51P THE *IN VIVO* CARDIOVASCULAR PHARMACOLOGY OF THE ADENOSINE ANTAGONIST ZM241385 IN THE CAT AND DOG: COMPARISON WITH THEOPHYLLINE

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ZM241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5- ylamino]ethyl)phenol), is a novel, non-xanthine, A_{2a} selective adenosine receptor antagonist (Poucher et al, 1994). *In vivo* oral activity in the spontaneously hypertensive rat and vascular selectivity in the anaesthetised dog have been confirmed (Keddie et al, 1994). This communication compares the *in vivo* pharmacology of ZM241385 and theophylline in the cat and dog.

In conscious cats with indwelling jugular vein and carotid artery catheters ZM241385 (1- 10 mg kg^{-1} p.o. in polyethylene glycol 400, [PEG 400] within a gelatin capsule) attenuated the diastolic blood pressure falls to exogenous adenosine (i.v.) in a dose dependent manner (maximum inhibition 93 ± 11%, 2 hours post 10 mg kg^{-1}, n=7). Inhibition was observed for up to 9 and 12 hours respectively, following 1 and 3 mg kg^{-1} ZM241385. By comparison theophylline (3 mg kg^{-1} p.o., n=5) produced a maximum inhibition of 48 ± 9%, 2 hours post dosing with no significant activity at 6 hours (31 ± 14%).

In anaesthetised cats ZM241385 administration (1-10 mg kg^{-1}, i.d. in PEG 400, n=3) produced a rapid (within 15 min.) and prolonged (up to 4 hours) attenuation of depressor responses to 2-chloroadenosine (i.v.). A maximum rightward shift of the dose response curve(DRC) in excess of 100-fold was observed at 15 min. and a 12-fold shift remained 4 hours following 3 mg kg^{-1}. Theophylline (6 mg kg^{-1}, i.d., in PEG 400, n=3) produced maximum rightward shift of only 11-fold. Similarly in the anaesthetised dog ZM241385 (3 and 10mg kg^{-1} i.d., in PEG 400, n=4) produced maximal rightward shift of the depressor DRC to exogenous adenosine in the pump perfused denervated hind limb of up to 13-fold at 15 min. post dosing. Significant inhibition (mean dose ratio = 6.7) remained at 6 hours post dosing (10 mg kg^{-1}). Theophylline (6mg kg^{-1}, i.d., in PEG 400, n=4) by comparison produced only a weak adenosine antagonism (max.mean dose ratio = 2.4 at 45 min.) which was not significantly different from control.

Several alkylxanthines including theophylline and enprofylline have positive inotropic actions unrelated to their adenosine antagonist actions (Collis et al 1984). In the barbiturate anaesthetised dog ZM241385 demonstrated no positive inotropic action at doses up to 10mg kg^{-1} i.v.(n=3) yet demonstrated greater potency than theophylline in antagonising adenosine depressor responses. Theophylline at 10 and 20mg kg^{-1} (n=4) demonstrated positive inotropic activity (32 ± 3 and 70 ± 11% increase in $LV^{dP}/_{dt}$ max.).

In conclusion, ZM241385, the selective A_{2a} adenosine receptor antagonist, shows oral activity in conscious cat, demonstrates good duration of action in both cat and dog following intraduodenal dosing and unlike theophylline, is devoid of positive inotropic action.

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52P THE *IN VIVO* CARDIOVASCULAR PHARMACOLOGY OF ZM241385, A NOVEL, NON-XANTHINE, ADENOSINE ANTAGONIST

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ZM241385 (4-(2-[7-amino-2-(2-furyl) [1,2,4] triazolo [2,3-a] [1,3,5] triazin-5-yl amino] ethyl) phenol) is a novel, non-xanthine, A_{2a} selective adenosine receptor antagonist (Poucher et al, 1994). This communication describes the selectivity and oral activity of the compound *in vivo*.

Vascular selectivity was assessed in female beagles (12 - 18kg), anaesthetised with sodium pentobarbitone (45 - 50 mg kg^{-1}, i.v.) and artificially ventilated. The right hindlimb was denervated and perfused by pump at a constant flow. Adenosine (0.001 - 3 mg kg^{-1}) was administered into the left ventricle via a catheter advanced from the left common carotid artery. Nitrobenzyl thioinosine (0.5 mg kg^{-1}, i.v.) was given to inhibit purine transport. The bradycardia and hind limb vasodilatation produced by adenosine were measured before and after administration of ZM241385 (0.3 - 3 mg kg^{-1}, i.v. in 50% polyethylene glycol 400 / 50% 0.1M NaOH). Potency was expressed as the dose required to produce a two-fold rightward shift of the adenosine dose response curve (DR_2).

Oral activity and duration were assessed in conscious, spontaneously hypertensive rats (SHR) which had catheters implanted in the jugular vein and carotid artery 24 hours previously for administration of adenosine and measurement of blood pressure respectively. Systemic blood pressure and pulse rate responses to adenosine (1 mg kg^{-1} min^{-1}, i.v.) were measured before and after ZM241385 (1-10 mg kg^{-1}, p.o. in polyethylene glycol 400).

In the dogs, ZM241385 produced parallel rightward shifts of the adenosine dose response curve in the perfused hind limb (DR_2 = 0.02 ± 0.004 mg kg^{-1}, n= 9 dogs) whilst demonstrating negligible antagonism to the bradycardic response (DR_2 = 2.8 ± 1.7 mg kg^{-1}, n=6 ie. only those dogs demonstrating dose ratios above unity at 1 and/or 3mg kg^{-1}).

In conscious SHR, mean arterial blood pressure (MABP) was 175 ± 3 mmHg at rest with the adenosine infusion resulting in a mean decrease of MABP of 69 ± 3 mmHg. ZM241385 (1-10mg kg^{-1} p.o.) resulted in a dose dependent attenuation of the blood pressure responses to adenosine infusion. At 1, 4 and 6 hours post dosing of ZM241385(10mg kg^{-1}) the decrease in MABP to adenosine was inhibited by 43 ± 9, 49 ± 5, 44 ± 7 % respectively (mean ± sem, n=11-14, $P<0.01$). The bradycardia produced by adenosine was not inhibited by ZM241385.

In conclusion, these results show that ZM241385, the selective A_{2a} adenosine receptor antagonist, is a selective adenosine receptor antagonist *in vivo* and shows oral activity with a long duration of action in the rat.

Poucher S.M.,Collis M.G., Keddie, J.R. *et al* (1994).C109 Brighton B.P.S.

53P ANALYSIS OF THE PHARMACOLOGICAL PROFILE OF HUMAN BRAIN A_{2b} ADENOSINE RECEPTOR TRANSFECTED INTO CHINESE HAMSTER OVARY (CHO-K1) CELLS

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The present classification of adenosine receptors (Fredholm *et al.*, 1994) allows their division into subtypes which are coupled to inhibition (A_1 and A_3) or stimulation of adenylyl cyclase activity (A_{2a} and A_{2b}). We have recently characterized the A_2 adenosine receptor mediating a stimulation of cAMP accumulation in human embryonic kidney (HEK 293) cells as being of the A_{2b} subtype on the basis of agonist potency and antagonist affinity (Cooper *et al.*, 1995). We now report the properties of an A_{2b} adenosine receptor which has recently been cloned from human brain (Pierce *et al.*, 1992).

[^{3}H]-cAMP accumulation was monitored in [^{3}H]-adenine-pre-labelled Chinese hamster ovary (CHO-K1) cells, transfected with the human brain A_{2b} adenosine receptor, at 37°C for 15 minutes in the presence of the phosphodiesterase inhibitor rolipram (100 µM) according to a previously published procedure (Peakman & Hill, 1994). Antagonist affinities were calculated using increasing antagonist concentrations in the presence of a fixed concentration of 5'-N-ethylcarboxamidoadenosine (NECA, 10 µM, Peakman & Hill, 1994). Data (means ± SEM) presented were derived from experiments conducted on at least three separate cell preparations and were initially expressed as [^{3}H]-cAMP production as a percentage conversion from total [^{3}H]-adenine nucleotides.

Basal accumulation of [^{3}H]-cAMP was 0.25 ± 0.03 % conversion. The maximal response to NECA generated a stimulation of [^{3}H]-cAMP to 2.71 ± 0.22 % conversion. Adenosine analogues evoked a concentration-dependent stimulation of [^{3}H]-cAMP accumulation with the rank order of potency: NECA (1.4 ± 0.1 µM) > adenosine (2.5 ± 0.4 µM) > 2-chloroadenosine (5.6 ± 1.0 µM). CGS 21680 failed to generate a significant response at concentrations up to 10 µM. Maximal responses to the agonists compared to 100 µM NECA were 34 ± 6 and 61 ± 6 % for adenosine and 2-chloroadenosine, respectively. Adenosine receptor antagonists antagonised NECA-evoked cAMP generation with the rank order of affinity: xanthine amine congener (13 ± 1 nM) > 8-cyclopentyl-1,3-dipropylxanthine (75 ± 21 nM) > PD 115,199 (151 ± 9 nM).

Antagonist affinities at the transfected human A_{2b} receptor are therefore very similar to our recent observations of the antagonist profile at A_{2b} adenosine receptors of intact HEK 293 cells (Cooper *et al.*, 1995). However, the potencies of the agonists, adenosine in particular, was increased at the transfected receptor compared to the A_{2b} receptor native to HEK 293 cells. Whether these differences in agonist potency reflect differences in receptor expression levels must await the development of a radioligand binding assay for the A_{2b} receptor.

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54P A_2 ADENOSINE RECEPTOR RELAXATION OF GUINEA-PIG ISOLATED, PRE-CONTRACTED TRACHEAL RINGS

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Adenosine receptors may be divided into subtypes (Fredholm *et al.*, 1994) which are coupled to inhibition (A_1 and A_3) or stimulation of adenylyl cyclase activity (A_{2a} and A_{2b}). However, adenosine receptor responses in smooth muscle are better characterised on a pharmacological basis rather than a biochemical one. For example, we have recently investigated the A_2 adenosine receptor which relaxes the guinea-pig isolated, pre-contracted aorta, and defined it as of the A_{2b} subtype on the basis of agonist potency and antgonist affinity (Alexander *et al.*, 1994). We report here that the pharmacological profile of the A_2 receptor mediating relaxation of guinea-pig tracheal rings (Collis *et al.*, 1989) is similar to but distinct from either the A_{2b} receptor we have previously described in the guinea-pig, or the A_{2a} receptor in the guinea-pig striatum defined by [^{3}H]-CGS 21680 binding (Alexander, this meeting).

Guinea-pig (200-600 g, either sex) tracheal rings (4/5) were attached to each other and to an isometric transducer with cotton suture and pre-contracted with 0.3 µM carbachol. Cumulative concentration-relaxation curves to 5'-N-ethylcarboxamidoadenosine (NECA) were carried out in the absence and presence of adenosine receptor antagonists to calculate antagonist affinities (Alexander *et al.*, 1994). All data (means ± SEM) presented were from experiments conducted on at least three different preparations.

NECA was more potent than 2-chloroadenosine at inducing concentration-dependent relaxations (EC_{50} values of 0.46 ± 0.04 and 6.1 ± 1.0 µM, respectively). However, the maximal relaxation elicited by 2-chloroadenosine was greater than that for NECA (208 ± 38 % vs 73 ± 7 % contractile response). Comparison of the effects of adenosine and it's analogues at 10 µM showed the following magnitudes of relaxation: 2-chloroadenosine (75 ± 16 %) = NECA (69 ± 16 %) > CGS 21680 (11 ± 2 %) = adenosine (6 ± 4%).

PD 115,199 and xanthine amine congener were almost equipotent as antagonists of the NECA-induced relaxations with calculated K_i values of 27 ± 8 and 43 ± 11 nM, respectively. The putative A_{2a}-selective antagonist CP 66713 was of similar affinity to the A_1-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (285 ± 89 and 316 ± 114 nM, respectively).

The agonist potency data are therefore similar to our previous observations of A_2 adenosine receptor relaxation of the isolated aorta (Alexander *et al.*, 1994). However, the antagonist affinities, in particular the affinity of PD 115,199, imply that the tracheal A_2 receptor is distinct from the A_2 receptors we have previously described in guinea-pig cerebral cortex or aorta (Alexander *et al.* 1994) or neostriatum (Alexander, this meeting). Alternatively, these results might be explained by the presence of a mixed population of A_{2a} and A_{2b} adenosine receptors in this tissue.

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55P A_{2a} ADENOSINE RECEPTORS IN THE GUINEA-PIG NEOSTRIATUM: CYCLIC AMP GENERATION AND [^{3}H]-CGS 21680 RADIOLIGAND BINDING

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We have recently reported on the pharmacological profile and signal transduction characteristics of A_1 and A_{2b} adenosine receptors in the guinea-pig CNS (Alexander *et al.*, 1994a; 1994b; Hernández *et al.*, 1993). The present report sets out to define the characteristics of [^{3}H]-CGS 21680 binding, a purported A_{2a}-selective ligand (Jarvis *et al.*, 1989; Lupica *et al.*, 1990), to particulate preparations from the guinea-pig neostriatum, and examines the potential coupling of A_{2a} receptors to cAMP generation in the same tissue.

Radioligand binding was conducted using particulate preparations (20 000 g for 15 minutes x 3) of guinea-pig neostriatum at room temperature for 60 minutes at pH 7.4 in the presence of 10 mM $MgCl_2$, 1.2 U/ml adenosine deaminase and 0.01 % Triton X-100 in a total volume of 200 µL. [^{3}H]-cAMP accumulation in [^{3}H]-adenine-labelled neostriatal slices was carried out as previously described (Alexander *et al.*, 1994a). All data presented (means ± SEM) are from experiments conducted on at least three different preparations.

[^{3}H]-CGS 21680 bound with high capacity and affinity to guinea-pig neostriatal membranes (B_{max} 1326 ± 232 pmol/mg protein; K_i 23 ± 2 nM). Adenosine receptor antagonists competed for [^{3}H]-CGS 21680 binding with the rank order of affinity: PD 115,199 (37 ± 2 nM) > 8-cyclopentyl-1,3-dipropylxanthine (305 ± 40) > xanthine amine congener (1206 ± 160).

In guinea-pig striatal slices, the non-selective adenosine receptor agonist NECA was able to elicit a stimulation of cAMP levels to 2820 ± 848 % control, with an EC_{50} value of 2.0 ± 0.6 µM. CGS 21680, however, failed to elicit a stimulation of [^{3}H]-cAMP accumulation at concentrations up to 10 µM (173 ± 50% control). In the presence of the phosphodiesterase inhibitor rolipram (10 µM), responses to 10 µM NECA and 10 µM CGS 21680 were unchanged (NECA 2204 ± 604 %, CGS 21680 110 ± 5 % control).

These results indicate that the binding site labelled by [^{3}H]-CGS 21680 in guinea-pig neostriatal membranes is similar to that in the rat (Jarvis *et al*, 1989) - the A_{2a} receptor. However, the lack of stimulation of cAMP levels in the intact slice by CGS 21680 suggests that A_{2a} receptors are not efficiently coupled to adenylyl cyclase activity in the guinea-pig neostriatum. Furthermore, these results suggest that the affinity of the three xanthine-based antagonists described here may be used to define subtypes of adenosine receptor in the guinea-pig. That is, 8-cyclopentyl-1,3-dipropylxanthine is A_1-selective (Alexander *et al.*, 1994a), PD 115,199 is A_{2a}-selective, while xanthine amine congener is A_{2b}-selective (Alexander *et al.*, 1994b).

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56P ANTAGONIST AND ANTIHYPERTENSIVE PROFILES OF THE ANGIOTENSIN AT_1 RECEPTOR ANTAGONIST, GR138950

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Angiotensin AT_1 antagonists, like GR117289 and GR138950, reduce diastolic blood pressure (DBP) in renal artery ligated hypertensive (RALH) rats. However, this effect is not temporally correlated to their antagonist activity against AII-induced pressor responses (Hilditch *et al.*, 1994; 1995). In these experiments, we have compared the antihypertensive profile of GR138950 in conscious RALH rats with its antagonist profile against AI in conscious normotensive (NT) rats, and with data obtained previously using AII. In addition, angiotensin AT_1 antagonist activity was examined *ex-vivo* in aortae removed from RALH rats or NT rats treated with vehicle or GR138950.

RALH rats or NT rats were prepared as described previously (Hilditch *et al.*, 1994). After obtaining control measurements of DBP, vehicle (1ml kg^{-1} ia) or GR138950 (1mg kg^{-1} ia) was administered to RALH rats and DBP was measured 1, 5, 24 and 48h later. In NT rat studies, pressor responses to AI were obtained before and after vehicle or GR138950 treatment. In separate studies, rats were treated with vehicle or GR138950 and culled 1, 5, 24 and 48h later. Their aortae were removed and set up in isolated organ baths (Krebs solution; indomethacin 30µM; 1g tension at 37°C). Individual tissues were treated with a single concentration of AI (10^{-10}-10^{-4}M; Eglème *et al.*, 1990). Vehicle administration had no significant effect on DBP (<5mmHg; P>0.05). GR138950 reduced DBP in RALH rats, the maximum effect occurring 5h after administration (62mmHg; P<0.05) and the effect lasted for up to 48h. Vehicle administration had no effect on AI or AII-induced pressor responses (Agonist Dose-Ratio <2). GR138950 inhibited responses to both AI and AII but was more potent against AI than against AII-induced pressor responses. Maximum antagonism occurred 1h after administration. However, antagonism was not evident at 24h against AII-induced pressor responses whereas the pressor effect of AI was still inhibited markedly 24h after administration and, to a much lesser degree, at 48h (Table 1). AI concentration-effect curves in aortae isolated from RALH rats or NT rats were almost abolished 1 and 5h after treatment with GR138950. There was some recovery at 24h which was almost complete at 48h after administration compared to vehicle-treated-rats.

Table 1: GR138950-induced antagonism against pressor responses produced by administration of AI or AII (*Hilditch *et al.*, 1995) in NT rats. Results shown are geometric means (95% confidence limits; n=7) of the AI or AII dose-ratios, 1-48h after GR138950 administration.

Hours	1	5	24	48
AI	1406 (457-4322)	1183 (403-3468)	99 (59-165)	4 (2-8)
AII*	76 (43-133)	42 (30-58)	2 (1-4)	-

Overall, these results show that GR138950 exerts more marked and more prolonged antagonism of the pressor responses elicited by AI than by AII. Consequently, the time-course of the blockade of AI correlates better with the antihypertensive profile of GR138950. Thus, the renin-angiotensin system in vascular smooth muscle may be implicated in this phenomenon.

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57P IDENTIFICATION OF ANGIOTENSIN RECEPTORS IN THE NORMAL RAT KIDNEY EPITHELIAL CELL LINE, NRK 52E

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Angiotensin II (AII) stimulates sodium reabsorption from the renal proximal tubule via a Na^+-H^+ antiport on the epithelial membrane (Harris & Young, 1977). The rat kidney cell line NRK 52E has characteristics of tubular epithelium (De Larco & Todaro, 1978), but the expression of AII receptors by these cells is not reported. We have studied this using radioligand binding and estimation of intracellular calcium ion levels (Ca^{2+}_i) and cyclic AMP (cAMP).

NRK 52E cells (passage 28 to 32) were grown in Dulbecco's modified Eagle medium plus 10% foetal calf serum. Cells were harvested, stored at -70°C and on thawing, were homogenised and the resulting membranes suspended in buffer (50mM Tris, 5mM $MgCl_2$, 0.1mgml^{-1} phenylmethylsulphonylfluoride, 0.5% bovine serum albumin, 0.1% bacitracin). Membranes (248µg protein, 0.2ml) were incubated for 90min at room temperature with 0.2nM ^{125}I Sar^{1}Ile^8 AII (sarile) in the presence or absence of the AT_1 receptor antagonist, losartan, or the AT_2 receptor ligand, PD123177 (0.01 and 1µM). Non-specific binding was determined in the presence of 3µM AII. Poor specific binding was obtained using lower concentrations of membranes or sarile. In separate studies, cells were loaded with fura 2-AM (2µM) and Ca^{2+}_i estimated in the absence and presence of AII (0.1-10µM) from fluorescent images recorded at 510nm, following excitation at 350/380nm. Ca^{2+}_i was expressed as a ratio of fluorescence intensity. Other cells were preincubated in serum-free medium plus 3-isobutyl-1-methyl xanthine (300µM) in the absence and presence of forskolin (1µM) and AII (1µM). Cyclic AMP (expressed as pmoles cAMP per mg protein (pmol mg^{-1})) was measured by enzyme linked immunoassay.

Specific binding of sarile was 53±7% of total binding (1.2±0.4% of total counts added). In the presence of losartan (0.01 and 1µM), specific binding was significantly reduced ($P<0.05$, Student's t- test) by 42±9% and 101±1%, respectively (n=4). Binding was also reduced 21±11% and 34±9% ($P<0.05$) by PD123177 at 0.01 and 1µM, respectively (n=4). In fura 2-AM loaded cells, AII failed to elicit a rise in Ca^{2+}_i (ratio after AII 10µM = 0.20±0.01, n=3) but ionomycin (10µM) increased Ca^{2+}_i in all cells tested (ratio = 2.88±0.09). AII did not affect either basal cAMP levels (control 19.1±10.9, +AII 11.4±4.1 pmol mg^{-1}, n=6) or forskolin-stimulated cAMP levels (control 47.5±10.5, +AII 54.3±15.0 pmol mg^{-1}, n=6).

These results suggest that NRK 52E cells express a low density of AII receptors, which are predominantly of the AT_1, losartan sensitive subtype, although the effect of PD123177 may indicate a small population of AT_2 receptors. The receptors do not appear coupled to generation of intracellular calcium or modulation of cAMP levels.

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58P EFFECTS OF LOSARTAN ON HAEMODYNAMICS IN CONSCIOUS, VASOPRESSIN-DEFICIENT HYPERTENSIVE RATS

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Vasopressin-deficient, hypertensive (DI/H) rats show apparently normal vasodilator function (Gardiner *et al.*, 1994), so other factors are likely to be responsible for the maintenance of the hypertension. In the absence of vasopressin, mean arterial blood pressure (MAP) can become dependent on the renin-angiotensin system, particularly in the volume deplete state (Gardiner *et al.*, 1989). We have therefore assessed the effects of the angiotensin (AT-1) receptor antagonist, losartan, on haemodynamics in conscious male DI/H rats and age-matched normotensive controls (DI/N rats) (Gardiner *et al.*, 1994), in the volume replete state and following volume depletion induced by s.c. injection of polyethylene glycol (PEG; Carbowax 20M, 30% in 5 ml isotonic saline (Gardiner *et al.*, 1989)). Rats (5-6 months old, 400-450g) were instrumented with pulsed Doppler flow probes and intravascular catheters to monitor regional haemodynamics, and s.c. catheters for the administration of PEG. All surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.) and experiments began at least 24 h after the last surgical procedure (catheterization). Saline (5 ml) or PEG, warmed to body temperature, was injected s.c. over a period of 4-5 min, and losartan (10 mg kg^{-1} i.v.) was administered 5 h later. The results are summarized in Table 1.

In the volume replete state, losartan caused a fall in MAP associated with renal and mesenteric vasodilatations in both groups; the mesenteric vasodilator response to losartan was significantly greater in DI/N rats than in DI/H rats ($P < 0.05$; Mann Whitney U-test). Sixty min after administration of losartan in the volume deplete state, both groups showed similar falls in MAP, and increases in HR and renal and mesenteric vascular conductance, but the hindquarters vasodilator response to losartan was significantly ($P < 0.05$) smaller in the DI/H rats. Thus, in the volume replete and volume deplete state there is no evidence of an increased dependence of cardiovascular status on the renin-angiotensin system in DI/H, compared to DI/N, rats.

Table 1. Cardiovascular variables before (C) and 60 min after losartan (L). Values are mean ± s.e. mean; * $P < 0.05$ Friedman's test. R = renal, M = mesenteric, H = hindquarters vascular conductance (VC; units = kHz mm Hg^{-1} 10^3).

Volume replete

	DI/N (n = 7)		DI/H (n = 8)	
	C	L	C	L
HR (beats min^{-1})	334 ± 8	379 ± 13*	349 ± 14	364 ± 17*
MAP (mm Hg)	118 ± 3	108 ± 3*	166 ± 6	151 ± 7*
RVC (units)	53 ± 13	66 ± 15*	47 ± 6	58 ± 8*
MVC (units)	92 ± 6	123 ± 11*	81 ± 11	93 ± 12*
HVC (units)	39 ± 4	44 ± 9	24 ± 1	27 ± 3

Volume deplete

	DI/N (n = 9)		DI/H (n = 9)	
	C	L	C	L
HR (beats min^{-1})	384 ± 12*	463 ± 13*	338 ± 14	382 ± 13*
MAP (mm Hg)	122 ± 2	90 ± 3*	153 ± 4	124 ± 4*
RVC (units)	51 ± 6	77 ± 9*	40 ± 5	60 ± 7*
MVC (units)	70 ± 4	103 ± 7*	55 ± 5	76 ± 5*
HVC (units)	37 ± 4	60 ± 5*	20 ± 2	26 ± 3*

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59P EFFECT OF THE NON-PEPTIDE ANGIOTENSIN II RECEPTOR ANTAGONIST, LOSARTAN, ON RENAL FUNCTION IN 2K 2C GOLDBLATT HYPERTENSIVE RATS

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Antihypertensive therapy with angiotensin converting enzyme (ACE) inhibitors in renovascular hypertensive individuals or patients with renal artery stenosis carries a high risk of acute renal failure and they are contraindicated. It is likely that the raised circulating angiotensin II levels in these individuals elevates blood pressure but within the kidney causes a preferential efferent arteriolar vasoconstriction to maintain filtration at the glomerulus. Recently, we have used the 2K2C Goldblatt hypertensive rat in which the renin-angiotensin system is activated and renal function is renin-dependent as a model to show that reduction of blood pressure to normotensive levels with the ACE inhibitor, captopril, but not the non-specific vasodilator, hydralazine, caused renal failure. The question addressed in this study was whether the non-peptide angiotensin II receptor antagonist losartan would similarly act to depress kidney function in this model.

Male Wistar rats, 150-170g, were anaesthetised (N_2O/O_2/halothane) and 0.25mm diameter silver clips applied to both renal arteries close to the aorta. A normal sodium diet (0.32% Na^+ content) was fed for four weeks and they were supplied with a no sodium diet (I.C.N.) and distilled water for a further two weeks. The animals were then put into metabolic cages and received, by gavage, either vehicle (distilled water 0.3ml/100g), captopril (30mg/kg), losartan (30mg/kg) once per day or hydralazine (35mg/kg) every 12h for four days. Systolic blood pressure was measured by tail cuff plethysmography each day and at the end of this period the animals were sacrificed and an arterial blood sample taken for the estimation of plasma urea and creatinine.

The data show that hydralazine caused a marked depression in blood pressure but had no effect on plasma urea and creatinine. By contrast, reduction of blood pressure to the same extent with both losartan and captopril resulted in marked increases in plasma levels of both urea and creatinine, indicative of acute renal failure. These findings reinforce the concept that angiotensin II has an important role in the maintenance of glomerular filtration rate, particularly in this model of hypertension with activation of the renin-angiotensin system. They further suggest that the non-peptide angiotensin II antagonists should be used with caution therapeutically in patients with renovascular hypertension.

Table 1

Group	Systolic blood pressure (mmHg)				Plasma	
	Control	+24h	+48h	+72h	Urea (mmol)	Creatinine (µmol)
Vehicle (n=7)	273±13	273±11	279±10	266±17	7.3±0.5	44.7±1.3
Hydralazine (n=6)	297±3	153±9***†††	207±9***†††	192±9**†††	8.9±0.9	64.5±3.9
Captopril (n=6)	287±7	177±30***††	165±29**†††	182±31***††	43.1±0.9†††	208.2±30.7†††
Losartan (n=7)	270±4	180±17**††	146±15***†††	132±12***†††	52.3±6.7†††	174±22†††

* P<0.05 ; **P<0.01; ***P<0.001 compared to control. †† P<0.01 and ††† P < 0.001, compared to vehicle using Student's 't' test .

60P APPARENT ENHANCEMENT OF ENDOTHELIN-1 EFFECTS BY ET_A ANTAGONISTS IN THE RAT ELECTRICALLY STIMULATED VAS DEFERENS

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Endothelin-1 (ET-1) and endothelin-3 (ET-3) both potentiate the twitch response of the rat electrically stimulated vas deferens (RVD) but the effects of ET-3 are more easily antagonised by the selective ET_A antagonist BQ123 than those of ET-1 (Eglezos et al. 1993). Previous, unreported experiments at ZENECA had shown that BQ123 (1µM) enhanced the response of the RVD to ET-1. The aim of this study was to determine, by using four structurally diverse ET_A antagonists, whether the anomalous enhancement of ET-1 by BQ123 was produced by specific ET_A antagonist effects (as opposed to effects on ET-1 breakdown etc.) and whether similar enhancement could be seen vs. ET-3.

RVD were mounted for isotonic recording in 20ml organ baths containing oxygenated Krebs solution at 32° C. and stimulated every 10s by single 3ms rectilinear pulses at just maximal voltage. Two cumulative partial concentration response curves (CRCs) to either ET-1 or ET-3 were constructed (the second 150 min after the first) on each tissue. The second curve was either used as a control or constructed after a contact time of 30 min in the presence of an antagonist. The anagonists were tested vs. ET-1 and ET-3 and all four were found to enhance ET-1 responses at concentrations that antagonised ET-3 responses. No concentration of any antagonist was found to enhance ET-3. When tested at concentrations 10-30 times higher than those producing enhancement, the antagonists antagonised the effects of ET-1. All results are shown in Table 1.

We conclude from these results that the enhancement of ET-1 by the antagonists is due to a specific antagonism at the ET_A receptor and can be explained by ET-1 producing two opposing effects in the RVD, a potentiation of the twitch, only antagonised by high concentrations of ET_A antagonists and a smaller, opposing and more easily antagonised, inhibition of the twitch, which is antagonised by concentrations of antagonist similar to those required to antagonise the potentiating effects of ET-3. We believe that ET-3 is probably also producing an inhibitory effect that is not apparent in these experiments as both the inhibitory and excitatory effects are equally antagonised by the antagonists

Table 1 . Concentration ratios of 2nd. cf. 1st. CRC

Antagonist	vs. ET-1	vs. ET-3
Control	2.02±0.39	0.88±0.11
BQ123 1µM	0.4±.0.04 *	9.8±0.97 *
FR139317 0.1µM	-	1.75±0.56
FR139317 1µM	0.43±0.06 *	4.3 ± 1.44
FR139317 30µM	13.15 ± 3.98 *	-
Ro46-2005 30µM	0.5 ± 0.15*	15.8 ± 2.9 *
BMS182874 3µM	0.42 ± 0.41*	3.98 ± 1.3
BMS182874 30µM	2.93 ± 0.41	46 ± 18 *

* P<0.05 for unpaired Students "t" test comparing antagonists CRCs to relevant control.

Eglezos, A., Cucchi, P., Patacchini, R. et al. (1993) *Br. J. Pharmacol*,**109**,736-738.

61P SELECTIVE PROTEOLYTIC ACTIVATION AND DEGRADATION OF ETs AND BIG ETs IN PARENCHYMAL STRIPS OF THE GUINEA-PIG LUNG

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ET-1 is formed by an ET-converting enzyme (ECE) from a 38 or 39-amino acid precursor by cleavage between Trp^{21}-Val^{22}. A similar process may well form ET-2 and ET-3, from bET-2_{1-37}, bET-2_{1-38} or bET-$3_{1-41.\ amide}$. Several peptidases have ECE-like activity, but a membrane-bound phosphoramidon-sensitive neutral metalloendoprotease is the one that has been most studied, resulting in its recent purification, cloning and expression.

Here, we have compared the effects of the endothelin precursors (bET-1_{1-38}, bET-2_{1-38} and bET-$3_{1-41amide}$) and their respective mature peptides on parenchymal strips of the guinea-pig lung, in the absence or presence of three protease inhibitors.

Guinea-pigs (300-400g) were killed by cervical dislocation. The heart and lung were then excised and placed in Krebs' solution (37°C; 95%O_2:5%CO_2) . Strips of peripheral parenchyma were prepared, mounted in 10 ml organ baths and connected to auxotonic force-displacement transducers under a tension of 1.5 g, and allowed to stabilize for 1h with washing every 15 min.

In a first set of experiments, contractions induced by 10, 100 and 1000 nM of ET-1, ET-2, ET-3, bET-1_{1-38}, bET-2_{1-38} and bET-$3_{1-41amide}$ were recorded. In a second set of experiments, tissues were incubated with phosphoramidon, DL-thiorphan, or captropril (300 μM) for 30 min before addition of the peptides. Only one response to a single agonist was recorded on each strip. The contractile responses were compared to the contraction induced by 80 mM KCl.

ET-1, ET-2 and ET-3 (10, 100 and 1000 nM) induced similar concentration-related contractions (ET-1; 12.8±2.7, 22.8±2.6, 33.9±3.0: ET-2; 9.6±3.9, 18.8±1.8, 30.5±1.9: ET-3; 11.4±1.2, 18.5±1.9, 30.1±2.9%). Big ET-1_{1-38} (6.4±1.3, 25.3±2.6, 57.2±5.1%) and bET-2_{1-38} (2.6±0.7, 21.6±3.4, 58.6±4.5%) also produced similar concentration-related contractions. Big ET-$3_{1-41.\ amide}$ was inactive (n=5-9).

Contractions induced by 1000 nM of bET-1_{1-38} or bET-2_{1-38} were greater than those induced by the same concentrations of the respective mature peptides. Phosphoramidon (300 μM) inhibited the contractions induced by the three concentrations of bET-1_{1-38} by 100, 90, 32%, respectively. DL-thiorphan also inhibited the contractions induced by 10 and 100 nM bET-1_{1-38} by 100 and 48% while potentiating the last concentration used by 150%. Phosphoramidon or DL-thiorphan similarly inhibited contractions induced by bET-2_{1-38} and potentiated contractions induced by ET-1 (100, 1000 nM) by 33 and 197% and 63 and 219%, respectively. Similarly, contractions induced by ET-2 were also potentiated in the presence of either metalloendoprotease inhibitors by 81, 67, and 135%, and by 66, 39, and 53%, respectively. Phosphoramidon or thiorphan also potentiated contractions induced by ET-3 by 71, 60 and 99%, and by 58, 53 and 76%, respectively. Captopril had no effect on contractions induced by either agonists.

Thus, bET-1 and bET-2 are similarly potent in contracting parenchymal strips of the guinea-pig lung while big ET-3 is inactive suggesting that the ECE which converts bET-3 is not present and that at least two distinct ECE activities exist, one selective for bET-1 and bET-2 and one for bET-3. Phosphoramidon and DL-thiorphan, but not captopril, inhibited the contractions elicited by bET-1 and bET-2 but DL-thiorphan was less active, suggesting that a non-selective enzymatic process is involved in conversion of bET-1 and bET-2 in addition to a phosphoramidon-sensitive ECE. Big ET-1 and bET-2 also induced much higher contractions than their corresponding mature peptides. Both metalloendoprotease inhibitors, but not captopril, similarly potentiated contractions induced by ET-1, ET-2 or ET-3 to the level of those evoked by bET-1 and bET-2 indicating that only mature ET isopeptides and not their precursors are suceptible to degradation by metalloendoproteases.

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62P ENDOTHELIN-INDUCED MODULATION OF TISSUE PLASMINOGEN ACTIVATOR (tPA) SECRETION BY HUMAN CULTURED UMBILICAL VEIN ENDOTHELIAL CELLS

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The endothelins (ET-1, ET-2 and ET-3) are a family of peptides that possess vasoconstrictor and mitogenic properties. They exert their effects via actions at distinct receptor sub-types; ET_A receptors being selective for ET-1 and ET-2, whilst ET_B receptors bind ET-1, ET-2, ET-3 and sarafotoxin 6c (S6c) with similar affinities (Douglas *et al.*, 1994). Recently, ETs have also been reported to depress tPA secretion by endothelial cells (Kaji *et al.*, 1992). Since tPA is an important mediator of fibrinolysis, ETs may suppress fibrinolytic activity thereby leading to localised fibrin deposition, endothelial cell damage and subsequent development of chronic vascular disease (Handt *et al.*, 1994; Ross 1993). The aims of this study were: (i) to evaluate the effects of ET-1 and ET-3 on tPA secretion by endothelial cells; (ii) to identify the ET receptor sub-types involved by using a selective ET_B receptor agonist (S6c), plus mixed and selective receptor antagonists (SB209670, bosentan, BQ123 and BQ788; see Douglas *et al.*, 1994).

Human umbilical vein endothelial cells (HUVECs) were cultured in 24 well-plates in Medium 199 containing 30 µg/ml endothelial cell growth supplement, 5 IU/ml heparin, 2% foetal calf serum and protease inhibitors (100 µg/ml leupeptin, 250 µg/ml bacitracin and 0.1 mM AEBSF). The cells were incubated with varying concentrations of agonists and/or antagonists/vehicle for 24 h at 37°C. The amounts of tPA and its fast acting inhibitor, PAI-1, released into the culture medium over this period were then determined using ELISA kits.

TPA and PAI-1 concentrations in HUVEC conditioned medium, following 24 h incubation in the absence of ET receptor agonists and antagonists, were 0.31±0.03 and 26.4±2.6 ng/µg cell protein respectively (n=8 experiments). ET-1, ET-3 and S6c each produced concentration-related inhibition of tPA secretion. Maximal inhibition of tPA secretion for ET-1 (100 nM), ET-3 (3 nM) and S6c (100 nM) was 39%, 25% and 35%, respectively (calculated from means of 4 replicate wells per control/treatment group). No significant change in the amounts of PAI-1 secreted were detected. Surprisingly, the mixed receptor antagonists, SB 209670 (0.01-10 µM) and bosentan (0.1-100 µM) failed to reverse the activity of exogenous ET-1 (100 nM) on tPA. Indeed SB209670 (0.01-10 µM) & bosentan (1-100 µM) given alone depressed significantly tPA secretion by up to 44% and 36% respectively. The selective ET_B antagonist, BQ788 (100 nM), also reduced tPA secretion by 55% whereas the selective ET_A antagonist, BQ123 (10 nM-10 µM), had no effect. The reason for the similar activities of the agonists and antagonists is unknown; but, in light of the findings with S6c and BQ788, it is likely that ET_B receptors mediate these effects. It is possible that endogenous ET elevates tPA secretion from HUVECs above basal levels, whereas higher concentrations of exogenous ETs (acting via an alternative pathway) oppose this stimulation. Similar stimulatory /inhibitory mechanisms have recently been described for cyclic AMP formation following binding of ET-1 to ET_A receptors (Sokolovsky *et al.*, 1994).

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63P THE ENDOTHELIN ANTAGONIST FR139317 ATTENUATES THE CEREBROVASCULAR EFFECTS OF N^G-NITRO-L-ARGININE METHYL ESTER *IN VIVO*

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Inhibition of nitric oxide (NO) synthesis results in a decrease in local cerebral blood flow (LCBF) *(Kelly et al., 1994)*, suggesting that NO provides cerebrovascular dilator tone *in vivo*. Although the endothelium also has the capacity to produce endothelin, there is as yet no evidence that this vasoconstrictor substance has any physiological role in the regulation of LCBF. The purpose of the present study was to determine whether endogenous endothelin (ET) might be involved in producing the decreases in LCBF observed following treatment with N^G-nitro-L-arginine methyl ester (L-NAME).

Male Sprague-Dawley rats (n = 20) were anaesthetized (2% halothane in 70% N_2O: 30% O_2) for the stereotactic placement of unilateral guide cannulae into the striatum. Ten days after implantation, a needle was inserted into the guide cannulae of the conscious, partially restrained animals, for the infusion of either the specific ET_A-receptor antagonist FR139317 (10^{-6}M in CSF) or mock CSF alone. The intrastriatal infusions commenced at a rate of $1\mu l.min^{-1}$ for 60s prior to the i.v. injection of either L-NAME (35mg.kg^{-1}) or saline (n = 5 in each of the four treatment groups), and were continued ($0.2\mu l.min^{-1}$) for the following 20min, at the end of which LCBF was measured using the [^{14}C]-iodoantipyrine autoradiographic technique *(Sakurada et al., 1978)*. Data (presented as mean ± S.D.) were analysed using Scheffé's test with levels of significance set at $P < 0.05$.

Striatal LCBF was significantly reduced (-28%) following i.v. injection of L-NAME in those rats infused with CSF into the striatum. Intrastriatal infusion of FR139317 alone had no significant effect upon striatal LCBF, although a small increase from control (+14%) was noted. However, when the two treatments were combined, the effects of L-NAME upon LCBF were attenuated within the FR139317-injected striatum. In these animals striatal LCBF (80 ± 3ml.$100g^{-1}$.min^{-1}) was not significantly different from that following FR139317 infusion alone (85 ± 12ml.$100g^{-1}$.min^{-1}), nor from CSF-infused control rats (75 ± 6ml.$100g^{-1}$.min^{-1}), but was significantly higher than in the L-NAME injected, CSF-infused group (54 ± 3ml.$100g^{-1}$.min^{-1}). L-NAME induced similar levels of hypertension in both CSF (MABP = 171 ± 8mmHg) and FR139317-treated (169 ± 8mmHg) groups. There were no other significant differences in physiological parameters between any of the groups.

