIFFERENTIAL EFFECTS OF CYCLOSPORIN A ON HISTAMINE RELEASE INDUCED BY VARIOUS SECRETAGOGUES FROM MAST CELLS AND HUMAN BASOPHILS

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H_2O_2, the principal reactive oxygen metabolite, has effects on a variety of cellular functions including histamine release (HR) from rat peritoneal mast cells (RPMC) (Ohmori *et al.*, 1980) and guinea-pig rectocolonic mucosal cells (RCMC) (Peh *et al.*, 1992). We have studied the effect of cyclosporin A (CsA), an immunosuppressive agent with anti-allergic effects (Ezeamuzie & Assem, 1990) on HR induced by either A23187 or an H_2O_2 system from RCMC, RPMC and human basophils (HB).

RCMC, obtained by collagenase dispersion of the guinea pig colon, were incubated for 30 min with H_2O_2 (10 mM) + NaI (10 mM) + horseradish peroxidase (HRP, 0.25 units/ml) to induce HR after CsA had been added at 0 - 30 min before the inducer; the CsA incubation time for RCMC treated with A23187 (10 μM, 30 min) was 5 min. RPMC were incubated with CsA for 30 min, followed by a further 30 min incubation with A23187 (10 μM) or H_2O_2 (0.1 mM) + NaI (1 mM). HB, isolated from heparinized blood samples by methylcellulose sedimentation of red blood cells, were incubated with CsA (30 min) prior to incubation with A23187 (1 μM), H_2O_2 + NaI, or H_2O_2 + NaI + HRP at various concentrations for 30 min. Optimal doses of each inducer selected from dose-response curves were used for all experiments. Histamine was assayed fluorometrically.

Table 1. Inhibition of histamine release (%, mean ± s.e.mean, n=2-8, triplicate samples)

	Rectocolonic mucosal cells (RCMC).		Rat peritoneal mast cells (RPMC).		Human basophils (HB)
	A23187	H_2O_2/NaI/HRP	A23187	H_2O_2/NaI	A23187
CsA (M)					
10^{-8}	-11.8 ± 2.5	-	38.9 ± 6.2	-	32.1 ± 4.6
10^{-7}	25.4 ± 4.7	-	67.9 ± 1.9	25.5 ± 2.5	41.7 8.8
10^{-6}	65.0 ± 6.8	-3.2 ± 1.3	63.2 ± 6.0	31.2 ± 5.2	57.5 ± 9.5
10^{-5}	64.9 ± 9.1	-	-	53.6 ± 5.5	66.2 ± 14.6

* combined results from different experiments with various CsA incubation times; all showing a lack of inhibition by CsA.

The results showed that CsA produced a marked and dose-related inhibition of A23187-induced HR from RCMC, RPMC or HB. H_2O_2 + NaI-induced HR from RPMC was inhibited by CsA whereas H_2O_2 + NaI + HRP-induced HR from RCMC was little or not affected. H_2O_2 + NaI or H_2O_2 + NaI + HRP at various concentrations found to stimulate HR from RPMC or RCMC did not induce HR from HB. The lack of response of HB to H_2O_2 + NaI or H_2O_2 + NaI + HRP may reflect functional differences between HB and mast cells. In conclusion, the present study indicates that the action of CsA on HR differs in relation to the secretagogues and cell types used.

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478P A COMPUTER-AIDED LEARNING PROGRAM TO TEACH THE PRINCIPLES OF RESPIRATORY FUNCTION TESTING AND DIAGNOSIS

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The computer-aided learning program presented is designed to teach the principles of respiratory function testing and adopts a case-centred approach to teach, by investigation, the use of these tests in the diagnosis of common respiratory disorders. It is highly interactive, written in Borland C++ for IBM-compatible computers (EGA, VGA graphics cards) and is aimed at undergraduate students on a range of medical or health-related courses.

Students select options from an easy-to-use menu which includes an explanation of the investigative methods used to assess respiratory function and the opportunity to obtain typical function test results from a number of clinical cases covering a range of common respiratory diseases. In the METHODS section a combination of text and high-resolution colour graphics and features such as animation are used to describe the principles of **spirometry** (FVC, FEV_1), **reversibility studies, peak expiratory flow**, measurement of **lung volumes** (measurement of FRC by the Helium dilution test and calculation of other lung volumes such as TLC and Residual Volume), and **transfer factor** (using a single breath carbon monoxide test).

In order to perform the respiratory function tests students must first select either a **normal** subject (and define a range of subject parameters: sex, height and age) or one of the available **clinical case studies** which have been chosen to cover a range of common respiratory disorders including obstructive respiratory disease, emphysema and fibrosis. The **TESTS** menu has the same options as the METHODS menu except that students may now simulate performing, and obtain results from, the standard respiratory function tests. For normal subjects the results of all tests are available with the exception of reversibility and are based on predicted normal values. For the clinical cases the student is presented with a brief patient history and a description of presenting symptoms and may obtain clinically accurate results from all of the available tests for a patient suffering a specific respiratory disorder. These results may then be compared with predicted normal values at the press of a key and should aid in the diagnosis.

479P PREPARATION OF SPARK-ETCHED NODES FOR FAST CYCLIC VOLTAMMETRY

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Fast cyclic voltammetry (FCV) has been used to study regulation of monoamine neurotransmitter overflow in vivo and in vitro (Millar, et al., 1985; O'Connor & Kruk, 1992). Carbon fibre electrodes have been typically trimmed (i.e., cut) to the desired length by compression fracture with microforceps. We recently presented data comparing the electrochemical characteristics of cut versus spark-etched electrodes (Williams, et al., 1992). Spark-etching of the electrodes reduces the rate of attrition of electrodes during construction and reduces the variability between electrodes with respect to length and electrochemical characteristics. We now present a video demonstration of the method used which fully illustrates the manufacture of spark-etched electrodes.

Carbon fibre electrodes were made as described by (Armstrong-James & Millar, 1979). Glass capillary tubes (2.0 mm, Clark Electromedical Instruments) were each loaded with a single carbon fibre (7 μm) and pulled in a Narashige electrode puller. Stainless steel wire, dipped in conductive silver paint was passed into the glass tube such that the wire made contact with the carbon fibre on the internal aspect of the pulled taper. The other end of the wire was connected to a cambion pin to allow electrical connection. The portion of the carbon fibre protruding from the external aspect of the tapered glass was then trimmed to length (30-40 μm) using a fine tungsten electrode connected to a high voltage source under a light microscope.

Electrodes were tested electrochemically in vitro using the method described by Millar & Barnet (1988), with a Ag/AgCl reference electrode, in 0.1 Molar phosphate buffered 0.9% w/v NaCl solution, pH 7.4 (PBS). The applied potential consisted of a 1.5 cycle, 100 Hz triangular ramp scanning between -1.0 and +1.4 V relative to the Ag/AgCl reference electrode at a voltage scan rate of 480 Vs^{-1}. The scan was applied at 2Hz and between scans the potential was maintained a 0 V. Signals were fed into a Nicolet 310 digital storage oscilloscope, and measurement made from hard copies of signals.

The spark-etching process is more reproducible than compression fracture, and results in electrodes which are more comparable in length and electrochemical characteristics.

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ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

1P **Manahan-Vaughan D, Rowan MJ & Anwyl R** Agonist and antagonist effects of 5-HT_{1A} receptor ligands on excitatory synaptic transmission in the rat hippocampus *in vivo*

2P **VanderMaelen CP & Braselton JP** (-)-Penbutolol antagonizes 8-OH-DPAT-induced inhibition of rat 5-hydroxytryptaminergic dorsal raphé neurons

3P **Spokes RA, Mansell HL, Forster EA, Hartley JE, Reilly Y, Bissue N, Middlefell VC, Fletcher A & Cliffe IA** The differential pharmacology of the (-) and (+) stereoisomers of WAY 100339 at 5-HT_{1A} and α_2-adrenoceptors in the rat

4P **Barrett RP & Rowan MJ** The effects of the 5-HT_{1A} ligand MDL 73005EF on the spatial learning impairment produced by either gepirone or atropine

5P **Routledge C, Gurling J, Forster EA, Wright I, Fletcher A & Dourish CT** Antagonism of presynaptic and post-synaptic 5-HT_{1A} receptors *in vivo* by the selective 5-HT_{1A} receptor antagonist, WAY100135

6P **Bruinvels AT, Palacios JM & Hoyer D** Pharmacological characterization and localization of 5-HT_{1B}- and 5-HT_{1D}-like recognition sites in rat brain

7P **Sharma A & Fone KCF** 5-HT agonist regulation of 5-HT_{1C} receptors

8P **Beckett SRG, Marshall PW & Marsden CA** Intra-periaqueductal grey administration of mCPP potentiates a chemically-induced defence response

9P **O'Connor JJ & Kruk ZL** Effect of 21 days fluoxetine on 5-HT autoreceptor function in rat dorsal raphé and suprachiasmatic nucleus *in vitro*

10P **Cadogan AK, Kendall DA, Marsden CA & Tulloch I** Effects of paroxetine on 5-HT_2 receptor-mediated phosphoinositide hydrolysis and 5-HT_2 receptor binding in guinea-pig brain

11P **Neuman RS & Rahman S** Activation of 5-HT_2 receptors enhances depolarization of neocortical neurons by N-methyl-D-aspartate

12P **Weight FF, Fan P & Visentin S** Cocaine antagonizes 5-HT activation of 5-HT_3 channels in neurones from rat nodose ganglion

13P **Clement ME & McCall RB** Effects of kainic acid micro-injections into the cat medullary lateral tegmental field on the sympatholytic action of 8-OH-DPAT

14P **Anderson IK, Gardiner SM, Widdop RE, Bennett T, Martin GR & Ramage AG** Regional haemodynamic effects of 5-HT i.c.v. in conscious Long-Evans and Brattleboro rats

15P **Ong EGP & Ramage AG** Further investigation of the role of central 5-HT_{1A} receptors in the reflex vagal bradycardia elicited by i.v. phenylbiguanide in anaesthetized rats

16P **Fone KCF & Rivest R** Effect of intrathecal calcitonin gene-related peptides on thyrotrophin-releasing hormone and 5-HT agonist-induced motor behaviours

17P **Iredale PA, Martin KF, Hill SJ, Alexander SPH & Kendall DA** ATP is able to increase $[Ca^{2+}]_i$ in N1E-115 cells, possibly via activation of an atypical P_2 receptor

18P **Williams RJ & Kelly E** Ethanol-induced changes in G-protein α-subunit expression in NG108-15 cells are not mediated by adenosine

19P **Veale MA, Williams RJ & Kelly E** Characteristics of iloprost-induced loss of $G_s\alpha$ protein in NG108-15 cells

20P **Wheeler SV, Jane SD, Chad JE & Foreman R** Toxin sensitivity and membrane localisation of the nAChR: the effect of a β_2/β_4 hybrid subunit

21P **Evans AM & Martin RJ** Morantel: an agonist and a slowly dissociating blocker of nicotinic-acetylcholine channels from *Ascaris suum*?