These results suggest that the cerebrovascular constriction which follows treatment with L-NAME is mediated (in part at least) via ET_A receptors, for which ET itself is the endogenous ligand. The small increases in flow observed following intrastriatal injection of the ET_A-receptor anatgonist FR139137 indicate that there may be a tonic release of endogenous ET, the effects of which may only become apparent when NO dilator tone is removed. Although there is evidence of an interaction between NO and ET at a biochemical level, this study identifies a functional interaction which may be of importance in the regulation of LCBF.

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64P NECA AND R-PIA ACTIVATE DISTINCT SITES TO CAUSE RELAXATION OF THE ISOLATED RAT AORTA

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Adenosine and some of its analogues have been previously shown to relax the rat isolated aorta, but the degree of antagonism of agonist dose response (E/[A]) curves was agonist dependent (Lewis *et al.*, 1994). This observation is not consistent with activation of a homogeneous population of receptors. In order to elucidate further the nature of the receptor populations in this tissue we investigated the interaction between two adenosine analogues, 5'-(N-ethylcarboxamido)adenosine (NECA) and N^6-R-phenylisopropyl adenosine (R-PIA), and the antagonists 8-sulphophenyltheophylline (8-SPT, a non selective P_1 receptor antagonist, see Fredholm *et al.*, 1994) and PD 115,199 (an antagonist selective for A_{2a} over A_{2b} receptors, Bruns *et al.*, 1987). CGS 21680 (2-[p-(2-carbonyl-ethyl)-phenylethylamino]-NECA) has been reported to be a selective A_{2a} receptor agonist (see Fredholm *et al.*, 1994), however in the rat aorta this compound behaves as a partial agonist, only eliciting approximately 66% of the maximal response to NECA with a $p[A_{30}]$ of 6.3 (Lewis *et al.*, 1994). Therefore, in this study we were also able to use CGS 21680 as an antagonist. Male Wistar albino rats (University of Surrey strain, 200-250g) were killed by cervical dislocation, the aorta excised and 6mm rings set up in 3.5ml organ baths. Tissues were contracted with a near maximal dose of phenylephrine (0.1μM) and then cumulative relaxatory E/[A] curves were constructed in the absence or presence of antagonist. $p[A_{50}]$ estimates were obtained by logistic curve fitting, and where possible pA_2 values were estimated from the dose ratios obtained. However, in the case of NECA in the presence of CGS 21680, E/[A] curves were not fully defined and had no inflexion point therefore it was not possible to estimate a $p[A_{50}]$ value. The $p[A_{50}]$ data are summarised in Table 1. The pA_2 value calculated for 8-SPT versus NECA (5.3) is in agreement with its reported affinity at A_2 receptors (see Fredholm *et al.*, 1994) and that obtained for PD 115,199 versus NECA (8.0) is consistent with its affinity at A_{2a} receptors (Bruns *et al.*, 1987). These data coupled with the substantial rightward shift of NECA E/[A] curves obtained in the presence of CGS 21680 indicate that NECA induces responses via A_{2a} receptors. However, E/[A] curves to R-PIA were not significantly displaced by any of the compounds. 8-SPT (50μM) would be expected to inhibit responses mediated by A_1,A_{2a} and A_{2b} receptors, therefore it is unlikely that any of these receptor subtypes are involved in the responses elicited by R-PIA. Responses to R-PIA were also resistant to blockade by PD 115,199 and CGS 21680, confirming that A_{2a} receptors do not mediate R-PIA induced relaxations. In conclusion, NECA activates A_{2a} receptors in the rat aorta whereas R-PIA activates an as yet undefined site to cause relaxation.

Table 1. $p[A_{50}]$ values of agonists alone or in the presence of 8-SPT, PD 115,199 or CGS 21680.

Treatment	NECA $p[A_{50}]$	R-PIA $p[A_{50}]$
Control	6.86±0.11	5.88±0.10
+8-SPT (50μM)	5.86±0.05 *	5.60±0.06
Control	6.61±0.25	5.67±0.13
+PD 115,199 (0.1μM)	5.54±0.19 *	5.33±0.08
+PD 115,199 (1μM)		5.30±0.07
Control	6.55±0.20	5.44±0.15
+CGS 21680 (1μM)	<4.5 *	5.25±0.07

*Significantly different from control value, $p<0.05$.

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65P INDUCTION OF NITRIC OXIDE SYNTHASE BY LIPOPOLYSACCHARIDE IN RAT AORTIC SMOOTH MUSCLE CELLS IN CULTURE AND ITS INHIBITION BY AMINOGUANIDINE

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Compromised cardiovascular function caused by septic shock is the most common cause of death in intensive care units (Parrillo, 1990). Lipopolysaccharide (LPS) from Gram negative bacteria promotes inducible nitric oxide synthase (iNOS) expression in many cell types as part of the host defence mechanism. Recent evidence suggests that excessive production of nitric oxide (NO) in sepsis causes relaxation of smooth muscle throughout the vascular system resulting in severe hypotension (Koide *et al.*, 1994). We have followed increased NO production from rat aortic smooth muscle cells (RASMC) in culture treated with different LPS concentrations and the modulation of this release by a selective inhibitor of iNOS, aminoguanidine (AG) (Hasan *et al.*, 1993).

Confluent, second passage RASMC in 12 well plates were exposed to LPS (serotype 055:B5 from *E. coli*) and/or AG dissolved in Medium 199 supplemented with 10 % foetal bovine serum. Medium was sampled every 24 h and assayed for nitrite, the stable breakdown product of NO, using the Griess reaction. After sampling, medium above the cells was completely replaced with fresh drug solutions.

LPS dose-dependently stimulated nitrite production from the cells at concentrations of 0.1, 1, 10, 100 and 1000 μg/ml. Nitrite was detectable after 24h exposure to LPS and maximum production for each concentration was seen after 48 h (23±3 nmol/ml/24h (n=6) from cells treated with 1000 μg/ml LPS). At all doses, nitrite production remained elevated, or decreased slightly, in the following 72 h period studied. Cell number in these cultures exposed to 100 and 1000 μg/ml LPS for 120 h were significantly lower than untreated controls ($p<0.05$, Student's unpaired t-test). Cells exposed to 100 μg/ml LPS and 100 μM AG for 24 h produced 57 ±11 % of the nitrite concentration found in cultures exposed to LPS alone (n=6, $p<0.01$, paired Students t-test), but after treatment over a further 24 h with fresh solutions this inhibition was no longer apparent. In cells that had been pre-treated with 100 μM LPS for 48 h prior to addition of 100 μM AG, inhibition by AG was apparent for 24 h (40±11% nitrite produced compared with LPS alone, n=6, $p<0.01$) but, after this time, nitrite production by the AG treated cells had returned to the levels seen in cultures exposed to LPS alone. These results indicate that the potency of AG as an inhibitor of iNOS in RASMC was reduced upon exposure of the cells to AG beyond 24 h. The mechanism behind this remains to be established and if other iNOS inhibitors were found to have similar properties, this could have implications for the use of such compounds in the treatment of septic shock.

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66P EFFECTS OF PROPRANOLOL AND L-NAME ON β-ADRENOCEPTOR-MEDIATED RELAXATION IN RAT CAROTID ARTERY

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Atypical β-adrenoceptors, or β_3-adrenoceptors, characterized by their resistance to classical β-adrenoceptor antagonists e.g. propranolol and by their sensitivity to synthetic agonists e.g. BRL 37344 (Arch *et al.*, 1984) or ZD2079 (Grant *et al.*, 1994), are known to mediate smooth muscle relaxation in the gastrointestinal tract (e.g. McLaughlin & MacDonald 1990; 1991) and lipolysis (Arch *et al.*, 1984). Recently evidence was obtained for the presence of atypical β-adrenoceptors in a vascular smooth muscle preparation, the rat isolated common carotid artery (Oriowo, 1994). The present experiments were carried out to further investigate the properties of β-adrenoceptors in this preparation.

Male Wistar rats were injected with a lethal dose of sodium pentobarbitone and the common carotid arteries removed and carefully cleaned. Ring preparations (4 mm) were suspended in Krebs physiological salt solution (PSS) under 1g of tension. The PSS was maintained at 37°C, gassed with 95% O_2/5% CO_2 and contained EDTA (30 μM) and ascorbic acid (30 μM) to prevent oxidation of isoprenaline. Prazosin (0.1 μM) and yohimbine (0.1 μM) were also present to prevent α-adrenoceptor mediated effects of isoprenaline. After an equilibration period of 1 h the artery rings were constricted with the thromboxane-mimetic U46619 and cumulative concentration curves to agonists carried out. In studies with antagonist or L-NAME, two CRCs were performed, the second CRC in the presence of antagonist or L-NAME. Paired tissues acted as time controls.

U46619 produced a concentration-dependent contraction of the artery rings. A concentration of U46619 of 0.01 μM, which produced approximately 70% of the maximum response was chosen for subsequent experiments. Isoprenaline produced a concentration-dependent relaxation of the U46619-constricted vessels with a pD2 of 6.63±0.17 (n = 31). Propranolol (1 μM) produced a shift in the isoprenaline CRC of approximately 65 fold (pD_2s before and after propranolol: 6.25±0.22, 4.44±0.43, n = 6, $P<0.01$). ZD 2079 also produced a concentration-dependent relaxation of U46619-constricted vessels with a pD_2 of 4.99±0.45 (n = 5) compared with an isoprenaline control value of 5.88±0.31 ($P>0.05$, n=5). CGP 12177A (Mohell & Dicker, 1989) failed to produce relaxation in 4 experiments. L-NAME (100 μM) significantly reduced responses to isoprenaline e.g. maximum responses to isoprenaline (300 μM) reduced to 43 ± 13 % ($P< 0.01$, n = 7).

The effects of propranolol and ZD2079 are consistent with a mixed population of classical and atypical β-adrenoceptors mediating relaxation to isoprenaline in rat carotid artery although the lack of effect of CGP 12177A does not support the presence of β_3-adrenoceptors. Part of the response to isoprenaline appears to be mediated by nitric oxide.

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67P TRANSIENT CHANGES IN INDUCIBLE NITRIC OXIDE SYNTHASE ACTIVITY DURING CONTINUOUS INFUSION OF LIPOPOLYSACCHARIDE (LPS) IN CONSCIOUS RATS

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It is well established that a bolus injection of LPS causes expression of inducible nitric oxide synthase (iNOS) in various tissues (e.g., Salter *et al.*, 1991). However, the effect of continuous infusion of LPS has not been studied. We have now measured iNOS activity (as the Ca^{2+}-independent production of ^{14}C-citrulline from ^{14}C-arginine (Rees *et al.*, 1995)), in tissues obtained from male, Long Evans rats 2, 6 or 24h after onset of i.v. infusion of LPS (E coli serotype 0127 B8, 150 μg kg^{-1} h^{-1}). Animals had i.v. catheters implanted under sodium methohexitone anaesthesia (40 mg kg^{-1} i.p.) 24h before LPS infusion started; during this time catheters were perfused with sterile saline (0.4 ml h^{-1}) containing heparin (15 units ml^{-1}). Rats were anaesthetised with halothane (5% in oxygen) and killed by decapitation before tissues were collected. Activity of iNOS in tissues obtained from animals receiving saline for 24 h was taken as control. Table 1 summarises the results.

Table 1. iNOS activity (pmol min^{-1} mg^{-1} protein) in tissues from rats (n = 4-8) receiving saline (control) or LPS. Values are mean ± s.e. mean. *P < 0.05 versus control (ANOVA followed by Dunnett's test).

		LPS		
	Control	2 h	6 h	24h
Aorta	0.58±0.09	0.56±0.27	3.76±0.94*	2.32±0.93
Heart	0.30±0.23	0.59±0.20	3.04±0.29 *	0.91±0.48
Lung	0.62±0.37	44.83±6.03*	43.28±7.77*	1.02±0.32
Spleen	0.78±.056	12.56±2.32	50.75±7.24*	0.66±0.35
Liver	14.61±2.42	22.43±3.63	28.75±3.33*	12.78±2.88

All tissues showed high iNOS activity at 6 h; however, by 24 h iNOS activity was not significantly elevated. In previous studies we have shown that continuous infusion of LPS (at the dose used here) in conscious rats produces haemodynamic changes that increase between 6 and 24 h (Gardiner *et al.*, 1992, 1994; Waller *et al.*, 1994). Hence, the present results indicate that changes, other than just increases in iNOS activity, may play a role in the haemodynamic abnormalities at later times in septic shock. Furthermore, the finding that iNOS activity was not above control level, in any tissue studied, 24 h after the onset of LPS infusion, suggests that induction of iNOS by LPS is not a phenomenon that can be maintained indefinitely, in spite of the continuous presence of the inducing stimulus.

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68P EFFECT OF S-METHYLISOTHIOUREA ON NEURONAL NITRIC OXIDE SYNTHASE, NOCICEPTION AND BLOOD PRESSURE IN THE MOUSE

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Inhibition of nitric oxide synthase (NOS) with L-N^G nitro arginine methyl ester (Moore et al., 1991) or 7-nitro indazole (7-NI, Moore et al., 1993) elicits antinociception in the mouse most probably by inhibition of nitric oxide formation in the dorsal horn of the spinal cord. Recently, a number of substituted S-isothiourea compounds with potent NOS inhibitory activity have been described (Garvey et al., 1994; Southam et al., 1995). We have now compared six isothiourea compounds as inhibitors of mouse spinal cord NOS activity in vitro and have examined S-methylisothiourea (SMT) for antinociceptive activity and ability to influence blood pressure in the mouse.

Mouse (male, LACA, 20-30g) spinal cord homogenates (1,000 x g) were prepared and NOS enzyme activity determined as the conversion of [^{3}H] L-arginine to [^{3}H] citrulline as described previously (Moore et al., 1993). The order of potency with mean IC_{50} values (μM, n=6) was isopropyl isothiourea (0.66)>ethyl isothiourea (2.06) >SMT (2.56)>aminoethyl isothiourea (31.46)> pentyl isothiourea (78.71) >acetamidoisothiourea (1252). SMT (50 mg kg^{-1}, i.p., 15 min before subplantar injection of 5 μl 10% formalin) did not alter either the early (0-5 min, 87.7±6.1 s, c.f. 92.7±7.0 s in saline-injected control animals, n=17, P>0.05) or late phase (15-30 min, 72.7±13.0 s, c.f. 89.0±12.4 s, n=17, P>0.05) hindpaw licking response.Pretreatment with SMT (50 mg kg^{-1}, i.p., killed 30 min later) did not inhibit spinal cord NOS activity measured ex vivo (2.47±0.66 pmol citrulline mg $protein^{-1}$ 15 min^{-1},c.f. 2.31±0.47, n=9, P>0.05) and failed to influence formalin-induced increase in hindpaw weight (72.6±4.9 mg, c.f. 62.3±3.9 mg, n=9, P>0.05). In urethane (10 g kg^{-1}, i.p.) anaesthetised mice (resting mean arterial pressure, MAP, 47.1±3.3 mm Hg, n=14) administration of SMT (10 and 50 mg kg^{-1}, i.p.) caused a rapid and sustained increase in MAP (e.g. 27.4±12.3% and 63.1±6.8% increase over baseline after 5 min, n=7).

These results confirm the ability of selected S-isothiourea compounds to inhibit neuronal NOS in vitro. Furthermore, SMT increases MAP in the mouse most likely by inhibition of endothelial NOS activity. SMT failed to inhibit formalin-induced hindpaw licking in the mouse perhaps as a consequence of limited access across the blood brain barrier.

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69P INHIBITION OF Na^+-H^+ EXCHANGE AND Na^+-K^+ ATPASE ON THE CELL INWARD TRANSPORT OF L-DOPA IN RAT RENAL TUBULES

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The current view of the renal dopaminergic system is that of a local non-neuronal system constituted by epithelial cells of proximal convoluted renal tubules rich in aromatic L-amino acid decarboxylase (AAAD) activity and using circulating or filtered L-DOPA as a source for dopamine (Soares-da-Silva, 1994). More recently, studies in isolated rat renal tubules have shown that accumulation of L-DOPA in this preparation occurs through saturable and non-saturable mechanisms; at a concentration of the substrate approaching half saturation, diffusional uptake accounts for 36% of the total accumulation of L-DOPA (Soares-da-Silva et al., 1994). The present work has evaluated the role of sodium on the saturable uptake of L-DOPA in isolated rat renal tubules and determined the effect of inhibition of Na^+-K^+ ATPase with ouabain and inhibition of Na^+-H^+ exchange with lithium chloride (Mahnensmith & Aronson, 1985) on the rate L-DOPA uptake. The kinetics and the characteristics of the cellular uptake of L-DOPA were studied in experiments performed in suspensions of rat renal tubules obtained from benserazide-treated animals. Incubation of renal tubules at 4° C in the presence of increasing concentrations of L-DOPA results in a linear and concentration-dependent accumulation of the substrate. The kinetic characteristics of the saturable component of L-DOPA uptake, derived from the total amount of L-DOPA accumulated in renal tubules at 37°C, subtracted from the values obtained in experiments conducted at 4° C, were as follow: V_{max}=12.9±2.5 nmol mg protein^{-1} h^{-1} and K_m=279.4 µM (95% confident limits; 139.1, 561.3). The diffusion constant for the non-saturable component of L-DOPA accumulation was 0.0013 µmol^{-1} (0.0011, 0.0016). The effect of reducing the concentration of sodium in the incubation medium (from 137 mM to 107 mM, maintaining the osmolarity with 30 mM choline chloride) was a significant decrease (48% reduction) in the accumulation of L-DOPA in renal tubules incubated with 250 µM L-DOPA; when 30 mM sodium was substituted by lithium the uptake of L-DOPA was found to be reduced by 75%. Ouabain (0.1, 0.5 and 1.0 mM) was found to produce a concentration dependent decrease in the formation of dopamine (15% to 68% reduction). In conclusion, it is suggested that the tubular uptake of L-DOPA is a sodium-dependent mechanism and is sensitive to manoeuvres which tend to dissipate the cellular gradient for sodium.

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70P LACK OF EFFECT OF L-ARGININE AND OF ITS DECARBOXYLATION PRODUCT, AGMATINE, ON THE CONVERSION OF L-DOPA TO DOPAMINE IN RAT RENAL TUBULES

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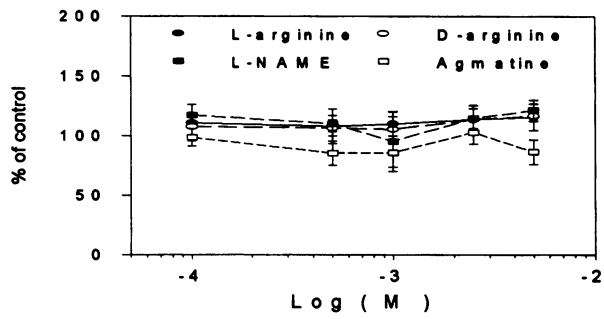
Previous work has shown that the hypertensive response resulting from long-term (14 days) administration of N^G-nitro-L-arginine methyl ester (L-NAME; 50 µg ml^{-1} in drinking water) was accompanied by an increased urinary excretion of dopamine (DA) and enhanced activity of renal aromatic L-amino acid decarboxylase (AAAD); the administration of L-arginine to L-NAME-treated rats prevented the increase in renal AAAD activity (Soares-da-Silva et al., 1994). Apart from being used in the formation of nitric oxide, L-arginine has recently been demonstrated to undergo decarboxylation by arginine decarboxylase in the mammalian brain and kidney to agmatine (Li et al., 1994; Reyes et al., 1994). In brain, this reaction was inhibited by its end product agmatine (Li et al., 1994). The hypothesis we decide to test has considered the possibility that the decrease in the renal formation of dopamine from added L-DOPA in L-arginine plus L-NAME-treated rats might be related to a competitive effect of L-arginine or its decarboxytion product, agmatine, on AAAD. For this purpose, the effects of L- and D-arginine, L-NAME and agmatine, were examined on AAAD activity determined in homogenates of renal tubules. Incubation of homogenates of isolated renal tubules, in conditions of COMT (tolcapone 1 µM) and MAO (pargyline 100 µM) inhibition, with L-DOPA (10 to 5000 µM) resulted in a concentration-dependent formation of dopamine. The kinetic parameters of AAAD activity were as follows: V_{max}=214.3±30.3 nmol mg protein^{-1}h^{-1}; K_m=1849.7±172.3 µM. In experiments performed with the aim to test the effects of L- and D-arginine, L-NAME and agmatine on the decarboxylation of L-DOPA (2 mM), homogenates of renal tubules were incubated with increasing concentrations (0.1 to 5.0 mM) of the compounds to be tested. As shown in the figure, no significant change was observed on the rate of L-DOPA decarboxylation.



In another set of experiments, 2 mM agmatine was found not to affect the decarboxylation of increasing concentrations (5 to 1000 µM) of L-DOPA. By contrast, 50 µM benserazide completely abolished the decarboxylation of L-DOPA in homogenates of renal tubules. It is concluded that L- and D-arginine do not compete with L-DOPA for decarboxylation in homogenates of isolated rat renal tubules. Furthermore, neither L-NAME nor agmatine appear to affect the activity of renal AAAD and the formation of dopamine.

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71P CELL INWARD TRANSPORT AND DECARBOXYLATION OF L-DOPA IN Caco-2 CELLS IN CULTURE

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There is evidence to suggest that dopamine may have a role in the intestinal regulation of water and electrolyte absorption (Finkel et al., 1994). The digestive tract, namely its duodenal and ileal portions, have also been shown to be endowed with a considerable ability to decarboxylate L-DOPA to dopamine (Vieira-Coelho & Soares-da-Silva, 1993). Caco-2, human colon adenocarcinoma derived cell line, undergo enterocyte differentiation in culture (Pinto et al., 1983). This cell line has been used as a model for studies on the intestinal absorption and metabolism of drugs. The present work reports on the kinetics and characteristics of cellular uptake of L-DOPA and its decarboxylation to dopamine in Caco-2 cells in culture. Caco-2 cells (ATCC HTB-37) were grown at 37° C in a humidified atmosphere (5% CO_2) on 75 cm^2 or 2 cm^2 plastic culture clusters (Costar 3276 and 3524, respectively). The cells were used at passage 22 and 23; after 7 days, the cells formed a monolayer and each cm^2 cell monolayer contained about 70 µg of cell protein. In uptake studies, Caco-2 cells were preincubated (30 min) with Hanks medium with added pargyline (100 µM), tolcapone (1 µM), in order to inhibit the enzymes monoamine oxidase and catechol-O-methyltransferase, respectively, and benserazide (50 µM), an aromatic L-amino acid decarboxylase inhibitor. Determination of initial rate of uptake was performed in experiments in which Caco-2 cells were incubated with L-DOPA (250 nM) during 1, 3, 6, 12, 30, 60 and 120 min; L-DOPA was applied from the apical cell border. The parameters of an exponential saturation equation ($C_i/C_o=k_{in}/k_{out}.(1-e^{-Kout.t})$) were fitted to the experimental data; C_i and C_o represent the intracellular and extracellular concentration of the substrate and t the incubation time. The analysis revealed for L-DOPA a rate constant of total inward transport (k_{in}) of 17.4 µl mg protein^{-1} min^{-1}, a rate constant of total outward transport (k_{out}) of 0.8 µl mg protein^{-1} min^{-1} and an equilibrium factor of accumulation (C_i/C_o) of 99.9±6.3 (n=5). Saturation experiments were performed in Caco-2 cells incubated for 6 min with increasing concentrations of L-DOPA; non-linear analysis of the saturation curve revealed a K_m of 72.8±4.1 µM and a V_{max} of 1.4±0.1 nmol mg protein^{-1} min^{-1} (n=5). Homogenates of Caco-2 cells prepared in Borax buffer with added pargyline (100 µM) and tolcapone (1 µM) were used to determine the activity of the enzyme aromatic L-amino acid decarboxylase (AAAD). Homogenates were incubated for 15 min with increasing concentrations of L-DOPA (0.1 to 5 mM). At the end of the incubation period, the reaction was stopped by addition of 250 µl 2M perchloric acid. The kinetics of AAAD determined in homogenates of Caco-2 cells showed a V_{max} of 20.4±1 nmol mg protein^{-1} h^{-1} and a K_m of 1.0±0.1 mM (n=4). It is concluded that Caco-2 cells in culture take up L-DOPA and decarboxylate it to dopamine.

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72P INHIBITION OF MONOAMINE OXIDASE AND (I_2) IMIDAZOLINE AFFINITY IN RAT KIDNEY

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I_2 imidazoline receptors have been suggested to be linked to monoamine oxidase as some monoamine oxidase inhibitors displace a component of I_2 receptor binding (Olmos *et al.*, 1993; MacKinnon *et al.*, 1995) and *in vivo* studies have shown that I_2 receptors are down-regulated by the selective MAO inhibitor clorgyline (Olmos *et al.*, 1993). In addition, I_2 receptors show a similar autoradiographical distribution to MAOB (Saura *et al.*, 1992 and see MacKinnon *et al.*, 1995). A recent study has shown that the I_2 selective compound 2-(2-benzofuranyl)-2-imidazoline (2-BFI) increases noradrenaline release in rat frontal cortex and hippocampus, an effect that was suggested to be due to inhibition of MAO (Lalies & Nutt, 1995). This study investigates the effect of some imidazoline compounds on MAOA and MAOB activity in rat kidney homogenates, and compares their potency to their affinity for I_2 receptors in the same tissue. I_2 affinity was determined from the inhibition of [^{3}H]-RS-45041-190 binding to rat kidney membranes (MacKinnon *et al.*, 1995). MAOA and MAOB activity were determined as described by Li *et al.* (1992). Inhibition of enzyme activity is given in Table 1.

In the present study, the selective MAOA inhibitor clorgyline and the selective MAOB inhibitor selegiline inhibited the A and B isoforms of the enzyme respectively with high potency (Table 1), however, only clorgyline showed high affinity for a proportion (35%) of [^{3}H]-RS-45041-190 binding. In addition no correlation was found between affinity for I_2 receptors and inhibition of MAO activity in rat kidney as RS-45041-190 had only modest potency (pIC_{50} = 6.12 and 4.47 for MAOA and MAOB repectively) and idazoxan was completely ineffective. The low potency of RS-45041-190 and idazoxan for MAOA and MAOB inhibition suggests that affinity for I_2 receptors does not correlate with functional activity at either of the MAO isoenzymes in rat kidney. Although I_2 compounds did not affect MAO activity *per se* the possiblity remains that they are involved in a more regulatory role of enzyme function.

	MAOA (pIC_{50})	MAOB (pIC_{50})	I_2 (pIC_{50})
clorgyline	7.75±0.06	4.58, 4.70$	8.60±0.27*
selegiline	5.12, 5.26$	6.79±0.06	5.25±0.20
RS-45041-190	6.12±0.10	4.47±0.06	8.39±0.04
idazoxan	<4.00	<4.00	7.85±0.03
guanabenz	6.59±1.26	4.42±0.07	7.23±0.07
clonidine	<4.00	<4.00	5.64±0.16

Table 1. Inhibition of MAOA and MAOB and I_2 affinity in rat kidney *affinity for 35% specific binding, $ n=2.

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73P THE EFFECT OF NIFEDIPINE ON THE ACIDOSIS-INDUCED CORONARY CONSTRICTION IN THE ISOLATED PERFUSED RAT HEART

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As a consequence of myocardial ischaemia, acidosis occurs. While the effects of pH changes on cardiac muscle are well documented (Orchard & Kentish 1990), the effect of pH changes on vascular smooth muscle, especially the fine resistance vessels within the heart, are less well understood. In order to address this, a modified constant flow Langendorff-perfused isolated rat heart was used and coronary perfusion pressure (CPP) used to monitor coronary resistance

Hearts from male Wistar rats were perfused at 10 ml.min.$^{-1}$ with Krebs-Henseleit solution pH7.4. Developed tension (DT), at a resting tension of 2g, was measured via a tension transducer attached to the apex of the heart. This signal also triggered a heart rate meter (HR). CPP was measured via a pressure transducer attached to a side arm on the aortic cannula. The superior mesenteric bed (SMB) was perfused at 5 ml.min.$^{-1}$ with the coronary effluent and perfusion pressure increased with phenylephrine (PE) infusion (final concentration 96μM) (data expressed as mean$\pm$s.e.mean, n=4). Statistics performed using paired t-test unless stated otherwise.

Following 20 minutes equilibration CPP was 79$\pm$8mmHg. On perfusing with modified Krebs pH6.8 (metabolic acidosis achieved by reducing [HCO_3^-], CPP transiently decreased to 64$\pm$7mmHg then rapidly increased to 156$\pm$9mmHg after 5 minutes ($p<0.001$); DT, control 7.2$\pm$0.5g, displayed a transient increase to 8.7$\pm$ 0.6 g which rapidly fell to a steady state of 3.0$\pm$0.3g ($p<0.001$). Perfusion with pH6.8 also caused a negative chronotropic response, control HR 303$\pm$6bpm decreased to 258$\pm$11bpm ($p<0.05$). On reperfusion at pH7.4, a further transient decrease in DT was observed with levels returning to control pH7.4 values within 5 minutes.

Acidosis had no effect on basal mesenteric perfusion pressure, but caused vasodilation in PE-preconstricted SMB from 130$\pm$10mmHg to 93$\pm$9mmHg ($p<0.05$) followed by a partial recovery to 108$\pm$16mmHg.

To investigate whether the cardiac and vascular effects were separate, cardiac contractility was blocked by increasing [K_o] from 5.9mM to 15mM Under these conditions, CPP was not significantly affected. Acidosis, however, still caused a vasoconstriction from 63$\pm$4mmHg to 163$\pm$14mmHg ($p<0.001$). A similar result was obtained if cardiac contractility was surpressed by pacing the hearts at 16 Hz.

Nifedipine (1-100 nM) progressively inhibited the acidosis-induced vasoconstriction. In the prescence of 100 nM nifedipine, CPP only increased from 48$\pm$3mmHg to 60$\pm$9mmHg with acidosis which was significantly different from control values ($p<0.05$; Mann-Whitney test).

This data confirms that metabolic acidosis dilates the rat SMB (Austin & Wray 1993). However, the increase in CPP following acidosis, and its inhibition by nifedipine, indicates an acidosis-induced coronary constriction mediated by L-type calcium channels. This is a surprising result as L-type calcium channels in porcine coronary arteries have been shown to be inhibited by acidosis (Klöckner & Isenberg 1994).

D.A.W. is a School Of Pharmacy & Pharmacology research student.

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74P ELECTROPHYSIOLOGICAL EFFECTS OF LOOP DIURETICS ON VENTRICULAR CARDIOMYOCYTES ISOLATED FROM RABBIT MYOCARDIUM

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The beneficial effects of loop diuretics in relieving pulmonary congestion and acute pulmonary odema are well established and are generally attributed to their potent diuretic action. These drugs have important cardiovascular haemodynamic effects which may precede any demonstrable diuretic effect. There has been limited investigation into the direct cardiac effects of these drugs; it is reported that furosemide has a negative inotropic effect in isolated rabbit heart (Feldman *et al.*, 1987) and inhibits cardiac L-type Ca^{2+} currents (Shimoni, 1991). The aim of the present study was to examine the electrophysiological effects of the loop diuretics, furosemide, bumetanide and torasemide, in ventricular cardiomyocytes, using a perforated patch clamp technique in which nystatin, a polyene antibiotic, forms voltage-insensitive ion pores in the membrane patch without dialysing the cytoplasmic factors (Zhou *et al*, 1993).

Ventricular cells were isolated by enzymatic dissociation from adult, male New Zealand White rabbits (2.5-3kg) as previously described (Varro *et al.*, 1991). Cells were perfused with a solution of the following composition (mM): NaCl 137; KCl 5.4; $CaCl_2$ 3; $MgCl_2$ 1.2; HEPES 5; Glucose 10 (pH 7.4, 35°C). Action potentials (APs) were elicited by the application of depolarizing pulses, of 6-12ms duration, in current clamp mode using an Axopatch 1D patch-clamp amplifier. The internal pipette solution contained (mM): K-gluconate 120; KCl 20; $MgCl_2$ 2; HEPES 5; nystatin 150mg.ml^{-1} (pH 7.2). In voltage-clamp mode, cells were held at -40mV to inactivate the Na^+ current and voltage steps of 200ms duration were applied every 5 s. Following stabilisation, recordings were made from depolarizing pulses applied in 10mV increments from -40 to +60mV, in the absence and presence of increasing concentrations of either furosemide ($3x10^{-6}$M-$3x10^{-4}$M), bumetanide (10^{-7}M-10^{-5}M) or torasemide (10^{-6}M-10^{-4}M) applied to the bath at 5 min intervals. Current-voltage relationships were constructed and the peak value expressed as mean ± s.e.m. Furosemide did not affect the duration of the AP over a concentration range of 10^{-5}M-$3x10^{-4}$M (398±36 ms at 90% of repolarization). Neither furosemide (n=14), bumetanide (n=11) nor torasemide (n=10) produced any significant effect on peak Ca^{2+} current amplitudes of -1.9±0.2nA, -2.9±0.4nA and -2.3±0.1nA, respectively. Moreover, furosemide (10^{-5}M, n=6) did not have any short-term effect on peak inward Ca^{2+} current when examined at 5 sec intervals (table) over a 5 min period.

Time (sec)	0	5	10	15	20	25	30
Furosemide(10^{-5}M; nA)	-2.51±0.38	-2.50±0.38	-2.50±0.37	-2.50±0.38	-2.48±0.38	-2.48±0.39	-2.46±0.39
Control (nA)	-2.51±0.43	-2.49±0.41	-2.49±0.42	-2.47±0.40	-2.47±0.43	-2.47±0.40	-2.51±0.42

In summary, loop diuretics did not have any effect on the duration of the AP or on the L-type Ca^{2+} current and therefore are unlikely to have any relevance when considering either the therapeutic or arrhythmogenic actions of these drugs.

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75P CHARACTERIZATION OF VENTRICULAR CARDIOMYOCYTES ISOLATED FROM RABBITS WITH EPIRUBICIN-INDUCED HEART FAILURE

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Experimental models of heart failure are useful for studying the pathology of the disease and valuable information regarding the effects of therapeutic agents and the cellular mechanisms responsible can be obtained. Anthracyclines such as epirubicin and doxorubicin are effective anti-neoplastic agents which have dose-related cardiomyopathy as a major side effect. Doxorubicin-induced cardiomyopathy has been extensively studied in humans, and in animals it is a model of low output heart failure. In this study we have investigated morphological, mechanical and electrophysiological characteristics of ventricular cardiomyocytes from rabbits following chronic administration of epirubicin. Male New Zealand White rabbits (8 weeks) were treated with twice weekly injections of epirubicin (4mg/kg/week) or saline, for 6 weeks, followed by a wash-out period of 2 weeks. Serum levels of lactate dehydrogenase (LDH) and creatine kinase (CK) were measured at the beginning and end of treatment. Left ventricular tissue was examined for structural cardiac lesions by scanning electron microscopy. Ca^{2+}-tolerant ventricular myocytes were isolated from rabbit hearts using Langendorff perfusion with collagenase. Measurements of cellular dimensions were obtained from video-recorded images (x400 magnification). Action potentials (APs) and L-type Ca^{2+} currents were recorded using a whole-cell patch-clamp technique and durations of AP (APD) at 90% of repolarization and peak Ca^{2+} currents determined. Cardiomyocyte function was examined during unloaded, electrically-stimulated (0.5 Hz) contraction. Contraction amplitude, expressed as % cell shortening (%δl), was investigated under basal conditions and in the presence of isoprenaline, over the concentration range 1 nM to 1 μM. All data are given as the mean ± standard deviation.
In heart-failed rabbits there were marked increases in serum levels of LDH (278 ± 120 U/L to 1101 ± 212 U/L (n=5)) and CK (271 ± 83 U/L to 1020 ± 197 U/L); scanning electron microscopy of left ventricular tissue indicated vacuolation, oedema and myofibrillar damage, compared to controls. There was no difference in the mean cell length, width and area between myocytes from the normal and diseased groups (135 ± 25 μm vs 147 ± 30 μm; 24 ± 6 μm vs 21 ± 8 μm; 3045 ± 411 μm^2 vs 3293 ± 383 μm^2, n=575 and 700 cells respectively). There was no difference in the mean cell length:width ratio in control and diseased groups (6.1 ± 1.9 vs 7.1 ± 2.7). In the latter however, there was a greater spread of values around the mean and a significant increase (13 to 34%, n=8 and 10 experiments, respectively) in the proportion of "stretched" cells of l:w ratio > 8.0, the value one standard deviation greater than the mean obtained for control myocytes. The APD cells from the heart-failed group was prolonged (396 ± 192 ms, n=58) compared to the control group (321 ± 183, n=51)($p<0.05$), but no differences were noted in the L-type Ca^{2+} current between the two groups (peak amplitudes were -2.16 ± 0.7 and -2.13 ± 0.6 nA, respectively, n=18). Myocytes from the heart-failed group had greater basal contractile amplitude (11.9±0.8 %δl, n=8) than controls (8.2±0.9 %δl, n=9) but had similar maximum responses which were 19.1 ± 1.9 and 17.3 ± 1.7 %δl, respectively. There was no significant change in the EC_{50} obtained with isoprenaline for myocytes from the healthy (31 ± 8.2 nM) and diseased groups (66 ± 37.5 nM).
Epirubicin-induced heart failure produced no significant increase in cardiomyocyte area due to hypertrophy. However, there was an increase in basal contraction possibly due to more efficient coupling of electrical stimulation, and a prolongation of the APD indicating changes in cell membrane properties. In conclusion, compared with control myocytes, cardiomyocytes isolated from the hearts of rabbits with epirubicin-induced heart failure were more heterogeneous and had significantly different electromechanical and electrophysiological properties.

76P VASOCONSTRICTOR AND VASODILATOR RESPONSES OF 5-HYDROXYTRYPTAMINE IN THE RAT PULMONARY ARTERY: EFFECTS OF SELECTIVE RECEPTOR ANTAGONISTS

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5-Hydroxytryptamine (5-HT) constricts the pulmonary vasculature of the rat however the pD_2 is relatively high. Several possibilities could account for this observation. In the cat pulmonary vasculature Neely et al. (1993) reported that 5-HT acts on an inhibitory receptor at low concentrations but induces contraction at high concentrations. Since 5-HT can apparently interact directly with α_1-adrenoceptors at high concentrations (Feniuk & Humphrey, 1989) another possibility is that the contractile response to 5-HT is mediated via the α_1-adrenoceptor.
The present study investigated these possibilities by examining the contractile responses to 5-HT in the first branch pulmonary artery in the absence and presence of ritanserin a selective 5-HT_2 antagonist, methysergide a non selective 5-HT antagonist and prazosin a selective α_1-adrenoceptor antagonist. The ability of 5-HT to induce relaxation was examined in artery rings preconstricted with phenylephrine (PE) or L-N^{ω}-nitro arginine methyl ester (L-NAME). Male Wistar rats (250 - 300g) were killed by intraperitoneal injection of sodium pentobarbitone (100 mg Kg^{-1}). Artery rings (1.2 - 1.5 mg) from the first branch pulmonary artery were suspended on stainless steel hooks in Krebs physiological salt solution (37°C) under a resting tension of 1 g and gassed with a mixture O_2:CO_2 (95%/5% v/v). In these experiments the tissues were allowed to equilibrate for 1 hour then two concentration response curves (CRCs) to 5-HT were performed, the second in the presence of the antagonist. Paired tissues acted as time controls. Results are the mean values ± the standard error of the mean. Comparisons between means were analysed using the Students paired T-test. 5-HT (1μM - 1mM) produced a concentration-dependent contractile response. Ritanserin (10^{-7}M) produced a small shift in the CRC to 5-HT (pD_2 before and after ritanserin: 4.99 ± 0.12; 4.5 ± 0.22, n = 8, $p < 0.05$). Methysergide (10^{-7}M) also produced a rightward shift in the CRC to 5-HT (pD_2 before and after methysergide: 5.12 ± 0.11; 4.78 ± 0.07, n = 9, $p < 0.01$). Prazosin (10^{-10} - 10^{-7} M) produced a concentration dependent non competitive inhibition of the contractile response to 5-HT. In artery rings preconstricted with PE (EC_{60} - EC_{75}) the response to 5-HT was biphasic, initially (1nM - 1μM) augmented the contractile response, then at higher concentrations 5-HT (10μM - 1mM) induced relaxation. In the presence of L-NAME (100μM) 15 out of 19 ring preparations developed tone. The mean increase in tone (78.5 ± 18.6 mg/mg) was approximately 25% of the maximum response to PE and in these arteries 5-HT (10^{-9} - 10^{-7}M) induced a prolonged relaxation. At higher concentrations 5-HT (1μM - 1mM) induced contraction. In some arteries the relaxation was preceded by a transient contraction. The relaxation was unaffected by the selective 5-HT_4 antagonist GR 113808A (10^{-7}M) (Gale et al., 1994). The results show that 5-HT has the ability to act on an inhibitory receptor at low concentrations. This response could act as a physiological antagonist of the contractile response induced by 5-HT. Although ritanserin shifted the CRC to 5-HT, suggesting that a component of the response may involve the 5-HT_2 receptor, the observed shift is not consistent with a single population of 5-HT_2 receptors. That prazosin produced a potent inhibition of the CRC to 5-HT may indicate an involvement of the α_1-adrenoceptor in the response.