22P **Haworth D, Culbert J-A & Thomson DS** (18-36) Neuropeptide Y is a potent rat mast cell degranulator

23P **Engel G, Kusterer K, Buchheit KH, Bruns C, Schade A & Usadel KH** Cytoprotection by octreotide against ethanol-induced lesions in the rat gastric mucosa

24P **MacDonald A & Lamont M** Relative contributions of β-adrenoceptor subtypes to isoprenaline-induced relaxation of rat distal colon

25P **Welsh NJ, Shankley NP & Black JW** The role of histamine in response to electrical field stimulation of the vagus in isolated stomachs from mouse, guinea-pig and rat

26P **Rubino A, Ralevic V & Burnstock G** Inhibitory effect of adenosine on vasodilation by sensory-motor nerves via prejunctional A_1 receptor in the rat mesenteric arterial bed

27P **Ralevic V, Rubino A & Burnstock G** Prejunctional modulation of sensory-motor nerve-induced vasodilatation of the rat mesenteric arterial bed by [Met^5]enkephalin

28P **Shahidullah M & Wilson WS** Effects of timolol, terbutaline and forskolin on aqueous humour formation and cyclic AMP content of ciliary processes in the bovine perfused eye

29P **Warren JB & Coughlan ML** Increasing intracellular cAMP suppresses bradykinin-induced oedema in rabbit skin only in the absence of vasodilation

30P **Field JL, Hall JM & Morton IKM** The nature of the bradykinin receptors involved in phosphatidylinositol hydrolysis and functional responses in the smooth muscle of the guinea-pig taenia caeci

31P **Farmer SG, DeSiato MA & Broom T** Effects of thiorphan and captopril, and bradykinin receptor antagonists, in ferret isolated tracheal smooth muscle

32P **Farmer SG, DeSiato MA & Broom T** Effects of kinin receptor agonists and antagonists in ferret isolated tracheal smooth muscle: evidence that bradykinin-induced contractions are mediated by B_2 receptors

33P **Bakhle YS, Tan BT & Bell C** Substance P and neurokinin A in a rat strain with a genetic neurotrophic defect

34P **Pennefather JN, Zeng X-P, Hall S & Burcher E** The stimulant actions of mammalian tachykinins on rat uterus are mediated by NK_2 receptors and are enhanced by peptidase inhibitors

35P **Bhogal R & Sheldrick RLG** The effects of islet amyloid polypeptide and pituitary adenylate cyclase activating polypeptide on guinea-pig tracheal smooth muscle *in vitro*

36P **Bishop LA, Gerskowitch VP, Hull RAD, Shankley NP & Black JW** Characterisation of the CCK_A and gastrin receptors coupled to muscle contraction in guinea-pig fundus using receptor desensitisation

37P **Hutchinson SP, Hollywood MA, Burke EP, Allen JM & McHale NG** Effects of 5-HT on spontaneous contractility in isolated bovine mesenteric lymphatics

38P **MacLean CM & Edwardson JM** Effects of genistein on exocytosis and protein phosphorylation in the exocrine pancreas of the rat

39P **Waschulewski IH, Hall DV, Kern HF & Edwardson JM** Effects of the immunosuppressants cyclosporin A and FK506 on exocytosis in the rat exocrine pancreas *in vitro*

40P **Deegan P, Basinger M, Jones M & Hande K** Atrial natriuretic peptide reduces the nephrotoxicity, but not the anti-tumour activity, of cisplatin

41P **Mitchell SC, Zhang AQ, Ayesh R & Smith RL** Trimethylamine N-oxide as a source of dimethylamine

42P **Ferry DR, Russell MA, Kerr DJ & Cullen MH** P-glycoprotein possesses allosterically coupled drug acceptor sites

43P **Ward PS, Vetterlein O, Bodmer M & Foulkes R** Is the intravenous route the only therapeutic route for monoclonal antibodies?

44P **Smyth EM, Brazil DP & Keenan AK** Interactions between atrial natriuretic factor and calcium in bovine pulmonary artery endothelial cells

45P **Griffin A & Webber SE** Effects of potassium channel openers on tracheal submucosal gland secretion and epithelial transport

46P **Challiss RAJ** Inhibition of histamine-stimulated phosphoinositide hydrolysis in bovine tracheal smooth muscle by BRL 38227 and isoprenaline

47P **Griffin A, Webber SE & Scott RH** K^+-currents recorded from cultured cells isolated from ovine tracheal submucosa

48P **Collier ML, Ward JPT & Aaronson PI** Charybdotoxin- and apamin-sensitive potassium currents in smooth muscle cells isolated from bovine trachealis

49P **Ward JPT, Taylor SG & Collier ML** The novel benzopyranol potassium channel activator BRL55834 activates two potassium channels in bovine airway smooth muscle

50P **Hirst SJ, Barnes PJ & Twort CHC** Inhibition of serum-induced proliferation by phorbol ester and protein kinase inhibitors in growth-arrested cultured rabbit tracheal smooth muscle cells

51P **Francischi JN, Conroy DM, Maghni K & Sirois P** Inhibition of Sephadex-induced leukocyte infiltration into guinea-pig lungs by rapamycin

52P **Giembycz MA, Belvisi MG, Miura M, Perkins RS, Kelly J, Tadjikarimi S, Yacoub MH & Barnes PJ** Soluble cyclic nucleotide phosphodiesterase isoenzymes from human tracheal smooth muscle

53P **Belvisi MG, Miura M, Peters MJ, Ward JK, Tadjikarimi S, Yacoub MH, Giembycz MA & Barnes PJ** Effect of isoenzyme-selective cyclic nucleotide phosphodiesterase inhibitors on human tracheal smooth muscle tone

54P **Rabe KF, Tenor H, Dent G, Schudt C, Liebig S & Magnusson H** Functional and biochemical characterisation of PDE isoenzymes regulating inherent tone in human peripheral airways

55P **Dent G, Giembycz MA, Rabe KF, Barnes PJ & Magnussen H** Inhibition of human alveolar macrophage respiratory burst by theophylline: association with phosphodiesterase inhibition

56P **Browne SE, Muir J, Everitt BJ, Robbins TW & McCulloch J** The acute effects of infusions of AMPA, NMDA and muscimol into the basal forebrain on cerebral function in the rat

57P **Cartmell J, Curtis AR, Kemp JA, Kendall DA & Alexander SPH** Second messenger responses to the isomers of 1-aminocyclopentane-1,3-dicarboxylate in guinea-pig cerebral cortical slices

58P **Fox GB, O'Donnell RA, Swayne GTG & Wyllie MG** Inhibition of increased locomotor activity in the mouse as a marker of N-methyl-D-aspartate (NMDA) receptor antagonism

59P **Gill R & Lodge D** The neuroprotective effects of a combination of MK-801 and NBQX in focal ischaemia

60P **Kenny BA, Calder C & Brown CM** Neuroprotective properties of phenytoin in a mouse model of focal cerebral ischaemia

61P **Doyle KM & Shaw GG** Is there a real polyamine antagonist?

62P **Bevan KA, Newman AH, Bowery NG & Tortella FC** Mechanism of the anticonvulsant action of dextromethorphan analogues in rat brain

63P **Morton AJ, Page GK & Tucker LM** Glutamate-induced changes in nicotinamide adenine diphosphonucleotide diaphorase activity in primary cultures of embryonic rat glia derived from cerebellum and cerebral cortex

64P **Whitton PS, Biggs CS, Pearce BR & Fowler LJ** Regional effects of dizocilpine on dopamine and its metabolites in rat hippocampus and striatum

65P **Daly SA & Waddington JL** Effects of clozapine on behavioural responses to the new selective D_1 dopamine receptor agonist A 68930: involvement of a D_1 receptor subtype?

66P **Smith LA, Kebabian J, Britton D, Jenner P & Marsden CD** A77636, a full D_1 dopamine agonist, reverses MPTP-induced motor deficits in common marmosets

67P **Martorana MG, Dickson M, Fox PK, Gemmell DK, Connick JH & Nicholson CD** Antimuscarinic activity increases the functional expression of D_1 receptor antagonism

68P **Pilowsky L, Costa DC, Ell P, Verhoeff P & Kerwin RW** A ^{123}I-IBZM single photon emission tomography study of *in vivo* dopamine receptor occupancy in typical antipsychotic responders and nonresponders

69P **Pilowsky L, Costa DC, Ell P, Verhoeff P & Kerwin RW** A single photon emission tomography study of *in vivo* dopamine receptor occupancy for typical antipsychotics and the atypical drug clozapine in schizophrenia

70P **Clapham J & Kilpatrick GJ** Thioperamide, a selective histamine H_3 receptor antagonist, inhibits amphetamine- and apomorphine-induced locomotor activity in the mouse

71P **Candy JM, Oakley AE, Morris CM, Love G, Perrott H & Edwardson JA** Increased iron in the neuromelanin-containing neurones in the substantia nigra zona compacta in Parkinson's disease

72P **Colado MI, Murray TK & Green AR** Chlormethiazole prevents 5-HT loss in rat brain four days after MDMA administration

73P **Suman-Chauhan N, Webdale L, Meecham K, Hill DR, Kneen C, Pritchard M, Hunter J & Woodruff GN** [^{3}H]PD 140376: a novel radioligand for CCK-B/gastrin receptors

74P **Wood PC, Wojcikiewicz RJH & Nahorski SR** Aluminium inhibits muscarinic agonist-stimulated inositol 1,4,5,-trisphosphate production and calcium release in permeabilised SH-SY5Y human neuroblastoma cells

75P **Alexander SPH, Curtis AR, Kendall DA & Hill SJ** The affinity of 8-cyclopentyl-1,3-dipropylxanthine at A_1 adenosine receptors of guinea-pig cerebral cortex

76P **Arias-Montaño JA & Young JM** Can Ni^{2+} be used to define the component dependent on Ca^{2+} entry in histamine-induced inositol phosphate formation in brain?

77P **Perretti M, Croxtall JD & Flower RJ** Endogenous lipocortin-1 mediates dexamethasone inhibition of interleukin-1-induced neutrophil accumulation *in vivo*

78P **Smith JA & Turner NJ** Oxidised low-density lipoprotein inhibits endothelium-dependent relaxation through activation of protein kinase C

79P **Boughton-Smith NK, Evans SM, Whittle BJR & Moncada S** Dexamethasone inhibits endotoxin-induced vascular permeability and nitric oxide synthase in the rat intestine

80P **Swierkosz TA, Zembowicz A & Vane JR** The nature of endothelial cell-dependent vasorelaxations induced by N^G-hydroxy-L-arginine

81P **Szabo Cs, Thiemermann C, Mitchell JA & Vane JR** Activation of constitutive and cytokine-inducible NO synthase accounts for the reduced pressor response to noradrenaline in endotoxin shock

82P **Gross SS, Levi R, Lowry SF & Moldawer L** LPS induces nitric oxide synthase in the cultured vascular smooth muscle by a mechanism which is independent of TNF and IL-1

83P **Warren JB & Coughlan ML** Nitric oxide synthase inhibitors prevent the delayed vasodilator response to ultraviolet light in the rat

84P **Mitchell JA, Kohlhass KL, Matsumoto T, Warner TD, Pollock JS, Forstermann U, Schmidt HHHW & Murad F** NO synthase activity in the lung is species-dependent

85P **Ward JK, Fox AJ, Miura M, Tadjikarimi S, Yacoub MH, Barnes PJ & Belvisi MG** Modulation of cholinergic neurotransmission by nitric oxide in human airway smooth muscle

86P **Smith AD & Muir TC** The effects of nitric oxide and nitrovasodilators on spontaneous electrical and mechanical activity in the rabbit distal colon

87P **Graham AM & Sneddon P** Investigation of purines and nitric oxide as putative neurotransmitters in rabbit isolated anococcygeus

88P **Macarthur H, Hecker M & Vane JR** Thapsigargin differentiates between EDRF and prostacyclin release induced by shear-stress or agonists

89P **Jeremy JY, Mikhailidis DP & Thompson CS** Opposite changes of adrenoceptor- and muscarine-receptor-linked prostacyclin synthesis by the aorta and bladder of the diabetic rat

90P **Yeardley HL, Coleman RA, Clayton JK, Marshall K & Senior J** A comparison of the inhibitory effects of prostanoid EP_2 receptor agonists and β_2-adrenoceptor agonists on human myometrium from pregnant donors

91P **Armstrong RA & Talpain E** Characterisation of the PGE receptor on human promyelocytic leukemia (HL-60) cells differentiated with dimethylsulfoxide into neutrophil-like cells

92P **Matthews JS, Armstrong RA, Woodward DF & Jones RL** Characterisation of the PGE receptor on human macrophage-like cells through binding studies conducted on a purified plasma membrane preparation

93P **Gwilt M, Norton B & Henderson CG** Mechanism of potassium efflux during low flow ischaemia in perfused guinea-pig hearts *in vitro*

94P **Henderson CG, Gwilt M, Orme JP & Rourke JD** K^+ channel blockers, K^+ efflux and ventricular fibrillation in ischaemic perfused guinea-pig hearts

95P **Sanders A, de Silva R, Myers R, Manjil LG, Ashworth S, Camici PG, Curtis MJ & Mann GE** Ischaemia and reperfusion induced changes in rat myocardial peripheral type benzodiazepine receptors

96P **Rees SA & Curtis MJ** The I_{Kl} blocker, RP58866, is an effective antifibrillatory agent in isolated rabbit hearts

97P **Wainwright CL, Parratt JR, Tweddel AC, Martin W & Cobbe SM** Adenosine and an A_2 agonist, CGS21680, reduce arrhythmias and suppress white cell accumulation in the porcine ischaemic myocardium

98P **Warren JB & Wilson AJ** Importance of adenylate cyclase-mediated vasodilation in the rabbit increases with decreasing vessel size

99P **Berman RS & Martin W** Hydrogen peroxide is the mediator of endothelial barrier dysfunction induced by xanthine oxidase and hypoxanthine

100P **Thompson M, Westwick J & Woodward B** Ischaemia/reperfusion modifies the effects of endothelins and vasodilators in isolated rat hearts

101P **Wilkes LC & Boarder MR** Endothelin stimulates vascular smooth muscle phospholipase D: attenuation by tyrosine kinase inhibitor ST271

102P **Hay DWP & Luttmann MA** Endothelin (ET) receptors mediating ET-1-induced contraction in guinea-pig and human pulmonary tissues

103P **Warner TD, Allcock GH, Corder R & Vane JR** BQ123 and different isolated tissue preparations reveal heterogeneity in the receptors mediating contractions to endothelin-1

104P **McMurdo L, Thiemermann C, Corder R & Vane JR** Characterisation of an ET_A receptor antagonist in the anaesthetised rat

105P **Weishaar RE, Wallace AM, Kiser LA, Britton LW, Ferraris VA & Sim MF** An assessment of flosequinan's direct effect on human arterial, venous and cardiac muscle: comparison with milrinone

106P **Carr RD, Fraser-Rae L, Killingback PG, Hallam C & Harper ST** Pharmacological profile of FPL 66564: the first ultra short-acting angiotensin-converting enzyme inhibitor (USACEI) in the rat

107P **Wilkinson GF & Boarder MR** Evidence for two distinct receptors for ATP on bovine aortic endothelial cells, both linked to phospholipase C activation

POSTER COMMUNICATIONS

108P **Cadogan AK, Wright IK, Coombs I, Marsden CA, Kendall DA & Tulloch I** Repeated paroxetine administration in the rat produces a decreased [^{3}H]ketanserin binding and an anxiolytic profile in the elevated X-maze

109P **Beckett SRG, Curwen JO, Marshall PW & Marsden CA** The haemodynamic effects of intra-periaqueductal grey administration of 8-OHDPAT on a chemically-induced defence response

110P **Robson L, Gower AJ & Marsden CA** Differential age-related changes in 5-HT agonist-induced behaviours in the Hooded Lister rat

111P **Ge J, Barnes NM, Cheng CHK, Costall B & Naylor RJ** Interaction of (R)- and (S)-zacopride and 5-HT_3/5-HT_4 receptor ligands to modify extracellular levels of 5-HT in the rat frontal cortex

112P **Cheetham SC, Martin KF, Viggers JA, Phillips I & Heal DJ** Effect of repeated anxiolytic drug administration on 5-HT_{1A} receptor binding in rat frontal cortex and hippocampus

113P **Leathley MJ & Goudie AJ** Effects of the 5-HT_2 antagonist, ritanserin, on benzodiazepine withdrawal-induced weight loss in rats

114P **Scott G, Luscombe GP & Mason R** The effects of BTS 54 505 on 5-HT and noradrenaline responses in the rat dorsolateral geniculate nucleus

115P **Ebenezer IS & Tite R** Different effects of 8-OH-DPAT on food intake in male and female rats

116P **Ormandy GC, Wilson DJ, Wren P, Barrett VJ & Prentice DJ** Comparison of the 5-HT_{1D}-like receptors in the saphenous veins and CNS of the dog and rabbit

117P **Yocca FD, Nowak HP, Bucci DJ, Carter RB & Mahle CD** Species differences in multiple [^{3}H]5-carboxamidotryptamine-sensitive (5-HT_{1D}-like) binding sites in vertebrate brain

118P **Bill DJ, Brown G, Fletcher A & Hughes IE** 8-OH-DPAT-induced hypothermia in the mouse may be mediated by a reduction in 5-HT release onto postsynaptic 5-HT_{1C} receptors

119P **Newberry NR, Watkins CJ, Reynolds DJM, Leslie RA & Grahame-Smith DG** Pharmacology of the 5-HT-induced depolarization of the ferret isolated vagus nerve

120P **Elliott P & Wallis DI** The pharmacology of responses evoked in lumbar motoneurones of the neonate rat spinal cord by descending stimulation

121P **Templeton AGB, MacMillan JB, McGrath JC & Whittle MJ** The effect of oxygen on the human umbilical artery

122P **Tuladhar BR, Costall B & Naylor RJ** Potentiation of the relaxation response to 5-methoxytryptamine in the rat ileum with monoamine oxidase inhibitors

123P **Lewis CA & Broadley KJ** Comparison of the bronchoconstriction by 5-HT and methacholine in conscious guinea-pigs: a possible reflex action by 5-HT

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125P **Waikar MV, Ford APDW, Hegde SS & Clarke DE** DAU 6285: a probe for the 5-HT_4 receptor in rat oesophagus and monkey bladder