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77P PROSTAGLANDIN E_1 OR PROSTAGLANDIN E_0 ATTENUATES THE REPERFUSION INJURY IN THE ISCHAEMIC RABBIT HEART *IN VIVO*

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Prostaglandin E_1 (PGE_1) and its active metabolite 13, 14-dihydro-PGE_1 (PGE_0) are vasodilator prostaglandins which inhibit platelet and neutrophil function *in vitro*. We have previously reported that infusion of PGE_1 or PGE_0 (0.1-1.0 μg $kg^{-1}min^{-1}$ starting 10 min prior to LAL occlusion and continued throughout the experiment) reduce myocardial infarct size after coronary artery occlusion in the anaesthetised rabbit in a dose related manner (Hide *et al.*, 1994). Here we investigate whether PGE_1 or PGE_0 reduce infarct when given 5 minutes prior to reperfusion of the ischaemic myocardium.

New Zealand white rabbits were premedicated with Hypnorm (0.1 ml kg^{-1}, i.m.). General anaesthesia was then induced (30 ml kg^{-1}, i.v.) and maintained with sodium pentobarbitone. After tracheotomy, the animals were ventilated with room air. A left intercostal thoracotomy was performed and the first antero-lateral branch of the left coronary artery (LAL) was occluded for 60 min followed by 120 min of reperfusion. Left ventricular pressure (LVP), mean arterial pressure (MAP) and heart rate (HR) were continuously recorded. Five min prior to the onset of reperfusion (i.e. after 55 min of LAL occlusion) different groups of animals were treated with vehicle (in 0.9% NaCl), PGE_1 or PGE_0 (1.0 μg $kg^{-1}min^{-1}$ administered at a rate of 0.05 ml/min, i.v.) At the end of the experiment the LAL was reoccluded and Evans blue dye (2% w/v) was injected into the left ventricle (LV) to determine area at risk (AR). Infarct size (IF) was determined by incubation of AR with nitro-blue tetrazolium (NBT; 0.5 mg/ml for 20min).

The mean values for AR (% LV) were not significantly different between groups. PGE_1 or PGE_0 caused a reduction in IF (expressed as a % of AR) from 62±6% (control; n=8) to 37±4%* (n=8) and 45±5%* (n=7) respectively (*$p<0.05$; unpaired Students *t*-test). PGE_1 or PGE_0 had no significant effect on heart rate but did produce a significant reduction in MAP and a transient reduction in pressure rate index (PRI), an index of myocardial oxygen consumption (Baller *et al.*, 1980).

Thus, PGE_1 or PGE_0 cause a reduction in myocardial infarct size in the anaesthetised rabbit when administered as an infusion starting 5 min prior to the onset of LAL reperfusion (t = 55 min). The mechanism of the cardioprotective effect of PGE_1 or PGE_0 is still unclear, but may well involve the inhibition of neutrophil activity.

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78P CO-INDUCTION OF NITRIC OXIDE SYNTHASE AND CYCLO-OXYGENASE ACTIVITY IN HUMAN INTERNAL MAMMARY ARTERY

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Nitric oxide (NO) and prostacyclin (PGI_2) are released by endothelial cells and act together to inhibit platelet function and relax vascular smooth muscle. NO is formed in endothelial cells by a cell-specific isoform of NO synthase (eNOS) whilst PGI_2 is formed by a constitutive isoform of cyclo-oxygenase (COX; COX-1). NO and PGI_2 are also released by vascular cells following the induction of different isoforms of NOS (iNOS) and COX (COX-2) respectively. We have investigated the effects of a combination of cytokines (interleukin-1β, 5 U/ml; tumour necrosis factor α, 10 U/ml; interferon γ, 200 U/ml) and bacterial lipopolysaccharide (LPS; 10 μg/ml) on the induction of NOS and COX activity in segments of human internal mammary artery.

Arteries were collected and placed into Tyrodes' solution for transport at 4°C. The vessels were dissected free of connective tissue and cut in to 3-6 mm lengths. Segments were placed into 24 well culture plates containing DMEM plus 10% foetal calf serum (2 ml). After equilibration (1 h) the medium was replaced and drugs or vehicles added. Medium was removed after 0, 24 and 48h, and PGI_2 concentration measured by radioimmunoassay for its hydrolysis product 6-keto $PGF_{1\alpha}$, (Mitchell et al., 1993a). Segments of artery were stored at -80°C until NOS (Mitchell et al.,1993b) and COX (Vane et al., 1994) activity was measured in tissue homogenates.

Intact tissue segments released 6-keto $PGF_{1\alpha}$ following incubation in culture medium (0 h, 0.0043 ± 0.0004 μg/g; 24 h, 0.78 ± 0.15 μg/g; 48 h, 1.3 ± 0.23 μg/g). This release was increased when the mixture of cytokines and LPS was included in the culture medium (0 h, 0.0038 ± 0.0008 μg/g; 24 h, 1.07 ± 0.22 μg/g; 48 h, 2.42 ± 0.36 μg/g). In tissue homogenates, NOS activity (Fig 1) was induced in tissues incubated only with the cytokine mixture. However, COX activity (Fig 2) was induced both in the presence and absence of cytokines. The cytokine induced NOS activity at 48 h was completely independent of calcium (in the presence of calcium, 7.2 ± 3.0 nmole/g; in the absence of calcium, 6.3 ± 2.0 nmole/g; n=4) and greatly reduced by L-NAME (1 mM; to 0.9 ±0.3; n=4).

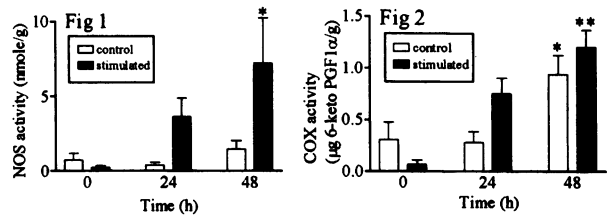

*Fig 1 and 2 show changes in NOS (n=3-4 patients) and COX (n=3-7 patients) activities in homogenates of human internal mammary artery following exposure of intact tissue to cytokines and LPS (filled columns). * denotes significance of $p<0.05$ and ** of $p<0.01$ as determined by Analysis of Variance.*

The induction of NOS and COX has been demonstrated in animal vessels following *in vivo* or *in vitro* administration of LPS. However, the induction of these enzymes in human tissue is less well documented. COX, but not NOS, activity was induced under control organ culture conditions. However, in the presence of cytokines relatively large amounts of calcium-independent NOS was induced. These observations support a role for NO in the hypotensive crisis associated with septic shock.

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79P BRADYKININ IN SPRAGUE DAWLEY RAT ISOLATED HEARTS

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Bradykinin (BK) is reported to be cardioprotective (Parratt, 1994). We recently reported lower kinin release and more arrhythmias during ischaemia in isolated hearts from spontaneously hypertensive rats than from Wistar Kyotos (Ahmad et al, 1995). Contrary to one earlier report (Baumgarten et al, 1993), we found no increase in BK release following coronary artery occlusion. To assess the effect of strain of animal on these findings, we have studied isolated hearts from Sprague Dawley rats, perfused with Krebs solution (10 ml min^{-1}), with and without BK (320 pg ml^{-1}). At 5 min after the start of BK perfusion, the left descending artery was occluded for 30 min, followed by 30 min reperfusion. Results are reported as mean (s.e. mean) and analysed using Mann-Whitney and Wilcoxon tests (significance at $P<0.05$).

The perfusion pressure in the BK perfused hearts was lower ($P<0.001$) than controls at all time points, indicating increased coronary flow. Although the mean ventricular ectopic beats were less in the BK-treated group (191±67) compared with controls (249±72), this did not reach significance. The effect of ischaemia and reperfusion on kinin release by isolated rat hearts was also determined. The perfusate samples were collected in chilled polypropylene tubes containing EDTA and aprotinin (1 mg & 500 KIU per ml respectively), stored at -20 °C until extracted with 60% acetonitrile using Sep-Pak Vac C18 cartridges and radioimmunoassayed for bradykinin (Moshi et al, 1992). The rates of kinin release (pg min^{-1}) immediately before (483±100), 10 min after occlusion (561±68), 30 min after occlusion (528±54) and immediately after reperfusion (398±39) were not significantly different. In a non-occluded control group perfused only with Krebs solution, the rate of kinin release (pg min^{-1}) at 0 min (287±44), 60 min, (299±71) and 180 min (293±53) did not differ significantly. Neither occlusion nor reperfusion had a significant effect on the rate of kinin release. The fact that the rate of kinin release remained constant for up to 180 min implies that it was due to de novo synthesis and not washout. Paralellism tests on the antibody curves showed the released kinin to be BK and not T-kinin or des-Arg-BK.

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80P EFFECTS OF RYANODINE AND CYCLOPIAZONIC ACID ON RATE OF BEATING OF GUINEA-PIG ISOLATED ATRIAL PREPARATIONS

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It is well known that cardiac pacemaking is determined by the interaction of a number of ionic currents across the plasma membrane (Irisawa *et al.*, 1993). In recent years cytosolic calcium has been recognised as an important regulator of electrical activity in many cell types (e.g. Campbell, 1983) and it was therefore of interest to investigate whether modification of sarcoplasmic reticulum (SR) function (and consequently cytosolic Ca^{++} transients) could affect heart rate under normal conditions.

The effects of ryanodine or cyclopiazonic acid (CPA, an inhibitor of the SR Ca^{++} ATPase) on guinea-pig heart rate were investigated using isolated atrial preparations (in either a 50 ml organ bath or a flow chamber) bathed in oxygenated balanced salt solution (35°C, 2.5 mM Ca^{++}). Heart rate was determined from the intervals between action potentials recorded either with surface electrodes (organ bath) or intracellular microelectrodes (flow chamber).

In four atrial preparations (organ bath) exposure to 10 μM ryanodine consistently reduced the resting heart rate over a 10 to 15 min period. The mean reduction was 41±10% of the pre-drug level. In three further preparations 10 μM ryanodine applied in a flowing solution reduced the resting heart rate by 40±8%. When these two sets of observations were pooled the mean reduction in heart rate in the presence of ryanodine was 40±6 % ($P<0.001$). In four preparations exposure to 30μM cyclopiazonic acid reduced heart rate by 28±2 % ($P<0.01$).

Our observations are consistent with the hypothesis that calcium released from the SR may be involved in the regulation of ionic currents that determine heart rate; for example, calcium released from the SR might enhance diastolic depolarisation, and this may be suppressed in the presence of ryanodine or CPA. Possible currents that may be influenced by cytosolic Ca^{++} include: L-type calcium current (e.g. Gurney *et al.*, 1989), the delayed rectifier potassium current (Tohse *et al.*, 1987), hyperpolarization-activated non-specific cation current (Hagiwara and Irisawa, 1989) and sodium/calcium exchange current (Irisawa *et al.*, 1993).

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81P EFFECT OF SOCIAL INTERACTION TESTING AND ONDANSETRON ON 5-HT LEVELS IN THE RIGHT AND LEFT HEMISPHERES OF YOUNG AND AGED RATS

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We have previously reported the existence of differential *ex vivo* 5-hydroxytryptamine (5-HT) levels in the right and left hemispheres of the rat frontal cortex and hippocampus (Cheng *et al.*, 1993). The present study aims to investigate whether there are differences between the right and left hemispheres in the levels of 5-HT in the frontal cortex and hippocampus of young and aged rats in responding to social interaction testing with ondansetron or vehicle treatment.

Aged (20 months) and young (5 months) male Lister-Hooded (Bradford University Bred) naive rats received ondansetron (0.1-10 $\mu g\ kg^{-1}$) or vehicle (saline) at 1 ml kg^{-1} i.p., 40 min prior to the social interaction test (see Barnes et al., 1992 for details). Following the 10 min test, rats were killed by cervical dislocation and the brain areas were dissected for the analysis of 5-HT content using HPLC-ECD as described in Barnes *et al* (1992).

5-HT levels were significantly higher in the left frontal cortex and in the right hippocampus as compared to the other hemisphere in both aged and young rats following social interaction testing with ondansetron or vehicle treatment. In the hippocampus, ondansetron at doses of 10 $\mu g\ kg^{-1}$ in aged and 1.0 $\mu g\ kg^{-1}$ in young rats significantly reduced the 5-HT levels by 48%/31% and 56%/31% (compared to the respective vehicle values, L/R) in both the left and right hemispheres respectively. In the left frontal cortex of the aged rats, ondansetron caused a significant reduction in the 5-HT levels only at 0.1 $\mu g\ kg^{-1}$ (49%) (Table 1). Ondansetron (1.0 and 10 $\mu g\ kg^{-1}$) also significantly increased the social interaction (sec, vehicle: 42.6±5; Ondansetron 1.0 µg: 131±8.2; 10 µg: 129±7, n = 6 pairs).

The results indicate lateralised differences in 5-HT levels between the hemispheres of the frontal cortex and hippocampus following the social interaction paradigm with or without ondansetron treatment. However the neurochemical lateralities were not affected by age. It remains to be determined whether the changes in 5-HT levels in the hippocampus reflect a direct effect of ondansetron within each hemisphere or a compensatory response to changes in balance between hemispheric function.

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Table 1. Effect of ondansetron (0.1-10 $\mu g\ kg^{-1}$ i.p.) treatment in the social interaction test on the levels of 5-HT (pg mg^{-1} wet weight) in the left (L) and right (R) hemispheres of the young and aged rat brain.

		Young				Aged			
Area:		Frontal Cortex		Hippocampus		Frontal Cortex		Hippocampus	
Treatment ($\mu g\ kg^{-1}$)		L	R	L	R	L	R	L	R
Veh (saline)		142±28^{++}	25±6	152±28	248±29^{+}	185±36	34±8^{+}	166±35	284±33^{+}
Ondansetron	0.1	98±19^{++}	30±9	134±23	212±26^{+}	94±19*	40±17^{+}	146±23	234±13^{+}
	1.0	128±40^{++}	25±7	67±10*	171±13^{++}*	112±36	23±5^{++}	102±20	221±15^{++}
	10.0	105±31^{++}	35±9	99±19	220±18^{++}	139±36	30±8^{++}	86±25*	195±16^{++}*

Data represent the mean±SEM n = 6. Significant differences compared to the vehicle control values and between the left and right hemispheres are indicated:*P<0.05 and $^{+}$P<0.05, $^{++}$P<0.01 respectively (ANOVA followed by Dunnett's t test).

82P CASCADE ANALYSIS IDENTIFICATION OF LOW-LEVEL ELECTROCHEMICAL SIGNALS *IN VIVO*

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When monoamine neurotransmitters are measured in the rat frontal/parietal cortex by fast cyclic voltammetry (FCV), peak levels of 5-10nM are detected in response to a maximal electrical stimulation (100 pulses, 100 Hz, 10 mA). This is far lower than for other nuclei where FCV has been used and where signals of c5nM have been detected in response to a minimal stimulation. A problem is that, with such a small signal, it is very difficult to identify a compound by a *single* cyclic voltammogram (CV): in other nuclei, one is able to obtain a clear CV by increasing the level of the stimulus. We have therefore used an alternative approach to help identify the electrochemical signal.

Instead of single CVs, identification of compounds is made by collecting entire sequences of voltammograms following a stimulation. These are displayed as a 'cascade' in which temporal trends may be observed. Small peaks (that would be undetectable on single scans) may thus be discriminated from the background noise and the overall shape used to aid identification. This is particularly valuable for the detection of 5-hydroxytryptamine (5-HT) which generates multiple oxidation peaks (Stamford et al, 1990) that have different timecourses.

Figure 1 shows a cascade of consecutive CVs (anodic scan shown) taken during an electrical stimulation (100p, 100Hz, 0.3ms, 10mA) in a rat frontocortical slice. There are two peaks (labelled A & B), of which B is larger in amplitude and has a faster timecourse than A. This is consistent with the measured substance being 5-HT.

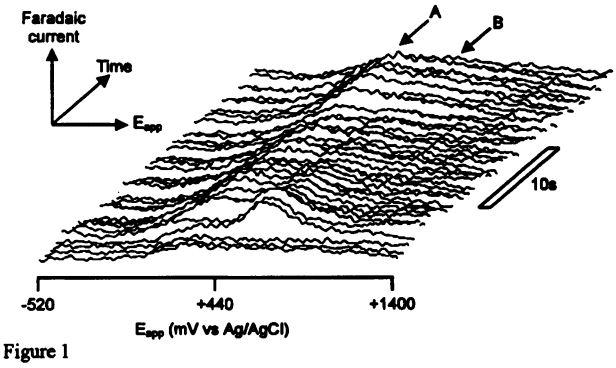

Figure 1

Individual CVs may confuse the identification of time-dependent electrochemical signals, such as 5-HT, by failing to show all oxidation and/or reduction peaks. Furthermore peak resolution on single CVs is much more susceptible to electrical noise.

We conclude that the use of *cascades* of CVs can aid the identification of small electrochemical signals in circumstances where *individual* CVs taken at different time points may have different shapes and fewer peaks. This approach is of particular value in the frontal cortex.

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83P ASSESSMENT OF *IN VIVO* BASAL 5-HT LEVELS IN THE LEFT AND RIGHT HIPPOCAMPUS OF FREELY MOVING RATS

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We have previously demonstrated in the majority of animals studied that a lateralised basal release of acetylcholine occurs in the hippocampus of either the right or left hemisphere (Cheng *et al.*, 1994). In the present study, we investigate whether similar interhemispheric differences exist in the basal release of 5-hydroxytryptamine (5-HT) in the rat hippocampus using the *in vivo* microdialysis technique.

Microdialysis probes (4 mm dialysis membrane) were implanted into chronically indwelling bilateral cannula located in the hippocampus (co-ordinates:Ant. +2.0, Vert. +8.0, Lat. ±4.8; Paxinos & Watson, 1982) of male Lister-Hooded rats (375-500 g) 16 h prior to the experiment. Probes were perfused with artificial cerebrospinal fluid at a rate of 2.0 μl min^{-1}. After a 60 min stabilisation period, 20 min samples were collected and immediately analysed for 5-HT by HPLC-ECD as described in Cheng *et al* (1993).

The basal 5-HT levels ranged from 0.78 to 3.58 pg 40μl^{-1} in the left hippocampus and 0.54 to 3.85 pg 40μl^{-1} in the left. In the 21 animals investigated, 8 had less than a 30% difference in levels of 5-HT between the left and right hippocampus, 7 had 36-158% higher 5-HT levels in the left hemisphere whilst 6 had 36-137% higher levels in the right hippocampus (Figure 1). Perfusion of tetrodotoxin (1 μM) via the microdialysis probe into the hippocampus for a period of 60 min at time (t) = 0 min caused a significant reduction ($P<0.01$, ANOVA;Dunnett's t test) in basal 5-HT levels maximally by 82% in the left (L) and 79% in the right (R) at t = 60 (% of basal levels at t = 0: 88±24 (L); 108±8 (R) and at t = 60: 16±5 (L); 23±8 (R); mean±S.E.M., n = 4).

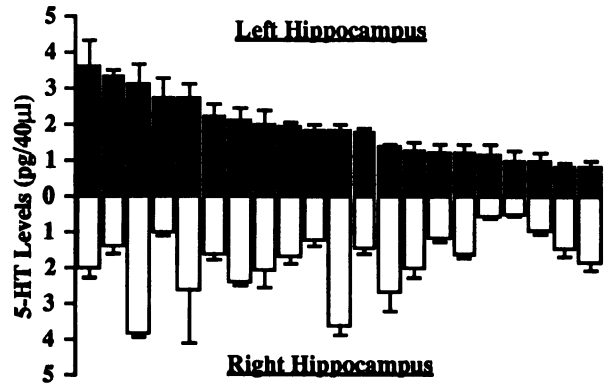

Figure 1. Extracellular levels of 5-HT in the right and left hippocampus of 21 rats. Each value represents the mean±S.E.M. of the basal levels obtained from four to five determinations in each rat.

The results indicate that lateralised basal 5-HT levels in the hippocampus exist in over 50% of the animals in the present study and that a relatively large component of the quantified 5-HT in both left and right hippocampus was neuronal in origin. The significance of the present findings to experimental design of sampling of 5-HT in microdialysis studies and the effects of drugs on serotonergic function in each hemisphere of the brain remains to be determined.

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84P EFFECT OF IONTOPHORETIC APPLICATION OF THE 5-HT_{1A} ANTAGONIST WAY100635 ON NEURONAL FIRING IN THE GUINEA-PIG DORSAL RAPHÉ NUCLEUS

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Following intravenous administration, the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) reversibly inhibits neuronal firing in the guinea-pig dorsal raphé nucleus (DRN) by an action at pre-synaptic 5-HT_{1A} somatodendritic autoreceptors. The 5-HT_{1A} antagonists, N-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide dihydrochloride (WAY100135, Fletcher *et al.*, 1993) and N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(pyridinyl) cyclohexanecarboxamide trichloride (WAY100635, Fletcher *et al.*, 1994) both antagonise the inhibitory effect of 8-OHDPAT on DRN neurones and the more potent antagonist WAY100635 also induces a dose-related increase in basal firing rate (Mundey *et al.*, 1994a; Mundey *et al.*, 1994b) when given intravenously. In the present study, 5-HT_{1A} selective compounds were iontophoretically applied into the DRN in the guinea-pig to establish the direct effects of WAY100635 on 5-HT neurones in this nucleus.

Male Dunkin-Hartley guinea-pigs (320-450 g, n=11) were anaesthetised with urethane (1.3 g kg^{-1} i.p.) and neuronal activity in the DRN (taking stereotaxic coordinates from lambda; A +0.7-1.0; L 0.0; V -6.5-7.5 mm, Mundey *et al.*, 1994b) was recorded using extracellular seven barrelled glass micropipettes, from which drugs were iontophoretically applied. Putative 5-HT neurones were identified by their slow, regular firing pattern (0.5-4.0 spikes sec^{-1}), reversible inhibition (76 ± 4%, mean ± s.e.mean) by 8-OHDPAT (10 mM, 10-25 nA, 11 of 11 cells tested, each in a separate animal) and histological confirmation of cell localisation to the DRN by iontophoretic application of pontamine sky blue.

When applied simultaneously, WAY100635 (15 mM, 10 nA, 4 of 4 cells tested in separate animals) completely prevented the inhibition of firing of DRN neurones normally produced by 8-OHDPAT (10 nA). Furthermore, WAY100635 also completely returned the firing of all neurones in the DRN which were already inhibited by 8-OHDPAT (10 nA, 3 of 3 cells) to the original basal rate. In contrast, the effect of WAY100635 (10 nA) on basal firing rate was inconsistent, only producing a slight increase (<20 %) in 2 of 6 neurones tested. In addition, the inhibition of DRN firing (by 35 and 46% in 2 of 2 neurones) produced by the 5-$HT_{1D/1A}$ agonist sumatriptan (15 mM, 15 nA) was unaltered by WAY100635.

The current iontophoretic data confirms that DRN neurones in the guinea-pig, like those in the rat, are inhibited by activation of somatodendritic 5-HT_{1A} receptors and also suggests that these neurones may possess inhibitory 5-HT_{1D} receptors. The inconsistent effect of the 5-HT_{1A} antagonist on basal neuronal firing rate implies that there is little 5-HT_{1A} mediated serotonergic inhibitory tone on DRN neurones in the anaesthetised guinea-pig.

We would like to acknowledge Wyeth Research (U.K.) Ltd for providing WAY100635.

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85P 5-HT_{1A} RECEPTORS TONICALLY REGULATE DOPAMINE SYNTHESIS AND RELEASE IN THE RAT STRIATUM, *IN VIVO*

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Interactions between CNS dopamine (DA) and 5-hydroxytryptamine (5-HT) mechanisms are well documented. Agonist studies suggest that 5-HT_{1A} heteroreceptors decrease (Johnson *et al.*, 1993) or increase (Ahlenius *et al.*, 1990; Benloucif *et al.*, 1993) striatal DA turnover *in vitro* and *in vivo*. These studies also implicate 5-HT_{1A} receptors in the tonic regulation of striatal DA turnover, however, the lack of selective 5-HT_{1A} antagonists has made the investigation of this phenomenon difficult. We have used a silent and selective 5-HT_{1A} receptor antagonist, WAY-100635, (Fletcher *et al.*, 1994) to examine the *in vivo* relationship between 5-HT_{1A} receptors and basal DA synthesis and release in the striatum. As an adjunct, striatal 5-HT synthesis and release were also studied.

Male Sprague-Dawley rats (280 - 350 g, Charles River) were used. Dialysis studies - a 4 mm probe, perfused with artificial CSF at 1.0 µl/min, was inserted into the striatum of an unrestrained animal, using a guide cannula implanted the previous day. After a 3h stabilisation period, 20 min samples were collected and analysed for 5-HT, DA, 5-HIAA and DOPAC using HPLC-ED. Three samples taken before s.c. vehicle or WAY-100635 (1.0 mg/kg) administration, were used to calculate a pre-injection control value. Results were expressed as a % of this control. Synthesis rate studies - animals received a single s.c. injection of vehicle or WAY-100635 (1.0 mg/kg), the brain was removed 1 or 3h later and the striatum dissected on ice. DA and 5-HT synthesis rates were assessed by measuring tissue 3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) levels using HPLC-ED after L-aromatic amino acid decarboxylase inhibition by NSD-1015 (100 mg/kg, i.p.) given 45 min before the rats were killed.

WAY-100635 significantly increased both the extracellular concentration and synthesis rate of DA in the striatum 1h after administration, but had no significant effect 3h after administration, table 1. These findings provide compelling *in vivo* evidence for tonic 5-HT_{1A} receptor inhibition of striatal DA synthesis and release.

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Table 1. Striatal L-DOPA and 5-HTP concentrations and *in vivo* DA, DOPAC, 5-HT and 5-HIAA concentrations after WAY-100635 (1.0 mg/kg s.c.) administration

		1 hour vehicle	1 hour WAY-100635	3 hour vehicle	3 hour WAY-100635
Dialysis	DA	100.0 ± 4.2 %	122.8 ± 7.3 % **	93.3 ± 4.8 %	91.0 ± 6.5 %
	DOPAC	84.2 ± 4.0 %	99.2 ± 3.8 % *	67.9 ± 7.3 %	75.4 ± 8.2 %
	5-HT	91.8 ± 12.2 %	91.9 ± 7.9 %	69.3 ± 9.1 %	61.8 ± 10.7 %
	5-HIAA	82.4 ± 2.9 %	92.1 ± 6.3 %	74.0 ± 5.8 %	85.0 ± 6.3 %
Synthesis rate	L-DOPA	161.4 ± 13.6 fmole/mg	245.2 ± 19.5 fmole/mg *	144.5 ± 8.2 fmole/mg	131.8 ± 6.3 fmole/mg
	5-HTP	19.9 ± 1.8 fmole/mg	23.2 ± 1.9 fmole/mg	19.4 ± 1.5 fmole/mg	19.3 ± 1.0 fmole/mg

Significance was determined using ANOVA followed by post hoc analysis. Values are mean ±s .e.m. n = 5 - 12. *p<0.05, **p<0.01,

86P BEHAVIOURAL SENSITIZATION AND DOPAMINERGIC FUNCTION IN THE SHELL OF THE RAT NUCLEUS ACCUMBENS

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Behavioural sensitization to psychomotor stimulants is used as an animal model in studies of dependence (Robinson & Becker, 1986). A role for dopamine (DA) in the development and expression of sensitization is indicated by the abolition of sensitization with drugs which interfere with DA function (Towell *et al.*, 1987). In an earlier study, we showed that changes in DA function occured in the core of the nucleus accumbens (NAc) following sensitization (Muscat *et al.*, 1993). We have now examined the effects of sensitization on DA function in the NAc shell. Behavioural sensitization (assessed by measurement of locomotor activity in open field) following administration (ip) of either 0.4mg/kg quinpirole (QUIN) or 1mg/kg (+)- amphetamine (AMP) on days 3, 6 & 9 was induced in groups of rats as previously described (Muscat *et al.*, 1993), time matched controls received 0.3 ml saline (SAL). On day 12, brain slices from these rats were prepared and electrically stimulated DA release from the shell was measured using fast cyclic voltammetry (Wieczorek & Kruk, 1994).
D_2 autoreceptor sensitivity was measured by the ability of QUIN, when superfused over the slice, to inhibit DA overflow evoked by a single pulse (1p: 0.1ms, 20V) or by 4p / 50Hz. 4p / 50Hz stimulation was used to demonstrate whether a receptor reserve might mask changes in the number or sensitivity of D_2 autoreceptors after sensitization. The degree of inhibition was expressed as a % of pre-drug control responses. In slices from SAL controls, superfusion with increasing concentrations of QUIN produced a dose - dependent inhibition of DA overflow evoked by 1p and 4p / 50Hz. The inhibitory dose response curve for QUIN using 4p / 50Hz was displaced to the right of that obtained with 1p. The IC_{50} value (concentration of QUIN required to reduce DA release by 50% of pre-drug control values) for 1p (4.3 ± 0.5nM) was significantly less than for 4p / 50Hz (7.63 ± 1.58 nM,n=5 P<0.05). Sensitization with either QUIN or AMP did not significantly alter the IC_{50} values for QUIN for either 1p or 4p / 50Hz stimulation. However, after AMP or QUIN sensitization, the maximum inhibition of DA release by QUIN using 4p / 50Hz, was 78.3 ±2.1%(n=3) and 78.1 ± 4%(n=4) respectively which were significantly (P<0.05) lower when compared to SAL controls (98.8± 1.4%inhibition). Sensitization did not alter the maximum inhibition of DA release by 1p when compared to SAL controls.
Stimulation with 25p / 50Hz produced maximum DA release. Sensitization with either AMP or QUIN resulted in no significant change in DA release evoked by 25p / 50Hz, compared to SAL controls (1850 ± 300 nM (AMP); 1816 ± 216 nM (QUIN) & 1552 ± 205 nM(SAL)).
Behavioural sensitization was induced by 3 exposures to AMP or QUIN. In the shell, sensitization cannot be explained by increased DA overflow or reduced D_2 sensitivity but our results indicate that there maybe a reduction in the number of D_2 autoreceptors which may contribute to the phenomenon of sensitization

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87P CHARACTERISATION OF THE MUSCARINIC RECEPTOR SUBTYPES INVOLVED IN THE HABITUATION RESPONSE IN THE RAT

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Numerous studies have demonstrated that muscarinic M_1 receptors play a key role in learning and memory; also a role for muscarinic receptors in habituation of exploratory behaviour is likely, since pre-treatment with either scopolamine or atropine sulphate attenuates this behaviour (Platel and Porsolt,1982). This study sets out to compare the effects of a range of cholinergic antagonists on habituation of exploratory behaviour in the rat. The effectiveness of central muscarinic blockade was determined by the degree of antagonism of oxotremorine-induced tremor in the mouse.

The habituation apparatus comprises of a circular arena with a central perspex cylinder and three symmetrically mounted infra-red beam transmitters with corresponding receivers. Male rats (Charles River) 100-200g and mice (CD1) 25-30g were used throughout these studies. In the habituation studies rats were dosed, via the tail vein, with vehicle or test compound (12 animals per group), 15 minutes prior to exposure to the activity chamber on day 1. Activity was measured in each animal for five minutes on Day 1 and again 24 hours later. Day two activity counts were analysed for differences between vehicle and test compound using the Mann Whitney U test. In the oxotremorine assay mice were dosed, via the tail vein 10 minutes prior to dosing with oxotremorine (0.2mg.kg^{-1} i.v.). After 5 minutes the animals tremor was scored (0-3). Results are shown in Table 1

The ability of scopolamine and atropine, but not the quaternary atropine methyl nitrate, to block habituation in the rat and oxotremorine-induced tremor in the mouse confirms that these activities are mediated through central muscarinic receptors. Based on the high potency of trihexyphenidyl at M_1 and himbacine at M_4 receptors (Dorje *et al* 1991), these receptors could play a role in both processes. As zamifenacin (Wallis et al 1993) freely penetrates the brain, as determined by whole body autoradiography (Smith and Beaumont, 1994), its lack of activity argues against a major role for M_3 receptors in the habituation response.

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Table 1

Compound	Atropine sulphate	Scopolamine	Trihexyphenidyl	Himbacine	Zamifenacin	Atropine methyl nitrate
Minimum effective dose (habituation) (mg.kg^{-1})	1.0	0.1	0.03	1.0	>10.0	>3.0
IC_{50} Tremor (mg.kg^{-1})	0.19	0.02	0.45	0.68	5.6	>1.0

88P EFFECT OF SCOPOLAMINE ON *IN VIVO* ACETYLCHOLINE RELEASE FROM THE LEFT AND RIGHT HIPPOCAMPUS OF FREELY MOVING RATS

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Differential *in vivo* acetylcholine (Ach) levels in the hippocampus have been demonstrated between hemispheres of the rat brain (Cheng *et al.*, 1994). The increase of Ach release in response to systemic administration of scopolamine, an anticholinergic drug, has been shown to be greater in rat hippocampus compared to rat frontal cortex (Toide, 1989). The present study examines the effect of scopolamine on the extracellular levels of Ach in the hippocampus in each hemisphere of the rat brain.

Microdialysis probes were implanted into chronically indwelling cannulae located in the dorsal hippocampus (Ant. +2.0, Vert. –8.0 and Lat. ±4.8; Paxinos & Watson, 1982) of male Lister Hooded rats (375-500g) 16 hours prior to the experiment. Probes were perfused with artificial cerebrospinal fluid containing 2 µM neostigmine at a rate of 2.0 µl min^{-1}. After 100 min stabilisation, 20 min samples were collected simultaneously from the left and right hippocampus and immediately analysed for the content of Ach by HPLC-ECD (Mark *et al.*, 1992). After the determination of four basal levels, scopolamine, 1 mg kg^{-1} was administered intraperitoneally at time (t) = 0 min.

Scopolamine (1.0 mg kg^{-1}, i.p.) caused significant increases in Ach levels to between 287-343% of the basal levels in the left and 317-414% in the right hippocampus. There was no significant difference in the magnitude of the increase in Ach release to scopolamine challenge between the left and right hemispheres of the hippocampus (Table 1). In addition, central administration of tetrodotoxin (1 µM) via the microdialysis probe for a 40 min period at time = 0 significantly reduced the Ach levels maximally by 68% and 74% in the left (L) and right (R) hippocampus respectively (% basal Ach levels at t = 0: 106±6 (L); 97±5 (R) and at t = 40: 34±5 (L); 26±4 (R); mean±S.E.M., n = 8)

The results suggests that a relatively large component of the quantified Ach release in both hippocampi was neuronal in origin and scopolamine did not exert a differential effect on the Ach levels in the left and right hippocampus of the rat.

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Table 1. Effect of scopolamine on extracellular levels of Ach in the right and left hippocampus of the rat

Time (min)	0	20	40	60	80	100	120	140	160	180
Left Hippocampus	96±10	337±37*	343±89*	287±54*	186±18	172±31	112±21	119±11	114±15	101±11
Right Hippocampus	98±8	334±47*	414±96*	317±52*	198±47	161±30	117±25	125±28	118±25	106±19

Data represent the mean±SEM (% of basal levels), n = 7. Significant differences compared to the basal levels are indicated as *P<0.01 (ANOVA followed by Dunnett's t test). There was no significant difference in response to scopolamine treatment between the left and right hippocampus (P>0.05).

89P RS-45041-190: A POTENT, SELECTIVE LIGAND FOR I_2 IMIDAZOLINE RECEPTORS, WITH EFFECTS SUGGESTING A POSSIBLE ROLE FOR THESE RECEPTORS IN THE MODULATION OF APPETITE

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Imidazoline receptors have been subdivided into I_1 and I_2 subtypes (Ernsberger, 1992). The I_1 subtype appears to be involved in central cardiovascular control (Ernsberger *et al.*, 1990) but the role of the I_2 subtype remains unclear. In this study we have investigated the binding characteristics and CNS pharmacology of RS-45041-190 (4-chloro-2-(imidazolin-2-yl)isoindoline), a high-affinity I_2 receptor ligand (MacKinnon *et al.*, 1995). Our CNS functional studies were selected on the basis of the neuroanatomical distribution of binding sites for this agent (MacKinnon *et al.*, 1995).

Binding studies: [^{3}H]-idazoxan binding to I_2 receptors on kidney membranes from rat, rabbit, dog and baboon was evaluated as described previously (MacKinnon *et al.*, 1993). Details of binding assays for other receptors are given elsewhere (Brown *et al.*, 1993). Male Sprague-Dawley rats (200-330 g) were used for the *in vivo* studies. *Central cardiovascular control:* Under pentobarbitone anaesthesia, rats received stereotaxic injections of RS-45041-190 (5 ng-50 µg in 10 µl; n = 5) into a lateral cerebral ventricle. Blood pressure and heart rate were monitored via a carotid arterial catheter. *Thermoregulation:* The effect of RS-45041-190 (1 mg kg^{-1}, s.c.; n = 6) or saline (1 ml kg^{-1}, s.c.; n = 6) on rectal and tail skin temperature (measured with thermocouples) was investigated in conscious, lightly-restrained rats (ambient temperature 18.5-20 °C). *Food and water intake:* Rats were singly housed with free access to their normal pellet diet and water, which were weighed immediately before injecting the rats with RS-45041-190 (1, 10 or 25 mg kg^{-1}, i.p.; n = 10 per group) or saline (1ml kg^{-1}, i.p.; n = 10). The food and water were re-weighed 4h post-dose. *Activity:* Rats were dosed with RS-45041-190 (10 mg kg^{-1}, s.c.; n = 6) or saline (1 ml kg^{-1}, s.c.; n = 6), and their activity in an unfamiliar cage measured 40 min later over a 9 min period, using infrared beam activity meters.

RS-45041-190 showed high affinity for I_2 receptors labelled by [^{3}H]-idazoxan on rat (pK_i = 8.66 ± 0.09), rabbit (pK_i = 9.37 ± 0.07), dog (pK_i = 9.32 ± 0.18) and baboon kidney (pK_i = 8.85 ± 0.12), but had very low affinity for α_2-adrenoceptors on rat cerebral cortex (pK_i = 5.7 ± 0.09). RS-45041-190 showed low affinity (selectivity ratio > 1000) for other adrenoceptors, dopamine, 5-HT and muscarinic receptors, and dihydropyridine binding sites. At the doses indicated above, RS-45041-190 had no effect on blood pressure or heart rate, rectal or tail skin temperature, water intake or activity. However, RS-45041-190 significantly increased food consumption in rats after doses of 10 and 25 mg kg^{-1}, i.p. ($P < 0.05$; comparison with saline-treated group using Dunnett's *t*-test), as shown in Table 1.

Table 1: Effect of RS-45041-190 on food intake in sated rats

treatment (i.p.; n=10)	*4h food intake (g kg^{-1})*	
saline (1 ml kg^{-1})	5.9 ± 1.6	
RS-45041-190 (1 mg kg^{-1})	6.6 ± 1.1	
RS-45041-190 (10 mg kg^{-1})	11.0 ± 0.8	($P < 0.05$)
RS-45041-190 (25 mg kg^{-1})	11.9 ± 1.3	($P < 0.05$)

RS-45041-190 is therefore a high-affinity, selective I_2 imidazoline receptor ligand, and its conspicuous effect on food intake may suggest a role for these receptors in the modulation of appetite. However, in the absence of a selective I_2 agonist it is unclear whether this ligand is an agonist or an antagonist.

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90P EFFECTS OF IMIDAZOLINE$_2$ (I_2) SITE LIGANDS ON FOOD AND WATER INTAKE IN THE RAT

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The α_2-adrenoceptor antagonist idazoxan has high affinity for non-adrenoceptor binding sites called imidazoline$_2$ (I_2) sites (Ernsberger, 1992). Idazoxan increases food and water intake in rats whereas selective α_2-adrenoceptor antagonists, which do not bind to I_2-sites, increase water but not food intake (Jackson et al., 1991). This suggests that the effects of idazoxan on food intake may be mediated by I_2-sites. In this study we have examined the effects of two analogues of idazoxan, RX821029 (2-(1,3-benzodioxanyl)-2-imidazoline) and RX801077 (2-(2-benzofuranyl)-2-imidazoline), on food and water intake in the rat. These compounds have 136 and 1550-fold selectivity for I_2-sites over α_2-adrenoceptors in rat brain (Hudson et al., 1995).

Subjects were individually-housed male Wistar rats (250-350g; n=6-7) which had free access to powdered rat diet and water. Experiments were performed during the light period. Feeding jars and water bottles were measured at the time of i.p. drug administration and after 2 and 4 h. Low doses of RX821029 and RX801077 (1, 3 mg/kg) had no effect on food or water intake. Cumulative food and water intakes of rats treated with RX821029 (10 mg/kg) were significantly greater than controls throughout the experiment. RX801077 (10 mg/kg) significantly increased food intake in the first 2 h following injection. However, cumulative food intake was similar to controls at the 4 h reading. RX801077 (10 mg/kg) did not significantly alter water intake. In a separate study, rats were injected daily with RX801077 (10 mg/kg) for 7 days. Food and water intake was measured in the 2 h after injection on days 1, 4 and 7. There was no evidence of tolerance to the hyperphagic effects of RX801077 (day 1: vehicle 1.9±0.4, RX801077 6.2±1.1*; day 4 : vehicle 1.6±0.5, RX801077 6.9±0.9*; day 7: vehicle 2.3±0.6, RX801077 9.3±1.5*; units as in Table 1). Surprisingly, RX801077 significantly increased water intake (g/kg) on days 4 and 7 (day 4: vehicle 6.6±0.6, RX801077 15.8±2.5*; day 7: vehicle 10.9±0.9, RX801077 28.7±4.3*).