126P **Shaw LA & Coker SJ** Combined administration of a thromboxane antagonist (ICI 192,605) and a 5-HT_2 antagonist (ICI 170,809) markedly reduces reperfusion-induced arrhythmias

127P **Matthew JD, Gaw AJ & Wadsworth RM** Evidence for nitrergic vasodilation in the sheep middle cerebral artery

128P **Vercruysse I, Vermeulen A, Belpaire F, Massart DL & Dupont AG** Inhibition of the metabolism of propranolol by nicardipine in rats: an *in vitro* study

129P **Brownrigg N, Garcia R, Jessup R, Lee V, Tunstall S & Wayne M** ICI D1542: a potent thromboxane A_2 (TXA_2) synthase inhibitor and receptor antagonist

130P **Menys VC** The effects of ICI D1542, a thromboxane A_2 synthase inhibitor and TP receptor antagonist, on collagen-induced human platelet degranulation and aggregation

131P **Laycock SK, Kane KA, McMurray J & Parratt JR** The purine-xanthine oxidase system does not depress myocardial function through a free radical mechanism

132P **Tweedie D, Kane KA & Henderson CG** Glibenclamide, but not UK-66,914, abolishes the ischaemia-induced shortening of the effective refractory period in perfused guinea-pig hearts

133P **Kelso EJ, McDermott BJ & Silke B** The role of phosphodiesterase inhibitors in regulating cyclic AMP and contractile function in isolated ventricular cardiomyocytes

134P **Marshall PW, Bramley J & Briggs I** The effects of ICI D7288, a novel sino-atrial node modulating agent, on guinea-pig isolated atria

135P **Valenzuela C, Pérez O, Casis O, Duarte J, Delpón E, Pérez-Vizcaino F & Tamargo J** Effects of lisinopril on contractility and electrophysiological properties in guinea-pig cardiac preparations

136P **Delpón E, Valenzuela C, Pérez O, Pérez-Vizcaino F & Tamargo J** Electrophysiological interactions between desipramine and imipramine on guinea-pig papillary muscles

137P **Piacentini L, Wainwright CL & Parratt JR** The antiarrhythmic effect of preconditioning, in rat isolated hearts, does not involve A_1 adenosine receptors

138P **Sun W, Wainwright CL & Parratt JR** The BK_2 antagonist, Hoe 140, does not attenuate the antiarrhythmic effect of ischaemic preconditioning in anaesthetised rats

139P **Oldham AA, Allott CP, Major JS, Smith CFC, Luke RWA, Roberts DA, Bradbury RH & Pearce RJ** Substituted 4-benzyloxyl-naphthyridine derivative: a novel, potent and orally-effective angiotensin II receptor antagonist

140P **Mo N, Wallis DI & Watson A** Depolarization induced by angiotensin in putative cardiac neurones of rat stellate ganglia

141P **Weishaar RE, Kirker ML, Wallace AM, Britton LW, Ferraris VA & Sim MF** Relationship between inotropic activity and phosphodiesterase inhibition for flosequinan, BTS 53 554 and milrinone

142P **Fry CH & Sim MF** Electromechanical effects of flosequinan on isolated cardiac ventricular muscle

143P **Evans HG, Shah AM & Lewis MJ** The characteristic effects of endothelium-derived relaxing factor (EDRF) on myocardial contraction are mimicked by interleukin-1β

144P **Paul W, Gresele P, Bianchi G & Page CP** Effect of defibrotide (DEF) on platelet accumulation in the rabbit

145P **Bulloch JM & McGrath JC** Evidence for increased purinergic contribution in hypertensive blood vessels exhibiting co-transmission

146P **Malkhandi J & Keen M** Effect of GTP concentration on agonist affinity estimates obtained using the receptor inactivation technique

147P **Carnell AJ & Williams RG** Hexamethonium-resistant vagally-evoked inhibitions of gastric smooth muscle tone in the rat: the effect of short term 6-hydroxydopamine treatment

148P **Lung MA** Action of sympathomimetic drugs on parasympathetic salivary secretion in dogs

149P **Suman-Chauhan N, Daum P, Hill DR & Woodruff GN** CCK-B/gastrin receptor-mediated changes in intracellular calcium in AR42J cells

150P **Shepheard SL, Cook DA, Williamson DJ, Hurley CJ, Hill RG & Hargreaves RJ** Inhibition of neurogenic plasma extravasation but not vasodilation in the hindlimb of the rat by the non-peptide NK-1 receptor antagonist (±)RP67580

151P **Woods M & Baird AW** Endogenous peptidases attenuate bradykinin-evoked ion transport in guinea-pig gallbladder

152P **Patacchini R, Maggi CA & Giachetti A** Activity of SR 48968 at smooth muscle NK_2 tachykinin receptors of hamster, rat and rabbit species

153P **Wijetunge S, Hughes AD & Sever PS** Amlodipine-induced inhibition of calcium channel currents in single smooth muscle cells isolated from rabbit ear artery

154P **Robertson SJ, Pennington AJ, Evans AM & Martin RJ** Pyrantel activated single-channel currents in the nematode parasite *Ascaris suum*

155P **Kurenny DE, Chen H & Smith PA** Analysis of muscarinic inhibition in the C-cells of bullfrog sympathetic ganglia

156P **Wheeler SV, Jane SD, Chad JE & Foreman RC** Subunit dependence of 43KDa protein-mediated clustering of nicotinic acetylcholine receptors (nAchRs) in *Xenopus* oocytes

157P **Gallagher A, McGlynn H & Ryan MP** Flow cytometric assessment of renal cellular injury in response to gentamicin or hypoxia/reoxygenation

158P **Purkiss JR, Wilkinson GF & Boarder MR** UTP and ATP stimulate bovine adrenal medullary endothelial cells by acting on a nucleotide receptor

159P **Green KL, Foong WC & Keysell GR** Treatment of an experimental arthritis with intra-articular ^{90}yttrium, methotrexate or chlorambucil

160P **D'Orleans-Juste P, Lehoux S, Sirois P & Plante GE** Phosphoramidon-sensitive effects of human big-endothelin-1 on plasma extravasation in conscious rats

161P **Mattera GG, Eglezos A, Cucchi P, Renzetti AR & Mizrahi J** Phosphoramidon-sensitive conversion of human proendothelin *in vivo*: effect on pressure and plasma prostacyclin

162P **McCreath G, Hill SJ & Hall IP** Regulation of histamine-induced inositol phospholipid hydrolysis by protein kinase C in cultured human umbilical vein endothelial cells

163P **Hu DE & Fan T-PD** Anti-angiogenic action of suramin and hydrocortisone in rats

164P **Hicks R & Hemati AA** Demonstration of myofibroblast contractility in fibrotic lungs of chromium and metal-fume pretreated rats

165P **Adams D, Nicholson CD & Challiss RAJ** Modulation of agonist-stimulated inositol phosphate accumulation in airway smooth muscle by isoenzyme-selective phosphodiesterase inhibitors

166P **Doolan C & Keenan AK** An epithelium-derived cytosolic inhibitor of cAMP-dependent protein kinase activity

167P **Kelly M & Keenan AK** Modulation of ANF receptor-effector coupling in endothelial cells by protein kinase C

168P **Tomkinson A, Lewis S, Raeburn D & Karlsson J-A** The effect of isoprenaline, administered as an aerosol or as a dry powder, on methacholine-induced bronchoconstriction in the anaesthetized pig

169P **Wang JCC & Lung MA** Action of histamine on nasal circulation and airway resistance in dogs

170P **Riccio MM & Page CP** Involvement of sensory nerves in 15-HPETE-induced airways hyperresponsiveness in normal rabbits

171P **Sasaki M Herd CM & Page CP** The effect of heparin on PAF-induced airway hyperresponsiveness and cellular infiltration in neonatally immunised rabbits

172P **Perretti F & Manzini S** PAF-induced bronchial hyperresponsiveness in anaesthetized guinea-pigs is dependent upon capsaicin-sensitive mechanism(s)

173P **Nally JE, McCall R, Young LC, Wakelam MJO & Thomson NC** Mechanical and biochemical responses to endothelin-1 and endothelin-3 in bovine bronchial smooth muscle

174P **Claphàm JC, Good DM & Trail BK** Effects of BRL 55834 on neurally-mediated bronchoconstriction in the guinea-pig

175P **Davies MP, McCurrie JR & Wood D** Effect of potassium channel openers on gastrointestinal transit in the mouse

176P **Hamilton CA, McCulloch J, Godfrey EG, Dominiczak M & Reid JL** Effect of chronic exposure of cultured endothelial cells to low density lipoproteins on phosphatidylinositol metabolism

177P **Dowell FJ, McMurray J, Hamilton CA & Reid JL** Which free radical/reactive oxygen species is important in damaging endothelial function?

178P **Godfrey EG, Stewart J, Dominiczak M, Hamilton CA, McMurray J, Dargie JH & Reid JL** Inhibition of human LDL cholesterol oxidation

179P **Smith JA, Morgan J & Leake DS** Bovine aortic endothelial cells in culture can oxidatively modify low-density lipoproteins

180P **van der Graaf PH, Welsh NJ, Shankley NP & Black JW** Efficacy of idazoxan at α_1-adrenoceptors in rat aorta and small mesenteric artery

181P **Sithers AJ, Marshall RW & Spriggs TLB** Functional evidence for heterogenous distribution of α-adrenoceptor subtypes in the rat vas deferens

182P **Davies AR, Marshall RW & Spriggs TLB** α_{1A}-adrenoceptors in rat vas deferens: there, but where?