The results of this study agree with the concept that the stimulatory effects of idazoxan on food intake may be mediated, at least in part, via I_2-sites and suggest that these sites may provide a novel target for the drug treatment of eating disorders. The effects of I_2-ligands on water intake warrant further investigation since RX801077, which has higher selectivity for I_2-sites than RX821029, significantly increased water intake after chronic, but not acute, administration.

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Table 1 Effect of I_2-site ligands on food and water intake in the rat

	RX821029				RX801077			
	Food intake		Water intake		Food intake		Water intake	
	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h
Vehicle	2.0±1.0	4.3±1.1	6.0±1.8	8.4±1.9	1.4±0.8	3.1±1.4	8.3±0.9	11.4±1.0
1 mg/kg	4.1±0.7	5.7±0.9	7.8±1.0	10.7±1.5	2.6±1.1	4.9±1.0	8.5±0.8	11.7±0.9
3 mg/kg	1.6±0.6	2.8±0.8	6.1±0.9	8.1±1.1	1.8±0.7	3.7±1.3	7.0±1.3	11.2±1.6
10 mg/kg	8.8±1.1*	9.6±0.8*	17.5±1.8*	20.4±1.8*	7.5±1.6*	8.0±1.7	11.9±3.8	15.5±3.6

Results are expressed as mean cumulative intakes (g/kg rat weight) ± s.e.mean, *P<0.05 vs vehicle (ANOVA and Dunnett's test).

91P THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR ANTAGONISTS MK-801 AND CGS 19755 INCREASE SUCROSE CONSUMPTION IN THIRSTY RATS

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NMDA receptor antagonists enhance ingestion of food and sucrose solution in rats (Wirtshafter & Trifunovic, 1988; Bednar et al, 1994). To examine the mechanism of this action, the effects of a competitive and a noncompetitive NMDA receptor antagonist, CGS 19755 (CGS) and MK-801 (MK), respectively, were compared to amphetamine (amp), which also increases feeding in rats (Evans & Vaccarino, 1987). To determine whether the action of MK might be indirectly mediated by activation of the 5-HT_{1A} receptor (Hutson et al. 1988), the effect of the 5-HT_{1A} receptor antagonist, WAY-100635 (WAY), (Fletcher et al., 1994), was examined alone and in combination with MK.

Lister hooded rats (200-270g, n=7/9) were deprived of water for 22.5h per day for 3-5 days before testing. Following two daily 20min periods of access to 5% sucrose, the rats were tested: 30min (amp) or 60min (MK, CGS, WAY) after drug treatment (s.c.) rats were given 20min access to 5% sucrose. Data were analysed using ANOVA followed by a post-hoc Dunnett's test.

MK (0.03 mg/kg) and CGS (3.0 mg/kg) significantly increased sucrose intake. This effect does not appear to be due to a stimulant action of NMDA receptor antagonists as low doses of amphetamine had no effect on sucrose intake and doses above 0.3 mg/kg reduced intake. Thus, the effects of MK and CGS on sucrose ingestion may be due to an enhancement of hunger, thirst and/or palatability. WAY, did not affect sucrose intake when administered alone nor did it block the effect of MK (0.03 mg/kg). These data provide no behavioural evidence of an interaction between a non-competitive NMDA receptor antagonist and 5-HT_{1A} receptor blockade.

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Table 1. The effect of MK-801, CGS 19755, amphetamine & WAY-100635 on sucrose consumption (g±SEM). * =$P<0.05$ cf. vehicle

Drug	mg/kg	Sucrose (g)	Drug	mg/kg	Sucrose (g)	Drug	mg/kg	Sucrose (g)
Saline		14.1 ± 0.52	Saline		16.1 ± 0.41	Saline		13.7 ± 0.68
MK	0.003	14.5 ± 0.51	CGS	1.0	16.3 ± 0.68	amp	0.03	12.7 ± 1.77
MK	0.01	15.1 ± 0.94	CGS	3.0	18.4 ± 0.55*	amp	0.10	13.2 ± 0.60
MK	0.03	17.7 ± 0.64*	CGS	10.0	16.9 ± 0.98	amp	0.30	13.9 ± 0.46
CGS	3.0	17.0 ± 0.53*	MK	0.03	18.4 ± 0.25*	MK	0.03	19.4 ± 0.87*
Saline		15.3 ± 0.80	Saline		14.1 ± 0.36	Saline		14.0 ± 0.50
amp	0.3	13.1 ± 0.35	WAY	0.03	14.7 ± 0.65	MK .03		16.9 ± 0.65*
amp	1.0	8.2 ± 1.30*	WAY	0.30	13.3 ± 0.54	MK .03+ WAY	0.03	16.6 ± 0.43*
amp	3.0	0.6 ± 0.11*	WAY	3.0	13.6 ± 0.36	MK .03+ WAY	0.30	17.1 ± 0.76*
MK	0.03	18.9 ± 1.04*	MK	0.03	18.2 ± 1.31*	MK .03+ WAY	3.0	16.6 ± 0.50*

92P BEHAVIOURAL EFFECTS OF ADENOSINE RECEPTOR LIGANDS DURING ETHANOL WITHDRAWAL

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Decreases in adenosine uptake may be involved in the behavioural effects of ethanol. Tolerance occurs to this effect and there is evidence of changes in adenosine systems after chronic ethanol intake. The present study investigated the effects of the adenosine receptor A1 selective agonist, CPA and the A1 antagonist, DPCPX during ethanol withdrawal.

Male TO mice (25-30g) were administered ethanol by liquid diet. Ethanol treated mice received control diet for 3 days, then 7 days 8% v/v ethanol diet. Control groups were pair-fed control diet. CPA, (0.1 mg/kg), DPCPX (2.5 mg/kg), both drugs or vehicle (Tween 80, 0.5%) were injected, i.p., 10 min (CPA) or 15 min (DPCPX) before testing. The CPA dose was in the middle of the range for depression of locomotor activity. Measurements of locomotor activity were made for 10 min by breaking of infra-red beams, in separate groups of mice, starting 2h, 4h or 6h from withdrawal. Responses to handling were then rated on a scale of 0-5.

At 2h, CPA significantly decreased locomotor activity in ethanol treated animals ($P<0.05$) but this effect was not significant in control mice. At 4h, locomotor activity was decreased in ethanol treated animals after vehicle injections ($P=0.02$), and the activity after CPA was significantly lower than in controls ($P<0.05$). CPA at the 6h interval decreased locomotor activity in controls ($P < 0.05$) but not in ethanol treated animals. DPCPX decreased these effects of CPA, with no effect by itself on locomotor activity. Handling-induced behaviour scores were increased in vehicle treated mice undergoing withdrawal, at each time interval ($P< 0.01$; Mann Whitney U-test, cf. controls). At the 2h interval, CPA significantly increased the handling scores in control animals ($P<0.01$), but decreased the ratings in animals undergoing ethanol withdrawal ($P<0.05$). No significant effects were seen at the other times, although the mean control value was increased at 6h. The actions of CPA at 2h was prevented by DPCPX, which had no effect alone. At the 4h interval DPCPX caused a small, but significant ($P<0.05$) increase in the scores during ethanol withdrawal.

These results provide further evidence that a complex sequence of changes occurs during ethanol withdrawal (Watson and Little, 1995) and that in the early phase of withdrawal these may involve alterations in adenosine systems. Increases in A1 receptor binding have been reported after chronic ethanol (Daly et al.,1994) and adenosine may be released as seizures begin. The increased binding may explain the greater effect on locomotor activity after ethanol but does not explain why CPA increased handling scores in controls but decreased them during ethanol withdrawal.

Watson, W.P. and Little, H.J. (1995) J. Pharmacol. Exp.Ther., 272, 870 - 884
Daly et al. (1994) Brain Res., 650, 153 -156

Table 1. Locomotor activity (L.A.) = mean ± s.e.m. Handling responses (H.R.) = medians, interquartile ranges. Controls = C; ethanol=E

Time	C/ vehicle	C/CPA	C/DPCPX	C/CPA/DPCPX	E/ vehicle	E/ CPA	E/ DPCPX	E/CPA/DPCPX
L.A. 2h	2080 ±513	1110±148	3353±741	2795±576	2101±260	569±112†	1740±144	948±276
H.R. 2h	0 (0,0)	2 (2,3)**	0.5 (0,2)	0(0,1)	3 (3,3)**	0.5 (0,2) †	3 (1,3)	4 (3,4)
L.A. 4h	2322±459	1327±107	2044±266	2112±377	1003±137*	720±176•	1138±181	1025±224
H.R. 4h	0 (0,1)	0.5 (0,2)	0(0,0)	0(0,0)	3 (2,3)**	3 (3,4)	4 (3,4)†	3 (2,4)

* P<0.05; **P<0.01 cf. controls +vehicle; †P<0.05 cf. ethanol +vehicle;• P<0.05 cf. control + CPA

93P EFFECTS OF CAM1028 ON ETHANOL WITHDRAWAL SIGNS

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Repeated administration of ethanol results in physical dependence, characterised by withdrawal signs upon removal of the ethanol. In rodents, these signs include tremors, anxiety and seizures. The CCK_B receptor antagonist CI988 has recently been reported to block some seizures when studied at 16h after ethanol withdrawal (Field *et al.*, 1992). Withdrawal signs occur at different times; handling hyperexcitability peaks around 3h after withdrawal, audiogenic seizures at 7h and N-methyl-DL-aspartate (NMDLA) sensitivity changes are maximum at 16h (Watson and Little, 1995). The present study investigated the effects of the selective CCK_B antagonist, CAM1028, on withdrawal signs.

Male TO mice (25-30g) were administered ethanol by liquid diet. Ethanol-treated mice received control diet for 3 days, then 7 days 8% v/v ethanol diet. Control groups were pair-fed control diet. Behavioural ratings were made by an observer blind to the drug treatment. CAM1028 (0.1 and 3mg/kg) or saline were injected, s.c., on withdrawal (t=0h) and 2h 20min later; handling-induced hyperexcitability was rated hourly on a scale of 0-5 (Littleton *et al.*, 1990) for 12h (n=10). In the remaining experiments, CAM1028 (0.1-3mg/kg) was injected, s.c., on withdrawal and 40min before tests. Handling hyperexcitability was rated 7h or 16h after withdrawal. Mice withdrawn for 16h were then infused, i.v., with NMDLA (70mg/ml) and the threshold dose required to elicit a clonic seizure was calculated (n=12). Different mice were individually exposed to an electric doorbell noise for 60s; latencies for onset of wild-running and clonic seizures were noted (n=18-20).

Ethanol withdrawal increased handling scores in vehicle treated mice ($P<0.001$; nonparametric analysis of variance) and 3mg/kg of CAM1028 slightly reduced these scores over the 12h period ($P<0.01$). Lower doses had no effect. Handling scores were slightly reduced by 0.1mg/kg CAM1028, 7h after ethanol withdrawal ($P<0.05$; Mann Whitney U-test) whilst at 16h, only a dose of 3mg/kg had a protective effect ($P<0.05$; see Table 1). Only the highest dose of 3mg/kg ($P<0.05$; Mann Whitney U-test) increased the latency to clonic seizures 7h after removal of ethanol, with a similar effect on latency to wild-running. The threshold dose of NMDLA (mg/kg) was decreased 16h after withdrawal ($P<0.01$; Student's t-test) and 1mg/kg and 3mg/kg of CAM1028 prevented this effect ($P<0.001$; see Table 1).

These results demonstrate that CAM1028 had some effects on the behavioural signs of ethanol withdrawal, but these were not pronounced except for the NMDLA effect.

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Watson, W.P. and Little, H.J. (1995) J. Pharmacol. Exp. Ther. 272, 870-884

CAM1028 dose:-	Con. diet + Saline	Eth. + Saline	Eth.+ 0.1mg/kg	Eth.+ 0.3mg/kg	Eth.+ 1 mg/kg	Eth.+ 3 mg/kg
Handling score (7h)	0.5 (0-2)	3.5 (3-4)††	3 (3-3)*			3 (3-4)
Clonic seizure latency (7h)	-	32.9 ± 4.8	42.5 ± 5.3	39.8 ± 5.0	42.4 ± 5.1	48.7 ± 4.0*
Handling score (16h)	1 (0-1)	2 (2-3)††	2 (1-1.5)	3 (2.5-3)	2 (1-2)	1 (1-2)*
NMDLA threshold (16h)	161.1 ± 18.3	95.4 ± 8.6†	103.5 ± 9.5	115.9 ± 7.0	156.0 ± 11.7**	164.5 ± 11.3**

Table 1: Handling-induced hyperexcitability is expressed as median and interquartile ranges; NMDLA threshold (mg/kg) and latencies (s) are expressed as mean ± s.e.m. †$P<0.01$, ††$P<0.001$ cf. control group, *$P<0.05$, **$P<0.001$ cf. ethanol alone.

94P ACTIONS OF BICUCULLINE AND 4-AMINOPYRIDINE ON GENERAL ANAESTHESIA INDUCED BY ETHANOL OR KETAMINE

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The mechanism of general anaesthesia is not fully understood. Some central stimulant compounds have been suggested to decrease anaesthesia (e.g. Dolin et al., 1988) but there has been no systematic study of which compounds are effective. The following study was designed to examine the action of drugs which produce hyperexcitability via different mechanisms, on the loss of righting reflex caused by either ethanol or ketamine.

Male TO mice (25-35g) were administered i.p. with 4-aminopyridine (4-AP), N-methyl D,L aspartate (NMDLA), bicuculline or saline vehicle as indicated in the table. The doses of these drugs represented the dose which would cause seizures in 50% of previously untreated animals.

Dose-response curves were constructed, by an observer blind to the drug treatment, by injecting separate groups of 10 mice with ethanol (20% in saline i.p., 2.8 - 3.8 g/kg) or ketamine (80 - 160 mg/kg i.p.). At least 4 doses were used for each curve. The number of mice with lost righting reflex was counted 10 min after the anaesthetic injection which corresponded with the peak duration of anaesthesia. For each dose of convulsant drug, the dose of anaesthetic required to cause loss of righting reflex in 50 % of the animals was calculated by the method of Lichfield and Wilcoxon (1949).

The results shown below, indicate that bicuculline antagonised the anaesthesia due to ethanol but not that due to ketamine. The excitatory amino acid NMDLA had no effect on anaesthesia due to either agent. 4-AP antagonised both anaesthetics when it was given 5 min prior to the anaesthetic injection. However, when 4-AP was given 20 min before the ethanol, this drug caused a significant potentiation of ethanol-induced anaesthesia. Although pharmacokinetic effects cannot be ruled out until central drug concentrations are measured, it appears that the antagonism of anaesthesia by these convulsant drugs operates via specific mechanisms, and that the interaction may be different for each anaesthetic agent.

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Litchfield, J.T. and Wilcoxon, F. (1949) J. Pharmacol. Exp. Therap., **96**, 94-113.

Key: * $P < 0.05$

Drug	Ethanol Pretreatment (min)	Ethanol ED50 (mg/kg)	Ethanol 95% limits	Ketamine Pretreatment (min)	Ketamine ED50 (mg/kg)	Ketamine 95% limits
Saline	5	3.60	(3.43-3.78)	5	112	(98 - 128)
Bicuculline 2.9 mg/kg	5	3.93	(3.75 - 4.12)*	5	110	(95 - 165) N.S.
NMDA 300 mg/kg	5	3.51	(3.30 - 3.74) N.S.	5	110	(96 - 124) N.S.
Saline	5	3.24	(3.15 - 3.34)	5	112	(98-128)
4-AP	5	3.67	(3.56 - 3.78) *	5	149	(139 - 160) *
Saline	20	3.60	(3.43-3.78)	20	106	(96 - 117)
4-AP 8 mg/kg	20	3.04	(2.84 - 3.24)*	20	133	(107 - 164)*

95P HYPERALGESIA AND FEVER IN RATS FOLLOWING i.c.v. ADMINISTERED LIPOPOLYSACCHARIDE: EFFECTS OF BRADYKININ B_1 AND B_2 RECEPTOR ANTAGONISTS

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Bradykinin (Bk) and des-Arg^9 Bk are formed rapidly following tissue injury or inflammation and induce their effects via the activation of B_2 or B_1 receptors, respectively. Apart from their well known actions in the periphery, these peptides are also active in the CNS where they stimulate neural and neuroglial tissues to synthesise and release other pro-inflammatory mediators (Walker, *et al.* 1995). The present study examined whether inhibition of kinin activity could alleviate some effects of CNS inflammation.

We have investigated the development of thermal hyperalgesia and fever following i.c.v. injections of *E. Coli* lipopolysaccharide (LPS). Rats (170-190 g) received a single injection of LPS (200 ng) in 10 µl of sterile saline into a lateral ventricle of the brain under enflurane anaesthesia. Body temperature (BT) was monitored via a rectal probe before and at 2 h intervals following LPS treatment. Thermal (Ugo Basile Plantar Test) hind paw withdrawal thresholds (PWT) were measured before and at 4, 6, 8 and 24 h following LPS treatment. Data were analysed using ANOVA and post-hoc comparisons (*t* test, Tukey, Dunnett's) and significant differences are reported for $P < 0.05$ (n = 6/group). BT and PWT are expressed as the change from pre-LPS baseline (mean ± s.e. mean). LPS-induced increases in BT and decreases in PWT were maximal 6 h after injection. LPS produced a mean BT increase of 1.2 ± 0.2 °C and a mean decrease in PWT of -5.4 ± 1.3 s following i.c.v. administration but there was no change in BT or PWT when this dose was administered s.c., or when sterile saline was injected i.c.v. LPS-induced fever was inhibited by the B_2 receptor antagonist HOE 140 (30 pmol: 0.1 ± 0.2 °C) when co-administered i.c.v., but not systemically (30-300 nmol, s.c.). HOE 140 inhibited LPS-induced decreases in PWT (30 pmol: -0.2 ± 0.5 s) when given i.c.v. HOE 140 (300 nmol/kg) also inhibited LPS-induced decreases in PWT (-0.03 ± 0.9 s) when administered s.c., but lower doses (30 & 100 nmol/kg) were ineffective. LPS-induced fever and decreases in PWT were also inhibited by 10 nmol of indomethacin (dissolved in 2 % Na_2CO_2 and then buffered to pH 7 with NaH_2PO_4) i.c.v. (mean BT increase: 0.2 ± 0.1 °C; mean PWT decrease: -0.1 ± 0.9 s), and by a dose of 30 µmol/kg (mean BT change:-0.2 ± 0.1°C; mean PWT change: 0.9 ± 0.6 s) . Co-administration of the B_1 receptor antagonists, des-Arg^9-Leu^8 Bk (0.1-1 nmol) or des-Arg^{10} HOE 140 (0.1-1 nmol) i.c.v. had no effect on LPS-induced fever or decreased PWT. These experiments demonstrate that administration of endotoxin to the CNS induces the development of hyperalgesia and fever and that these responses involve the activity of kinins, via the stimulation of B_2 receptors, and the formation of prostanoids.

Walker K., Perkins M. & Dray A. (1995). *Neurochem. Int.* **26**, 1-16.

96P INTERLEUKIN-1 RECEPTOR ANTAGONIST INHIBITS IL-1 FEVER IN THE BRAIN IN RATS

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Interleukin-1 (IL-1) is an important inflammatory mediator and a potent endogenous pyrogen. IL-1 expression is induced in brain by a variety of stimuli, including peripheral or central injection of lipopolysaccharide (LPS), and many of its effects, including induction of fever, are ascribed to direct actions within the CNS (Rothwell & Luheshi, 1994). The pyrogenic responses to peripheral injections of IL-1 can be inhibited by co-injection of the endogenous competitive IL-1 receptor antagonist (IL-1ra), but the effectiveness of IL-1ra within the brain is disputed. Previous work has suggested that IL-1ra inhibits some central IL-1 actions (Bluthe *et al.*, 1992), but not fever or thermogenesis (Kent *et al.*, 1992). The aim of the present study was to investigate further the effects of IL-1ra in the CNS on the pyrogenic actions of exogenous IL-1α or β, and endogenous IL-1 induced by peripheral injection of LPS in conscious free moving animals.

Male, Sprague Dawley rats (250-300g) were implanted with temperature-sensitive radiotransmitters (Data Sciences, St. Paul, USA) in the abdominal cavity and intracerebroventricular guide cannulae, under pentobarbitone (60mg/kg) anaesthesia. Animals were injected with maximal effective doses of IL-1α (5ng) or IL-1β (5ng) icv to induce fever and co-injected with IL-1ra (2.5µg/rat, icv). Another group of animals was injected with LPS (100µg/kg, ip) to induce fever and injected with IL-1ra (100µg/rat or 200µg/rat, icv) twice (1h and 2h) after LPS. Body temperature was monitored continuously by remote radiotelemetry (Data Sciences, St. Paul, USA). Data are presented as mean±SEM and statistically analysed using MANOVA.

Animals injected with IL-1α or IL-1β icv developed fever with rapid onset (30 min), which reached maximal values 38.6±0.1°C and 39.4±0.1°C, respectively after 3h, compared to 37.3±0.2°C in control animals. Coinjection of IL-1ra significantly attenuated fever induced by IL-1β (37.7±0.4°C at 3h, $P<0.01$) or IL-1α (37.1±0.1°C at 3h, $P<0.01$). Injection of LPS resulted in fever which reached 38.7±0.2°C after 3h, compared to 37.2±0.1°C in control animals. This fever was not significantly modified by IL-1ra (2x100µg/rat 1h and 2h after LPS, 38.3±0.15°C after 3h). In contrast, animals treated with 2x200µg/rat IL-1ra at 1 and 2h exhibited a significantly reduced fever (37.9±0.15°C at 3h, $P<0.05$). These data suggest that IL-1ra can inhibit the pyrogenic actions of exogenous IL-1α and ß in the brain in free moving animals at a dose ratio of 1:500. The effect of icv injection IL-1ra on fever induced by ip LPS, presumably reflects the actions of brain IL-1, although 160-fold higher dose was required to inhibit the febrile response.

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97P FEVER AND INTERLEUKIN-6 RESPONSES TO SYSTEMIC OR LOCALISED (AIR POUCH) INJECTION OF LIPOPOLYSACCHARIDE

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Systemic injection of lipopolysaccharide (LPS) has been extensively used in experimental animals as an inflammatory stimulus to study fever and the involvement of circulating cytokines, such as interleukin-6 (IL-6) (LeMay *et al* 1990). However, normal systemic injection limits the study of important cytokine relationships and interactions during the induction of fever. We have recently shown that administration of turpentine subcutaneously (sc) into an air pouch is a useful procedure for studying fever (Miller *et al* 1994), and cytokines have been detected in the pouch in response to LPS (Ferrándiz and Foster 1991). The aim of this study was to investigate the kinetics of IL-6 production within the air pouch and plasma following injection of LPS sc. The resultant fever and changes in plasma IL-6 activity were compared with those induced by intraperitoneally (ip) administered LPS.

LPS (100μg/kg) (dose determined by preliminary studies) or 0.3ml sterile saline for controls (n=4-8) was injected either ip or sc into a six day old air pouch in male, Sprague-Dawley rats (200-300g). Air pouches were formed under anaesthesia as described earlier (Miller *et al* 1994). Core temperatures were monitored using remote, radio-telemetry via pre-implanted abdominal transmitters. To investigate the kinetics of the circulating and locally produced IL-6, animals were killed at specific time-points after injection of LPS. Blood was collected by cardiac puncture, and plasma prepared. In animals injected sc, the pouch was lavaged with 1ml of sterile saline. The lavage fluid was extracted and centrifuged for two minutes at 13000rpm and the supernatant collected. Plasma and pouch fluid samples were assayed for IL-6 using the B9-hybridoma cell-line. Data are expressed as mean$\pm$SEM and were analysed using ANOVA, with a 5% significance level.

LPS induced fever peaked at 3h (38.7$\pm$0.2°C, compared with 37.1$\pm$0.2°C at 0h, $p<0.001$) when injected sc or 4h (38.3$\pm$0.2°C, compared with 37.1$\pm$0.1°C at 0h, $p<0.0001$) when injected ip. Circulating IL-6 concentrations peaked 3h after ip injection (5765$\pm$942IU/ml, compared with 7$\pm$1IU/ml at 0h, $p<0.001$), or 2h (1346$\pm$447IU/ml, compared with 35$\pm$21IU/ml at 0h, $p<0.05$) after sc injection, thus preceding the fever peak. IL-6 concentration within the pouch fluid increased earlier (0.5h), with a 25-fold higher peak IL-6 concentration (33885$\pm$7129IU/ml after 3h, compared with 96$\pm$20IU/ml at 0h, $p<0.01$) than was observed in the plasma of the same animals. The results of this study show that similar fevers can be induced by systemic and local injection of LPS (100μg/kg), despite lower concentrations of circulating IL-6 being induced after the sc route, which suggests that locally produced cytokines may be important in the induction of fever.

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98P THE EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITION ON EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE LEWIS RAT

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Nitric oxide (NO), a potent cytotoxic and vasodilator molecule, is implicated in the pathogenesis of multiple sclerosis and its animal counterpart experimental allergic encephalomyelitis (EAE) (Mitrovic *et al*, 1994). The use of the nitric oxide synthase (NOS) inhibitors aminoguanidine and N^G-monomethyl-L-arginine to modify the course of EAE via suppression of NO formation has however produced conflicting results (Ruuls *et al.*, 1994; Cross *et al.*, 1994). Consequently we have examined the effects of aminoguanidine and another NOS inhibitor, 7-nitroindazole (Babbedge *et al*, 1993) on disease progression and central nervous system (CNS) nitrogen metabolite levels in the Lewis rat model of EAE.

Male Lewis rats (200-300g) inoculated for EAE were administered either aminoguanidine (AG) (200mg/kg) or vehicle (Veh) s.c. and 7-nitroindazole (7-NI) (10mg/kg) or vehicle i.p. once daily from days 1 to 13 and days 7 to 11 post-inoculation (P.I.) respectively. Animal weights and disease symptoms were recorded daily. Symptoms were scored on a severity scale from 0 to 5. NO levels within CNS cytosol preparations were measured as the nitrite breakdown product by the Griess reaction (Green *et al.*, 1982).

Administration of both aminoguanidine or vehicle lead to significant reductions in the CNS nitrite levels of EAE-inoculated animals: EAE-diseased 407±80, AG 74±34, Veh 27±12 µmoles nitrite/mg protein in cerebellum (Mean ± S.E.M., n=6; $p<0.01$, Mann Whitney U Test). 7-nitroindazole and vehicle also had similar effects upon CNS nitrite levels: EAE-diseased 407±80, 7-NI 33±18, Veh 62±29, µmoles nitrite/mg protein in cerebellum (n=6; $p<0.01$, Mann Whitney U Test). However, weight changes and the course of the disease were not significantly altered following administration of the NOS inhibitors ((Day 12 P.I. Mean weight loss in g ± S.E.M. AG 9.5±1.6, AGVeh 7.2±2.2, 7-NI 4.3±2.5, 7-NIVeh 4.5±1.0, Day 12 P.I. Mean clinical score ± S.E.M. AG 4.2±0.3, AGVeh 3.5±0.7, 7-NI 2.2±0.5, 7NIVeh 2.3±0.3, n=6).

In conclusion, treatment with NOS inhibitors or vehicle markedly reduced NO levels within the CNS tissue from rats with EAE, but did not affect the neurological development of the disease. Such results illustrate the need for appropriate vehicle controls when studying *ex vivo* NO production.

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99P RECEPTORS MEDIATING TACHYKININ-EVOKED DEPOLARISATIONS OF THE NEONATAL RAT SPINAL CORD: EVIDENCE FOR A 'SEPTIDE-SENSITIVE' RECEPTOR

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Considerable evidence supports a role for both substance P and neurokinin A in nociceptive processing in the spinal cord. However, there are conflicting reports regarding the importance of the different tachykinin receptors. Here we have investigated the contribution of NK_1, NK_2 and NK_3 receptors to tachykinin-evoked depolaristions of the neonatal rat spinal cord *in vitro*. The spinal cord and tail from 1 day old rat pups were perfused separately with Krebs solution at room temperature. Drugs were perfused onto the spinal cord, and ventral root potentials (VRPs) measured using a suction electrode. Agonist responses were expressed as a percentage of the maximal response to a noxious heat stimulus applied to the tail.

The activities of the NK_1 receptor selective agonists substance P methyl ester (SPOMe), [Sar^9]-SP-sulphone and septide, the NK_2 selective agonist [β-Ala^8]-NKA(4-10), and the NK_3 selective agonist senktide, are shown in Table 1. The NK_1 receptor antagonist RP67580 was a more potent antagonist of the NK_1 agonists than CP-96,345 although both were more active against septide than [Sar^9]-SP-sulphone (Table 1). Indeed, a significantly higher pK_B value was obtained for antagonism by RP67580 of responses to septide (7.67 ± 0.04; n=9) compared to [Sar^9]-SP-sulphone (7.18 ± 0.05; n=9; P<0.01). In both cases Schild analysis indicated competitive antagonism. RP67580 also inhibited responses to [β-Ala^8]-NKA(4-10) (pA_2=7.5), although a shallow Schild regression (slope = 0.44 ± 0.05) indicated an action of [β-Ala^8]-NKA(4-10) at a site additional to the NK_1 receptor.

Table 1. Activities of tachykinin receptor agonists and the NK_1 antagonists RP67580 and CP-96,345 in the rat spinal cord. Each case shows mean (with 95 % CL) from 4-6 preparations).

Agonist	*EC_{50} (nM)*	*Antagonist IC_{50} (nM)*	
		RP67580	*CP-96,345*
SPOMe	7.2 (4.4-10.9)	16.0 (10.7-23.4)	840 (510-1,400)
[Sar^9]-SP-sulphone	3.09 (1.6-5.1)	58.0 (41.0-89.0)	790 (500-1,170)
septide	4.6 (1.8-9.3)	19.8 (8.9-37.0)	370 (270-510)
senktide	12.0 (5.1-23.4)	inactive	
[β-Ala^8]-NKA(4-10)	228.2 (138-350)		

The NK_2 receptor antagonist MEN 10,376 (1 μM) partially inhibited responses to [β-Ala^8]-NKA(4-10) with a maximal reduction of 25.6 ± 7.3 % (n=9), and to septide (30.2 ± 5.6 % inhibition, n=4). MEN 10,376 did not affect responses to senktide or [Sar^9]-SP-sulphone. In contrast, the non-peptide NK_2 antagonist SR 48,968 (1 μM) produced a maximal 48 ± 7.7 % inhibition of responses to [β-Ala^8]-NKA(4-10) whilst having no effect against responses to septide.

These data suggest that NK_1 and NK_3 receptors mediate depolarisations of the neonatal rat spinal cord with a lesser involvement of NK_2 receptors. Moreover, they suggest the presence of two NK_1 receptor populations, showing preference for [Sar^9]-SP-sulphone and for septide, at which RP67580 shows different affinities.

100P INHIBITION BY CAPSAZEPINE AND RUTHENIUM RED OF THE PRO- AND ANTI-NOCICEPTIVE EFFECTS OF OLVANIL

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We previously reported the antinociceptive effect of olvanil in the phenyl-p-quinone (ppq)-induced mouse abdominal constriction model (MAC) and a pro-nociceptive effect in the 50°C mouse hot-plate model (Davey *et al.*, 1994). In the present study, we have compared the inhibitory actions of capsazepine, a vanilloid receptor antagonist, with a non-selective cation channel blocker, ruthenium red, in these models.

Male ICR mice were randomly distributed into groups of 10 and dosed sc with olvanil (2-3mgkg^{-1}) or vehicle (1% ethanol, 1% Tween 80) 180 min prior to testing in either the mouse hot-plate (50°C), or MAC (2.5mgkg^{-1} ppq ip) model. In some mice, capsazepine (100mgkg^{-1} sc in 10% ethanol, 10% Tween 80), ruthenium red (4mgkg^{-1} sc in 0.9% NaCl) or the appropriate vehicle was given 15min pre- or 150min post-olvanil. Nociceptive thresholds were measured in both tests by recording the latency to first reaction to the nociceptive stimulus. Percentage antinociception in the MAC model was determined using the following formula:

$$\% = \frac{T-C}{600-C} \times 100$$

Where T = test latency (s), C = mean control latency (s), 600 = cut off latency (s). Data were analysed using Students 't' test. Values are stated as mean ± s. e. mean. P values < 0.05 were considered to be significant.

In both tests neither capsazepine nor ruthenium red had any intrinsic activity. In the hot plate model, olvanil (3mgkg^{-1}) produced a decrease in nociceptive threshold (8.4 ± 0.4s, P<0.005) which was significantly different (P<0.005) from control (22.4 ± 2.1s). Capsazepine dosed 15min prior to olvanil significantly reversed (P<0.002) this lowering of threshold (14.3 ± 1.8s). However, when given 150min post-olvanil, capsazepine (11.0 ± 0.8s) had no effect against the significant threshold lowering effect (10.0 ± 0.4s versus control 27.4 ± 2.1s). Ruthenium red,.administered before olvanil, significantly attenuated (14.8 ± 2.0s, P<0.03) the lowering of threshold by olvanil (9.2 ± 1s versus control 30.4 ± 1.7s). Furthermore, ruthenium red dosed 150min post-olvanil also reversed (12 ± 1.2s) the pro-nociceptive effect of olvanil (8.5 ± 0.6s versus control 28.2 ± 2.5s , P<0.02).

In the MAC model, capsazepine given post-dose had no effect on olvanil but, with pre-dosing, reduced the antinociceptive effect of olvanil (2mgkg^{-1}) from 68.1 ± 13.1 % to 9.2 ± 1.7 % (P<0.004). Ruthenium red dosed 15min prior to olvanil significantly (P<0.001) lowered antinociception from 83.9 ± 8.2 % to 11.5 ± 15.0 %. Moreover, ruthenium red dosed 150min post-olvanil (2mgkg^{-1}), completely abolished the antinociception from 77.7 ± 13.7 % to 0.34 ± 8.6 % (P<0.001).

In conclusion, pre-dosing with the antagonists, capsazepine and ruthenium red, blocked the effects of olvanil in both models. In addition, the established pro-nociception and antinociception induced by olvanil were only inhibited by ruthenium red. These observed differences could be important in the understanding of the operation of the capsaicin sensitive ion channel.

Davey, P.T., Hamilton, T.C., & Sanger, G.J. (1994) Br. J. Pharmacol. **112**, 606P.

101P DEXTROMETHORPHAN POTENTIATES THE ANTIPARKINSONIAN ACTION OF L-DOPA IN RESERPINE-TREATED MICE

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In Parkinson's disease (PD) the primary loss of nigrostriatal neurones results in a secondary hyperactivity of glutamatergic neurones in the basal ganglia. It has been hypothesised that compounds which block the N-methyl-D-aspartate (NMDA) receptor-ion channel might therefore be used in conjunction with L-DOPA to relieve parkinsonian akinesia. Most such compounds are poorly tolerated by man. However, dextromethorphan (DEX) is a well-tolerated antitussive with weak NMDA antagonist activity, which suggests it might be useful in the treatment of PD (Montastruc et al., 1994). This study examines the antiPD activity of DEX in the reserpine-treated mouse.

Groups of 6-14 normal or 24 h reserpine-treated (5 mg/kg) mice received DEX and/or L-DOPA (plus 100 mg/kg benserazide), the D_1 agonist SKF 38393 or the D_2 agonist RU 24213. Motor activity was then monitored for 2 h by means of a microwave doppler sensor.

Normal mice gave a 2 h motor count of 3327±832. DEX (5-20 mg/kg i.p.) was without effect, whilst 40 mg/kg induced hyperlocomotion (21218±6005 counts, $P<0.05$) and stereotypy in a small fraction (4/14) of mice. DEX 80 mg/kg gave rise to modest ataxia but no hyperactivity (5770±1595 counts). Twenty four h reserpine-treated mice were totally akinetic (2 h counts 106±46). Fluent locomotion was reinstated by 150 mg/kg i.p. L-DOPA (1529±444, $P<0.05$) and 30 mg/kg i.p. SKF 38393 (4359±1034 counts), while more stilted locomotion was induced by 5 mg/kg s.c. RU 24213 (851±329 counts). DEX (10-40 mg/kg) was without effect on its own in reserpine-treated animals. However, 20 or 40 mg/kg DEX markedly potentiated the motor response to L-DOPA (motor counts 5791±2880 and 6175±1721 respectively, $P<0.05$), and the higher dose also facilitated SKF 38393 (13444±2232 counts, $P<0.05$).

These findings demonstrate that DEX has no antiPD activity of its own in monoamine-depleted mice, but is capable of enhancing that of L-DOPA without discernible side effects. This action of DEX could be attributable to its weak NMDA receptor blocking action. The mechanism by which DEX facilitates the motor response to L-DOPA in reserpine-treated mice could be due, at least in part, to an increase in the postsynaptic efficacy of D_1 but not D_2 receptor stimulation. Alternatively, DEX may enhance the biotransformation of L-DOPA and/or dopamine release.

Montastruc, J.L., Fabre, N., Rascol, O. *et al.* (1994) *Mov. Disord.* 9, 242.

102P CHARACTERISATION OF AN EPITOPE TAGGED FORM OF THE D_2 DOPAMINE RECEPTOR

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The D2 dopamine receptor is an important site of drug action and has been studied intensively in the recent past. It is now important to study the detailed structure and regulation of this receptor and this will necessitate the preparation of large amounts of the pure receptor. In order to facilitate this we have prepared an epitope tagged form of the D2(long) dopamine receptor and expressed this in insect cells (Sf 21).

The FLAG epitope (DYKDDDDK) was engineered on to the N-terminal of the receptor as described in Sanderson and Strange (1994) and expressed in Sf21 cells. The recombinant receptor was characterised using the binding of [^{3}H]spiperone in saturation and competition assays. Effects of the receptor on adenylyl cyclase activity were determined after labelling cellular ATP pools with [^{3}H]adenine and with separation of [^{3}H]cAMP on dowex and alumina columns. The recombinant D2 dopamine receptor containing the epitope tag (D2-F) was expressed at high levels in the Sf21 cultures at 30 h after infection and saturation analyses with [^{3}H]spiperone showed a Bmax of 1.8±0.1pmol/mg and Kd of 0.30±0.03nM (mean±S.E.M., 6 observations). The D2-F was characterised further in competition analyses with a series of selective antagonists and the following Ki values were obtained: (+)-butaclamol: 1.36±0.12nM, haloperidol: 10.8±1.4nM, (-)-sulpiride: 108.4±11.0nM, mean ± S.E.M., 3 observations. These were in excellent agreement with the values obtained for the native receptor expressed in Sf21 cells (Woodcock et al 1994) indicating that the addition of the FLAG tag had not altered the ability of the receptor to bind ligands. In order to assess the functional activity of the receptor we examined the effect of dopamine on the levels of cyclic AMP in the cells after stimulation by forskolin (10μM). Whereas forskolin raised cAMP levels over the basal level (by 10.9±0.5fmol/10^6 cells), in the presence of 0.5 μM dopamine a reduction in stimulation was seen (5.9±0.4fmol/10^6 cells). This effect of dopamine was reversed by the antagonist (+)-butaclamol (1μM) (10.9±0.8fmol/10^6 cells) (all values mean±range, 2 observations).

In conclusion, the epitope tagged form of the D2 dopamine receptor studied here retains the properties of the native receptor and, in addition, is functional in that it can mediate inhibition of adenylyl cyclase in the Sf 21 cells. The epitope tagged form of the D2 receptor should facilitate the further characterisation of this important site of drug action.

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103P THE ROLE OF CONSERVED SERINE RESIDUES IN THE BINDING OF AGONISTS TO D_2 DOPAMINE RECEPTORS

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Three serine residues are conserved in the fifth putative transmembrane spanning region of each of the dopamine receptors and by analogy with work on the β_2-adrenergic receptor it has been suggested that these may be involved in the binding of agonists containing catechol moieties. In order to examine this hypothesis for the D2 dopamine receptor we have mutated these serine residues (Ser 193, 194, 197) to alanines and evaluated the properties of the mutant receptors.

Single point mutations were inserted in to the cDNA for the long form of the rat D2 dopamine receptor using the oligonucleotide directed mutagenesis method (Amersham International). The mutant cDNA's were expressed in COS-7 cells and the properties of the mutant receptors determined using ligand binding assays with [^{3}H]spiperone (as described in Woodward et al 1994). For assays with agonists 100μM GTP was present so that agonist binding to the free receptor could be measured.

Each of the mutant receptors bound [^{3}H]spiperone with a high affinity (Kd ~50pM) similar to that observed for the native receptor. The binding of a range of antagonists was tested in competition assays versus [^{3}H]spiperone and for most of these the affinities for the mutant receptors were very similar to those of the native receptor (Coley et al 1994) indicating that the mutation had not altered the conformation of the receptor. Agonist binding was tested in competition assays in the presence of 100μM GTP in order to determine the affinities of the agonists for the receptor uncoupled from G-proteins. The affinities for agonists including dopamine are given in Table 1 and show that all three mutations alter the affinity of the receptor to bind these agonists and that the effects of the mutations are different for the different agonists. The magnitude of the effects seen is consistent with the mutation disrupting hydrogen bonds between the serine residues and the ligands (presumably to the catechol hydroxyl groups).

From these data it can be concluded that there is no single mode of binding of all catechol-containing agonists to the D2 dopamine receptor and that each of the three conserved serine residues in the fifth putative transmembrane region of the receptor can participate in hydrogen bond interactions with agonists.

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Table 1. The binding of agonists to native and mutant D2 dopamine receptors. (Ki, nM, mean±S.E.M., three or more experiments)

	native	Ala 193	Ala 194	Ala 197
dopamine	1093±148	83740±2900	9846±804	2477±620
5,6-ADTN	903±215	18362±3800	3616±1003	7410±1504
6,7-ADTN	96±13	6373±1586	397±73	106±9

104P DIFFERENT EFFICIENCY OF G-PROTEIN COUPLING OF THE RAT D_{2L} AND D_3 DOPAMINE RECEPTORS EXPRESSED IN CHO CELLS

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It was proposed that, like D_2 dopamine receptors (DARs), the D_3 DAR would couple to a pertussis toxin sensitive G-protein. The initial reports of rat D_3 DAR expression in CHO cells indicated that no modulation of agonist binding by Gpp(NH)p was observed (Sokoloff *et al.*, 1990) though this has since been revised (Sokoloff *et al.*, 1993) in agreement with other studies (Castro & Strange, 1993 and Chio *et al.*, 1994). The D_3 DAR was shown to couple to the same functional responses in CHO cells as the D_{2L} DAR (Chio *et al.*, 1994) including inhibition of forskolin-stimulated cAMP accumulation. Similarly we have reported that the D_3 DAR expressed in CHO cells inhibits forskolin-stimulated cAMP accumulation (Hall & Strange, 1994). In this study we compare the coupling of rat D_{2L} and D_3 DARs expressed in CHO cells to similar levels.

The interaction of DARs with G-proteins was measured using three approaches; radioligand binding assay, [^{35}S]GTPγS binding assay and cAMP assay. Agonist binding, in membranes, was shown to be GTP sensitive. However, 0.1mM GTP induced a greater decrease in the affinity of dopamine for the D_{2L} DAR (pK_i=6.26±0.08, pK_{iGTP}=5.51 ±0.02 mean ± sem; n=3; K_{iGTP}/K_i=5.6) than that observed for the D_3 DAR (pK_i=8.21±0.07, pK_{iGTP}=7.99±0.05 mean ± sem; n=14; K_{iGTP}/K_i=1.7). In the [^{35}S]GTPγS binding assay a maximally stimulating concentration of dopamine, 100μM, promoted a greater percentage stimulation of [^{35}S]GTPγS binding in membranes containing D_{2L} DARs than those containing D_3 DARs, 44±4% and 17±3% (mean ± sem; n≥10) above basal, respectively. A similar difference in the maximal level of inhibition of 10μM forskolin-stimulated cAMP accumulation was observed for D_{2L} and D_3 DARs, 65±3% and 46±3% (mean ± sem; n≥3), respectively.

These data imply that the efficiency of coupling to G-proteins is lower for the D_3 DAR than for the D_{2L} DAR in CHO cells. This is entirely consistent with the results and conclusions of Chio *et al.* (1994). This difference in the efficiency of G-protein coupling is probably due to differences in the amino acid sequence of the D_3 DAR when compared to the D_{2L} DAR in the regions involved in coupling. The third intracellular loop has been identified as being critical in specifying G-protein coupling (Savarese & Fraser, 1992). This region has the least homology, 27%, of any intracellular region of the two receptors. This may indicate that *in vivo* D_{2L} and D_3 DARs couple to different G-proteins and that CHO cells may not express the appropriate G-proteins for efficient D_3 DAR coupling.

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105P EXPRESSION OF THE 5-HT_{2B} RECEPTOR PROTEIN IN THE RAT BRAIN

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The 5-HT_{2B} receptor is the most recent addition to the 5-HT_2 receptor family; being cloned initially from the rat stomach fundus (Foguet *et al.*, 1992) where it mediates 5-HT-induced contraction. More recently the 5-HT_{2B} receptor has been cloned in the mouse (Loric *et al.*, 1993) and human (Schmuck *et al.*, 1994; Kursar *et al.*, 1994) where the transcript was detected in the brain. However, there is conflicting evidence whether 5-HT_{2B} mRNA is present in the rat CNS (Flanigan *et al.*, 1995; Foguet *et al.*, 1992) and the absence of a specific high affinity ligand to distinguish 5-HT_{2B} from 5-HT_{2A} and 5-HT_{2C} receptors currently prevents detection of this receptor in the brain by ligand binding. To overcome this problem, we raised a polyclonal antiserum to the N-terminus of the rat 5-HT_{2B} receptor protein and used Western blotting and immunohistochemistry to establish expression in the rat brain.

A polyclonal antiserum raised in sheep to the N-terminal amino acids (MASSYKMSEQST + C) of the rat 5-HT_{2B} receptor (Foguet *et al.*, 1992) conjugated to bovine serum albumin, was purified by Sephadex chromatography to yield an immunoglobulin G (IgG) enriched fraction. For immunohistochemistry, male Lister Hooded rats (342-399 g) were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.p.) to enable intracardiac perfusion with 0.154 M saline followed by 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (PFA pH 7.4, 60 ml each). Brains were removed and fixed for 24 h in PFA at 4 ^{0}C. Coronal sections (100 µm) were cut on a vibratome and individually placed into immunobuffer (NaCl 12, KCl 0.005, Na_2HPO_4 900, Na_2HPO_4 1.5, merthiolate 0.1 mM and Triton X-100 0.3% (w/v)) with which all subsequent solutions were made. Endogenous peroxidase activity was removed with H_2O_2 before sequential incubation in 2% (v/v) rabbit serum containing 3% (w/v) casein (blocking buffer) for 24 h, and either Sephadex purified 5-HT_{2B} receptor antibodies (diluted 1 : 20, n = 4) or control pre-immune serum (n = 2) for 72 h, and biotinylated rabbit anti-sheep immunoglobulins (1 : 200 in blocking buffer, 24 h). 5-HT_{2B} positive cells were visualised using avidin/biotin and 3,3' diaminobenzidine (DAB). For Western blot analysis, membranes from selected rat brain regions (n = 4) were solubilised in 50 mM TRIS (pH 7.4, containing 5% (v/v) aprotinin and 15 mM PMSF) and protein (10 µg per lane) separated by SDS-PAGE prior to transfer onto nitrocellulose sheets. Immunoreactive proteins were visualised by sequential incubation with 5-HT_{2B} receptor or pre-immune IgGs (1 : 20, 12 h), peroxidase-conjugated secondary antibodies (1 : 200, 1 h) and DAB in the presence of nickel.

Immunohistochemistry revealed intense 5-HT_{2B} protein-LI on all Purkinje cells in the cerebellum and on multipolar and bipolar cell bodies in both the medial amygdala and the lateral septum. 5-HT_{2B}-LI was also seen in numerous fibres in the frontal cortex but was absent from all other brain areas including the striatum and hypothalamus. Western blot analysis of membranes derived from rat stomach fundus, frontal cortex, hippocampus, septum and cerebellum revealed two intense immunopositive bands (52 and 70 KDa). The lower molecular weight band is of a comparable weight to that of the cloned rat receptor protein (53.6 KDa) while the second band may represent a glycosylated form of the receptor, which is under investigation. Both bands were also present in membranes prepared from a human SH-SY5Y cell line from which the 5-HT_{2B} receptor was recently cloned (Schmuck *et al.*, 1994).

Brain regions showing positive immunohistochemical and Western blot staining with the current 5-HT_{2B} antiserum correlate with those previously found to contain mRNA (Flanigan *et al.*, 1995). This study demonstrates expression of the 5-HT_{2B} receptor protein in discrete rat brain regions and will assist establishment of receptor function in the CNS.

We acknowledge the BBSRC and SB for funding MSD.

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106P COMPARISON OF THE BINDING OF [^{125}I]-α-DENDROTOXIN TO HUMAN AND RAT BRAIN

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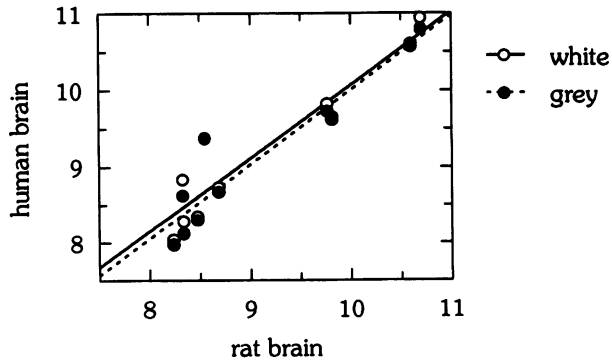
[^{125}I]α-dendrotoxin ([^{125}I]α-DTX) has been widely used to label voltage-gated potassium channels in rat brain but relatively little work has been performed using human tissue. Keighley *et al.* (1994) used autoradiography to demonstrate [^{125}I]α-DTX binding sites in human tissue and Schechter *et al.* (1994) reported that 4 peptide toxins displaced [^{125}I]α-DTX binding from human and rat hippocampus. We have extended these observations to provide a more extensive comparison of [^{125}I]α-DTX binding to human and rat brain.

Post-mortem samples of grey and white matter from 2 patients were obtained from the Multiple Sclerosis Society Tissue Bank. Homogenates were prepared as described for rat brain (Pryke *et al.*, This meeting) and used in competition studies with 0.2nM [^{125}I]α-DTX.

Ten peptide potassium channel blockers tested that displaced [^{125}I]α-DTX binding from rat brain (Pryke *et al.*, This meeting) also displaced [^{125}I]α-DTX from human brain with a similar rank order of potency (Figure 1). However, iberiotoxin did not displace [^{125}I]α-DTX from rat or human brain. Regression analyses of the data for human grey and white matter versus that for whole rat brain gave a good correlation ($r = 0.94$, $p < 0.01\%$ for both data sets). Therefore, it appears that [^{125}I]α-DTX binding sites in rat and human brain are pharmacologically similar.

Figure 1. pK_i values for peptide potassium channel blockers against [^{125}I]α-DTX binding for rat versus human brain. The peptides, in order of potency, were stichodactyla toxin > toxin I > α-DTX > kaliotoxin > δ-DTX > toxin K > β-DTX > mast cell degranulating peptide > charybdotoxin > γ-DTX.



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107P ANTAGONISM BY SR 142801, A NON-PEPTIDE NK_3 RECEPTOR ANTAGONIST, OF SENKTIDE-EVOKED INCREASE IN FIRING RATE IN GUINEA-PIG LOCUS COERULEUS SLICES

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SR 142801, the first potent and selective non-peptide antagonist described for the NK_3 tachykinin receptor displays a marked species-dependent profile exhibiting a higher affinity for human and guinea-pig NK_3 receptors than for rat NK_3 receptors (Emonds-Alt *et al.*, 1995). Electrophysiological studies in the guinea-pig locus coeruleus slice suggest that both NK_1 and NK_3 receptors modulate the activity of noradrenergic neurons in this brain area (Seabrook *et al.*, 1992 ; McLean *et al.*, 1993). We have used this model to study the effects of SR 142801 and SR 142806, its inactive enantiomer, on the response induced by senktide.

Guinea-pigs were anaesthetized with ketamine (200 mg/kg, i.p.) and 350 µm slices were prepared according to Henderson *et al.* (1982). Extracellular recordings were made at 35 °C from 122 spontaneously active neurons (mean firing rate: 1.5 ± 0.2 Hz).

In 78/122 cells to which senktide was applied for 1 min, a concentration-dependent increase in firing rate was observed with an EC_{50} of 26 nM (confidence interval 13.9-35.4 nM, significant level 0.05). For antagonism studies, we chose a concentration of 30 nM of senktide which allowed a reproducible response, with no tachyphylaxis if applied every 20 min. As shown in Table 1, SR 142801 antagonized in a concentration-dependent manner the excitation induced by senktide. This effect was progressive and needed 1 h to reach its maximum for every concentration tested. For the two active concentrations (5 and 50 nM) no recovery was observed after 2 h washout of SR 142801. SR 142806 was tested at a concentration of 50 nM and did not exhibit any effect (101 ± 6 %, n=3).

In conclusion, SR 142801 displays a potent stereoselective antagonist activity on senktide-induced increase in firing rate, further supporting the functional role of NK_3 receptors in the guinea-pig locus coeruleus.

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Table 1: Effect of SR 142801 on the senktide-induced response at different times of incubation (% of control ± s.e.mean.)

SR 142801 (nM)	Control	20 min	40 min	60 min
0.5	100 ± 18 (3)	98 ± 2 (3)	99 ± 3 (3)	97 ± 1 (3)
5.0	100 ± 17 (5)	94 ± 7 (5)	78 ± 7 (5)	67 ± 15* (5)
50	100 ± 30 (6)	61 ± 9 (6)	28 ± 8** (6)	7 ± 5** (4)

(n) = number of slices * P < 0.05 ** P < 0.01 (Student's paired t test).

108P A ROLE FOR VASOPRESSIN IN THE REGULATION OF GONADOTROPHIN SECRETION

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The secretion of adrenocorticotrophin by the anterior pituitary gland in stress is initiated by the synergistic action of two neuropeptides, corticotrphin releasing factor-41 (CRF-41) and arginine vasopressin (AVP), which are co-secreted by the parvocellular neurones originating in the paraventricular nucleus of the hypothalamus. CRF-41 also acts within the hypothalamus to inhibit the release of gonadotrophin releasing hormone (GnRH)[1] and has hence been implicated in the aetiology of infertility. Our original studies in Brattleboro rats suggest that AVP may also be important in this regard[2]. Accordingly in this study we have used an *in vitro* model[3] to examine the influence of AVP on the release of GnRH from isolated hypothalami. We have also investigated the effects of the AVP V_1 antagonist (1-(ß-mercapto-ß,ß-cyclopentamethylene propionic acid)-2-[O-methyl]tyrosine-AVP) administered centrally, on serum LH concentrations.

For *in vitro* studies groups of hypothalami (n=5-6), removed post mortem from adult male Sprague-Dawley rats were incubated in artificial cerebrospinal fluid with or without AVP (first incubation) and in the presence of 59mM K^+ with or without AVP (second incubation). GnRH (pg/ml) released into the medium was determined by radioimmunoassay. Values are means ± s.e.mean and statistical analysis was performed using Scheffe's test. AVP (10^{-8}M-10^{-6}M) had no significant effect on basal (first incubation) release but produced dose related decreases in K^+ stimulated GnRH release (104.1±9.9 vs 38.8±8.6 at 10^{-6}M, P<0.05).

For *in vivo* studies the vasopressin V_1 antagonist was injected (3µl) into the third ventricle of conscious adult male rats (200g) via an indwelling intracerebroventricular cannula (placed under sodium pentobarbitone [60mg/kg, i.p.] anaesthesia 7-10 days previously). Blood was collected 5 min later for LH (ng/ml) determination by radioimmunoassay. The antagonist (10-500ng) had no effect on serum LH concentrations compared with the vehicle treated control group (0.7±0.1 vs 0.6±0.1 for 500ng antagonist vs vehicle, n=7/6, P>0.05). An intraperitoneal injection of histamine (0.6mg/100g body weight) produced a significant increase in serum prolactin 5min after administration but had no effect on serum LH concentrations (0.6±0.1 vs 0.7±0.1 for basal vs histamine, n=6, P>0.05), in contrast to effects shown previously in younger animals[2]. These values were unaffected by administration of the AVP V_1 receptor antagonist (10ng, 500ng) 5 min before histamine (16.7±2.3 vs 11.9±3.4 ng/ml prolactin for 500ng antagonist vs vehicle, n=5, P>0.05; 0.6±0.1 vs 0.7±0.1 ng/ml LH for 500ng antagonist vs vehicle, n=6, P>0.05).

The *in vitro* results support our previous suggestion that AVP may have a role in the regulation of LH secretion. However, the failure of the AVP V_1 antagonist to influence serum LH concentrations may be age-related and therefore requires further investigation.

We are grateful to the Nuffield Foundation for financial support.

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109P STEREOSPECIFIC EFFECTS OF THE COMPETITIVE AND SELECTIVE AMPA ANTAGONIST LY293558 ON RAT SPINAL NEURONES *IN VIVO*

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The novel compound LY215490, (3RS, 4aRS, 6RS, 8aRS) - 6 - [2-(1 (2) H - tetrazole - 5 - yl) ethyl] decahydroquinoline - 3 - carboxylic acid, has been shown to be a selective and competitive AMPA (α - amino - 3 - hydroxy - 5 - methyl - 4 - isoxazolepropionate) antagonist in rat neocortical slices and in vivo in its ability to reduce excitation of rat spinal neurones evoked by the iontophoretic application of AMPA, but not that of NMDA (N - methyl - D - aspartate) (Ornstein et al., 1993). The compound has also been shown to be neuroprotective following middle cerebral artery occlusion in the rat (Gill and Lodge, 1994).

We have now studied, in pentobarbitone-anaesthetised female Wistar rats (200-230g), effects of the separated isomers on spinal neurones both by iontophoretic application and systemic administration. On 13 spinal neurones (-) LY293558 (5mM in 200mM NaCl; 3-10nA) reduced responses to AMPA by 73 ± 5% (mean ± s.e.m.) and those to NMDA by 6 ± 2%. In contrast (+) LY293559 (5mM in 200mM NaCl; 20-40nA) reduced responses to AMPA and NMDA by 38 ± 6% and 16 ± 4% respectively. Onset and recovery of effects was rapid with no obvious effects on action potential shape or amplitude.

The stereoselectivity was maintained following systemic administration while recording responses of spinal neurones to iontophoretic AMPA and NMDA. (-) LY293558 at 10, 5 and 2 mg/kg.iv. reduced responses to AMPA by 86 ± 7%, 49 ± 4% and 18 ± 2% respectively (n=6), with no effect on responses to NMDA. The onset of activity was gradual, with a peak effect seen approximately 30 minutes after administration. Where recovery was achieved this ranged between 1-2 hours depending on the dose level administered. (+) LY293559 at 40 mg/kg.iv. was inactive.

These results indicate that the activity of LY215490 lies in the (-) isomer and that LY293558 is a potent and selective AMPA antagonist following local application and systemic administration, having the potential as a therapeutic agent in those conditions where overactivity of AMPA receptors contributes to the pathology.

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110P FUNCTIONAL CHARACTERISATION OF GLUTAMATE RECEPTORS EXPRESSED FROM MOUSE AND FROG cDNAS

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Many glutamate receptor sub-units have been identified in the rat and mouse central nervous systems (CNS) by cDNA cloning (Hollman and Heinemann, 1994). We have sub-cloned N-methyl-D-aspartate sub-units from *Xenopus* CNS (XNR1 and mouse CNS (ε1, ε2, and ε3) into the expression vector pcDNAI-AMP (Invitrogen). The genes were expressed in *Xenopus* oocytes by injecting nuclei of stage IV, V and VI oocytes with 10nl or 20nl of 5μg/μl pcDNAI-AMP (Bourdelles et al., 1994). Electrophysiological recordings were made from day 1 post-injection using a two-electrode voltage clamp technique (Brackley et al., 1990).

Homo-oligomeric receptors for ε1 (n=47), ε2 (n=61), and ε3 (n=40) failed to respond to NMDA, and only one oocyte (n=78) injected with XNR1 pcDNA responded to NMDA (giving inward currents of only 6-8nA at -100mV). Functional receptors were routinely expressed in oocytes injected with XNR1 plus ε1, ε2, or ε3 pcDNAs. These hybrid receptors exhibited conventional pharmacological profiles. For example, responses to NMDA were potentiated by 1-50μM glycine, blocked in a voltage-dependent manner by 1 mM Mg^{2+}, competitively antagonised by 1μM AP-5 and non-competitively antagonised by 1μM Zn^{2+} and by 10-100nM dizocilpine (Fig. 1).

Most oocytes co-injected with XNR1 and ε1 pcDNAs gave very large currents (>1500nA on day 2) at -60mV in response to 100μM NMDA (with or without 10μM glycine) which reversed at ~5±5mV (SD: n=11). The EC_{50}s for XNR1 plus ε1 and XNR1 plus ε2 were ~27±13μM (n=4) and ~30μM (n=1) respectively. This is the first reported expression and pharmacological characterisation of ligand-gated receptors containing amphibian subunits.

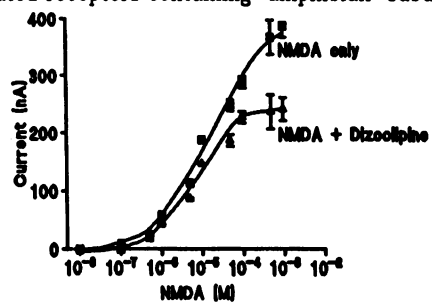

Fig. 1. Non-competitive antagonism by $5x10^{-6}$M dizocilpine of NMDA-evoked responses recorded from an oocyte injected with XNR1 plus ε1 pcDNA (holding potential:-40mV).

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111P INHIBITION OF HUMAN N-TYPE AND RAT P-TYPE VOLTAGE-DEPENDENT CALCIUM CHANNELS BY IFENPRODIL AND ELIPRODIL

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Ifenprodil and eliprodil are both non-competitive NMDA receptor antagonists which have been shown to inhibit neuronal calcium channel currents (Biton *et al.*, 1994, Church *et al.*, 1994). We have examined the effects of these agents on two defined subtypes of voltage-dependent calcium channels. Experiments were performed on recombinantly expressed human $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}3}$ subunits in HEK 293 cells which results in an ω–conotoxin-sensitive neuronal N-type voltage-dependent calcium channel (Williams *et al.*, 1992) and on ω-Aga IVa sensitive calcium channels (P-type) in acutely isolated cerebellar Purkinje neurones (Regan, 1991). Whole cell voltage clamp recording conditions were used to evoke calcium currents (I_{Ca}) in transfected HEK293 cells from a holding potential (Vh) of -90mV to a test potential (Vt) of +10mV and in cerebellar Purkinje neurons from Vh = -70mV to Vt = 0mV. Ifenprodil and eliprodil reversibly inhibited N-type voltage-dependent I_{Ca} in HEK 293 cells with IC_{50} values of 50μM and 10μM respectively. P type calcium channel currents were inhibited reversibly by ifenprodil and eliprodil with approximate IC_{50} values of 60μM and 9 μM respectively (I_{max} approximately 80% for ifenprodil and eliprodil). Current-voltage relationships constructed for N and P type VDCC were reversibly inhibited by ifenprodil and eliprodil with no observed shift in the peak of the curves. Current inhibitions produced by ifenprodil and eliprodil were not relieved by hyperpolarizations and did not show any apparent use-dependence. These results show that eliprodil is more potent than ifenprodil in blocking both the human N-type and the rat P-type voltage-dependent calcium channel but neither agent appears to show selectivity for either type of channel.

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112P EFFECTS OF LY293558 AND NBQX ON GLUTAMATE RECEPTOR RESPONSES IN RAT CEREBELLAR PURKINJE NEURONES AND HEK293 CELLS EXPRESSING THE HUMAN GLUR6 GLUTAMATE RECEPTOR

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The effects of the non-NMDA receptor antagonists (4S,4aR,6R,8aR)-6-[2-(1-(2)H-tetrazol-5-yl)ethyl] decahydroisoquinoline-3-carboxylic acid (LY293558) (Ornstein et al., 1992) and 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F)quinoxaline (NBQX) were investigated on non-NMDA receptor mediated responses in acutely isolated rat cerebellar Purkinje neurons and in HEK293 cells expressing human iGluR6. Cerebellar Purkinje neurons were isolated according to the method of Regan (1991) from the cerebellar vermis of 6-11 day old rats and whole cell voltage-clamp electrophysiology used to measure inward currents evoked by agonists in the absence or presence of antagonists. Inward currents were evoked by AMPA (1μM) in cerebellar Purkinje cells in the presence of 30μM cyclothiazide. AMPA-induced currents were inhibited by LY293558 in a concentration-dependent manner (IC_{50} value 1.1 ± 0.3μM). The inhibition produced by LY293558 was competitive with a K_i of 0.68 ± 0.18μM and Schild plot slope of 0.97 ± 0.15. The inhibitory effects of LY293558 and NBQX were compared on kainate-induced currents in rat cerebellar Purkinje neurons and HEK 293 cells expressing human iGluR6 glutamate receptors. LY293558 and NBQX inhibited 100μM kainate-induced currents in rat cerebellar Purkinje neurons with IC_{50} values of 0.45 ± 0.08 and 0.16 ± 0.03μM respectively. Inward currents evoked by kainate (1μM) in the presence of concanavalin A (250μg/ml) in HEK 293 cells expressing the human iGluR6 glutamate receptor were inhibited by NBQX with an IC_{50} value of 2.4 ± 0.4μM whereas LY293558 had no effect on these currents at concentrations up to 100μM.

These studies demonstrate that both LY293558 and NBQX blocked AMPA-preferring non-NMDA receptor mediated responses in cerebellar Purkinje neurons but only NBQX blocked the kainate-preferring non-NMDA receptor mediated responses in HEK 293 cells transfected with the human iGluR6 receptor ion channel.

Ornstein, P.L., Arnold, M.B., Augenstein, N.K., Lodge, D., Leander, J.D. and Schoepp, D.D. (1993) J. Med. Chem. **36**, 2046-2048.
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113P HETEROGENEOUS EFFECTS OF NMDA ON DOPAMINE RELEASE IN THE RAT CAUDATE PUTAMEN

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There is a massive corticostriatal glutamatergic input to the caudate putamen. Using fast cyclic voltammetry (FCV), we have shown that 20 μM NMDA in the absence of Mg^{2+} results in release of dopamine (DA) and inhibition of electrically-evoked DA release in the caudate putamen (CPu) of the rat (Iravani & Kruk, 1992). In the rat CPu at least two populations of DA-releasing sites could be identified. The terms 'hotspots' and 'coldspots' are used to designate sites in the CPu where increasing the frequency of pulses resulted in either a significant increase in the dopamine signal (hotspot) or no increase (coldspot) (Trout & Kruk 1992; Iravani & Kruk, 1995). The aim of the present study was to investigate the effects of NMDA on DA signals in the 'hotspots' and 'coldspots' in the rat CPu.

Experiments were performed in 350 μm coronal slices of rat CPu in a brain bath, superfused with artificial CSF (aCSF), saturated with a 95% O_2, 5% CO_2 mixture; electrically stimulated DA release was monitored by FCV (Trout & Kruk, 1992). 'Hotspots' and 'coldspots' were identified by comparing DA release evoked by single pulse (S1) with those evoked by trains of pulses (20 pulses at 10 Hz, S2; 20 Hz, S3; and 50 Hz, S4) applied every 5 min. Once a site was identified, normal aCSF was replaced with a Mg^{2+} free aCSF. Following stable S1 responses, 20 μM NMDA was added to the aCSF, and its effects on the background voltammetric signal and on the DA release evoked by S1 was investigated.

In the absence of Mg^{2+}, S1 stimulated DA release was equally increased in hotspots and coldspots. 20 μM NMDA evoked a transient rise in the background signal (maximal at 5 min) followed by a decline below the original baseline. The peak signals following NMDA in the 'coldspots' and 'hotspots' were equivalent to 105 ± 45 nM DA (n=12) and 21 ± 7.3 nM DA (n = 8) respectively. In the presence of 20μM NMDA, S1 stimulated DA release was attenuated, the effect being greater in coldspots than in hotspots (Figure 1).

The results show that there is a regional variation in the DA releasing ability of NMDA and its effect on electrically stimulated DA release in the rat CPu. The basis for this heterogeneity is not established, but may reflect the patch / matrix organisation of the CPu (Gerfen 1992), varying contribution of mesostriatal and nigrostriatal (A9 / A10) inputs to different subregions of the CPu (Gerfen et al 1987), or differential densities of glutamic acid receptors in the CPu (Dure et al 1992).

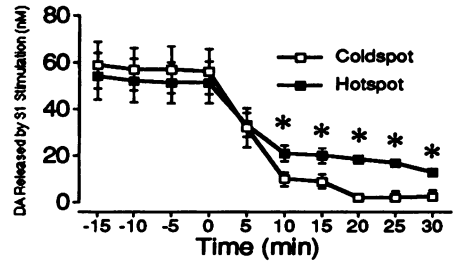

Figure 1. *The inhibitory effect of NMDA on single pulse (S1) evoked DA release in the 'coldspot' and 'hotspot' recording sites in the rat caudate putamen. At time = 0 min., NMDA 20μM was added. Each data point is the mean ± s.e.m. *P < 0.05, (n = 8-12), Student's paired t-test.*

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114P NITRIC OXIDE MODULATES BASAL AND NMDA-EVOKED RELEASE OF GABA IN VENTRAL HIPPOCAMPUS *IN VIVO*

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Nitric oxide (NO) is a messenger molecule in the periphery and central nervous system (CNS). Roles for NO in normal brain function have yet to be fully established. However, activation of glutamatergic N-methyl-D-aspartate (NMDA) receptors leads to increased synthesis of NO (Garthwaite, 1991). NO is associated with excitatory transmission (Garthwaite, 1991). However, it has recently been reported that NO increases GABA release in the striatum of anesthetised rats (Guevara-Guzman et al., 1994).

In the present study we have investigated the effects of infusion, via dialysis probes, of NMDA and the nitric oxide synthase (NOS) inhibitor L-nitro-arginine methyl ester (L-NAME) on GABA release in rat hippocampus of freely-moving rats. The effects of the NO donor drugs S-nitroso-N-penicillamine (SNAP) and sodium nitroprusside (SNP) on GABA release were also studied.

Concentric dialysis probes were implanted into the ventral hippocampus of male Wistar rats (280-320g) under chloral hydrate (400mg/kg) anaesthesia. The following day rats were dialysed (0.5μl/min) with artificial cerebrospinal fluid. Four samples were collected prior to drug infusion. GABA was determined using HPLC followed by fluorimetric detection. Basal levels of GABA were 0.24 ± 0.03μM (n = 52).

NMDA (10-1000μM) caused a concentration-dependent increase in dialysate GABA with a maximum increase of 219 ± 18 % at the highest NMDA concentration (mean ± s.e.mean, n = 5). When L-NAME (100μM) was infused for 60 min prior to co-infusion of L-NAME and NMDA (both 100μM), GABA was increased by 460 ± 59 % compared with 178 ± 9 % following 100μM NMDA alone. When 200μM L-NAME was infused with 100μM NMDA, GABA release was 327 ± 19 % above basal. In the presence of 1mM L-NAME, NMDA did not significantly increase dialysate GABA. In fact 1mM L-NAME decreased dialysate GABA to 63 ± 4 % of basal values.

SNAP, infused at 0.5mM, decreased extracellular GABA to 60 ± 7 % of basal values. In contrast, higher concentrations of SNAP (1.0 and 2.0mM) increased dialysate GABA by 820 ± 105 % above basal at the highest SNAP dose. SNP (0.5-2.5mM) produced concentration-dependent increases in extracellular GABA. The maximum response was 613 ± 75 % above basal following 2.5mM SNP.

These data suggest that the level of NOS activity and concentration of NO alters basal and NMDA evoked GABA release in the hippocampus. The biphasic response in dialysate GABA level following SNAP infusion supports these findings. These observations may in part explain conflicting reports that NO can have pro- and anticonvulsant effects.

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115P INABILITY OF MODULATORS OF THE NMDA RECEPTOR COMPLEX TO ALTER ^{3}H-DIZOCILPINE BINDING *EX VIVO* IN MOUSE BRAIN

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^{3}H-dizocilpine binding to rodent brain tissue *in vitro* provides a good indicator of the activation state of the NMDA receptor complex (Huettner *et al* 1988; Wong & Kemp 1991). To extend this to *ex vivo* conditions offers the advantage of being able to study the receptor state in a more physiological environment. Thus, in the present study, we have used an *ex vivo* ^{3}H-dizocilpine binding technique in mouse brain to examine whether the functional effects of different modulators of the NMDA receptor complex can be detected. Receptor autoradiography was used to detect subtle changes in the regional levels of binding.

Male CD1 mice (20-25g) were injected i.p. with saline or unlabelled test compound. After 15 min, 50μCi ^{3}H-dizocilpine (53.3Ci/mmol, 1nCi/ml) was injected i.v. (tail vein) 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.p.). Receptor autoradiograms were prepared, as reported by Price *et al* (1988), with slight modification. Cryostat sections (12μm) were washed twice for 45s in Tris HCl buffer (pH 7.4) and apposed to Amersham Hyperfilm with tritium standards for 4-6 weeks.

The pattern of distribution of ^{3}H-dizocilpine binding sites obtained using the *ex vivo* techique was the same as obtained *in vitro* in mouse and rat brain sections (Bowery *et al* 1988) with the highest densities occurring in the hippocampus and frontal cortex, lower levels in the caudate putamen and thalamus, and a very low density in the cerebellum. D-serine (100mg/kg) enhanced *ex vivo* ^{3}H-dizolcilpine binding in many brain regions with the greatest effect in the hippocampal CA3 region (22.8 ± 5.8% above basal, $p < 0.05$ ANOVA, n=5). By contrast, D-cycloserine (100mg/kg) produced a slight but significant decrease in the same brain region (11.3 ± 3.1% below basal, $p < 0.05$, n=6). L-serine (1-100mg/kg), 7-chlorokynurenic acid (1-10mg/kg), kynurenic acid (1-10mg/kg), ACBC (1-aminocyclobutane-1-carboxylic acid (1-100mg/kg), cycloleucine (1-100mg/kg), NMDA (1-30mg/kg), HA966 (3-amino-1-hydroxy-2-pyrrolidone), 1-10mg/kg), ifenprodil (1-10mg/kg), CGP 37849 (DL-[E]-2-amino-4-methyl-5-phosphono-3-pentenoic acid 1-30mg/kg) and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, 1-80mg/kg) produced no significant change in the level of binding. Longer periods (up to 60 min) between injection of modulator and ^{3}H-dizocilpine also failed to produce any modulation of the binding.

The fact that compounds which freely cross the blood brain barrier displayed no change in *ex vivo* binding was surprising since they all modulate ^{3}H-dizocilpine binding *in vitro* (Bakker *et al* 1991). Binding of dizocilpine appears to have complex kinetics indicating several intermediate states of the ion channel between fully closed and fully open (Cull Candy *et al* 1988; Javitt *et al* 1990) and ^{3}H-dizocilpine could gain access to its site of action *in vivo* even though the receptor complex is probably inactivated. In conclusion, these data suggest that *ex vivo* ^{3}H-dizocilpine binding does not represent a sensitive marker for modulation of the NMDA receptor complex under physiological conditions.

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116P LAMOTRIGINE ALTERS VERATRIDINE- BUT NOT K$^+$-EVOKED AMINO ACID RELEASE IN THE VENTRAL HIPPOCAMPUS OF THE RAT *IN VIVO*

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Lamotrigine (LTG) is a voltage- and use-dependent inhibitor of Type IIa sodium channels (Meldrum & Leach, 1994; Xie *et al.*, 1994). *In vitro* studies have shown that LTG inhibits veratrine induced aspartate and glutamate release, with no effect on the spontaneous or K$^+$- evoked release of these amino acids (Meldrum & Leach, 1994). These studies are consistent with the inhibition of voltage-dependent Na$^+$ channels.

In the present study we have tested this possibility using *in vivo* microdialysis to monitor extracellular amino acid levels in the ventral hippocampus following i.p. injection of LTG under depolarizing conditions. Male Wistar rats (250-300g) were implanted with concentric dialysis probes in the ventral hippocampus under chloral hydrate anaesthesia (400mg/kg). The following day the probes were perfused with artificial CSF at 0.5μl.min^{-1}. 50μM veratridine or 100mM K$^+$ was infused via the probes for 30min following the collection of 4x30min samples. At t=180min, LTG (10 & 20mg/kg) or vehicle (control) was injected i.p.. Then at t=270min,veratridine/K$^+$ was infused for a second 30mins, and then a final 4x30min samples were collected. Samples were analysed for amino acid content by HPLC. Basal dialysate amino acid levels were found to be: 4.3±0.8 (aspartate), 36.2±4.8 (glutamate), 49.2±6.3 (glutamine), 106±3.4 (taurine) and 2.9±1.0 (GABA) pmols/15μl dialysate ± s.e.mean. LTG significantly reduced veratridine-evoked release of aspartate, GABA and particularly glutamate, but failed to alter basal or K$^+$ evoked amino acid release (Table 1). These *in vivo* data support previous *in vitro* findings suggesting that LTG decreases amino acid release by an interaction with voltage dependent Na$^+$ channels.

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S.A. is a BBSRC CASE student with the Wellcome Foundation.

STIMULUS	% of basal levels(mean±s.e.mean;n=7)				
	ASPARTATE	GLUTAMATE	GLUTAMINE	TAURINE	GABA
Ver. + vehicle	205±36	324±63	54±7	154±16	1147±227
Ver. + LTG (10mg/kg)	206±36	379±42	61±6	154±9	1084±192
Ver. + LTG (20mg/kg)	*158±46	*89±23	67±10	105±15	*555±187
K$^+$ + vehicle	277±34	225±66	64±14	357±89	804±213
K$^+$ + LTG (20mg/kg)	336±29	255±54	53±19	474±65	977±123

Table1: The effect of LTG on extracellular amino acid levels under depolarizing conditions. Figures represent maximal changes observed generally 60 mins following the second veratridine/K$^+$ pulse.*$p<0.05$ LTG vs vehicle (Mann-Whitney U-test).

117P THE USE OF *CONUS* TOXINS TO ESTABLISH CALCIUM CHANNEL IDENTITY OF CA3-CA1 NEUROTRANSMISSION IN RAT HIPPOCAMPAL SLICES

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Recent debate has centred on the identification of calcium channels responsible for transmitter release at central synapses (Wheeler *et al*, 1994a; Dunlap *et al*, 1994; Wheeler *et al*, 1994b). The present study has used selective toxins to estimate the contribution of different calcium channel types to glutamate release at Schaffer collateral-CA1 synapses.

Extracellular recordings were made from 400μm transverse hippocampal slices obtained from female Wistar rats. Excitatory post-synaptic field potentials (field epsps) were recorded with 3-5MΩ electrodes filled with 3M NaCl, in response to Schaffer collateral nerve stimulation every 30s (20μs; 10-40V) with a bipolar tungsten electrode. Hippocampal slices were submerged in, and continuously superfused with, Krebs solution (in mM: 124 NaCl, 3 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgSO_4$, 1.25 NaH_2PO_4, 10 D-Glucose, 95% O_2/5% CO_2). Toxins were applied, at the stated concentrations, via the superfusate. Data were digitized, captured and analysed using Spike 2 software (CED, UK). Neurotransmission was estimated by measuring the slope of the rising phase of the averages of 4 field epsps. Data are presented as mean ± s.e.mean from n slices.

ω-Conotoxin GVIA (CgTx GVIA), a selective N-type Ca channel antagonist, produced a maximal 50% decrease in the epsp slope. Dose dependency was more apparent in the rate of block than in the degree of block: thus 100nM CgTx GVIA produced 29 ± 7 % inhibition (n=10) at the end of 20 min application. A further decrease of the epsp slope was often seen, however, in the initial stages of washout. Consequently, 40 min applications were used thereafter, with 46 ± 3 % (n=3) inhibition observed at this time. Application of 300nM CgTx GVIA generally produced no further inhibition (48 ± 2 % reduction n=5) to that observed with 100nM. No recovery was seen with prolonged washout (1-2 hr).

The non-selective Ca channel toxin ω-Conotoxin MVIIC (CmTx MVIIC), produced an irreversible dose-dependent inhibition of synaptic transmission, with 100 and 300nM producing 31 ± 4 % (n=3) and 67 ± 9 % (n=5) inhibition, respectively at the end of 40 min application. Application of CmTx MVIIC (300nM) following exposure to CgTx GVIA (300nM) reduced the epsp slope by 73 ± 4 % (n=3), whereas 300nM CmTx MVIIC followed by CgTx GVIA (100 or 300nM) reduced the epsp slope by 89± 6 %.

Thus it would appear that 40 -50 % of the release of glutamate at this synapse is due to the involvement of N-type Ca channels. The remaining channels would appear to be sensitive to blockade by CmTx MVIIC and represent the Q/P-type channel component.

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118P NON-SPECIFIC BLOCK OF VOLTAGE-DEPENDENT SODIUM AND CALCIUM CHANNELS IN GUINEA-PIG SENSORY NEURONES BY (±) CP 96,345 AND (±) CP 99,994

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Some of the recently discovered non-peptide antagonists of neurokinin receptors are reported to block ion channels (Wang et al., 1994). We have measured the effects of two compounds which are specific NK1 receptor antagonists, (±)CP 96,345 (Snider et al. 1991), and (±)CP 99,994 (see McLean et al., 1993), on action potential conduction in sensory nerves *in vitro* and also on the size of voltage-dependent sodium and calcium currents in voltage-clamped sensory neurones in culture.