183P **Aidulis DM & Pollock D** Inhibition of enhanced contractions to UK-14,304 by yohimbine, prazosin or a vasopressin antagonist in rat tail artery rings *in vitro*

184P **Jackson A, O'Brien H & Chess-Williams R** Effects of nifedipine on responses mediated by α_1-adrenoceptor subtypes

185P **Doubleday B, Reynolds GP & Chess-Williams R** Regulation of cardiac α- and β-adrenoceptors by the sympathetic nervous system

186P **Parkinson NA, Hughes AD & Sever PS** Pertussis toxin inhibition of α_2-adrenergic activity in human isolated resistance arteries

187P **Garcha RS, Hughes AD & Sever PS** Cytosolic heparin inhibits noradrenaline-induced Ca^{2+} release from intracellular stores in rat resistance arteries

188P **Smith JA, Evans RA & Turner NJ** Oxidised low-density lipoprotein does not inhibit endothelium-dependent relaxation by promoting superoxide anion generation

189P **Young DA, Aidulis DM & Pollock D** A comparison of the effects of vasorelaxants in vascular and non-vascular smooth muscle from spontaneously hypertensive rats

190P **Randall VA & Williams KI** Differential peptidergic and nitrergic involvements in NANC responses evoked by transmural stimulation and DMPP in the guinea-pig taenia caeci

191P **Brave SR, Gibson A & Tucker JF** The effect of nitrovasodilators and cGMP on Ca^{2+}-induced tension responses and Ca^{2+}-reloading in the mouse anococcygeus muscle

192P **Otter D, Warren MA & Chess-Williams R** Reduced vasodilator responses to carbachol in isolated aorta from streptozotocin diabetic rats

193P **Rae MG & Muir TC** Nitrovasodilators potentiate and inhibit spontaneous electrical and mechanical activity in the guinea-pig internal anal sphincter (gpIAS)

194P **Babbedge RC, Moore PK, Gaffen Z & Hart SL** L-N^G-nitro arginine p-nitroanilide (L-NAPNA): a selective inhibitor of nitric oxide synthase in the brain?

195P **Gibson A, Babbedge R, Bhat S, Brave S, Hart S, Hobbs A, Tucker J, Wallace P & Moore P** Loss of nerve-mediated responses and nitric oxide synthase activity in cold-stored anococcygeus muscles from mice

196P **Khatsenko O, Gross SS, Mitchell JA, Rifkind AB & Vane JR** Inhibition of cytochrome P-450 activity by nitric oxide *in vitro* and during endotoxin shock *in vivo*

197P **Shaw AM, Barkell E, Rourke S, Barfaraz AR, Pollock D & McGrath JC** A comparison of the effects of L-N^w-nitro arginine methyl ester and flurbiprofen on responses to vasocontrictors in the pulmonary circulation of the rat perfused lung

198P **Cristol JP, Thiemermann C, Mitchell JA, Walder C & Vane JR** Endogenous nitric oxide formulation supports renal blood flow after ischaemia-reperfusion injury

199P **Soares de Moura R, Leão MC, Lourenço CD, Monnerat Lemos AJ & Salvaro P** Captopril prevents hypertension developed by inhibition of nitric oxide synthesis induced by chronic treatment of rats with L-NAME

200P **Laight DW, Waterfall JF & Woodward B** Vasodilation to histamine in the rat isolated perfused kidney (IPK) preconstricted with K^+ is EDRF(NO)-independent and mediated by H_2 receptors

201P **Plane F, Pearson T & Garland CJ** Differences between endothelium-dependent relaxation to acetylcholine and A23187 in the rabbit isolated femoral artery

202P **Aldir HC, Lang D & Lewis MJ** A comparison of three nitric oxide synthase inhibitors on endothelium-dependent relaxation in rabbit aorta and on NO synthase activity in rabbit cerebellum

203P **Kenny BA & Sheridan RD** D-CPP and phenytoin protect against hypoxia-induced failure of synaptic transmission in the rat hippocampus *in vitro*

204P **Grimwood S, Struthers L & Foster AC** Modulation by polyamines of [^{3}H]L-689,560 binding to the glycine site on the N-methyl-D-aspartate receptor from rat cortex/hippocampus membranes

205P **Ellis Y & Davies JA** The effect of neuropeptides on potassium-stimulated release of glutamate from rat striatal slices

206P **Britton P, Whitton PS, Fowler LJ & Bowery NG** Effect of tetanus toxin on extracellular amino acid levels in rat hippocampus: an *in vivo* microdialysis study

207P **Jones MG & Lodge D** Effect of ethanol on responses of rat central neurones *in vivo* to excitatory amino acids

208P **Watson WP & Little HJ** The calcium channel antagonist, diltiazem, increases the severity of the ethanol withdrawal syndrome

209P **Biggs CS, Pearce BR, Fowler LJ & Whitton PS** Sodium valproate alters extracellular GABA levels in the rat ventral hippocampus *in vivo*

210P **Smith S & Sharp T** *In vivo* measurement of extracellular GABA in rat nucleus accumbens: improved methodology

211P **Pratt GD & Bowery NG** Autoradiography of GABA receptor binding sites in the dorsal vagal complex of the rat hindbrain

212P **Wieczorek W & Kruk ZL** Differential action of (+)amphetamine on electrically-evoked dopamine efflux in the rat caudate putamen and nucleus accumbens

213P **Davidson C & Stamford JA** Heterogeneity of the afferent dopaminergic innervation to the medial axis of the rat neostriatum: *in vitro* voltammetric data

214P **Iravani MM & Kruk ZL** Differential sensitivities of rat striatal D_2 dopamine autoreceptor sites

215P **Jorm CM & Stamford JA** Age-related differences in evoked noradrenaline efflux as measured by fast cyclic voltammetry in rat brain slices

216P **Palij J & Stamford JA** Control of noradrenaline efflux in the rat nucleus interstitialis stria terminalis by α_2 autoreceptors: *in vitro* voltammetric data

217P **Trout SJ, Patel J & Kruk ZL** Monoamine efflux evoked by electrical stimulation in rat brain slices containing substantia nigra and ventral tegmental area

218P **Williams JEG, Millar J & Kruk ZL** A comparison of cut and spark-etched electrodes for fast cyclic voltammetry

219P **Logan SD, Pickering AE, Gibson IC & Spanswick D** Multiple actions of noradrenaline on sympathetic preganglionic neurones *in vitro*

220P **Jenkinson S, Nahorski SR & Challiss RAJ** Lithium-sensitive accumulation of a novel inositol phosphate product of phosphoinositide turnover in rat cerebral cortex slices

221P **Safrany ST, Liu C, Potter BVL & Nahorski SR** The evaluation of L-chiro inositol trisphosphate analogues as selective inositol 1,4,5,-trisphosphate 5-phosphatase inhibitors

222P **Reynolds DJM, Taylor HMN, McQuade R, Grahame-Smith DG & Leslie RA** C-*fos* immunocytochemistry identifies brainstem neurones involved in the emetic action of loperamide in the ferret

223P **Salib RJ, Fone KCF & Bennett GW** Effect of thyrotophin-releasing hormone on high-affinity choline uptake in rat brain synaptoneurosomes

224P **Renzetti AR, Catalioto RM & Mizrahi J** Angiotensin II receptor subtypes on bovine cerebellum: characterisation by agonists, antagonists and dithiothreitol

225P **Rea LA & Wallis DI** Capsaicin desensitization of peripheral nociceptive fibres impairs sensitivity to ATP

226P **Wong CLJ** The effect of histamine receptor agonists and antagonists on restraint-induced antinociception in mice

227P **Allam D, Girdlestone D & Garret C** Effects of the substance P antagonist, RP 67580, on neuronal activity in the rat locus coeruleus

228P **Seabrook GR, Main MJ & Hill RG** Pharmacology of neurokinin receptors on neurones within brain slices of the guinea-pig locus coeruleus

229P **Meechan KG, Suman-Chauhan N, Kneen C, Hill DR, Hughes J, Hunter JC & Woodruff GN** [^{3}H]PD140376 binding to guinea-pig gastrin receptors

230P **Hadley AJ, Buckingham JC & Kendall MD** Selective effects of thymic peptides on the release of pituitary hormones by rat adenohypophysial tissue *in vitro*

231P **Kumari M, Cover PO, Hadley AJ, Flack JD & Buckingham JC** Peripheral and central effects of denbufylline on neuroendocrine function in the rat

232P **Wayman CP & Wilson JF** Nitric oxide mediates NMDA-stimulation of a-MSH release from rat hypothalamus

DEMONSTRATION

233P **Wright IK, Bickerdike MJ & Marsden CA** Preparation of reliable low-cost microdialysis probes

ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

234P **Fernyhough P, Diemel LT, Smith WJ & Tomlinson DR** Treatment with brain-derived neurotrophic factor reverses deficient nerve growth factor mRNA in sciatic nerve of streptozotocin-diabetic rats

235P **Bevan S, Rang H & Shah K** Capsazepine does not block the proton-induced activation of rat sensory neurones

236P **Dray A, Patel I, Naeem S, Rueff A & Urban L** Studies with capsazepine on peripheral nociceptor activation by capsaicin and low pH: evidence for a dual effect of capsaicin

237P **Perkins MN, Campbell EA, Davis A & Dray A** Antinociceptive activity of bradykinin B_1 and B_2 antagonists in two models of persistent hyperalgesia in the rat

238P **Yeats JC, Boddeke HWGM & Docherty RJ** Capsaicin desensitization in rat dorsal root ganglion neurones is due to activation of calcineurin

239P **Sutton KG, Dolphin AC & Scott RH** Argiotoxin-636 inhibits Ca^{2+}-dependent currents recorded from cultured neurones from rat dorsal root ganglia

240P **Sweeney MI & Dolphin AC** The $GABA_B$ receptor agonist (-)-baclofen and the 1,4-dihydropyridine Ca^{2+}-channel agonist Bay K8644 modulate the GTP-binding protein G_o via different mechanisms

241P **McKibbon VL, Hayward NJ, Poat J, Woodruff GN & Hughes J** Effect of dizocilpine and enadoline on immediate early gene expression following ischaemia

242P **Brugger F & Evans RH** Comparison of quinoxalinedione potency as depressants of the monosynaptic activation of spinal motoneurones from dorsal roots *in vitro*