Action potentials were measured using isolated de-sheathed guinea-pig vagus nerves in a grease-gap apparatus (Rang & Ritchie, 1988). Vagus nerves were stimulated supramaximally at 1 Hz to evoke compound action potentials and either (±)CP 96,345 or (±)CP 99,994 was added to the superfusate. Cumulative dose-response curves were constructed for the effect of the compounds on the amplitude of the TTX-sensitive action potential in 'A' fibres (conduction velocity at 20°C = 10.0 ± 1.1 m/s (mean ± s.e.m., n=8)). The compounds were allowed to act for at least 10 min at each concentration. Both (±)CP 96,345 and (±)CP 99,994 inhibited action potential amplitude with EC50s of 85.9 ± 15.0 μM (n=4) and 21.8 ± 3.2 μM (n=4) respectively.

Voltage-clamped sodium and calcium currents were isolated in adult guinea-pig dorsal root ganglion neurones using methods previously described for rats (Docherty, Robertson & Bevan, 1990; Arbuckle & Docherty, 1995).

Tetrodotoxin-resistant (TTX-R) sodium currents were activated during 10 ms steps to 0 mV from a holding potential (Vh) of -60 mV. TTX-R current was reduced only 12.8 ± 2.3% (n=10) by 1 μM TTX. An additional sodium current (TTX-S) which was reduced by 86.0 ± 12.2% (n=14) by 1 μM TTX was activated at 0 mV after a 485 ms conditioning pre-pulse to -100 mV. Either (±)CP 96,345 or (±)CP 99,994 was applied to cells at a concentration of 10 μM. (±)CP 96,345 inhibited TTX-R current by 59.2 ± 8.2% (n=5) and TTX-S current by 33.7 ± 14.3% (n=6). (±)CP 99,994 inhibited TTX-R current by 39.6 ± 8.5% (n=6) and TTX-S current by 65.0 ± 6.2% (n=6).

Calcium currents (no attempt was made to distinguish sub-types) were measured during 30 ms voltage steps to 0 mV from Vh = -90 mV. Calcium currents were reduced by 37.7 ± 7.9% (n=4) by (±)CP 96,345 and 12.4 ± 4.7% (n=4) by (±)CP 99,994.

The data agree with previous reports of non-specific actions of (±)CP 96,345 on other tissues (Wang et al., 1994) and also show that (±)CP 99,994 can block action potential conduction and activity of voltage-dependent ion channels in sensory neurones albeit at fairly high concentrations. These non-specific effects could contribute to the actions of the compounds *in vivo*, especially when high concentrations are administered locally.

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119P IS NS1619 A BK_{Ca} CHANNEL OPENER IN HUMAN BRONCHUS?

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Calcium-activated potassium channels are a group of channels that share a dependence on intracellular calcium ions for activity. The high density in bovine airway smooth muscle (Macmillan et al., 1994), and large conductance (>150pS) of the BK_{Ca} channels in airways smooth muscle suggests that they may be involved in the regulation of tone. We have used NS1619 (a BK_{Ca} channel opener, Olesen *et al*, 1994) and iberiotoxin (a BK_{Ca} channel inhibitor) to determine whether this channel is functional in isolated human airways.

Human lung samples were obtained from patients undergoing surgery for bronchial carcinoma. Bronchi from 3rd-6th order were cut into rings 3-4mm in length and mounted between two tungsten wires in Krebs' bicarbonate at 37°C bubbled with $95\%O_2/5\%CO_2$ under 2g tension. In some rings spontaneous tone developed, in others where it did not indomethacin (2.8μM) was added and thereafter tone was raised with either histamine (3μM) or LTD_4 (10nM). The relaxant agents NS1619 (1μM-100μM), levcromakalim (1nM-30μM) or isoprenaline (1nM-0.3μM) were added cumulatively to tissues which had stable spontaneous tone or the contractile response had reached a plateau. In experiments using NS1619 or levcromakalim, isoprenaline (0.3μM) was added at the end to determine maximal relaxant capacity. When studied iberiotoxin was added at least one hour before NS1619. BK_{Ca} channel activity was recorded from human bronchial smooth muscle (HBSM) cells (Clone Technics) in primary culture using standard patch clamp techniques.

NS1619 at concentrations of > 10μM produced relaxation in some but not all bronchi 15/22(9 patients) tested, and regardless of the spasmogen used. In all cases the relaxation was approximately 30% of that to isoprenaline. Iberiotoxin (100nM) had no effect on the ability of NS1619 to induce this small relaxation, and alone it produced a slowly developing contraction in 60% of tissues. In inside-out patches of HBSM cells BK_{Ca} channel activity was recorded from only 18% of the 60 patches studied. Isoprenaline-induced relaxation of bronchus was dependent on the spasmogen, pIC_{50} values were 8.63±0.06 (spontaneous tone) (n=6/3 patients), 7.87±0.04 (histamine) (n=5/3) and 8.02±0.05 (LTD_4) (n=4/2). Levcromakalim was studied only in tissues which developed spontaneous tone; pIC_{50} value 6.61±0.01.

These data show that NS1619 relaxed only a small number of human bronchi regardless of the spasmogen, at concentrations 30-fold greater than those effective in relaxing spontaneous tone of guinea-pig trachea (pIC_{50} 5.21±0.07; unpublished observations). The relatively low density of BK_{Ca} channels recorded from HBSM cells may provide a possible explanation for this lack of effect of NS1619 in human bronchus. The observation that iberiotoxin did not inhibit these small relaxant effects suggests that the mechanism of NS1619 is not via opening of BK_{Ca} channels. That iberiotoxin can induce contraction may imply that the channels are present, but activated, under these experimental conditions, thus making further channel opening impossible. In conclusion these results suggest that the weak relaxant effect of NS1619 in human bronchi is not via BK_{Ca} channels. The utility of BK_{Ca} channel activators as bronchodilators is therefore questionable.

The cell culture work by Greig Duncan is gratefully acknowledged.

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120P ACTIONS OF PHOTORELEASED cAMP ON CALCIUM CURRENTS AND DELAYED RECTIFIER POTASSIUM CURRENTS IN GUINEA-PIG ISOLATED VENTRICULAR MYOCYTES

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Single cells were isolated from guinea-pig ventricular muscle and studied using the whole-cell, patch-clamp technique. The bath solution was a balanced salt solution containing 2.5mM added $CaCl_2$ (36°C). Intracellular solutions contained 13mM KCl, 109mM K-glutamate, 10mM HEPES, 11mM EGTA, 5mM $CaCl_2$ (100nM free), 5mM NaOH and 14mM MgATP supplemented with 1mM caged cAMP, pH=7.2.. Cells were held at -40mV and stimulated at 6s intervals with alternating depolarising pulses (300ms to +10mV and 700ms to +40mV) to activate calcium (I_{Ca}) and potassium currents (I_K), respectively. Calcium currents were measured as peak inward current relative to current at 300ms. Potassium currents were measured as deactivating outward tail currents.

Photolysis of caged cAMP has been previously shown to cause an increase in I_{Ca} in ventricular myocytes (Kozlowski *et al.* 1994). In the present experiments, release of cAMP by a 1ms flash from a xenon lamp caused a simultaneous increase in I_K (Figure 1a) from 500pA ± 95pA to 724pA ± 130pA, n=8 cells. I_{Ca} (Figure 1b) increased from -3.29nA ± 0.58nA to -6.6nA ± 0.9nA, n=8 cells). Maximal effect on both I_K and I_{Ca} occurred between 6s and 24s after the flash. Decline of the cAMP induced response could be fitted by a single exponential. The time constant for the decline in I_{Ca} (77s ± 12s, n=8) appeared faster than that of I_K (203s ± 85s, n=8; $P<0.05$). The sensitivity of both currents to subsequent flashes of photoreleased cAMP was not altered. Inclusion of the phosphatase inhibitor, okadaic acid (1-25μM), in the cell dialysing solution caused a rapid runup of both I_{Ca} (as shown by Heschler 1988) and I_K. In 4 cells no response to photoreleased cAMP was observed. This effect may be attributable to decreased level of dephosphorylation resulting in maximal activation of I_K and I_{Ca}.

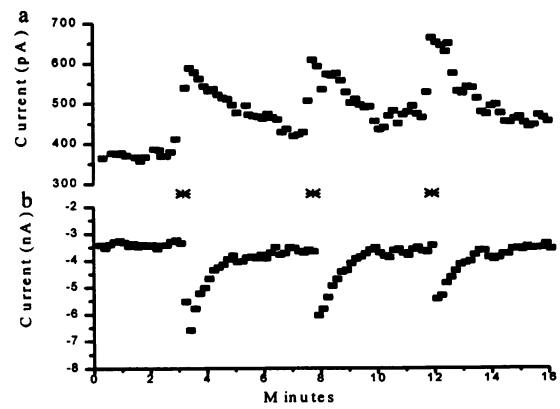

Figure 1. Increase in potassium currents (a) and calcium currents (b) in response to photo-released cAMP(*).

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121P INTERACTION OF RS-91309 WITH K_{ATP} BINDING SITES IN THE RAT AORTA

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Potassium channel openers (KCOs) open plasmalemmal K+ channels in a variety of tissues, causing membrane hyperpolarization, K+ efflux and tissue relaxation. These properties have prompted their development for possible use in the treatment of asthma, hypertension and ischaemic heart disease. KCOs are a diverse group of compounds, including the benzopyrans eg. cromakalim and the cyanoguanidines eg. pinacidil. Patch and whole-cell clamp studies show that the KCOs act on the ATP sensitive K+ channel (K_{ATP}). P1075 is an analogue of pinacidil and its tritiated derivative has recently been introduced by Quast *et al.* (1992) to study the binding of KCOs in rat aortic tissue.

Using a method essentially similar to Quast *et al.* (1992) this abstract discribes the binding of 3nM [^{3}H]-P1075 to rat aortic rings in tissue culture plates. Specific binding which represented 40% of total and was selectively displaced by KCOs. RS-91309 (N-[[2,2-dimethyl-4-(2-oxo-2H-pyridin-1-yl)-6-trifloromethyl-2H-1-benzopyran-3-yl]methyl]-N-hydroxylacetamide; Lochead *et al.* 1993), displaced specific binding of [^{3}H]-P1075 in rat aortic rings with a pKi of 7.66±0.07. Other compounds investigated had the following pKi values: RS-91714 (N-[[2,2-dimethyl-4-(2-oxo-2H-pyridin-1-yl)-6-pentafluroethyl-2H-1-benzopyran-3-yl]methyl]-N-hydroxylacetamide; Lochead *et al.* 1993) 7.11±0.26, P1075 9.22±0.08, Ro 31-6930 8.11±0.15, levcromakalim 7.35±0.34, RP-49356 6.75±0.08.

We obtained a B_{max} for P1075 binding to rat aortic rings of 9.95±2.26 fmoles/mg ww. and a Kd of 0.66±0.42nM. Addition of 40nM RS-91309 did not significantly alter the B_{max} although the Kd was significantly decreased to 4.70±2.38nM ($p<0.05$, unpaired one-tailed, Student's t-test). These data are consistant with a competitive interaction between RS-91309 and P1075.

RS-91309 induced relaxation in rat aorta precontracted with 20mM KCl which was antagonised by glibenclamide (apparent pK_B 7.63; schild slope 1.1) consistant with a K_{ATP} opener mechanism. Comparison of the pKi values for [^{3}H]-P1075 displacement, with pIC_{50} values for inducing relaxation in both rat aorta, indicated that relaxant and binding affinities for most KCOs tested was similar. However, RS-91309 and RS-91714 showed a 14 fold and 48 fold respective selectivity for their smooth muscle relaxant effects over their binding affinity.

The mechanism underlying the difference in binding affinity and relaxation potency could be due to allosteric interaction of the KCOs with a different channel binding site. However, this requires further study.

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122P THE INHIBITORY EFFECTS OF RUBIDIUM ON RESPONSES TO PINACIDIL IN THE MOUSE INTESTINE

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Rubidium (Rb) efflux has been used as a measure of potassium (K) efflux in experiments on K channel modulating agents. However, Rb inhibited the relaxant effect of potassium channel openers (KCOs) in rat aorta (Greenwood & Weston, 1993) and differentially affected responses to KCOs in human uterus (Criddle & Soares de Moura, 1994). In the present study, the effects of Rb on the relaxant action of pinacidil (PIN), an agent thought to open ATP-sensitive K channels, were investigated in mouse intestine contracted by electrical field stimulation (EFS).

Mucosa-free preparations derived from the distal ileum of male BKW mice were placed either in normal Krebs' (KPS) or Krebs' in which K was replaced by Rb (RbPS) under 0.5g tension (37°C, 95% O_2/5% CO_2) throughout the experiment. Maximal isometric twitch contractions were elicited by EFS in each solution (0.5Hz in KPS or 0.2Hz in RbPS since in this solution the higher frequency produced tetanus; supramaximal voltage). When twitch height was constant, cumulative concentration-response curves were performed to PIN (0.3-200μM); verapamil (VP; 10nM-3μM); papaverine (PA; 1-100μM) or their respective vehicles. This procedure was repeated following 20min incubation with a single concentration of the K_{ATP} channel blocking agent, glibenclamide (GBC, 0.1-3μM) or vehicle. Relaxant potency was expressed as geometric mean (with 95% confidence limits) of EC_{50} (the concentration required to reduce twitch heights by 50%). The effect of GBC on relaxant responses was expressed in terms of dose ratio; the difference between responses to relaxants in tissues exposed to KPS or RbPS and relaxant effects in the presence of GBC were compared using Student's unpaired and paired t-tests respectively.

There was a slight difference between initial twitch responses in tissues bathed in KPS or RbPS; twitch heights were 0.79±0.03g (n=19) and 0.64±0.03g (n=20, $P<0.01$) respectively, possibly due to the different stimulus frequencies used. The EC_{50} for PIN in KPS was lower than in RbPS: 3.2μM (2.7-4.0, n=12) vs 37.9μM (31.3-48.9, n=12. $P<0.001$). The antagonistic effect of GBC on relaxation caused by PIN was also different as shown by the respective dose ratios (GBC in μM, n=4 in all cases): in KPS: 2.8±0.6 (0.1); 9.2±3.3 (1.0), in RbPS: 2.4±0.5 (0.1); 2.0±0.3 (1.0). However, in RbPS, GBC 3μM caused no shift in the PIN concentration-response curve, the dose ratio was 1.1±0.1. Relaxation to VP did not differ in the two solutions with EC_{50}s of 47.4nM (25.9-71.6; KPS, n=4) and 36.3nM (29.7-44.7; RbPS, n=8). The relaxant effect of PA was not changed in RbPS as indicated by respective EC_{50}s, 10.7μM (8.2-14.0; KPS, n=7) and 16.0μM (10.8-21.8; RbPS, n=4). GBC (1μM) had no effect on either VP or PA concentration-response curves in either solution. GBC (0.1-10μM) alone had no effect on preparations in either solution (n=4).

In the present study substitution of K by Rb caused changes only in responses to PIN and GBC. This is consistent with the suggestions that Rb cannot effectively substitute for K in the K channels opened by PIN or that Rb may have an inhibitory action on the K channel itself. The effect of Rb appears to involve mainly K channels since neither GBC nor Rb itself showed any direct interactions with agents known to affect the L-type calcium channels or other relaxant mechanisms as indicated by the lack of effect of the RbPS on VP and PA responses in this tissue.

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123P RAT BLADDER POTASSIUM CHANNELS: EFFECTS OF POTASSIUM-CHANNEL MODULATORS

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The outward K-conductance in guinea-pig detrusor is partially Ca-sensitive and an inwardly-rectifying current has also been described (Klöckner & Isenberg, 1985). K-channel openers reduce the contractility of detrusor smooth muscle strips (Foster *et al.*, 1989) and in guinea-pig detrusor cells, levcromakalim induces a glibenclamide-sensitive current attributable to the opening of ATP-sensitive K-channels (K_{ATP}) (Bonev & Nelson, 1993). The objective of the present study was to assess the effects of the K-channel modulators levcromakalim (LK), tetraethylammonium (TEA), 4-aminopyridine (4-AP), and ciclazindol (CZ) on the outward K-currents present in the rat detrusor using the whole-cell voltage-clamp technique.

Smooth muscle cells of the rat detrusor were dispersed by enzymatic treatment and recordings of whole-cell K-currents were made under calcium-free conditions. The voltage-step protocol used induced a K-conductance comprising two main voltage-sensitive components and a non-inactivating current (I_{NI}). The voltage-sensitive currents had characteristics similar to those of the A-current ($I_{K(A)}$) and delayed-rectifier current ($I_{K(V)}$) described in rat portal vein myocytes (Noack *et al.*, 1992). In the majority of cells, stepping to test potentials negative of -60mV from a holding potential of -10mV induced an inwardly-rectifying current.

Exposure to LK (10μM) induced a glibenclamide-sensitive non-inactivating K-current ($I_{K(ATP)}$) of 100.2 ± 19.4 pA in magnitude and caused the cells to hyperpolarise by approximately 25mV. The K-channel inhibitors TEA (20mM) and CZ (100μM) inhibited both $I_{K(V)}$ and I_{NI} as well as the LK-induced current. On stepping to a test potential of +40mV, TEA and CZ inhibited $I_{K(V)}$ by 150.6 ± 34.3 pA and 212.9 ± 37.0 pA, respectively whilst these agents inhibited I_{NI} by 49.2 ± 13.7 pA and 58.6 ± 7.1 pA, respectively. 4-AP (5mM) attenuated $I_{K(V)}$ by 149.0 ± 59.5 pA and inhibited the LK-induced current but did not inhibit I_{NI}. All values represent mean ± s.e. mean, n=4-12.

These data demonstrate that the outward K-conductance of detrusor smooth muscle has several components due to the presence of a variety of K-channel sub-types including K_{ATP}. Further experimentation to characterise the nature of the inwardly-rectifying current in these cells is in progress.

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124P WHOLE-CELL POTASSIUM CONDUCTANCES FROM CONDITIONALLY IMMORTALIZED MESANGIAL CELLS FROM THE *H-2K*^{b}-tsA58 TRANSGENIC MOUSE

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Introduced by A.W.Cuthbert

Potassium conductances are universally acknowledged to be important in determination of cell resting membrane potential. This study reports the characteristics of the potassium conductance in mesangial cells from the *H-2K*^{b}-tsA58 transgenic mouse (Jat *et al.* 1991) using the whole-cell configuration of the patch clamp technique. The *H-2K*^{b}-tsA58 transgenic mouse harbours a temperature sensitive mutant of the SV40 T antigen under the control of the γ-interferon (IFN) inducible *H-2K*^{b} promoter and this allows the direct derivation of conditionally immortal cell lines by cell isolation procedures. Cells were cultured in permissive and non-permissive conditions (33°C +IFN and 37°C -IFN) and experiments were performed on cells from passage 8-15 in solutions buffered to pH 7.4 with Na-HEPES.

Whole-cell conductances (g) in symmetrical 100mM potassium gluconate/40mM KCl with 1μM pipette Ca^{2+} were 0.68±0.03nS (n=37) and 0.42±0.02nS (n=32) for cells grown in permissive and non-permissive culture conditions. These values are significantly different ($p<0.01$). In asymmetrical 100mM potassium gluconate and 40mM KCl/100mM sodium gluconate, 35mM NaCl and 5mM KCl both conductances could be inhibited by either 5mM Ba^{2+} or 10mM Cs^+ (g=0.22±0.02nS and 0.20±0.04nS respectively for cells grown in permissive conditions and 0.26±0.02nS and 0.25±0.04nS for cells grown in non-permissive conditions, n=4-6, $p<0.05$ vs controls). No voltage-activated currents could be detected and neither 100μM nor 1mM 4-aminopyridine (4-AP) had any effect on the conductances. However 10mM 4-AP inhibited the conductance in cells grown in permissive conditions (g=0.48±0.06nS, n=5 in the presence of 10mM 4-AP) but not in cells grown in non-permissive conditions. 1μM glibenclamide also inhibited the conductance in these cells (g=0.44±0.08nS in the presence of 1μM glibenclamide) but the effects of both 10mM 4-AP and 1μM glibenclamide were not additive. Furthermore 1mM ATP in the pipette solution decreased the conductance to 0.35±0.06nS, n=5. This suggests that there is an ATP-sensitive potassium conductance found in the cells when they are cultured at 33°C with IFN.

The Ca^{2+}-sensitivity of the conductance was assessed in the presence of 1μM glibenclamide and 1mM $LaCl_3$ to prevent Ca^{2+} entry into the cells. Increasing pipette Ca^{2+} concentration caused a dose-dependent increase in conductance in cells grown in permissive culture conditions from 0.29±0.03nS (10^{-8}M Ca^{2+}, n=6) to 0.51±0.04nS (10^{-3}M Ca^{2+}, n=6) and had an EC_{50} of 570nM. However no Ca^{2+}-sensitivity was observed in cells grown in non-permissive conditions. Apamin which inhibits SK_{Ca} was an effective inhibitor in cells grown in permissive conditions (IC_{50}=9.1nM) but not in cells grown in non-permissive conditions. This suggests that there may be SK_{Ca} channels in cells grown in permissive culture conditions and that these make up most of the rest of ATP-insensitive current.

This study suggests that there may be both ATP-sensitive potassium channels and SK_{Ca} channels in *H-2K*^{b}-tsA58 transgenic mouse mesangial cells grown in permissive culture conditions. However neither of these conductances can be demonstrated in cells grown in non-permissive conditions. Furthermore none of the cells showed the presence of any voltage-dependent currents.

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125P ANALYSIS OF AGONISM IN THE RAT AORTA: FURTHER EVIDENCE FOR HETEROGENEITY OF α_1-ADRENOCEPTORS

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Previously, our analysis of competitive antagonism suggested heterogeneity of α_1-adrenoceptors in the rat aorta (Van der Graaf *et al.*, 1993). Here we present further evidence for this hypothesis obtained from an analysis of agonism. After removal of the endothelium, 4mm ring segments of thoracic aorta from male Wistar rats (225-300g) were mounted in modified Krebs' buffer (2g basal tension, 37°C, gassed with 95%O_2/5%CO_2) with low (0.25mM) [Ca^{2+}] to prevent spontaneous phasic contractions. Tissues were calibrated with a 1μM phenylephrine (PE) sighter followed by a washout period of 30min. Tissues were then exposed to vehicle (6μl ethanol) or to 60nM phenoxybenzamine (PBZ) for 4 or 7min and washed for 30min. Following 90min incubation with 30μM cocaine and 6μM timolol, single concentration-effect (E/[A]) curves were obtained by cumulative dosing to noradrenaline (NA), in both vehicle (n=10) and PBZ-treated tissues, and to the following α_1-adrenoceptor agonists (see Hieble *et al.*, 1986) in vehicle treated tissues only: PE (n=8), methoxamine (METH, n=7), oxymetazoline (OXY, n=7), cirazoline (CIR, n=9), Sgd 101/75 (SGD, n=6), ST 587 (n=6) and SK&F 89748-A (SK&F, n=6). Effects were expressed as percentage of the PE sighting response. The agonist E/[A] data were fitted to the Hill equation to provide estimates (presented as mean ± s.e.mean) of the midpoint location ($p[A]_{50}$), upper asymptote (α) and midpoint slope (n_H; Table 1). PBZ pretreatment produced a significant rightward shift and depression of the upper asymptote of the NA E/[A] curve ($p[A]_{50}$=7.3±0.2 and 6.6±0.2; α=89±15% and 36±4% after 4 and 7min pretreatment with 60nM PBZ, respectively, n=5). Individual NA control and PBZ-treated E/[A] curves were fitted simultaneously to the operational model of agonism (Black *et al*, 1985) which provided estimates of the maximum achievable effect (E_m=195±29%) and slope of the occupancy-effect relation (n=0.8±0.2). The dissociation equilibrium constants (pK_A) and efficacies (log τ) for each agonist were then estimated by fitting the individual E/[A] curves to the operational model using the estimated values of E_m and n (Table 1). The relationship between the Hill slope, n_H, and the operational model parameters, τ and n, was then analysed as described by Black *et al.* (1985). It was found that with the exception of NA and PE, the E/[A] curves of all agonists tested were steeper than predicted by the operational model of agonism. However, the data could be quantitatively accounted for by the same two-receptor model we employed before to describe the complex antagonism of NA in this tissue (Van der Graaf *et al.*, 1993). These results therefore support our hypothesis that the rat aorta contains a heterogeneous α_1-adrenoceptor population.

Table 1 Analysis of α_1-adrenoceptor agonism in rat aorta

	$p[A]_{50}$	α(%)	n_H	pK_A	log τ
NA	7.8±0.1	137±6	0.88±0.04	7.1±0.2	0.46±0.07
PE	7.2±0.2*	123±5	1.01±0.08	6.6±0.2	0.27±0.04
METH	5.8±0.1*	115±7*	1.58±0.15*	5.0±0.1	0.34±0.09
OXY	6.5±0.1*	103±3*	1.91±0.29*	5.8±0.3	0.17±0.05
CIR	7.4±0.1	122±7	1.38±0.09*	6.7±0.2	0.32±0.05
SGD	5.8±0.1*	80±3*	1.43±0.05*	5.5±0.2	-0.16±0.06
ST 587	6.1±0.1*	55±5*	1.39±0.16	5.7±0.2	-0.44±0.08
SK&F	6.7±0.2*	84±4*	1.60±0.13*	6.4±0.2	-0.13±0.10

*P<0.05 compared to NA (Bonferroni corrected t-test)

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126P CHARACTERIZATION OF α_1-ADRENOCEPTOR SUBTYPES IN GUINEA PIGS

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Growing evidence suggests that the cloned α_1-adrenoceptor subtype previously designated α_{1c} represents the molecular correlate of the pharmacologically defined α_{1A}-adrenoceptor (Ford et al. 1994; Michel & Insel 1994), but some data in guinea pigs have indicated that the cloned receptor designated α_{1d} may represent the α_{1A}-adrenoceptor (Garcia-Sainz et al. 1992). Therefore, we have characterized the α_1-adrenoceptor subtypes in guinea pig spleen, kidney and cerebral cortex using [^{3}H]prazosin as the radioligand as previously described (Michel et al. 1993). Agonist competition experiments were performed in the presence of 100 µM GTP. Data are mean ± S.E. mean of 3-8 experiments.

Incubation with chloroethylclonidine (30 min at 37° C followed by two washout centrifugations) concentration-dependently reduced detectable α_1-adrenoceptors in all three tissues. While 1 µM chloroethylclonidine appeared to be more effective in spleen than in kidney or brain, 10 µM was similarly effective in all three tissues (70-90% inactivation).

5-Methylurapidil, methoxamine, (+)-niguldipine, noradrenaline, oxymetazoline, phentolamine, SDZ NVI-085 ((-)-(4aR,10aR)-3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2H-naphth[2,3,b)-1,4-oxazine HCl), (-)- and (+)-tamsulosin were tested in competition binding studies. In spleen all drugs competed for [^{3}H]prazosin binding with steep and monophasic curves yielding affinities (-log K_i, M) of 6.1, 3.7, 7.1, 6.0, 6.6, 7.7, 5.0, 9.0, and 7.0, respectively. Results in kidney were similar except for noradrenaline and (-)-tamsulosin which yielded biphasic curves with 35-40% high affinity sites (-log $K_{i\ high}$ 7.4 and 9.4, -log $K_{i\ low}$ 4.8 and 7.5, respectively). In brain all compounds had shallow and biphasic competition curves. Noradrenaline and (-)-tamsulosin had 50-51% high affinity sites (-log $K_{i\ high}$ 6.4 and 9.5, -log $K_{i\ low}$ 5.0 and 7.8). All other compounds recognized approximately 25% high affinity sites. Affinities at the low affinity sites were similar to those in spleen. Those at the high affinity site were 8.3 (5-methylurapidil), 5.5 (methoxamine), 8.9 ((+)-niguldipine), 8.0 (oxymetazoline), 8.9 (phentolamine), 6.2 (SDZ NVI-085), and 8.5 ((+)-tamsulosin).

We conclude that guinea pig tissues express an α_{1A}- and an α_{1B}-like adrenoceptor. However, the α_{1A}-like adrenoceptor in the guinea pig may be more chloroethylclonidine sensitive than that in other species, and the interaction of guinea pig α_1-adrenoceptors with noradrenaline and (-)-tamsulosin is not readily explained by current concepts of α_1-adrenoceptor subtype classification.

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127P NON-SURMOUNTABLE ANTAGONIST EFFECTS OF TAMSULOSIM ON THE α_{1A}-ADRENOCEPTOR-MEDIATED RESPONSES OF THE RAT AND HUMAN VAS DEFERENS

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The α_1-adrenoceptor antagonist tamsulosin acts as a competitive antagonist at the functional α_1-adrenoceptors of the human prostate and the rat spleen, but with a greater affinity for the prostatic receptor (Noble et al., 1994). The present study examines the actions of tamsulosin at the α_{1A}-adrenoceptors of the rat and human vas deferens (Furukawa et al., 1994) and the α_{1B}-adrenoceptors of the rabbit corpus cavernosum penis (Chess-Williams et al., 1994).

Strips of the corpus cavernosum penis from New Zealand White rabbits and human and rat epidiymal vas deferens were isolated and set up in gassed Krebs-bicarbonate solution at 37°C. Concentration-response curves to phenylephrine were constructed in the absence and presence of tamsulosin (0.1-3.0nM) after a 60 min antagonist equilibration period. All experiments were performed in the presence of cocaine (10μM), corticosterone (10μM) and propranolol (1μM). Control experiments were performed without the addition of antagonists.

On the human vas deferens tamsulosin (0.3-3.0nM) acted as a non-competitive antagonist. Tamsulosin (1nM) increased geometric mean EC_{50} values ($P<0.01$) from 10.1(4.4-22.9)μM to 116.3(57.4-236.0)μM but also reduced mean maximal responses (±SEM) by 51% from 1.58 ± 0.31g to 0.77 ± 0.18g (n=6)

Similar non-competitive effects were observed for tamsulosin on the rat vas deferens (1-10nM), tamsulosin (1nM) increasing EC_{50} values ($P<0.01$) from 9.0(7.0-11.6)μM to 35.9(21.2-60.7)μM and reducing maximum responses to phenylephrine by 31% ($P<0.05$) from 2.43 ± 0.14g to 1.67 ± 0.23g (n=6). The reponses of depolarised tissues (100mM KCl) to calcium were unaltered in the presence of tamsulosin (10nM) indicating that the drug was not exerting an action at voltage dependent calcium channels.

At the α_{1B}-adrenoceptors of the rabbit corpus cavernosum penis, tamsulosin (1-10nM) acted as a competitive antagonist. The antagonist caused parallel dextral shifts of phenylephrine concentration-response curves producing Schild plots with a slope similar to unity (0.85 ± 0.12) and a mean pK_B value (± SEM) of 9.2 ± 0.1.

Thus, although tamsulosin interacts competitively with prostatic α_1-adrenoceptors (Noble et al., 1994), these results demonstrate that the antagonist can have non-competitive effects on α_{1A}-adrenoceptor mediated responses.

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128P COMPARISON OF α_1-ADRENOCEPTOR SUBTYPES IN BOVINE BRAIN AND RAT LIVER

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The cloned bovine α_{1a}-adrenoceptor (previously designated α_{1c}) has been reported to differ from the α_{1A}-adrenoceptor found in other species with regard to chloroethylclonidine sensitivity and (+)-niguldipine affinity (Schwinn et al. 1990; Schwinn & Lomasney 1992). Therefore, we have compared α_1-adrenoceptor binding properties of bovine cerebral cortex with those of rat liver using [^{3}H]prazosin as the radioligand as previously described (Michel et al. 1993). Agonist competition experiments were performed in the presence of 100 µM GTP. Data are mean ± S.E. mean of 3-10 experiments.

Incubation with chloroethylclonidine (30 min at 37° C followed by two washout centrifugations) concentration-dependently reduced detectable α_1-adrenoceptors in both tissues. While 10 µM chloroethylclonidine almost completely inactivated α_1-adrenoceptors in rat liver, approximately 30% of receptors were resistant to inactivation in bovine brain.

In competition binding studies in rat liver 5-methylurapidil, methoxamine, (+)-niguldipine, noradrenaline, oxymetazoline, phentolamine, SDZ NVI-085 ((-)-(4aR,10aR)-3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2H-naphth[2,3,b)-1,4-oxazine HCl), (-)- and (+)-tamsulosin competed for [^{3}H]prazosin binding with steep and monophasic curves yielding affinities (-log K_i, M) of 6.3, 3.9, 6.9, 6.1, 6.6, 7.5, 4.9, 9.2, and 6.9, respectively.

In competition studies in bovine brain all of the above drugs except for noradrenaline competed for [^{3}H]prazosin binding with shallow and biphasic curves in which approximately 70% of all sites had high affinity for the test compounds. The calculated drug affinites (-log K_i) at the high affinity site were 8.5 (5-methylurapidil), 5.4 (methoxamine), 9.3 ((+)-niguldipine), 7.9 (oxymetazoline), 9.0 (phentolamine), 6.7 (SDZ NVI-085), 10.8 ((-)-tamsulosin), and 8.6 ((+)-tamsulosin). Drug affinities at the low affinity site were similar to those determined in rat liver. Noradrenaline competition curves were steep and monophasic with a -log K_i of 6.1.

We conclude that two α_1-adrenoceptor subtypes are present in bovine cerebral cortex. One them is pharmacologically similar to the α_{1B}-adrenoceptor in rat liver and to the cloned rat α_{1b}-adrenoceptor (Michel & Insel 1994). The other one is similar to the cloned bovine α_{1a}-adrenoceptor (Michel & Insel 1994) but has high affinity for (+)-niguldipine and is relatively insensitive to chloroethylclonidine.

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129P COUPLING OF AN ENDOGENOUS 5-HT_{1B}-LIKE RECEPTOR TO INCREASES IN INTRACELLULAR CALCIUM VIA A PERTUSSIS TOXIN-SENSITIVE MECHANISM IN CHO-K1 CELLS

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Chinese hamster ovarian cells (CHO-K1) express an endogenous 5-hydroxytryptamine $(5\text{-HT})_{1B}$-like receptor that is negatively coupled to adenylate cyclase through a pertussis toxin (PTX)-sensitive G_i/G_o protein (Berg *et al.*, 1994; Giles *et al.*, 1994). We have recently shown that the human adenosine A_1-receptor expressed in CHO-K1 cells stimulates calcium mobilization through a PTX-sensitive mechanism (Iredale *et al.*, 1994). The aim of this investigation was to determine whether the endogenous 5-HT_{1B}-like receptor is capable of coupling to phospholipase C (PLC) and hence calcium mobilization.

$[Ca^{2+}]_i$ was measured in CHO-A1 cells (CHO-K1 cells transfected with the human adenosine A_1-receptor, Townsend-Nicholson & Shine, 1992) and untransfected CHO-K1 cells. Cells were cultured in 75 cm^2 flasks in Dulbeco's Modified Eagles Medium/Nutrient F12 (1:1) supplemented with 10% foetal calf serum and 2 mM L-glutamine. Cells were split 1:10 and grown on 22 mm circular glass coverslips for 24 h before loading with 5 μM fura-2 as described previously (Marsh & Hill, 1994). $[Ca^{2+}]_i$ was monitored using a Nikon fluorescent microscope and the Applied Imaging Magical image analysis system as described previously (Marsh & Hill, 1994). Increases in $[Ca^{2+}]_i$ represent the mean ± s.e. mean of all (typically 15-20) cells in the microscopic field of view.

5-HT stimulated a concentration-dependent increase in $[Ca^{2+}]_i$ in CHO-A1 cells ($p[EC_{50}] = 8.07 \pm 0.05$; n=3). In the presence of extracellular Ca^{2+} (2mM) 5-HT (1 μM) increased $[Ca^{2+}]_i$ from 174 ± 17 nM to 376 ± 22 nM (n=12). The 5-HT_1-receptor agonists 5-carboxyamidotryptamine (5-CT; $p[EC_{50}] = 7.9 \pm 0.02$; n=3), RU24969 ($p[EC_{50}] = 8.1 \pm 0.07$; n=3) and sumatriptan ($p[EC_{50} = 5.9 \pm 0.11$; n=3) all elicited concentration dependent increases in $[Ca^{2+}]_i$. Similar maximal increases in $[Ca^{2+}]_i$ were obtained with each agonist. The selective 5-HT_{1A} receptor agonist 8-OH-DPAT (10 μM; n=4) did not stimulate increases in $[Ca^{2+}]_i$. Furthermore, 5-HT (1 μM) mediated increases in $[Ca^{2+}]_i$ were insensitive to the 5-HT_2 antagonists ritanserin (100 nM; n=8) and ketanserin (100 nM; n=9), and the 5-HT_3 antagonist LY-278,584 (1 μM; n=7). The response to 5-HT (100 nM) was antagonised by the non-selective 5-HT_1 antagonist methiothepin ($p[IC_{50}]$ 7.88 ± 3; n=3). Pretreatment with pertussis toxin (200 ng/ml for 4h) completely attenuated the response to 100 μM 5-HT (n=6). In untransfected CHO-K1 cells 5-HT (1 μM; 251 ± 20 nM to 469 ± 43 nM; n=3), RU24969 (1 μM; 205 ± 20 nM to 406 ± 23 nM; n=3) and 5-CT (1 μM; 174 ± 13 nM to 444 ± 33 nM; n=3) all stimulated increases in $[Ca^{2+}]_i$.

In summary, we have shown that the endogenous 5-HT_{1B}-receptor in CHO-K1 cells stimulates increases in $[Ca^{2+}]_i$ through a PTX-sensitive mechanism suggesting the involvement of G_i/G_o protein.

We thank the Wellcome Trust for financial support and Drs Andrea Townsend-Nicholson and John Shine for providing the adenosine A_1-receptor transfected CHO-K1 cells.

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130P CONTRACTILE ACTIONS OF THE MIXED 5-$HT_{2A/2B/2C}$ RECEPTOR ANTAGONIST ICI 169369 IN RAT STOMACH FUNDUS

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The mixed 5-hydroxytryptamine (5-HT)2A/2B/2C antagonist ICI 169369 is reported to be a potent but non-surmountable antagonist of 5-HT in rat stomach fundus (Blackburn *et al.*, 1988). The present study was initiated to investigate further the nature of the antagonism displayed by ICI 169369 in this preparation.

Rat stomach fundus was set up as previously described (Baxter *et al.*, 1994) except that all studies were conducted under isotonic conditions with tissues under a load of 1g. Two agonist concentration-effect curves were constructed in each preparation. The effects of antagonists were evaluated after an equilibration period of one hour between successive concentration-effect curves.

Cumulative addition of 5-HT evoked a concentration-dependent contractile response in rat stomach fundus yielding monophasic concentration-effect curves (pEC_{50} [95% C.L.] = 8.3 [8.1-8.5], n=5). ICI 169, 369 (0.1μM) caused an rightward displacement ± s.e.mean of 5-HT concentration-effect curves of 14.7 ± 0.7 -fold yielding an apparent pA_2 [95% C.L.] of 8.1 [8.1-8.2], n=5. At higher concentrations, ICI 169,369 (≥ 0.3μM) caused a contractile response in its own right. Concentration-effect curves to 5-HT, superimposed upon the elevated baseline response to ICI 169,369 were not shifted further to the right and the curve maxima were not reduced. The measurable location parameters of concentration-effect curves to carbachol were not influenced by the presence of ICI 169,369 (0.1-1μM). In separate experiments, the cumulative addition of ICI 169369 (0.1-1μM) yielded a steep, monophasic concentration-effect curve (pEC_{50} [95% C.L.], slope factor ± s.e.mean = 6.1 [5.9-6.3], 2.5 ± 0.3, n=6 respectively) with curve maxima equal to 110 ± 0.5 % of that achieved with 5-HT. Responses to ICI 169369 were not affected by the following ligands (at 1μM) which possess affinity for 5-HT2B receptors; SB 204741, mianserin, yohimbine and phenoxybenzamine (Baxter *et al.*, 1995). Responses to both ICI 169,369 and 5-HT were abolished, and those to carbachol attenuated, by the removal of calcium from the incubation media.

In conclusion, at low concentrations, ICI 169369 acts as surmountable antagonist of 5-HT in rat stomach fundus. At higher concentrations it is a contractile agent. This contractile response, like that to 5-HT, is dependent upon extracellular calcium but does not appear to be mediated by the 5-HT2B receptor. No direct evidence for non-surmountable antagonism at 5-HT2B receptors could be obtained in the present study.

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131P PRELIMINARY CHARACTERISATION OF 5-HYDROXYTRYPTAMINE (5-HT) RECEPTORS MEDIATING RELAXATION OF CAT JUGULAR VEIN

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5-HT may evoke vascular relaxation via activation of both 5-HT_1-like and 5-HT_{2B} receptors (*for references see* Ellis *et al* 1995). It has also been proposed that 5-ht7 receptors may mediate vascular relaxation (*see* Martin & Wilson 1995). The present study was designed to characterise the 5-HT receptors mediating relaxation of cat jugular vein.

Tissues were obtained from cats (either sex, 3.5-4.5 kg) previously used for acute *in-vivo* studies and stored in the fridge overnight. Ring segments of vessels (5mm in length) were suspended, under 0.75g tension, for isometric tension recording in oxygenated (95% O_2/5% CO_2) Kreb's solution at 37°C. All experiments were conducted in the presence of ketanserin (0.1μM) and indomethacin (3.0μM). Two cumulative relaxant agonist concentration-effect curves (1h apart) were obtained in each preparation and antagonists were incubated for at least one hour between successive concentration-effect curves.

In tissues with intact endothelium, 5-HT, BW 723C86 ([±]-1-[5-(2-thenyloxy)-1H-indol-3-yl]-propan-2-amine HCl), 5-CT and α-methyl 5-HT caused concentration dependent relaxations (pEC_{50} [95% CL], (n) = 8.8 [8.6-8.9], (5); 8.4 [8.2-8.7], (10); 8.5 [8.4-8.7], (3); 7.7 [7.6-7.9], (3) respectively). In the presence of L-NABE (Nω-nitro-L-arginine benzyl ester) 0.1 mM, concentration-response curves to 5-HT were of lower potency (pEC_{50} [95% CL] = 7.2 [6.8-7.5], n=3) and responses to BW 723C86 and α-methyl 5-HT were not distinguishable from tissue 'fade'. In contrast, responses to 5-CT were shifted only slightly (Mean concentration-ratio ± s.e.mean = 1.9 ± 0.16). The selective 5-HT_{2B} antagonist SB 204741 (Baxter *et al.*, 1995) caused concentration dependent rightward displacement of concentration-effect curves to BW 723C86 (pA_2 [slope] = 7.4 [0.61] n≥5). In vessels incubated with L-NABE (0.1 mM) and from which the endothelium had been removed by rubbing the vessel along a stainless steel wire, responses to 5-CT were antagonised by methiothepin, cyproheptadine, spiperone and ritanserin (pA_2 estimates determined by single point analysis [95% CL] = 8.9 [8.4-9.4], 7.3 [7.1-7.6], 7.3 [6.8-7.9], 7.3 [7.2-7.3] n≥3 respectively) but not the mixed 5-$HT_{2B/2C}$ antagonist, SB 200646 (Ellis *et al.*, 1995) or SB 204741 (both at 1.0μM).

In conclusion, the relaxant response to 5-HT in the cat jugular vein has two components. The high potency, nitric oxide dependent phase, appears similar to the 5-HT_{2B} receptor (Ellis *et al* 1995). The lower potency, nitric oxide independent phase possesses a similar pharmacological fingerprint to the 5-ht7-like receptor in rabbit femoral vein (Martin & Wilson 1995).

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132P 5-HT RECEPTOR SUBTYPE(S) IN THE ADULT RABBIT PULMONARY ARTERY

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5-Hydroxytryptamine (5HT) is known to be an important pulmonary vasoconstrictor and thought to be involved in pulmonary hypertension (Johnson & Georgieff, 1989). Evidence indicates that the vasoconstrictor effect of 5HT in systemic arteries is mediated mainly through $5HT_2$ receptor types (Saxena & Villalon, 1990). It has recently been demonstrated that $5HT_1$-like receptors may play a comparatively greater role in 5HT induced vasoconstriction in pulmonary arteries (PAs) (Templeton *et al.*, 1994; MacLean *et al.*, 1994). To our knowledge, 5HT receptor types have not been studied in the rabbit pulmonary vascular bed. In this preliminary study, we used 5HT agonists to determine the 5HT receptor subtype(s) involved in the 5HT-induced vasoconstriction of adult rabbit PAs. Adult rabbits were killed with sodium pentobarbitone. The left branch was dissected out and rings taken from both extra- and intra-pulmonary regions. These were mounted (under 1.5 and 1.25g tension respectively) in Krebs-filled (5ml) organ baths and bubbled with 16% O_2/6%CO_2 balance N_2. Cumulative concentration-response curves (CCRCs) were constructed for agonists which show different selectivities for $5HT_1$ and $5HT_2$ receptor classes. These were 5HT, 5-carboxamidotryptamine(5-CT), 8-hydroxydipropylaminotetralin (8OH-DPAT), sumatriptan and (±)α Methyl-5HT (αMe-5HT) (all 0.1nM-300μM). The results are shown in Table 1. These results show that in the rabbit pulmonary artery, the rank order of potency of 5HT agonists is 5HT>αMe-5HT>5-CT>8OH-DPAT. Sumatriptan was inactive. The same order of potency is seen in both extra- and intra-lobe regions of the pulmonary artery branch. This suggests that a '$5HT_2$ type' receptor is mainly involved in the 5HT-induced vasoconstriction in adult rabbit pulmonary arteries although some involvement of a '$5HT_1$ type' (not $5HT_{1D}$) cannot be ruled out. This work was supported by the Wellcome Trust & The Royal Society (London)

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Table 1. pEC_{50} values for 5HT agonists and their maximum response as a % of the maximum 5HT response in the same preparation. A Extra-lobe B Intra-lobe. n/n = number of ring preparations/number of animals.

Agonist	*A. pEC50*	*Max.*	*n/n*	*B.pEC50*	*Max.*	*n/n*
5-CT	5.8 ± 0.1***	70 ± 3.3	9/6	5.8 ± 0.1***	68 ± 4.5	8/6
8OH-DPAT	5.2 ± 0.1***	54 ± 3.4	7/5	5.5 ± 0.2***	56.5 ± 10	5/5
αMe-5HT	6.9 ± 0.1**	100	10/6	6.8 ± 0.1	100	5/5
Sumatriptan	no response	-----	5/5	no response	-----	6/6
5HT	7.4 ± 0.1	100	13/9	7.1 ± 0.1	100	18/12

*Statistical difference from 5HT pEC50, Student's unpaired t-test *P<0.05; **P<0.01; ***P<0.001*

133P THE INFLUENCE OF NITRIC OXIDE ON PULMONARY ARTERY RESPONSES TO 5-HT IN FOETAL, NEONATAL AND ADULT RABBIT

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5-Hydroxytryptamine (5-HT) is an important pulmonary vasoconstrictor and thought to be involved in pulmonary hypertension (Johnson & Georgieff, 1989). Nitric oxide (NO) is known to have a modulatory role in pulmonary vascular responses to circulating vasoconstrictors and neurotransmitters (MacLean *et al* 1993a & 1993b). Here we examined the vasoconstrictor responses to 5HT in foetal, neonatal and adult pulmonary artery (PA) rings and the effect of NO synthase inhibition on these responses. The rabbits were killed with sodium pentobarbitone. The main PA & left branch (extra- & intra-lobe regions) were dissected out. The rings were mounted under tension in Krebs-filled (5ml) organ baths and bubbled with 16% O2/6%CO2 balance N2 (3% O2/ 6% CO2/ balance N2 for foetal tissue). Foetal & neonatal intra-lobal arteries were mounted on a wire myograph under tension. Paired cumulative concentration-response curves (CCRCs) were constructed for 5HT (0.1nM-300μM) in the presence or absence of 100μM L-NAME. The results are shown in Table 1. The results show that the sensitivity of the vessels to 5HT varies with developmental age. Inhibition of NO synthase with L-NAME markedly potentiated the maximum response size in the majority of PAs studied and significantly increased the sensitivity in foetal and neonatal tissue. The neonatal pulmonary vasculature undergoes changes in 5HT sensitivity. These changes are dependent on developmental age and vessel location. NO plays an important role in modulating pulmonary vascular responses to 5HT in the foetal, neonate and adult rabbit.

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Table 1. *p*EC50 values for 5HT ±100μM L-NAME as well as maximum response in the presence of L-NAME as % maximum 5HT response in the same preparation. n/n = number of preparations/number of animals.

Preparation		*pEC50*		*n/n*	*Maximum response + L-NAME*
		CONTROLS	+ L-NAME		
Foetal	-main	6.5 ± 0.1	7.0 ± 0.1*	5/5	259 ± 60***
	-extra-lobe	6.9 ± 0.1††	7.1 ± 0.1	4/4	234 ± 3***
	-intra-lobe	7.1 ± 1.0	7.7 ± 0.3	3/3	100 ± 10
0-24h	-main	6.8 ± 0.1	6.8 ± 0.1	8/8	113 ± 5*
	-extra-lobe	6.5 ± 0.1†††	7.2 ± 0.1***	4/4	144 ± 18**
4 day	-main	6.8 ± 0.1	6.9 ± 0.1	5/5	106 ± 7
	-extra-lobe	6.7 ± 0.1†††	6.8 ± 0.1*	7/7	343 ± 94***
	-intra-lobe	7.7 ± 0.5	8.1 ± 0.2	5/4	254 ± 78***
Adult	-extra-lobe	7.4 ± 0.1	7.2 ± 0.2	5/5	116 ± 6*
	-intra-lobe	7.0 ± 0.1	7.0 ± 0.1	8/8	104 ± 4

*Statistical difference 1) from adult 5HT pEC50, Student's unpaired t-test ††P<0.01; †††P<0.001 2) from control 5HT pEC50 or maximum response Student's paired t-test *P<0.05; **P<0.01; ***P<0.001.*

134P ARE THERE SUBTYPES OF PROSTAGLANDIN DP RECEPTORS?

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The original classification of prostaglandin (PG) DP receptors was based predominantly on potency orders of the naturally occurring agonists (Kennedy *et al.*, 1982; Coleman *et al.*, 1984) and was later confirmed by the demonstration that BW A868C was a selective antagonist (Giles *et al.*, 1989). A range of the equilibrium dissociation constant (pK_B) estimates of BW A868C at DP receptors has been reported, using BW245C as a selective agonist: 9.3 in human platelet (Giles *et al.*, 1989), 9.1 in human myometrium (Fernandes & Crankshaw, in press), 8.7 in rabbit jugular vein (Giles *et al.*, 1989) and 8.5 in rabbit saphenous vein (Lydford *et al.*, in press). The possible existence of DP receptor subtypes has been suggested, based primarily on the different profiles of selective agonists (Woodward *et al.*, 1993). In preliminary studies, we have found that DP receptors appear to mediate relaxation of isolated dog major palatine artery, dorsal nasal vein and saphenous vein. In the present study, the antagonism of BW A868C was examined against BW245C and PGD_2 in an attempt to characterize the DP receptors in these vascular beds.

Isolated ring preparations were obtained from dogs (10 - 16 kg), male or female, killed by an overdose of Euthatal and set up for isometric force displacement recordings in modified Krebs buffer, oxygenated with 5% CO_2 in O_2 and maintained at 37 ± 0.5 °C. In all experiments indomethacin (2.8 μM) and GR32191B (5 μM) were included to prevent interference by cyclo-oxygenase products and TP receptor activation, respectively. The preparations were pre-contracted with a sub-maximal dose of KCl (50 mM). Antagonist pre-treatments, 60 min before the first dose of an agonist, were allocated according to a randomised block-design to minimise errors from non-biological sources. Relaxation curves of BW245C or PGD_2 in the absence and presence of BW A868C were recorded simultaneously. Concentration-effect data (E/[A] curve) were fitted using a logistic function which produced estimates of the upper asymptote (α), mid-point location ($p[A_{50}]$) and slope index (p). Parallelism was tested by one-way analyses of variance on the computed α and p estimates. If the criteria for simple competition had been satisfied a modified form of Schild equation was used to determine the pK_B.

In the dog dorsal nasal vein BW A868C, up to a concentration of 0.3 μM, displaced BW245C curves in a simple competitive manner. A pK_B estimate of 7.4 ± 0.1 (s.e., d.f.= 19) was obtained. Similar pA_2 estimates, were also obtained in the dog major palatine artery (7.6 ± 0.2, d.f.= 7) and saphenous vein (7.1 ± 0.1, d.f.= 11). BW A868C was also tested against PGD_2, in dog dorsal nasal vein and major palatine artery where the pA_2 estimates were found to be identical (7.3 ± 0.2, d.f. = 8 and 3).

These results show that in a number of dog vascular beds BW A868C, up to 0.3 μM in concentration, behaves as an antagonist with a significantly lower affinity than previously reported in other studies. This may be due to species variations or may be indicative of the existence of DP receptor subtypes. Further studies using a variety of antagonists and agonists in several species are necessary to resolve this issue.

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135P A PRELIMINARY STUDY OF THE EFFECTS OF PROSTAGLANDIN E_2 AND THE THROMBOXANE MIMETIC, U46619, ON THE HUMAN UMBILICAL ARTERY

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Prostaglandins have been found to play an important role in the initiation of labour. Although there is strong evidence to support this concept in certain species, it remains controversial in relation to human parturition. The aim of the present study is to investigate the effect of the thromboxane mimetic U46619 (11, 9-epoxymethano PGH_2) and prostaglandin E_2 (PGE_2) on human umbilical artery. The effect of PGE_2 is of particular interest since this compound is used clinically to induce cervical ripening at term. In this work the stable thromboxane mimetic U46619 was also used in conjunction with the thromboxane receptor (TP) antagonists, Bay u3405 and SQ 29548 (Coleman *et al.*, 1994).

Samples of human umbilical cord were obtained from full term pregnancies (all women gave written consent) and placed immediately into Krebs solution at room temperature. The cords were then transported to the laboratory within 60 minutes. The artery was carefully dissected of the surrounding Wharton's Jelly and cut into 5 mm rings. Care is required in the dissection as it is known that prostaglandins are released from damaged tissue (Piper & Vane, 1971) which can cause constriction of the human umbilical artery (Hillier & Karim, 1968). The rings of umbilical artery were suspended in Krebs solution containing indomethacin (2.79 μM) at 37°C in a 10ml organ bath and oxygenated with 2.5 % O_2/ 8 % CO_2/balance N_2. The tension of the tissue was maintained at 2g throughout the 2h equilibration period. Agonists were added directly into the organ bath in a cumulative fashion. Dose effect curves were constructed to U46619 and PGE_2. Where antagonists were used they were allowed to equilibrate for at least 30 minutes before the agonist dose-response curve was repeated. Schild analysis was used to calculate pA_2 values.

The thromboxane mimetic, U46619, was a potent constrictor agonist (EC_{50} = 4.0 x 10^{-7} M). Both of the thromboxane antagonists tested caused a progressive rightward shift of the dose response curve to U46619 with increasing concentration of antagonist. The pA_2 values below correspond with those quoted by Coleman *et al.*, (1994).

TP Antagonist	Dose Range	pA_2	Slope
Bay u3405	10^{-6} - 10^{-8} M	8.3	0.7
SQ 29548	10^{-6} - 10^{-8} M	8.6	0.9

PGE_2 produced constriction (EC_{50} = 8.7 x 10^{-6} M) of the tissue over a dose range 1 x 10^{-9}- 3 x 10^{-6} M but this dose effect curve was inhibited by both Bay u3405 10^{-7}M and SQ 29548 10^{-7}M. These results suggest that part of the PGE_2 response is mediated through the TP-receptor. This study is being extended to resolve the components of the PGE_2 response.

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136P CHARACTERISATION OF PROSTACYCLIN (PGI_2) AND PROSTAGLANDIN E_2 (PGE_2) RELEASE FROM HUMAN SAPHENOUS VEIN IN RESPONSE TO BACTERIAL LPS

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The release of PGI_2 is regulated by constitutive isoforms of phospholipase A_2 (PLA_2) and cyclo-oxygenase (COX; COX-1). However, certain cytokines, growth factors or bacterial lipopolysaccharide (LPS) can induce both PLA_2 and a novel isoform of COX (COX-2), (see Mitchell *et al.*, 1995). PGI_2 is the main COX metabolite released by vascular cells and has several protective roles in the cardiovascular system. Indeed, reduced PGI_2 production by damaged vessels is associated with diseases such as atherosclerosis. The selective induction of COX and PLA_2 in vascular preparations may compensate for a reduced capacity of vessels to make PGI_2. Using isolated segments of human saphenous vein, obtained from patients undergoing bypass surgery (59-76 yrs old; 2 female, 3 male), we have investigated the effects LPS on the release of PGI_2 and PGE_2.

Veins were collected in sterile phosphate buffered saline (PBS) containing penicillin (1000 IU.ml^{-1}) and streptomycin (1 mg.ml^{-1}; Pen-Strep). Under sterile conditions, vessels were cleaned, washed 4-6 times with the PBS containing Pen-Strep and cut into rings approximately 2 mm wide (15.5±0.6 mg wet weight). Rings were placed into separate wells of a 48 well culture plate containing 500 μl of Eagles complete DMEM supplemented with glutamine (2 mM) and Pen-Strep. In some experiments 10% foetal calf serum (FCS) was included in the culture medium. After 1h (37°C; 5% CO_2) the medium was replaced and drugs added. Medium was removed after 48h and prostaglandin concentration measured by radioimmunoassay for PGE_2 or 6-keto $PGF_{1\alpha}$ (the hydrolysis product of PGI_2), (Mitchell *et al.*, 1993).

LPS caused a significant increase in the production of both 6-keto $PGF_{1\alpha}$ and PGE_2 either in the presence or absence of FCS (Table1). FCS alone increased the release of 6-keto $PGF_{1\alpha}$ ($p<0.05$) but had no effect on production induced by LPS. In contrast the basal release of PGE_2 was unaffected by FCS, however, the release in response to LPS (100 μg.ml^{-1}) was increased ($p<0.05$).

DMEM	PGE_2	6-keto $PGF_{1\alpha}$	ratio
vehicle	50±8 (n=4)	68±5 (n=5)	0.6±0.06
LPS 10 (μg.ml^{-1})	289±138 (n=4)*	316±105 (n=5)*	1±0.2*
LPS 100 (μg.ml^{-1})	93±6.5 (n=3)*	207±55 (n=4)*	0.7±0.07
DMEM plus FCS			
vehicle	102±23 (n=4)	116±18 (n=5)	1±0.2
LPS10 (μg.ml^{-1})	420±207(n=4)*	244±63 (n=5)*	1.4±0.1
LPS 100 (μg.ml^{-1})	234±14 (n=3)*	340±82 (n=4)*	1.2±0.4

table 1: Release of prostaglandins (ng.ml^{-1}; 48 h) from human saphenous vein. Results show mean ± s.e. mean for n patients.
** denotes significant ($p<0.05$; Mann-Whitney U-test) differences compared to the relevant vehicle. The ratio shown is the ratio between the release of PGE_2 and 6-keto $PGF_{1\alpha}$ calculated in each determination measured.*

The ratio of PGE_2:6-keto $PGF_{1\alpha}$ released under the different conditions was not significantly changed by FCS. This ratio was significantly increased by LPS (10 μg.ml^{-1}; $p<0.05$) in the absence but not in the presence of FCS. The protein synthesis inhibitor, cycloheximide (1 μM), attenuated the LPS-induced release of both metabolites in the presence (6-keto $PGF_{1\alpha}$ by 45±3%; PGE_2 by 74±1%; n=3) or absence (6-keto $PGF_{1\alpha}$ by 33±12; PGE_2 by 59±6; n=3) of FCS. Indomethacin (30 μM) greatly reduced (by more than 95%) the LPS-stimulated release of both 6-keto $PGF_{1\alpha}$ and PGE_2 either in the presence or absence of FCS (n=3).

Here we show that LPS increases the release of both PGI_2 and PGE_2 by human saphenous vein, probably due to an induction of COX and/or PLA_2 enzymes. Many venous grafts fail because of atherosclerosis and restenosis, a process which is reduced in some experimental models by PGI_2. The identification of agents which increase the capacity of bypass vessels to release PGI_2, may ultimately be used to improve graft success.

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137P THE EFFECT OF ILOPROST PRETREATMENT ON THE SUB-CELLULAR LOCALIZATION OF IP PROSTANOID RECEPTORS AND $G_{s\alpha}$ IN NG108-15 CELLS

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Prolonged exposure of NG108-15 neuroblastoma x glioma cells to IP prostanoid receptor agonists results in a loss of IP receptors and $G_{s\alpha}$ from the cells (Kelly et al., 1990; MacKenzie and Milligan, 1990). Inhibition of protein synthesis with cycloheximide blocks neither the loss of the IP receptor (Kenimer and Nirenberg, 1981) nor of $G_{s\alpha}$ (Veale et al., 1992), suggesting that the observed down-regulation is due to increased breakdown of receptor and G protein. In this study we have used subcellular fractionation to investigate whether the IP receptors and/or $G_{s\alpha}$ are internalized during exposure to agonist.

Confluent NG108-15 cells (passage 16-25) were pretreated for 2h or 8h in Dulbecco's modified Eagle's medium containing 10μM iloprost or vehicle as control. Cells were then harvested and washed before being homogenized in 25mM TrisHCl, pH 7.4, containing 0.25M sucrose, 1mM EDTA and 0.5mM PMSF. Nuclei and unbroken cells were pelleted in a low speed spin and the supernatant was then centrifuged at 60,000 x g for 55min at 4°C on 35ml gradients self-generated from 20% Percoll. IP receptors were assayed using the specific binding of 1-10nM [^{3}H]-iloprost, measured in the presence of 100μM guanylylimidodiphosphate (Krane et al., 1994). Immunoblotting with the $G_{s\alpha}$-specific antiserum CS1 was used to detect $G_{s\alpha}$ (MacKenzie and Milligan, 1990).

In controls, the peak of [^{3}H]-iloprost binding activity was recovered from the middle of the gradient (fractions 6-22), co-localized with the binding of the muscarinic antagonist [^{3}H]-N-methyscopolamine, which was used as a plasma membrane marker. A single peak of acid phosphatase activity (a lysosomal marker) was found near the top of each gradient (fractions 32-36). The Percoll gradients were divided into two pools: a heavy pool (fractions 1-24) containing the plasma membrane marker and a light pool (fractions 25-36) containing the lysosomal marker. In control homogenates, the specific binding of 5nM [^{3}H]-iloprost was 34.9±14.9 fmol.mg protein^{-1}; 90% of this activity was recovered in the heavy pool (30.9±3.9 fmol.mg protein^{-1}) and no significant binding activity was detected in the light pool (2.5±2.9 fmol.mg protein^{-1}). Following pretreatment with iloprost for 2h and 8h, [^{3}H]-iloprost binding in homogenates was reduced by 45% and 75% to 19.3±9.8 and 8.5±1.2 fmol.mg protein^{-1}, respectively. Binding in the heavy pools was reduced by a similar degree to 14.2±4.8 and 5.4±2.0 fmol.mg protein^{-1}. Significant binding was not detected in the light pools, where the binding was 2.7±6.4 and 2.7±8.3 fmol.mg protein^{-1} following iloprost pretreatment for 2h and 8h respectively (all values mean±s.e.mean, n=4). Similarly, $G_{s\alpha}$ was detected in the cellular homogenates and the heavy pools from control cells and cells that had been pretreated for 2h or 8h with iloprost. There was a time dependent decrease in the amount of $G_{s\alpha}$ detected in these pools, but no $G_{s\alpha}$ was ever detected in the light pool.

Thus these data do not demonstrate an accumulation of IP receptors or $G_{s\alpha}$ in intracellular vesicles following pretreatment with iloprost. This may indicate that internalization does not occur. However, it is also possible that breakdown of internalized proteins is too rapid for them to be detected under these conditions or simply that the sub-cellular fractionation procedure does not adequately separate internalized vesicles from plasma membrane.

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138P THE EFFECT OF INHIBITORS OF INTERNALIZATION ON IP PROSTANOID RECEPTOR DOWN-REGULATION IN NG108-15 CELLS

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Prolonged exposure of NG108-15 neuroblastoma x glioma cells to IP prostanoid receptor agonists produces a pronounced loss of responsiveness to IP receptor agonists, which is accompanied by a loss of IP receptors (Kenimer and Nirenberg, 1981; Kelly et al., 1990). This IP receptor down-regulation is not blocked by inhibition of protein synthesis (Kenimer and Nirenberg, 1981) and can be partially inhibited by chloroquine (Krane et al., 1994), suggesting that it may be due to proteolysis of receptors in lysosomes. Concanavalin A, hypertonic medium and K^+-depleted medium have all been reported to inhibit receptor internalization in other systems (Toews et al., 1984; Yu et al., 1983; Cantau et al., 1988). In this study we have investigated whether the same manipulations reduce the loss of IP receptors produced by iloprost pretreatment, in an attempt to determine whether IP receptor internalization is necessary for down-regulation.

Confluent NG108-15 cells (passage 16-25) were pretreated for 17h in the absence or presence of 10μM iloprost. In controls this pretreatment was carried out in Dulbecco's modified Eagle's medium (DMEM); in parallel incubations the DMEM was supplemented with 0.25mg.ml^{-1} concanavalin A (con A), made hypertonic with 0.5M sucrose, or substituted with K^+-depleted medium, in which K^+ had been replaced with equimolar Na^+. Cells were subsequently harvested and washed, and [^{3}H]-iloprost binding activity was measured as previously described (Krane et al., 1994).

Neither con A nor hypertonic medium had any effect on the loss of [^{3}H]-iloprost binding produced by exposure to iloprost. In the absence of con A, the specific binding of 10nM [^{3}H]-iloprost, measured in the presence of 100μM guanylylimido-diphosphate (GppNHp), was reduced by 95%, from 31.2±13.5 fmol.mg protein^{-1} to 1.7±1.3 fmol.mg protein^{-1}; in the presence of con A, this binding was reduced by 84%, from 45.3±25.5 fmol.mg protein^{-1} to 7.1±5.6 fmol.mg protein^{-1} (each value mean±s.e.mean, n=3). Similarly, in hypertonic medium the specific binding of 10nM [^{3}H]-iloprost was reduced by 87%, compared with a reduction of 93% in controls. However, IP receptor down-regulation was markedly less than controls when iloprost pretreatment was carried out in K^+-depleted medium. The specific binding of 10nM [^{3}H]-iloprost, measured in the presence of 100μM GppNHp, was 42.2±6.8 fmol.mg protein^{-1} in DMEM controls and 45.7±5.4 fmol.mg protein^{-1} in K^+-depleted medium. Iloprost pretreatment reduced this value by over 80% in DMEM, to 7.0±4.9 fmol.mg protein^{-1}, but by only 20% in K^+-depleted medium, to 36.9±5.5 fmol.mg protein^{-1} (each value mean±range, n=2).

Thus, while the lack of effect of con A and hypertonic medium may tend to suggest that IP receptor internalization does not occur, these results appear to contradict the inhibition of receptor down-regulation observed in K^+-depleted medium and the role of internalization in IP receptor down-regulation remains to be determined.

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139P CHARACTERISATION AND COMPARISON OF MUSCARINIC RECEPTORS IN THE DOG CILIARY MUSCLE WITH ILEUM

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Pharmacological evidence has been provided for the presence of four muscarinic receptor subtypes (Caulfield,1993). This was based on selective inhibition of agonist responses by muscarinic antagonists such as pirenzepine (M_1), methoctramine (M_2) , 4-diphenylacetoxy-N-methylpiperidine methobromide (4-DAMP) (M_3) and himbacine (M_4). This study seeks to characterise the functional muscarinic receptor in the dog ciliary muscle, compared with that in the dog ileum.

Studies were conducted in Krebs gassed with 95%O_2 and 5%CO_2. Cumulative dose response curves to carbachol and acetylcholine were generated on ciliary muscle (37°C) and on ileum (32°C) respectively. Tissues were incubated for 20 minutes (zamifenacin 60 minutes on ileum) with increasing concentrations of antagonists prior to rechallenge with the agonists. Schild's analysis was used to determine pA_2 values (See Table 1. for values expressed as mean ± sem).

The relative pA_2 values of the standard selective antagonists (4-DAMP, methoctramine and pirenzepine) profiled in this study indicate that the receptors in both the ciliary muscle and ileum are of the M_3 subtype. This finding in the dog ciliary muscle is consistent with studies in bovine (Honkanen *et al* 1990) and human ciliary muscle (Woldemussie *et al*, 1993). Zamifenacin is a novel M_3 selective receptor antagonist (Wallis *et al*, 1994) on guinea pig tissues, and the data on the dog ileum are consistent with its high affinity for smooth muscle M_3 receptors. In contrast, in the dog ciliary muscle zamifenacin was at least two orders of magnitude less potent, thus raising the possibility that the functional M_3 receptor in the ciliary muscle may be different from that in the ileum.

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Table 1	Ciliary Muscle			Ileum		
Compound	n	pA_2	Slope	n	pA_2	slope
Atropine	5	8.48 ± 0.06	1.27 ± 0.16	5	9.3 ± 0.07 *	1.2 ± 0.08 *
Pirenzepine	6	6.67 ± 0.07	1.27 ± 0.16	6	7.0 ± 0.1	1.09 ± 0.05
Methoctramine	6	<5.5	-	6	6.85 ± 0.16	0.89 ± 0.11
4-DAMP	5	8.5 ± 0.09	1.11 ± 0.05	4	8.84 ± 0.08	0.98 ± 0.03
Zamifenacin	6	<6	-	4	8.64 ± 0.1	0.96 ± 0.12

* L-Hyoscyamine

140P CHARACTERISATION OF THE MUSCARINIC RECEPTOR SUBTYPE MEDIATING SALIVARY GLAND SECRETION AND TRACHEAL SMOOTH MUSCLE CONTRACTION IN THE RAT

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Functional studies in isolated tissues have shown that muscarinic receptor antagonists, such as parafluorohexahydrosiladifenidol and zamifenacin, have greater affinity for M_3 receptors in the ileum than at M_3 receptors in, for example, trachea or salivary gland (Eglen *et al.*, 1990; Wallis *et al.*, 1993). Additionally, ligand binding studies in salivary gland tissue (Nilverbrant & Sparf, 1988) have suggested that glandular M_3 binding sites and smooth muscle M_3 receptors may not be identical. We have determined the functional activity of a range of muscarinic antagonists in isolated rat trachea and parotid gland in order to determine whether M_3 receptors in these tissues can be differentiated functionally.

pA_2 values were derived from Schild analysis of cumulative dose response curves to carbachol of rat trachea spirals in the absence and presence of antagonist. The rate constant for ^{86}Rb efflux (K_{Rb}) from parotid gland was determined as described elsewhere (Gater *et al.*, 1982). Inhibition of carbachol (10^{-5}M)-evoked increase in K_{Rb} by antagonist was expressed as the negative log of the 50% inhibitory concentration (pIC_{50}). Tissues were maintained in Krebs-Henseleit solution (plus 10nM indomethacin; trachea) gassed with 95% O_2/5% CO_2, at 37°C. Antagonists were preincubated for 30 minutes.

pA_2 values on rat trachea indicated that the receptor mediating contraction of this tissue was the M_3 subtype (values of 9.1, 7.0 and 6.6 for 4-DAMP, pirenzepine and methoctramine, respectively). Additionally, antagonist rank-order potency on trachea was essentially similar to that on salivary gland (Table 1) and a plot of pIC_{50} *vs* pA_2 had a correlation coefficient of 0.98. Thus, we conclude that the muscarinic receptors mediating trachea smooth muscle contraction and salivary gland secretion in the rat are the M_3 subtype and cannot be differentiated functionally with the antagonists studied here.

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Table 1. Muscarinic receptor antagonist pA_2 values on rat trachea and pIC_{50} values on rat salivary gland. (95% confidence limits in parentheses) Atr (atropine), 4-D (4-DAMP), Tz (telenzepine), UH-AH (UH-AH 37), HSD (hexahydrosiladifenidol), Pro (procyclidine), pFHSD (parafluorohexahydrasiladifenidol), Dicy (dicyclomine), Pz (pirenzepine), Meth (methoctramine), AF-DX (AF-DX 116).

	Atr	4-D	Tz	UH-AH	HSD	Pro	pFHSD	Dicy	Pz	Meth	AF-DX
pA_2	9.6 (9.4-9.9)	9.1 (9-9.3)	8.2 (8.1-8.4)	8.1 (7.8-8.3)	8.0 (7.9-8.2)	8.0 (7.8-8.2)	7.6 (7.4-7.9)	7.4 (7.2-7.6)	7.0 (6.9-7.2)	6.6 (6.4-6.9)	6.4 (6.2-6.6)
pIC_{50}	8.1 (8-8.2)	7.7 (7.5-7.9)	6.7 (6.6-6.9)	6.5 (6.4-6.6)	6.5 (6.4-6.6)	6.4 (6.3-6.5)	5.8 (5.6-5.9)	5.2 (5.0-5.3)	5.4 (5.3-5.5)	4.4 (4.3-4.5)	4.8 (4.6-4.9)

Data from 4-9 separate determinations. Schild slopes not significantly different from 1, except methoctramine, 0.85 (0.74-0.97).

141P CHARACTERISATION OF A SENSITIVE BRADYKININ B_2 PREPARATION IN THE RABBIT SCIATIC VEIN

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The effects of bradykinin (BK) and peptidic antagonists have been described in a wide variety of isolated tissues leading to the classification of three BK receptors - B_1, B_2 and B_3 (Farmer *et al*, 1989) with the proposal of further subdivision of the B_2 class into B_{2A} and B_{2B} subtypes based on differences in potency of antagonists when tested on rabbit jugular vein and guinea pig ileum (Rhaleb *et al*, 1992). Here we describe the pharmacological characterisation of the BK receptor found in the rabbit sciatic vein.

Sciatic veins were removed from male New Zealand White rabbits (2.5-3.5kg) following overdose with pentabarbitone and exsanguination. Rings of vein (2-3mm wide) were mounted on hooks in modified Krebs solution (5.5mM glucose) containing 5μM captopril at 37°C, under 2g. wt. initial tension. Isometric responses to cumulative additions of BK were monitored and the effects of a range of antagonists were determined following 30 min incubation. Antagonist potency was assessed as pK_B's when limited activity over the test concentration range or non-surmountable antagonism prevented pA_2 analysis.

BK was found to act as a potent spasmogen of the sciatic vein (pD_2 =8.3) inducing well maintained contractions of rapid onset. Cumulative concentration response curves could be repeated at 30 min intervals without tachyphylaxis. The action of BK appeared to be independent of other mediators as responses were unaffected by atropine (3μM), indomethacin (3μM), mepyramine (3.5μM), capsaicin (10μM), yohimbine (1μM), propranolol (1μM), or ketanserin (10μM). The rank order of potency of standard peptidic antagonists [HOE-140 (pK_b 10.8) > NPC-16,731 (pA_2 9.3) > NPC-349 (pA_2 7.9) ≥NPC-567 (pA_2 7.4) ≥ adamantyl NPC-349 (pK_B 7.4) > desArg^9[Leu^8]-BK (pA_2 <6)] and the low efficacy of the selective B_1 agonist desArg^9-BK (pD_2 <6.3) suggest that the bradykinin receptor mediating contraction is of the B_2 subtype. Both HOE-140 and NPC-16,731 acted as non-surmountable antagonists, markedly depressing the maximum response to BK.

Antagonist data obtained in the rabbit sciatic vein correlated strongly (r=0.96) with that found in binding studies in guinea pig ileal membranes. Comparison with functional guinea pig ileum data showed that all antagonists were at least 10 fold more active in the rabbit sciatic vein, with the same potency rank order. On this basis, the sciatic vein may be most appropriately classed as B_{2A}. The high sensitivity to antagonists coupled with the direct action of BK and the convenience of cumulative dosing without tachyphylaxis gives the sciatic vein practical advantages over other B_2 preparations thus this tissue may be of use in the identification and study of new B_2 antagonists.

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142P SPECIES DIFFERENCES IN THE EFFECTS OF NK_2 RECEPTOR ACTIVATION ON RAT AND GUINEA-PIG BLADDER, *IN VIVO*

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Rat and guinea-pig bladder are innervated by capsaicin-sensitive primary afferent neurones which are characterised by high levels of tachykinin immunoreactivity (Holzer et al, 1982) and NK_2 receptor sites (Carstairs et al, 1993). In the anaesthetised guinea pig, NK_2 receptor-dependent bladder contractions are indirect and are abolished by agents which inhibit the efferent arm of the micturition reflex arc: atropine, [αβ-Me]ATP (Metcalfe et al, 1994) and hexamethonium (Metcalfe et al, 1995). Therefore, the effects of NK_2 receptor stimulation on the guinea pig bladder are mediated by activation of the micturition reflex. In the present study, we have compared the responses of the rat and guinea pig bladder to [βala^8]$NKA_{(4-10)}$ and have examined the site of action of this selective NK_2 receptor agonist.

Female Dunkin Hartley guinea pigs (350-450g) or male CD rats were anaesthetised with urethane (1.2g/kg i.p.), the carotid artery was cannulated for blood pressure recording, the left jugular vein cannulated for i.v. drug administration and the trachea cannulated for artificial ventilation. After tying off the external urethra, a catheter was inserted via the dome into the bladder for intraluminal pressure recording.

Initial *in vitro* studies confirmed that the NK_2 receptor agonist [βala^8]$NKA_{(4-10)}$ contracted rat and guinea pig bladder preparations, giving respective pEC_{50} values of 7.0±0.06 and 6.8±0.1 and maximal contractile responses of 2.7±0.17 and 1.3±0.21g tension (mean+s.e.mean, n=8). In the anaesthetised rat, administration of [βala^8]$NKA_{(4-10)}$ caused dose-dependent (ED_{50}=3.5μg/kg, range 1.4 - 6.5, n=4) graded bladder contractions which contrasted with the "all or nothing" biphasic bladder contractions seen in the anaesthetised guinea-pig (threshold dose = 1-5μg/kg). The NK_2 receptor antagonist SR48968 (1-300μg/kg) abolished guinea pig bladder contractions at doses of 30μg/kg and above and caused a dose-dependent inhibition of bladder contractions in the rat, giving an ED_{50} of 142μg/kg (range 103 - 204, n=4). Atropine (10-1000μg/kg) or hexamethonium (0.1-30mg/kg) treatment 5 min prior to [βala^8]$NKA_{(4-10)}$ (5μg/kg) had little or no effect on the bladder response in the rat (n=4). However, there was a dose-dependent inhibition of the guinea pig bladder response, giving ED_{50} values of 72μg/kg (range 29 - 140, n=4) with atropine and 0.58mg/kg (range 0.18 - 1.33, n=7) with hexamethonium.

In conclusion, there are clear species differences in the effects of NK_2 receptor activation on bladder pressure in rats and guinea-pigs. In the guinea pig, [βala^8]$NKA_{(4-10)}$ produces reflex contractions of the urinary bladder by an action on pre-ganglionic efferent or afferent NK_2 receptors. In contrast, NK_2 receptor activation in the rat results in direct contraction of bladder smooth muscle which is not modified by blockade of the micturition reflex arc. Since autoradiographic studies have identified NK_2 receptor sites on both bladder smooth muscle and capsaicin-sensitive afferent nerves in the bladder mucosa (Carstairs et al, 1993), it is likely that activation of these different NK_2 receptor populations underlies the direct and indirect effects, respectively, of [βala^8]$NKA_{(4-10)}$, seen in this study.

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143P THE ONTOGENY OF ADENOSINE RECEPTOR SUBTYPES IN THE RAT VAS DEFERENS

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The rat vas deferens is a tissue which has been used extensively for adenosine receptor research. We have recently shown that the adult rat vas deferens contains inhibitory prejunctional A_1 receptors, inhibitory postjunctional A_2 receptors (Hourani *et al.*, 1993) and excitatory postjunctional A_1 receptors (Hourani & Jones, 1994). Radioligand binding assays using the A_1 selective antagonist [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]-DPCPX) have also identified [^{3}H]DPCPX binding sites commensurate with A_1 receptors in the rat vas deferens (Peachey *et al.*, 1994). As part of a continuing project to study the ontogeny of purinoceptors in smooth muscle, we have investigated the development with neonatal age of the adenosine receptors in the rat vas deferens using a combination of radioligand binding and functional assays.

Isolated vasa deferentia from male Wistar albino rats (University of Surrey strain) aged 10, 15, 20, 25, 30, 40 days and adult were used, the day of birth being designated as day 1. Functional assays were carried out as previously described (Hourani *et al.*, 1993; Hourani & Jones, 1994). Prejunctional A_1 receptors were detected by measuring the inhibitory effect of N^6-cyclopentyladenosine (CPA) (0.01-10μM) on the nerve mediated contractions (field stimulation, Grass S48 stimulator, twin pulses of 1ms duration, 75 ms delay, 70V). Excitatory postjunctional A_1 receptors were detected by measuring the enhancement of KCl (35mM)-induced contractions following preincubation (1 min) with CPA (0.01-30μM). The inhibitory effects of 5'-N-ethylcarboxamidoadenosine (NECA) (0.01-30μM) on the KCl-induced contractions were used to show the presence of postjunctional A_2 receptors. Saturation binding assays were performed as previously described (Peachey *et al.*, 1994), using [^{3}H]DPCPX (0.05-10nM). Insufficient tissue was obtained from 10 day old animals for saturation assays to be performed, therefore homologous displacement assays were performed using a K_d concentration of [^{3}H]DPCPX (1nM) and unlabelled DPCPX (0.1-100nM).

CPA inhibited nerve mediated contractions from day 15 onwards, day 15 being the earliest age to respond to nerve stimulation, and potency values were constant across the ages studied (pD_2 6.5-7). NECA inhibited KCl induced contractions from day 10 onwards, potency values remaining constant across the ages studied (pEC_{25} 6.5-7). In contrast, CPA failed to enhance KCl-induced contractions at days 10, 15 and 20, with a small enhancement (<10%) being observed at day 25 and increasing with age up to day 40 to a value similar to that seen in the adult (~35%). Binding studies failed to detect binding at days 10, 15 and 20, but by day 25 the density of binding sites peaked (B_{max} 75.2±11.1 fmol/mg protein) declining at day 30 to a value commensurate with that found in the adult (~40 fmol/mg protein).