243P **Jones MG, Bond A & Lodge D** Antagonism of excitatory amino acid-evoked response of rat spinal neurones *in vivo* by 2,3-benzodiazepines GYKI 52466 and GYKI 53655

244P **Eaton DA, Salt TE, Sunter DC, Udvarhelyi PM & Watkins JC** Antagonism of nociceptive responses of rat thalamic neurones *in vivo* by novel antagonists of (1S,3R)-ACPD

245P **Jones RSG & Lambert CE** 4-aminopyridine-induced epileptogenesis in rat temporal lobe *in vitro*: effects of excitatory amino acid antagonists and anticonvulsant drugs

246P **Ripley TL, Morris J, Butterworth AR, Watson WP & Little HJ** Effects of competitive NMDA antagonists, given concurrently with ethanol, on the ethanol withdrawal syndrome and the development of tolerance to ethanol

247P **de Mendonça A & Ribeiro JA** Adenosine inhibits the NMDA receptor-mediated excitatory postsynaptic potential in the hippocampus

248P **Cunha RA, Vizi ES & Sebastião AM** Contribution of the catabolism of adenine nucleotides to the formation of endogenous extracellular adenosine in the rat hippocampus

249P **McCrea KE, Johnson M & Hill SJ** The effect of salmeterol on cyclic AMP accumulation in a neuronal cell line

250P **Chiu P, Cook SJ, Berry JL, Carpenter JR, Downing SJ, Small AM & Small RC** Activation of β_1- or β_2-adrenoceptors opens plasmalemmal K^+-channels in airways smooth muscle: evidence from $^{86}Rb^+$ efflux studies in bovine trachealis

251P **Nishikawa M, Mak JCW, Shirasaki H, Harding SE & Barnes PJ** Long-term exposure to noradrenaline in guinea-pig results in down-regulation of pulmonary β-receptors

252P **Nicholson CD, Shahid M, Bruin J, de Boer J, van Amsterdam RGM, Zaagsma J, Dent G, Giembycz MA & Barnes PJ** Org 20241, a cyclic nucleotide phosphodiesterase inhibitor for asthma

253P **Sheldrick RLG, Rabe KF, Magnussen H & Coleman RA** Further evidence for the involvement of NK_2-receptors in tachykinin-induced contraction of human isolated bronchus

254P **Holbrook M & Hughes B** The effect of rolipram and SK&F94120 on ozone-induced bronchial hyperreactivity to inhaled histamine in guinea-pigs

255P **Weg VB, Williams TJ, Lobb RR & Nourshargh S** Role of very late antigen-4 (VLA-4) in eosinophil accumulation *in vivo*

256P **Teixeira MM, Hellewell PG, McNiece C, Doenhoff MJ & Williams TJ** Local eosinophil accumulation and oedema formation induced by supernatants of homogenized cercaria of *Schistosoma mansoni* in guinea-pig skin

257P **Whalley ET, Loy SD, Modiferri D, Blodgett JK & Cheronis JC** A novel potent bis(succinimido)hexane peptide heterodimer antagonist (CP-0364) of bradykinin BK_1 and BK_2 receptors: *in vitro* and *in vivo* studies

258P **Peers SH, Duncan GS, Flower RJ & Perretti M** Release of interleukin-1 (IL-1), tumour necrosis factor-α (TNFα) activity and corticosterone (CCS) in experimental endotoxaemia in Lewis and Wistar rats

259P **Colville-Nash PR, Seed MP & Willoughby DA** Angiogenesis during the development of chronic granulomatous tissue as assessed by vascular casting *in vivo*

260P **Hu DE & Fan T-PD** The combined use of dehydroepiandrosterone and dexamethasone inhibits angiogenesis in the rat without causing immunosuppression

261P **MacIntyre DE, Meurer RD, Eirmann GJ, Renjilian R, Singer I, Kazazis D & Forrest MJ** Anti-inflammatory and pharmacokinetic properties of a murine anti-CD18 monoclonal antibody and its humanized counterpart in the rabbit

262P **Hollingsworth SJ, Anderson EM & Bennett A** Methotrexate/indomethacin interaction in normal and tumour cell lines: effects of folate concentration

263P **Thorin E, Hamilton CA, Godfrey EG, Dominiczak M & Reid JL** Oxidised low density lipoprotein suppressed bradykinin-induced prostacyclin release by cultured endothelial cells

264P **Snow HM, McAuliffe SJG, Jessup R, Wayne M & Noble MIM** Prevention of coronary thrombosis in the dog by inhibitors of platelet aggregation

265P **Belcher P, Drake-Holland AJ, Hynd JW & Noble MIM** Trimetazidine reduces myocardial infarct size, relative to area at risk, after temporary coronary artery occlusion in the rabbit

266P **Belcher P, Drake-Holland AJ & Noble MIM** Suppression of intracoronary thrombosis by trimetazidine without adverse haematological effects

267P **Cirillo R, Lidbury PS & Vane JR** The anti-ischaemic properties of cloricromene in the rabbit are not mediated by an anti-platelet activity

268P **Briand V & Galzin AM** Effects of substrate deprivation and hypoxia on the membrane integrity in cultured neonatal rat ventricular myocytes

269P **Tweedie D, Boachie-Ansah G, Kane KA & Henderson CG** Phentolamine attenuates hypoxia-induced abbreviation of the cardiac action potential

270P **Manning AS, Bruyninckx C, Hodeige D, Ramboux J & Chatelain P** SR 33589: a new amiodarone-like anti-arrhythmic agent

271P **Vogelsang M, Broede A, Schäfer E, Zerkowski H-R & Brodde O-E** Interaction between the adenylyl cyclase/cyclic AMP and the phospholipase C-inositol trisphosphate-diacylglycerol systems in the human isolated right atrium

272P **Schäfers R, Adler S, Daul A, Philipp T, Wenzel R & Brodde O-E** Effects of terbutaline in the human heart: *in vitro* and *in vivo* studies

273P **Summers RJ, Kompa AR & Russell FD** Regulation of β_1- and β_2-adrenoceptors in guinea-pig sino-atrial node after chronic (-)-isoprenaline infusion

274P **Vegh A, Papp J, Szekeres L & Parratt J** Are K_{ATP} channels involved in the profound antiarrhythmic effects of preconditioning in anaesthetised dogs?

275P **Dunne MJ, Jaggar JH & Harding EA** Modulation of ATP-sensitive K^+ channels in insulin-secreting cells by polymyxin B

276P **Stevens EJ, Carrington AL & Tomlinson DR** Prostacyclin release in experimental diabetes: effects of evening primrose oil

277P **Karet FE, Kuc RE & Davenport AP** Endothelin receptors in human kidney characterised using novel ligands BQ3020 and BQ123

278P **Cristol JP, Warner TD, Thiemermann C & Vane JR** The vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and renal vasculature are mediated via different receptors

279P **Plumpton C, Champeney R, Ashby MJ, Kuc RE, Hoskins SL & Davenport AP** Identification of endothelin isoforms and proendothelin-1 in the human heart by high performance liquid chromatography and radioimmunoassay

280P **Lawrence E & Brain SD** Extravascular big endothelin-1 and big endothelin-3 conversion in the hamster cheek pouch microvasculature

281P **Harrison VJ, Corder R, Khan N, Änggård EE & Vane JR** Studies of endothelin converting enzyme in cultured bovine aortic endothelial cells

282P **Gross SS, Aisaka K & Levi R** Vasoconstrictors release nitric oxide from the isolated perfused guinea-pig mesenteric vasculature in a concentration-dependent manner

283P **Mitchell JA, Kohlhass KL, Sorrentino R, Murad F, Warner TD & Vane JR** Induction of calcium-independent nitric oxide synthase in rat mesentery during sepsis does not impair the vascular response to vasoconstrictor agonists

284P **Lot TY & Wilson VG** Aminoguanidine, N^G-monomethyl-L-arginine and N^G-nitro-L-arginine methyl ester causes endothelium-dependent contractions of the porcine isolated splenic artery

285P **Vallance P, Fickling S, Leone A, Nussey S, Moncada S & Whitley G** Synthesis of N^G,N^G dimethylarginine by human endothelial cells

286P **Bogle RG, Baydoun AR, Pearson JD & Mann GE** Citrulline can restore the ability of activated macrophages to generate nitric oxide in the absence of extracellular arginine

287P **Paul W, Lawrence L, Douglas GJ, Schachter M & Page CP** Modulators of the L-arginine-nitric oxide pathway: differential effects on cutaneous permeability to bradykinin and histamine in the guinea-pig

288P **Szabó Cs, Thiemermann C, Mitchell JA & Vane JR** Nitric oxide mediates the vascular hyporeactivity to noradrenaline and decompensation in haemorrhagic shock

289P **Pons F, Williams TJ & Warren JB** Mechanisms underlying vasodilatation induced by *E. coli* lipopolysaccharide (LPS) in the rabbit cutaneous microcirculation

290P **Gardiner SM, Kemp PA & Bennett T** Regional haemodynamic changes during chronic infusion of lipopolysaccharide (LPS) in conscious rats

291P **Andrade SP, Buttery LDK, Springall DR, Polak JM, Hart IR, Moncada S & Piper PJ** Modulation of tumour blood flow by induction of nitric oxide synthesis

292P **Bridgewater M, Macneil HJ & Brading AF** Control of smooth muscle from the pig proximal urethra

293P **Millar J, Palij P, Davidson C, Jorm CM & Stamford JA** Simultaneous single unit recording and fast cyclic voltammetry in brain slices

294P **O'Connor JJ, Trout SJ & Kruk ZL** Modulation of stimulated 5-HT overflow in slices of the dorsal raphé nucleus of the guinea-pig studied using fast cyclic voltammetry

295P **Dexter DT, Jhanji R, Gross-Sampson MA, Muller DPR, Marsden CD & Jenner P** Regional dopaminergic terminal loss in the striatum of vitamin E deficient rats as assessed by [^{3}H]-mazindol binding