These results show that the prejunctional A_1 receptors and the postjunctional A_2 receptors are fully functional from days 15 and 10 respectively, the earliest days which could be tested using functional assays. However, the excitatory postjunctional A_1 receptors developed later, the response being detectable only from day 25. The A_1 binding sites identified using [^{3}H]DPCPX were also present from day 25, and this suggests that this binding assay detects only the postjunctional A_1 receptors and not those present on the nerve terminals.

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144P FURTHER CHARACTERISATION OF THE VANILLOID RECEPTOR IN THE RAT ISOLATED VAS DEFERENS

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A specific receptor (termed the vanilloid receptor) has recently been identified on the cell membrane of primary afferent neurones which recognises capsaicin and other natural pungent agents including resiniferatoxin (RTX, Szallasi & Blumberg, 1990). Stimulation of this receptor results in neuropeptide release and the sensation of pain. The vanilloid receptor is known to be present in the rat vas deferens (Maggi *et al.*, 1994). In the present study, this receptor has been further characterised using newly available pharmacological tools.

The rat vas deferens was set up for electrical field stimulation (efs, 0.2 Hz, 0.5 ms, 60V) and isotonic tension recording. Following a 60 min equilibration period, tissues were dosed cumulatively with increasing concentrations of capsaicin, RTX, olvanil or calcitonin gene-related peptide (CGRP). The time course of agonist addition was determined by the rate of onset and time to plateau of the agonist response. A single agonist concentration-effect curve was constructed in each tissue. The effects of antagonists were investigated in paired vas deferens following a 40 min equilibration period. Responses were expressed as % inhibition in the amplitude of electrically-evoked contractions.

Capsaicin (1 - 1000 nM) produced a concentration-dependent inhibition in the amplitude of efs (pEC_{50} 7.6 ± 0.03, n = 8). Responses to capsaicin peaked within 5 min (4.3 ± 0.1 min at EC_{50}, n = 6) and faded rapidly over a 30 min exposure period. Responses to capsaicin were mimicked by CGRP (0.1 - 100 nM, pEC_{50} 8.3 ± 0.04, n = 6). RTX (0.1 - 1000 pM) also inhibited efs (pEC_{50} 12.3 ± 0.2, I.A. 0.6 ± 0.03, n = 6). Responses to RTX were slow in onset (17.3 ± 0.2 min at EC_{50}, n = 6) and well maintained over a 30 min exposure period. Responses to capsaicin and RTX displayed mutual cross desensitisation. Olvanil (0.01 - 10 μM) produced a small, inconsistent inhibition of efs at high concentrations, but overall this effect was not significantly different from that of the solvent.

Responses to capsaicin were antagonised in a competitive manner by capsazepine (3 - 10 μM, pA_2 5.7 ± 0.2, n = 6) and in a non-surmountable manner by ruthenium red (1 - 10 μM, pD'_2 5.3 ± 0.2, n = 6). At these concentrations, capsazepine had no effect on efs, while ruthenium red produced a concentration-dependent inhibition of efs (35% inhibition at 10 μM). Capsazepine (3 - 10 μM) and ruthenium red (1 - 10 μM) also antagonised responses to RTX, however accurate analysis of this antagonism was not possible due to the slow equilibration rate of RTX. Responses to olvanil (n = 4 each) and CGRP (n = 6 each) were not antagonised by either capsazepine (10 μM) or ruthenium red (10 μM).

Further characterisation of the vanilloid receptor in the rat isolated vas deferens has demonstrated a clear difference in the rate of onset of responses between capsaicin and RTX. This is likely to be due to pharmacokinetic differences but the existence of multiple classes of receptor cannot be excluded. Thus, the lack of intrinsic activity of olvanil in the rat vas deferens is of particular interest in view of previous reports (Bevan & Docherty, 1993) that this compound is a full agonist in other systems.

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145P INVESTIGATION OF THE REVERSIBLE VASODILATOR RESPONSE AFTER LOCAL CUTANEOUS HEATING OF THE RAT TAIL

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We investigated the possibility that sensory neuropeptides are involved in the local reversible vasodilator response observed after local heating of rat tail skin. Male Wistar rats (230-280g) were anaesthetised with sodium pentobarbitone (50mg/kg; i.p.). Cutaneous blood flow in the tail was measured continuously at two sites (1cm diameter) by a dual channel Moor laser Doppler flow meter and expressed as arbitrary units (flux). One site could be heated by a thermostatically controlled skin heater, the other was used as a control. Heat at 44°C for 2.5 min was applied to the test site. A rapid increase in blood flow was observed, this reached a peak after about 2 min (from 31±7 flux to 83±24 flux; $P<0.01$, Student's paired *t* test; n=6) and returned to baseline within 15 min. Drug or vehicle was administered i.v. after the first heat stimulation and the stimulation was then repeated twice more. There was no significant change in blood flow at the unheated, control site and none of the drugs under investigation had any effect on basal blood flow at the concentrations studied.

Calcitonin gene-related peptide (CGRP) antagonist $CGRP_{8-37}$ and the tachykinin NK_1 receptor antagonist SR140333 were given at effective doses (Hall *et al.*,1995; Emonds-Alt *et al.*, 1993) 5 min prior to second stimulation. The nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) and the cyclo-oxygenase inhibitor indomethacin were both given 15 min before second stimulation.

Table 1. Effects of drugs on the heat-induced increase in blood flow expressed as mean±s.e.mean area under the curve of the heated site minus the control site (n=5-6).

	Stimulation 1 (in mm^2)	Stimulation 2 (in mm^2)	Stimulation 3 (in mm^2)
$CGRP_{8-37}$ (200nmol/kg)	292±49	326±62	285±30
Vehicle	327±40	341±66	312±37
SR140333 (100µg/kg)	228±32	207±52	277±39
Vehicle	230±48	285±32	257±33
L-NAME (1mg/kg)	230±38	155±16	93±21**
Vehicle	267±42	253±39	274±52
Indomethacin (3mg/kg)	368±40	116±40**	202±23*
Vehicle	262±63	283±68	212±58

* $P<0.05$; ** $P<0.01$ cf. stimulation 1 (Tukey-Kramer multiple comparison test).

There does not appear to be a sensory neuropeptide involvement in the reversible reactive hyperaemia caused by local heating of the rat tail skin. However, NO and prostaglandins may play a role.

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146P SURAMIN REVEALS TWO DISTINCT NEURONALLY-MEDIATED INHIBITORY JUNCTION POTENTIAL COMPONENTS IN THE GUINEA-PIG INTERNAL ANAL SPHINCTER (gpIAS)

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Conventional intracellular microelectrode and simultaneous mechanical recording techniques have revealed two distinct inhibitory junction potential (IJP) components in response to electrical field stimulation (1-5 pulses, 1-40 Hz, 0.1ms, supramaximal voltage) of non-adrenergic, non-cholinergic (NANC) nerves supplying the circular muscle of the gpIAS in the presence of atropine and phentolamine (each 1µM); one, large, fast and apamin (0.3µM)-sensitive; the other, slower, smaller and apamin-insensitive (Rae & Muir, 1994). The present study, also using conventional microelectrode and simultaneous mechanical recording techniques, sought to determine whether the proposed purinoceptor antagonist suramin (Hoyle *et al*, 1990), like the small conductance Ca^{2+}-activated K^+ channel blocker apamin (Capoid & Ogden,1989), could also split the IJP into its two components and allow analysis of the mediators responsible. Suramin reduced IJP amplitude. This effect was not significant at 10µM but maximal at 100µM (by 59.6±4.3% in response to a single pulse, n=8 tissues; $P<0.01$ for all frequencies). The remaining suramin-insensitive component was reduced significantly by the NO scavenger oxyhaemoglobin (HbO; 10µM; by 40.1±8.5% in response to a single pulse, n=5 tissues; $P<0.05$ at all frequencies except 40Hz), an effect reversible on washing. It was abolished by the NO synthase inhibitor L-NAME (100µM; n=5 tissues), but not by D-NAME (100µM; n=3 tissues) and partially restored by L-arginine (100µM; to 58.7±2.9% of control in response to a single pulse after 40 minutes, n=3 tissues). Exogenous ATP (100µM) also hyperpolarized (16.9±1.1 mV, n=5 tissues) and relaxed the tissue. Suramin reduced the mechanical responses to lower concentrations of ATP (1-3mM) but was less effective against higher concentrations of the nucleotide (9-30mM).

These results demonstrate that suramin, like apamin, can be used to reveal the mediators of NANC transmission in this tissue which are purinergic and nitrergic in nature.

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

147P EVALUATION OF THE ROLE OF NITRIC OXIDE IN HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS

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Mast cells, once activated, release preformed and newly generated mediators which can act as messengers in various physiological conditions. Nitric oxide (NO), which is principally generated from L-arginine (L-Arg), can play a role as an intra- and intercellular messenger. Salvemini *et al.* (1990) showed that sodium nitroprusside (NaNp) and L-Arg inhibited histamine release from, and increased cyclic GMP (cGMP) in rat mast cells.

The aims of the present work were to study (1) the effects of L-Arg, D-Arg and NaNp on histamine release from rat peritoneal mast cells (RPMC), (2) whether these compounds changed cGMP, oxyhaemoglobin fluorescence and nitrite production within RPMC and (3) the effects of L-Arg, D-Arg and NaNp on NADPH-diaphorase staining within RPMC using nitro blue tetrazolium dye.

RPMC were obtained by direct lavage and purified by density centrifugation over Percoll. cGMP was determined by radioimmunoassay (Amersham). Inorganic nitrite ions were assayed using a two-step diazotization reaction (Ignarro *et al.*, 1987). The method of Kelm *et al.* (1988), using the conversion of oxyhaemoglobin to methaemoglobin to measure nitric oxide production, was used. NADPH-diaphorase staining of the mast cells for nitric oxide synthase using nitro blue tetrazolium dye as described by Ursell (1993) was used.

L-Arg and D-Arg caused a time-dependent release of histamine. The histamine release was comparable in purified and non-purified RPMC. NaNp had no effect in either preparation. These results are presented in Table 1. Preincubation of RPMC with the NO synthase inhibitor L-NAME had no effect on histamine release induced by L-Arg and D-Arg.

Incubating RPMC with L-Arg, D-Arg or NaNp for up to 60 minutes caused no change in the amount of intracellular cGMP from the basal level of 2.8±0.12 pmol/10^6 cells (n=4). Incubation of purified or non-purified RPMC with the three compounds caused no change in the fluorescence of oxyhaemoglobin or the diazotization reaction. Staining of RPMC using NADPH-diaphorase histochemistry showed no change in stain intensity from unstimulated cells compared to those stimulated with L-Arg, D-Arg and NaNp, implying that the inducible form of NO synthase was not present. Removing extracellular and intracellular calcium using EDTA had no effect on the L-Arg induced release implies that the Ca^{2+}-calmodulin dependent constitutive form of NO synthase was not present.

In conclusion, the results presented in this study suggest that RPMC do not contain NO synthase, and on stimulation with the various agents do not produce the oxide. L-Arg and D-Arg, therefore, probably cause histamine release via another mechanism not involving NO, which requires further investigation.

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	L-Arg (30 min)	L-Arg (60 min)	D-Arg (30 min)	D-Arg (60 min)	NaNp (30 min)	NaNp (60 min)
Purified RPMC	7.9 ±2.4	22.4±3.2	6.7±3.4	12.8±2.9	1.2±2.8	1.5±3.1
Non-purified RPMC	12.3±2.2	26.8±2.7	9.4±2.3	14.6±2.7	1.8±2.5	2.3±2.1

The results are shown as percentage histamine release (mean±s.e.mean) induced by the various agents (2 mM) for 4 experiments done in triplicate.

148P DUROQUINONE, BUT NOT HYDROQUINONE, INHIBITS NITRERGIC RELAXATIONS OF THE MOUSE ANOCOCCYGEUS AFTER INHIBITION OF SUPEROXIDE DISMUTASE

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Recently, it has been suggested that NO released from nitrergic nerves in the bovine retractor penis may be protected by superoxide dismutase (SOD), thus explaining the resistance of nitrergic relaxations to superoxide anion-generating drugs (Martin *et al.*, 1994). In the present study, we have investigated this proposal in the mouse anococcygeus muscle using two quinones with differing superoxide anion-generating potential; duroquinone (DQ), which is strongly redox active, and hydroquinone (HQ), which redox cycles very poorly (Boersma *et al.*, 1994).

Mouse anococcygeus muscles were set up for the isometric recording of nitrergic relaxations (70V; 1ms pulse width) as described previously (Hobbs *et al.*, 1991). DQ was dissolved in dimethylsulphoxide and HQ in distilled water; control experiments revealed neither vehicle to have significant effect on drug- or nerve-induced responses at the concentrations employed. Results are expressed as mean ± s.e (n of at least 5), and statistical analysis was by Student's t test.

In control muscles, authentic NO (15μM) and nitrergic field stimulation (10Hz; 10s train) relaxed carbachol (50μM)-induced tone by 48±5% and 46±9% respectively; these were used as standard relaxant stimuli in further experiments. Both HQ and DQ (10-200μM) caused a concentration-dependent inhibition of NO-induced relaxations (52±10% and 55±7% inhibition at 100μM HQ and DQ respectively). DQ also inhibited nitrergic relaxations (11±1% and 32±5% inhibition at 50μM and 100μM DQ); however, HQ (up to 200μM) had no effect on nitrergic relaxations. The inhibitory effect of DQ (100μM) on nitrergic relaxations was reversed (by 89±11%) in the presence of SOD (250u ml^{-1}).

Following incubation with the SOD inhibitor diethyldithiocarbamate (DETCA; 3mM; 45min incubation followed by 10min washout) there was a 10-fold leftward shift of the DQ concentration-response curve against nitrergic relaxations (32±5%, 49±6% and 85±8% inhibition at 10μM, 20μM and 100μM DQ respectively). The inhibitory effect of 100μM DQ on nitrergic relaxations was partially reversed (by 31±7%) on addition of SOD (250u ml^{-1}) to the organ bath. In contrast, HQ (100μM) continued to have no effect on nitrergic relaxations even after incubation with DETCA (relaxation before HQ, 45±5%; relaxation in the presence of HQ, 46±5%; $P>0.05$). Indeed, HQ (100μM) partially reversed (by 44±8%) the inhibitory effect of DQ (100μM) on nitrergic relaxations.

These results support the proposal that SOD protects the nitrergic transmitter from inhibition by superoxide anion-generating substances, such as DQ. However, the differential effect of HQ on relaxations to exogenous NO and nitrergic stimulation requires another explanation, presumably because HQ does not readily generate superoxide anions (Hobbs *et al.*, 1991; Boersma *et al.*, 1994); perhaps some other antioxidant system is involved in protecting endogenous NO from HQ (Brave *et al.*, 1993; Gibson *et al.*, 1995).

E.L. has an MRC studentship.

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149P EFFECTS OF CHARYBDOTOXIN AND APAMIN ON RESPONSES OF ISOLATED GUINEA PIG CAECI TO PHOTOCHEMICAL ACTIVATION OF THE 'CAGED' NITRIC OXIDE DONOR $K_2[Ru(NO)Cl_5]$

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Immunohistochemical studies have shown nitric oxide synthase (NOS) to be present in myenteric plexus neurones from the guinea pig taenia caeci (Saffrey *et al*, 1992). Non-adrenergic, non-cholinergic (NANC) electrical field stimulation of this tissue *in vitro* produces relaxations which are inhibited by the nitric oxide synthesis inhibitors N^{ω}-nitro-L-arginine (NOARG) and N^{ω}-nitro-L-arginine methyl ester (NAME), suggesting a role for nitric oxide (NO) in these responses (Piotrowski *et al*, 1993, Piotrowski *et al*, 1994). Methylene blue appears to have little or no effect on NANC relaxations in the taenia, arguing against a major role for guanylyl cyclase in these responses (Piotrowski *et al*, 1993).

To investigate further the mechanisms of action for nitric oxide in this preparation, segments of taenia caeci 2cm long were removed from freshly killed guinea pigs (450-950g) and set up in 10ml isolated tissue baths maintained at 37°C, supplied with Kreb's solution containing guanethidine (10μM) and bubbled with 95%O_2/5%CO_2. Mechanical activity of the preparations was recorded isotonically using a resting tension of 0.5g. Following precontraction with carbachol (0.3-1.0μM), relaxations of the tissue were induced by photon activation of the nitric oxide donor $K_2[Ru(NO)Cl_5]$ (100μM) (Williams *et al*, 1993) using 10s pulses of light every 90s. Illumination was supplied via a liquid light guide (Cairn Research) from a tungsten-halogen light source. The responses obtained are subsequently expressed as means$\pm$sem of control relaxations.

In the absence of any other treatment, photochemically-induced relaxations of the taenia caeci were reproducible for periods in excess of 1 hour. These responses were slightly potentiated by charybdotoxin (50nM) (121.3$\pm$4.4%*, n=4), whilst apamin (300nM) produced a marked inhibition (56.3$\pm$5.8%*, n=4) of the light-evoked relaxations. In tissues which were precontracted with potassium chloride instead of carbachol, light-induced relaxations showed a marked dependence on potassium concentration, with abolition of responses observed at KCl concentrations in the range 30-50mM, tissue tone being submaximal at these concentrations.

These data suggest the involvement of potassium channels in the NO-evoked relaxations of guinea pig taenia caeci, and that an apamin-sensitive subpopulation of these channels (possibly SK_{Ca}) contributes to the relaxations. A further charybdotoxin-sensitive channel may also be involved.

(*=$p<0.05$ compared to pre-drug controls, Student's t-test)
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150P COMPARISON OF THE EFFECTS OF GUANYLIN AND *ESCHERICHIA COLI* HEAT STABLE ENTEROTOXIN ON HUMAN INTESTINAL MUCOSAL ELECTROLYTE TRANSPORT

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Guanylin is a naturally occurring peptide with a similar homology to Escherichia Coli heat stable enterotoxin (ST_a). Like the toxin, guanylin causes intestinal fluid and electrolyte secretion by elevation of cGMP, and subsequent secretion of chloride ions. It has been suggested that guanylin may be the endogenous ligand for ST_a receptors (Field & Semrad, 1993), and that it is released from mucosal paracrine cells, with subsequent binding to ST_a receptors on apical membranes of enterocytes (Li & Goy, 1993). This investigation was initiated to compare the actions of guanylin and ST_a on intact human ileal and colonic mucosa, and to determine whether the peptides might produce secretion when applied to the basolateral side of mucosal preparations, as has been observed in rat colon (Kachur *et al.*, 1992).

Macroscopically normal mucosa plus submucosa was obtained, by sharp dissection, from specimens of human colon or ileum resected at operation for Crohn's disease or cancer. Mucosal sheets were mounted in Ussing chambers and clamped at zero potential. Transmucosal short-circuit current (SCC) was continuously monitored and used as a measure of electrogenic fluid secretion. Guanylin and ST_a were dissolved in bovine serum albumin (BSA, 1mgml^{-1}), and applied cumulatively to the tissue with a 15min contact time for each concentration. Responses to guanylin and ST_a were expressed as a percentage of the maximum response to carbachol (10^{-4}M) or forskolin (25μM). After 60min equilibration, basal SCC was 36.9±3.3 and 68.0±8.4μAcm^{-2} for ileum (n=39) and colon (n=48) respectively. The effects of apical and basolateral application of guanylin (12.5-1000nM) and ST_a (1-80u/ml) to colonic and ileal mucosa are shown in Table 1. Apical application of guanylin or ST_a led to a rapid rise in SCC. When applied to the basolateral side, however, guanylin and ST_a were found to produce a smaller rise in SCC (Table 1), and in addition the response was significantly slower than that after apical application; the maximum response was not reached within the 15min contact time. Application of BSA control vehicle had no effect on SCC. The colonic response appeared independent of neural involvement, as it was unaffected by application of tetrodotoxin (3.2μM, $p>0.05$, n=3).

	Ileum		Colon	
	Guanylin	ST_a	Guanylin	ST_a
EC_{50}[a](Ap)	46.2nM (29.5-72.4)	2.91u/ml (2.43-3.49)	34.3nM (22.1-53.2)	3.10u/ml (2.79-3.45)
ΔSCC[b](Ap)	55.4±8.1	42.0±4.1	66.1±4.4	51.9±5.8
ΔSCC[b](Baso)	14.9±3.3*	21.3±3.9*	35.6±9.1*	18.3±3.6*

Table 1. Effect of apical (Ap) and basolateral (Baso) guanylin and ST_a on SCC of human ileal and colonic mucosa. [a]indicates EC_{50} on apical application, given as geometric mean (95% confidence limits), [b]indicates maximum increase in SCC (μAcm^{-2}) upon apical or basolateral application, given as mean±s.e.mean, *indicates $p<0.01$ (Mann-Whitney U test; apical vs. basolateral, all data are n≥3)

We have confirmed observations by Kuhn *et al.* (1994) that basal SCC of human colonic mucosa is increased by apical guanylin and ST_a. In addition a similar effect has been demonstrated using human ileal mucosa. In contrast to Kuhn *et al.* (1994), we have shown that basolateral application of either peptide to both ileal and colonic mucosa produces a rise in SCC. The reason for the discrepancy may be due to the slow onset of effect, and time taken to reach maximum effect, observed for basolateral application. The possibility that guanylin is crossing over from the basolateral to apical domain is currently being investigated.

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151P MEMBRANE CHOLESTEROL ALTERATIONS IN CULTURED WKY AND SHRSP VASCULAR SMOOTH MUSCLE CELLS ON EXPOSURE TO LDL, OX-LDL AND VITAMIN E

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The development of atherosclerosis is dependent on both environmental and genetic factors. Previous work (Thorin et al., 1995) suggested that increased free cholesterol content within the cell membrane may be an initiator of proatherogenic changes at the level of membrane physicochemical structure and calcium influx.. In the following study we have investigated the effects of LDL, OX-LDL, and vitamin E on free membrane cholesterol levels in a genetic model of hypertension.

On reaching confluence WKY and SHRSP vascular smooth muscle cells were incubated for 48 hours in medium 199 containing 20ug/ml LDL or OX-LDL. Further experiments were conducted with native LDL exposure in the presence and absence of 100uM vitamin E. Appropriate controls were used for each experiment. Following incubation, cells were scraped and free membrane cholesterol measured by enzymatic fluorescence assay determination. Protein content was measured using a modified Lowry assay and results calculated in 'ug cholesterol per mg protein'. Statistical analysis was carried out using repeated measures ANOVA and Bonferroni paired multiple comparison tests. Results were as shown in tables 1 and 2.

Small increases in free membrane cholesterol were observed following exposure to LDL and OX-LDL. Vitamin E abolished any LDL effect, but also reduced basal free membrane cholesterol levels by up to 55%, indicating that vitamin E has other effects in addition to antioxidant activity.

Table 1 Free Membrane Cholesterol Content

Cell Type	CONTROL	LDL	OX-LDL
WKY	5.67(1.10)	6.56(1.06)	6.82(1.34)
SHRSP	4.97(0.54)	6.30(0.71)*	6.72(0.75)*

Standard errors given in brackets
* Highlights significant differences from control ($p<0.05$)
Results calculated from 20-24 experiments, using 6-7 different primary cultures and a minimum of 3 different LDL/OX-LDL preparations per cell type

Table 2 Free Membrane Cholesterol Content

Cell Type	CONTROL	VIT E	LDL	LDL + VIT E
WKY	6.47(2.02)	2.92(1.31)*	7.61(2.74)	2.90(0.83)**
SHRSP	5.06(1.76)	2.61(0.91)	5.37(1.90)	2.32(0.73)**

Standard errors given in brackets
* Highlights significant difference from control ($p<0.05$)
** Highlights significant differences from LDL sample set ($p<0.05$)
Results calculated from 9-11 experiments, using 4 different primary cultures and a minimum of 3 different LDL/OX-LDL preparations per cell type

Thorin E., Hamilton C.A., Dominiczak A.F., Dominiczak M.H., Reid J.L., Atherosclerosis, (In Press)

152P RELAXANT EFFECTS OF 17β-OESTRADIOL ON RAT ISOLATED AORTA

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Oestrogens have been shown to reduce systemic vascular resistance (Magness & Rosenfeld, 1989), modulate responses of rabbit femoral arteries (Gisclard *et al.*, 1988) and to dilate rat tail artery by inhibiting calcium influx (Shan *et al.*, 1994). In the present experiments we investigated mechanisms involved in 17β-oestradiol (EST) relaxation in rat aortic rings contracted by different agonists.

Aortic rings 3-5 mm wide with or without endothelium were prepared from male Hooded Lister rats (200-300g, Bradford strain) and placed in Krebs solution containing 10 μM indomethacin under 2g tension 37°C; 95% O_2, 5% CO_2. Endothelium was removed from rings by rubbing the internal surface with moist cotton thread, removal of the endothelium being confirmed by loss of relaxation to acetylcholine.

Intact and de-endothelialised preparations were contracted with $PGF_{2\alpha}$ (10 μM) or KCl (80 mM) approximately EC_{80} concentrations giving responses of 2.3±0.06g and 1.91±0.09g respectively. Relaxation was expressed as % reversal of contraction. When contraction was stable, EST was applied for 40 minutes. EST caused concentration-related gradual relaxation in all tissues (Table 1).

Table 1: 17β-Oestradiol relaxation of contraction produced by $PGF_{2\alpha}$ or KCl expressed as % reversal of contraction (n≥4)

EST (μM)	% Relaxation $PGF_{2\alpha}$ (10 μM)	% Relaxation KCl (80 mM)
5	13.0 ± 1.5	16.8 ± 4.8
10	27.0 ± 1.8	26.7 ± 3.9
20	54.8 ± 3.9	59.8 ± 3.4

No differences in the effect of EST in intact or de-endothelialised preparations and no vehicle effects were observed. The relaxant effects of EST were unaffected by prior incubation of the tissue (15 mins) with L-NAME (10 μM) or methylene blue (10 μM) (n = 4).

To investigate interactions between EST and calcium mobilisation rings were incubated (2 hr) with Ca^{2+}-free Krebs containing 100 μM EDTA. KCl (80 mM) was added and after 15 mins incubation a cumulative Ca^{2+} concentration-response curve constructed. This was repeated in the presence of EST (20 μM) or nifedipine (NIF, 100 nM), (n = 4-8). EST shifted the Ca^{2+} concentration-response curve to the right and decreased E_{max} in a manner qualitatively similar to NIF. Thus EST appeared to have effects comparable to calcium channel blockade. In further experiments effects of EST (20 μM) on contractions produced by $PGF_{2\alpha}$ (50μM) in Ca^{2+}-free solution containing 100 μM EDTA were investigated. Rings were incubated for 2 hr prior to application of $PGF_{2\alpha}$ which caused a small contraction (0.9 ± 0.1g) in this solution; EST (20 μM) produced 54.2 ± 3.9% relaxation of contraction (n = 5).

Thus EST relaxes contractions produced by two agents in normal Krebs solution, this relaxation was independent of the endothelium, prostaglandin or nitric oxide release and was not affected by the guanylate cyclase inhibitor methylene blue. Although the relaxant effect of EST appeared to resemble that of NIF, relaxation was obtained in Ca^{2+}-free solution which eliminated possible calcium channel blockade. We concluded that while changes in intracellular calcium mobilisation cannot be discounted, the relaxant effect of EST may involve actions on the plasma membrane or on cellular enzyme activities independent of calcium. A mechanism accounting for such calcium-independent effects, involving inhibition of protein kinase C, has been suggested by Low *et al.* (1994).

Gisclard, V *et al* (1988) *J Pharmacol Exp Therap* 244, 19-22.
Low, AM *et al* (1994) *Br J Pharmacol* 112, 604-610.
Magness, RR, Rosenfeld CR (1989) *Am J Physiol* 256, E536-542.
Shan, J *et al* (1994) *Am J Physiol* 266, H967-973.

153P A TIME COURSE STUDY TO INVESTIGATE PULMONARY EOSINOPHIL ACCUMULATION AND BRONCHIAL RESPONSIVENESS TO HISTAMINE IN OVALBUMIN SENSITISED GUINEA PIGS

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Bronchial hyperresponsiveness (BHR) is a characteristic feature of asthma but the relationship between this and increased numbers of eosinophils in the bronchial mucosa is controversial (Djukanovic *et al.*, 1990; Gibson *et al.*, 1989). We have attempted to relate eosinophil accumulation with BHR (to histamine) in guinea pigs sensitized to ovalbumin (OA).

Male sensitized guinea pigs (Seeds *et al.*, 1991) were exposed to OA (100μgml^{-1} for 1h). Pulmonary inflation pressure (PIP) in response to i.v. histamine (1-50μgkg^{-1}) was measured and bronchoalveolar lavage (BAL) performed (Seeds *et al.*, 1991) at one of five time points following OA challenge (n=4-6 animals per time point). Results are expressed as mean ± s.e.mean and data was analysed by analysis of variance followed by Dunnett's test (*P < 0.05 vs. sham sensitized).

Exposure of OA sensitized animals to aerosolised OA produced a significant increase in eosinophil infiltration 24h later which was sustained at 48 and 72h (Figure 1). Challenge of OA sensitized animals with OA had no significant effect on the dose of histamine required to produce a 100% increase in PIP at any time point (Figure 2). These data show that allergen challenge of sensitized guinea pigs can elicit airway eosinophil accumulation without accompanying hyperresponsiveness.

K.H.Banner is supported by the M.R.C.

Figure 1

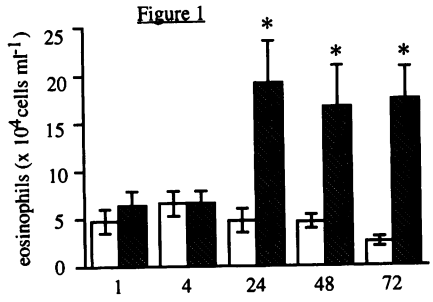
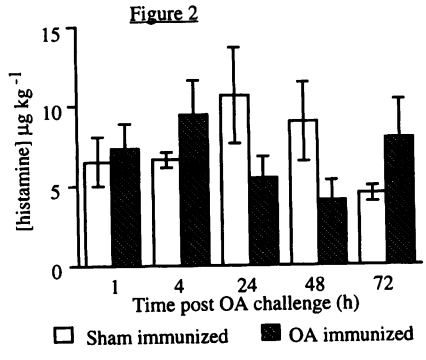


Figure 2



Djukanovic, R., Roche W.R., Wilson J.W. *et al.* (1990) *Am. Rev. Respir. Dis.* 142, 434-457.
Gibson, P.G., Denburg J., Dolovich J. *et al.* (1989) *Lancet* 1346-1348.
Seeds, E.A.M., Coyle, A.J., Page, C.P. (1991) *J. Lipid Med.* 4, 111-122.

154P REVERSAL OF PAF-INDUCED AIRWAY HYPERREACTIVITY BY THE POTASSIUM CHANNEL OPENER RS-91309 IS DISTINCT FROM ITS SPASMOLYTIC ACTIVITY IN THE GUINEA-PIG

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Platelet-activating factor (PAF) induces airway hyperreactivity in both experimental animals (Mazzoni *et al.*, 1985) and man (Cuss *et al.*, 1986). This study sought to investigate the effects of a novel potassium channel opener (KCO), RS-91309 (N-[[2,2-dimethyl-4-(2-oxo-2H-pyridin-1-yl)-6-trifloromethyl-2H-1 -benzopyran-3-yl]methyl]-N-hydroxylacetamide; US patent 5,250,547 Benzopyran derivatives), on PAF-induced airway hyperreactivity in the guinea-pig.

Guinea-pigs (400-600 g) were anaesthetized with phenobarbitone (100 mg kg^{-1} i.p.), pentobarbitone (30 mg kg^{-1} i.p.), paralysed with gallamine (10 mg kg^{-1} i.m.) and ventilated (8 ml kg^{-1}, 1 Hz) via a tracheal cannula. Changes in airway resistance (R_L, cm H_2O l^{-1} sec^{-1}) were monitored (PMS 8000, Mumed Ltd., U.K.; Chapman *et al.*, 1992). RS-91309 (1-300 μg kg^{-1} i.v.) administered prior to (6 min) histamine (range 2.5 μg kg^{-1}-7.5 μg kg^{-1} i.v. giving a 2 fold increase in R_L) dose-dependently and maximally inhibited increased R_L to histamine (ED_{50} 18 μg kg^{-1}) in normoreactive animals. From this dose-effect curve a non-spasmolytic dose of RS-91309 (5 μg kg^{-1} i.v. bolus) was selected for study in hyperreactive animals. Increases in R_L in response to a single dose of histamine (range 2.5 μg kg^{-1}-7.5 μg kg^{-1} i.v.) were measured before and after exposure to PAF (600ng kg^{-1} h^{-1} i.v. infusion). Maximal R_L to histamine was significantly enhanced following infusion of PAF, an effect that was fully reversed by RS-91309 (5μg kg^{-1} i.v. bolus) but not by vehicle (tween 2% w/v, glucose 5% w/w in saline 0.9%) (Table 1).

Table 1. Increased R_L to histamine (mean ± s.e.mean) prior to (A) and following (B) exposure to PAF in guinea-pigs treated with vehicle or RS-91309 (C).

	A	B	C
Vehicle (n=5)	146.3±33.3	406.9±66.8**	381.0±28.2**
RS-91309 (n=6)	224.8±39.0	465.4±48.8**	253.5±33.5##

(** p<0.01 vs A, ## p<0.01 vs B, Student's t test).

The present data demonstrates that RS-91309 reverses PAF-induced airway hyperreactivity to histamine by a mechanism that is unrelated to its spasmolytic activity. These observations concur with previous studies using other KCO's (Chapman *et al.*, 1992). Reversal of airway hyperreactivity by KCO's such as RS-91309 may provide a novel approach to the treatment of pulmonary disease that is characterized by airway hyperreactivity.

Chapman, I.D., Kristersson, A., Mathelin, G. *et al.* (1992) Br. J. Pharmacol. 106, 423-429.
Cuss, F.M., Dixon, C.M.S. & Barnes, P.J. (1986) Lancet. ii, 189-192.
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155P EFFECT OF THEOPHYLLINE ON ANTIGEN-INDUCED AIRWAY RESPONSES IN THE NEONATALLY IMMUNISED RABBIT

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Recent evidence suggests that theophylline may possess anti-inflammatory activity in addition to its ability to relax airway smooth muscle (Sullivan *et al*, 1994).

Two groups of 9 litter-matched NZW rabbits, immunised within 24h of birth and until 12 weeks of age (2.7-3.4kg) with *Alternaria tenuis* antigen (Ag) in Al(OH)3 gel (*i.p.*), were treated twice daily on days 1-3 with theophylline (3mg kg^{-1} *i.p*) or vehicle (5% DMSO in saline); (2ml kg^{-1}). On day 3, the concentration of inhaled aerosolised histamine required to provoke a 50% increase (PC_{50}) in total lung resistance (R_L) and 35% decrease (PC_{35}) in dynamic compliance (C_{dyn}) were determined and bronchoalveolar lavage (BAL) performed. On day 4, rabbits were given a final dose of theophylline or vehicle 1h prior to Ag aerosol (20,000 PNU ml^{-1} in saline for 20 min). On Day 5, PC_{50} and PC_{35} values were determined and BAL performed as on day 3 (Herd *et al*,1994).

Ag-induced acute bronchoconstriction was unnaffected by theophylline pre-treatment (% increase in R_L 30.2±4.6 Vs vehicle 36.2±4.4; % decrease in C_{dyn} 31.1±4.3 Vs vehicle 34.9±2.7).

Table 1. Histamine PC_{50} and PC_{35} values pre and post antigen.

Treatment	R_L PC_{50}(mg ml^{-1})		C_{dyn} PC_{35}(mg ml^{-1})	
	Pre	Post	Pre	Post
vehicle	15.5±1.3	6.5±1.2†	16.5±1.3	6.4±1.1†
theoph	18.7±1.1	10.2±1.3†	17.2±1.1	8.9±1.2†

† P<0.05 Vs Pre Ag value (paired t-test); n=9 each group.

Ag-induced airway hyperresponsiveness was evidenced in both vehicle and theophylline pre-treated rabbits by significantly reduced PC_{50} and PC_{35} values to inhaled histamine 24h post antigen. (Table 1).

Pre-treatment with theophylline did not alter basal lung function. (R_L 39.8±2.1 Vs.vehicle 41.4±1.8 cmH_2O^{-1} L^{-1} sec^{-1} and C_{dyn} 4.2±0.1 Vs vehicle 4.1±0.1 ml cmH_2O^{-1}).

Total leucocytes recovered in BAL 24h post Ag challenge were significantly increased in vehicle-treated rabbits only. Ag-induced increases in eosinophils were significantly reduced (57%) with theophylline pre-treatment (P=0.01). Neutrophils tended to be reduced in theophylline pre-treated rabbits 24h after Ag challenge (Table 2).

Table 2. Total and differential cell counts in BAL (10^4 cells ml^{-1}) pre and post Ag.

Treatment	Total		Mono		Neut		Eosin	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Vehicle	32.6 ±1.7	41.4† ±2.8	31.3 ±1.5	31.0 ±2.5	1.2 ±0.3	5.8† ±1.0	0.1 ±0.1	4.7† ±0.9
theoph	30.5 ±2.0	33.2# ±1.3	29.7 ±2.1	27.5 ±1.6	0.7 ±0.2	3.7† ±0.7	0.1 ±0.1	2.0†# ±0.3

†P<0.05 Vs Pre; #P<0.05 Vs post vehicle (Wilcoxon test), *n*=9 per group.

These data demonstrate that, with this dosing regime, theophylline attenuates Ag-induced pulmonary eosinophilia, whilst having no effect on Ag-induced acute bronchoconstriction or airway hyperresponsiveness in the immunised rabbit.

Herd, C.M. *et al.* (1994) *Br. J. Pharmacol.* 112, 292-298.

Sullivan, P. *et al.* (1994) *Lancet.* 343, 1006-1008.

156P BTS 67 582 LOWERS GLUCOSE IN GLIBENCLAMIDE-RESISTANT STREPTOZOTOCIN DIABETIC RATS

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We have previously shown that BTS 67 582 (1,1-dimethyl-2-[2-(4-morpholinoophenyl)]guanidine monofumarate) is a novel glucose lowering agent in normal animals and does not displace [^{3}H]-glibenclamide in ligand binding studies (Kaul et al., 1995). BTS 67 582 has been further evaluated in a model of streptozotocin-induced diabetes in the rat.

Female Sprague Dawley rats (160-230 g) were injected with streptozotocin (50 mg/kg iv). After 10 to 14 days plasma glucose was determined (Beckman Glucose Analyser 2) in rats starved overnight. Rats were then administered a large dose of glibenclamide (100 mg/kg po, 5 ml/kg) together with a glucose load (1 g/kg po) and plasma glucose measured 3 hours later. Only those animals which demonstrated a fasting plasma glucose greater than 15 mmol/l and in which plasma glucose values were raised at 3 hours compared to the basal values were accepted for further study and were defined as glibenclamide resistant. BTS 67 582 was then evaluated in these glibenclamide resistant animals. Rats were administered a glucose load 30 minutes after dosing with drug or vehicle (0.2% agar). Blood samples were taken and plasma analysed for glucose content and insulin by RIA (Isopharm). Data from several experiments were pooled and analysed by two way analysis of variance.

In vehicle dosed animals, plasma glucose in glucose primed animals increased from 22.6 ± 0.3 to 35.3 ± 0.5 mmol/l 1hr after dosing (n=80). Plasma glucose fell thereafter to 26.1 ± 0.7 mmol/l at 3.5 hr after dosing. BTS 67 582 caused significant reductions in plasma glucose values compared to vehicle dosed animals at all doses tested (William's test p<0.01). Reductions in plasma glucose 1 hr after dosing of 12.6% (n=16), 18.3% (n=31), 16.6% (n=46) and 19.2% (n=34) relative to control were observed at 36.7, 73.4, 146.7 and 293.5 mg/kg respectively. Glibenclamide (100 mg/kg po) caused a small but not significant (p≥0.09, unpaired Students t test) fall of 4.7%, 6.8% and 8.9% in plasma glucose relative to the vehicle dosed group at 1, 2 and 3.5 hr after dosing respectively (n=44).

In a separate study BTS 67 582 (293.5 mg/kg po) significantly raised plasma insulin levels compared to vehicle in glucose loaded rats 1 hr after dosing (10.6 ± 2.3 and 22.5 ± 3.7 µU/ml, for vehicle and BTS 67 582 respectively, n=8, p<0.01). Glibenclamide (100 mg/kg po) also raised plasma insulin levels though this did not reach significance (16.3 ± 2.0 µU/ml, n=7, p>0.05).

BTS 67 582 causes significant reductions in plasma glucose in streptozotocin diabetic rats selected for their insensitivity to glibenclamide. Although an extrapancreatic effect of BTS 67 582 cannot be ruled out, it is likely that its greater insulin releasing activity compared to glibenclamide, underlies its glucose lowering effect in this model.

Kaul, C.L., Marita, A.R., Dickinson, K. & Jones, R.B. (1995) Br. J. Pharmacol., in press.