296P **Needham PL, Cowan A, Skill MJ & Heal DJ** Contrasting effects of repeated reserpination on behavioural and biochemical correlates of D_1 and D_2 receptor interaction

297P **Patel S, Marwood R, Emms FA, Knowles MR, McAllister G & Freedman SB** Pharmacological studies on the human dopamine D_3 receptor expressed in stable cell lines

298P **Baldwin HA, Colado MI, Murray TK & Green AR** Chlormethiazole, but not dizocilpine, inhibits dopamine release in rat striatum induced by high dose methamphetamine

299P **Jones DNC & Holtzman SG** The influence of naloxone upon locomotor activity induced by a range of psychomotor stimulants

300P **Del Rio J, Pérez-Otaño I, Oset C, Herrero MT, Luquin MR & Obeso JA** Compared neurotoxic effect of chronic and acute MPTP treatment on neurotransmitter systems of the basal ganglia of the cynomolgous monkey

301P **Pearce RKB, Collins P, Jenner P & Marsden CD** Basic fibroblast growth factor (bFGF) intraventricular infusion in the MPTP-treated marmoset

302P **de Ceballos ML, Fernandez A, Jenner P & Marsden CD** Alterations in neuropeptides in basal ganglia in Parkinson's disease and incidental Lewy body disease

303P **Morris CM, Candy JM, Edwardson JA, Bloxham CA & Smith A** Haemopexin immunoreactivity in neurones in the human brain

304P **Davenport AP, Molenaar P & Kuc RE** BQ123 and BQ3020 reveal endothelin ET_A and ET_B receptor subtypes in human cardiac ventricle and rat cerebellum

305P **Widdop RE, Gardiner SM, Kemp PA & Bennett T** Central administration of EXP3174 or PD123319 inhibits effects of central angiotensin II in conscious rats

306P **Taylor AD, Flower RJ & Buckingham JC** The inhibition by lipocortin 1 of ACTH secretion evoked *in vitro* by the calcium channel agonist, BAY K8644

307P **Loxley HD, Flower RJ & Buckingham JC** Inhibitory effects of human recombinant lipocortin 1 and dexamethasone on the secretion of corticotrophin releasing factor-41 (CRF-41) in the rat

308P **Al-Qatari M & Taberner PV** Effect of chronic ethanol treatment on energy balance in gold thioglucose-induced obesity

309P **Siarey RJ, Hendrikse NH, Long SK & Evans RH** Effect of sumatriptan on synaptic transmission in the rat superior cervical ganglion *in vitro*

310P **Gupta P, Lattimer N & Rhodes KF** Characterisation of a 5-HT receptor which mediates N^G-nitro-L-arginine methyl ester-resistant relaxation of guinea-pig isolated jugular vein

311P **Seager JM & Garland CJ** Responses to 5-HT in strips of rabbit mesenteric artery permeabilized with staphylococcus α-toxin

312P **Coleman J, Rhodes KF & Ward TJ** A relationship between the structures of some N-alkyl-1,4-dihydro-4-oxoquinolines and their insurmountability as nicotinic and 5-HT_3 receptor antagonists

313P **King BF & Sanger GJ** Facilitation of noncholinergic neurotransmission by renzapride (BRL24924) in circular muscle of guinea-pig ileum

314P **Schoemaker RG, Du XY, Bax WA, Bos E & Saxena PR** Contractile responses to 5-HT in atrial but not in ventricular trabeculae: a study in porcine and human hearts

315P **Fozard JR, Menninger K, Tapparelli C & Dunning BE** Cardiovascular and metabolic effects of CGS 21680, a selective adenosine A_2 receptor agonist, in the Rhesus monkey

316P **Meghji P, Pearson JD & Slakey LL** Regulation of extracellular adenosine production by ectonucleotides of rat coronary microvascular endothelial cells

317P **Cardona-Sanclemente LE & Born GVR** Increased uptake of fibrinogen into arterial walls of unrestrained conscious rats caused by adrenaline or angiotensin II

318P **Forster L, Stewart-Lee A, Nourooz-Zadeh J, Änggård E & Ferns G** Probucol inhibits monocyte adhesion to the vascular endothelium *in vitro* and *in vivo*

319P **Demiryürek AT, Wainwright CL, Wadsworth RM & Kane KA** Characterisation of free radical production by porcine leukocytes

320P **Charalambous K & Hoult JRS** Ionophore-induced calcium toxicity in rat peritoneal leukocytes: suppression of superoxide generation and granular enzyme secretion

321P **Paterson SJ, Cunningham JR & Neal MJ** Mu opioids increase light-evoked release of acetylcholine from rabbit retina

322P **Burford NT, Tobin AB & Nahorski SR** Agonist binding and G-protein activation in CHO cells expressing M_1, M_2 or M_3 muscarinic receptors

323P **Hussain JF, Kendall DA & Wilson VG** A possible linkage of non-adrenoceptor, imidazoline binding sites labelled by [^{3}H]-idazoxan to K^+ channels and G-proteins

324P **Dasso LLT & Taylor CW** Alpha$_1$-adrenoceptors and vasopressin receptors share a common pool of G proteins in rat hepatocytes

325P **Burt RP, Chapple CR & Marshall I** Functional identification of an α_{1A}-adrenoceptor in rat epididymal vas deferens and an α_{1B}-adrenoceptor in rat spleen

326P **Munavvar AS & Johns EJ** The role of α_1-adrenoceptor subtypes in the regulation of renal haemodynamics in experimental hypertension

327P **Marshall I, Burt RP, Andersson PO, Chapple CR, Greengrass PM & Wyllie MG** Human α_{1C}-adrenoceptor: functional characterization in prostate

328P **Gaw AJ, Hills DM & Spraggs CF** Characterisation of cholecystokinin receptors mediating cardiovascular effects in the pithed rat

329P **Bennett A, Bishai P & Stamford IF** Differential blockade of CCK_B/gastrin receptor antagonists in guinea-pig stomach and ileum muscle

330P **Haworth D, Culbert J-A, Edmonds AE & Thomson DS** Effect of neuropeptide Y on cardiac output and organ vascular conductances in syrosingopinised rats

331P **Michel MC & Kötting A** Rapid desensitization of adrenaline- and neuropeptide Y-stimulated Ca^2 mobilization in HEL-cells

332P **Dickenson JM, White TE & Hill SJ** The effect of cAMP on histamine H_1-receptor-mediated increases in $[Ca^{2+}]_i$ in the hamster vas deferens cell line DDT_1MF-2

POSTER COMMUNICATIONS

333P **Beart PM & Loiacono RE** Microinjection of the nitric oxide donor nitroprusside into the hippocampus results in a dose-dependent neuronal degeneration

334P **Hernández F & Kendall DA** Cyclic GMP responses to NMDA in adult guinea-pig cerebellar slices

335P **Harris NC & Davies J** Cortically-evoked monosynaptic responses in the cat red nucleus are antagonised by AP5 but not by AP7

336P **Porter RHP & Roberts PJ** Activation of glutamate metabotropic receptors (mGluR) by sulphur-containing excitatory amino acids

337P **Nakamura-Craig M & Follenfant RL** Analgesic effects of lamotrigine in an experimental model of neuropathic pain in rats

338P **Scott G, Cheetham SC, Mason R, Luscombe GP & Heal DJ** [^{3}H]Dizocilpine (MK-801) binding parameters in rat hippocampus are strikingly altered by changes in buffer concentration

339P **Cartmell J, Kemp JA, Alexander SPH & Kendall DA** Endogenous adenosine regulates the apparent efficacy of 1S,3R-ACPD inhibition of forskolin-stimulated cyclic AMP accumulation in rat cerebral cortical slices

340P **Gardiner SM & Bennett T** Regional haemodynamic responses to dizocilpine maleate (MK-801) in conscious Long-Evans and Brattleboro rats

341P **Iravani MM & Kruk ZL** Effect of NMDA receptor blockade on dopamine overflow in rat caudate putamen

342P **Grimwood S, Struthers L & Foster AC** Autoradiographical analysis of the binding of [^{3}H]L-689,560, an antagonist of the glycine site on the N-methyl-D-aspartate (NMDA) receptor, to rat brain

343P **Carletti R, Ratti E, Gaviraghi G & Bowery NG** Modulation of [^{3}H]dizocilpine binding *in vivo* by ligands for the NMDA receptor complex in mouse brain

344P **Curtis AR & Alexander SPH** Inhibition of 1-aminocyclopentane-1S,3R-dicarboxylate-stimulated phosphoinositide turnover by L-serine-*O*-phosphate

345P **Herdon H** Characterisation of 3,4-diaminopyridine-stimulated endogenous glutamate release from rat hippocampal slices

346P **Laing RJ, Morice AH & Jakubowski J** Reactivity of the rat middle cerebral artery in controls and following ischaemia

347P **Hogg S, Barnes JM, Costall B, Kelly ME & Currie JR** ACE inhibitors reverse the cognitive deficit induced by carotid occlusion

348P **Harder JA, Kelly ME & Currie JR** The influence of the number of daily trials on the acquisition of the Morris water maze task by rats

349P **Davis D & Morinan A** Differences in levels of anxiety in three outbred rat strains

350P **Stanford SC, Gettins D & Baldwin HA** Non-noxious stress and the benzodiazepine inverse agonist, FG7142, have opposing effects on noradrenaline release in rat frontal cortex

351P **Williamson D, Jackson A & Tricklebank MD** The benzodiazepine receptor agonists FG 8205 and diazepam may exert their anticonvulsant action via different mechanisms

352P **Pringle AK, Gardner CR & Walker RJ** The benzodiazepine receptor ligand RU34347 has biphasic actions in the *in vitro* rat cerebellar slice

353P **Goudie AJ & Leathley MJ** Assessment of the dependence potential of the imidazopyridine anxiolytic, alpidem, in rats

354P **Cross AJ, Misra A, Sandilands A & Green AR** Inhibition by chlormethiazole of the harmaline-induced increase in cyclic GMP content in mouse cerebellum

355P **Zeegers A, Leeuwin RS, van Wilgenburg H & Kolossvary E** Cardiac actions of benzodiazepines

356P **Phillips HJ, Blackburn TP, Duffy C, Holmes S & Wood MD** Lack of effect of delta subunit antibodies on $GABA_A$ receptor function in rat cerebellar membranes

357P **Houston AJ, Wong JCL & Ebenezer IS** The effects of systemic administration of baclofen on post-prandial and histamine-induced water intake in rats

358P **Mercy LC, Krane A & Keen M** Phorbol ester treatment does not affect adenosine A_2 or prostacyclin IP receptor function in NG108-15 cells

359P **Clavell DMJ, Watson WP, Ripley TL & Little HJ** Tolerance and cross-tolerance to the sedative effects of dihydropyridines: different patterns of development with felodipine and nitrendipine

360P **Muge DK & Livingston A** The relationship of analgesia produced by xylazine hydrochloride and plasma levels in normal sheep

361P **Menargues A, Obach R & García-Sevilla JA** Effects of aging and chronic clorgyline and desipramine on CNS postsynaptic α_2-adrenoceptors mediating mydriasis in the rat

362P **Crespi F, Corti C, Benedetti R, Ratti E & Marien M** Further evidence that melatonin is monitored *in vivo* by voltammetry

363P **Chong NWS, Evans JE & Sugden D** N-Bromoacetyl 5-methoxy-tryptamine: an irreversible melatonin agonist?

364P **Kay IS & Davies WE** The effects of nimodipine on salicylate ototoxicity in the anaesthetized rat

365P **Pertwee RG, Stevenson LA, Fernando SR & Corbett AD** The production of cannabinoid tolerance in preparations of mouse vas deferens and of myenteric plexus-longitudinal muscle of mouse small intestine

366P **Gurden JM, Penny DM & Watts IS** Development of a model of acute hindlimb ischaemia in the anaesthetised rabbit: effect of pentoxifylline

367P **Hawley J, Rubin PC & Hill SJ** Agonist-induced inositol phospholipid hydrolysis in cultured smooth muscle cells from human umbilical artery

368P **Murphy TV, Cross KMA, Dunning PM & Garland CJ** Differential ability of various vasodilators to relax rat aortae contracted with noradrenaline, phorbol 12,13-dibutyrate and phorbol 12-myristate 13-acetate

369P **Baker GM, Richardson AR, Smith JW & Wilson KA** Changes in reactivity of isolated aorta from young spontaneously hypertensive rats (SHRs)

370P **Lukovic L, Jones CR, Petty MA & de Jong W** α_1- and β_2-adrenoceptors and endothelin receptor density in rat heart after ischaemia and prolonged reperfusion

371P **Thomson DS, Haworth D, Edmonds AE & Carroll JA** Isoprenaline infusion in rats: effect on density and number of ventricular β_1- and β_2-adrenoceptors

372P **Growcott JW, Green M & Holloway B** Atypical β-adrenoceptor antagonist profile of ICI D7114 in guinea-pig and rat isolated ileum

373P **Arnold IR, Mistry RJ & Barnett DB** Regulation of rat cardiac β-adrenoceptor density and coupling to adenylate cyclase following treatment with xamoterol or isoprenaline *in vivo*

374P **Brightling CE, Baker AJ & Fuller RW** Isoprenaline inhibits thromboxane B_2 release from U937 cells

375P **Wenham D & Marshall I** α_1-adrenoceptor subtype(s) in rat thoracic aorta defined using abanoquil and other selective antagonists

376P **Bryant CE, Elliott J, Clarke KC & Soydan J** Characterisation of α-adrenoceptors in the hind limb venous system of the horse

377P **Ribas C, Miralles A & García-Sevilla JA** Long-term treatment with clorgyline accelerates the turnover of brain α_2-adrenoceptors in normotensive (WKY) but not in hypertensive (SHR) rats

378P **Pimoule C, Graham G, Arbilla S & Langer SZ** Characterization of human α_2-adrenergic receptor stably expressed in transfected Chinese hamster ovary cell-lines

379P **Cook SJ, Chiu P, Berry JL, Carpenter JR, Downing SJ & Small RC** Activation of β_1- or β_2-adrenoceptors opens plasmalemmal K^+ channels in airways smooth muscle: evidence from mechanical and electrophysiological studies in guinea-pig trachealis

380P **Krusenberg J, Rübben H, Philipp T, Michel MC & Brodde O-E** α_1-, α_2- and β-adrenoceptor subtypes in human and rat kidney

381P **BoSmith RE, Briggs I, Danks P & Sturgess NC** Actions of ICI D7288 on sinoatrial node cellular electrophysiology: whole-cell currents

382P **Briggs I & Heapy CG** Actions of ICI D7288 on sinoatrial node cellular electrophysiology: action potentials

383P **Rouse W & Johnson IR** Haemodynamic actions of ICI D7288, a novel sino-atrial node modulator

384P **Poucher SM, Brooks R, Pleeth RM, Conant AR & Collis MG** Reduction of myocardial infarction in the ferret by inhibition of purine transport using (s-(p-nitrobenzyl)-6-thioinosine (NBII)

385P **Fox RM, Markham A & Morgan RM** Nitrendipine- and bepridil-induced calcium release from heart mitochondria associated with depolarisation of the membrane

386P **Sammut IA, Markham A & Morgan RM** Acyl compound-mediated modulation of Ca^{2+} transport in rat heart mitochondria *in vitro*

387P **Baird M, Gordon JF, McGrath JC & Spedding M** Etomoxir maintains endothelial function, as measured by acetylcholine relaxation, in rabbit saphenous vein preconstricted with KCl and maintained at 0% oxygen *in vitro*

388P **Tanda K, Williams FM & Williams TJ** Investigation into the role of free radicals in plasma protein leakage in ischaemic/reperfused rabbit myocardium *in vivo*

389P **Cicala C & Page C** The effect of lipopolysaccharide on platelet accumulation in the pulmonary vasculature of the rat

390P **Savineau JP, Robertson TP, Gonzales De La Fuente P, Twort CHC, Marthan R & Ward JPT** Effect of protein kinase C stimulation on tension and calcium concentration in the rat pulmonary artery: modulation by forskolin

391P **Wanstall JC & O'Donnell SR** Inhibition by pinacidil of *in vitro* hypoxic pulmonary vasoconstriction in isolated perfused lungs from pulmonary hypertensive and normotensive rats

392P **Thompson JS, Sheedy W & Morice AH** Neutral endopeptidase inhibitor, SCH 42495, attenuates the development of hypoxic pulmonary hypertension in rats

393P **Gater PR, Paciorek PM, McKean JC, Wilson K, Brewster M & Waterfall JF** Inhibition by potassium channel openers of cholinergic bronchoconstriction in the guinea-pig

394P **Fairbairn SM, Lees P, Page CP & Cunningham FM** Intravenous PAF causes neutrophil recruitment and airway dysfunction in allergic horses

395P **Eaton JD, Stevens EJ, Willars GB & Tomlinson DR** Mechanisms of angiotensin II-induced prostacyclin release in isolated perfused rat lung

396P **Howlett DR & Longman SD** Identification of a binding site for [^{3}H]-cromakalim in vascular and bronchial smooth muscle cells

397P **Pogue JE, McHarg AD & Wallis RM** Bronchodilator and cardiovascular effects of potassium channel openers in the anaesthetised dog

398P **Kelly PAT, Ritchie IM, Wright AK & Arbuthnott GW** Oligaemia induced by direct injection of N^G-nitro-L-arginine methyl ester into the rat striatum

399P **Quine L, Mitchell JA, Gross SS & Vane JR** An LPS-inducible activity in vascular smooth muscle cytosol which converts glyceryl trinitrate to free nitrite

400P **Martinez-Cuesta MA, Whittle BJR & Moncada S** Endogenous nitric oxide regulates motility of the rat isolated duodenum

401P **Oldroyd SD, Haylor JL, El Nahas AM & Morcos SK** Effect of L-nitroarginine methyl ester on the depression of renal function due to contrast media in the isolated perfused rat kidney

402P **Waller J & Wood D** Evidence for modulation of motor responses by inhibitory neurotransmitter in the rat anococcygeus muscle

403P **Kemp PA, Gardiner SM, March JE, Bennett T & Rubin PC** Hindquarters vasodilator effect of $MgSO_4$ in conscious rats: possible involvement of nitric oxide

404P **Paul W, Douglas GJ, Lawrence L, Page CP & Schachter M** L-N^G-nitro arginine methyl ester (L-NAME) reduces plasma protein leakage in the skin of the guinea-pig and rabbit

405P **Stewart-Lee A, Ferns GAA, Nourooz-Zadeh J, Shu-Rui L, Forster L & Ånggård EE** Vitamin E protects against impairment of endothelium-mediated relaxation in the cholesterol-fed rabbit carotid artery

406P **Shaw AM, Barfarez AR, Elbishti W, Pollock D & McGrath JC** The effect of L-N-nitro arginine methyl ester and sodium nitroprusside on 5-HT- and KCl-induced responses in the perfused lung and pulmonary arteries of the rat

407P **Plane F & Garland CJ** Comparison of the effects of nitric oxide and BRL 38227 on membrane potential and tone in the rabbit isolated basilar artery

408P **Tomlinson A, Appleton I & Willoughby DA** Temporal expression and cellular distribution of NADPH diaphorase staining in the chronic granulomatous tissue of the murine croton oil-induced air pouch

409P **Sorrentino R, Warner TD, Mitchell JA, Pinto A & Vane JR** L-NAME reveals an increased sensitivity to the vasoconstrictor effect of U46619 in the mesenteric arterial and venous muscles from rats treated with LPS

410P **Ford AJ & Longridge DJ** The time course of lipopolysaccharide-induced disseminated intravascular coagulation in the rabbit

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