British Journal of Pharmacology

April 1993

Volume 108 Proceedings Supplement 1P–318P

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1P EFFECT OF NITRIC OXIDE IN AQUEOUS SOLUTION, AND 8-BROMO-GUANOSINE-3':5'-MONOPHOSPHATE, ON CONTRACTION OF ISOLATED GUINEA-PIG CARDIAC VENTRICULAR MYOCYTES

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We have shown previously that the contraction of cardiac myocytes can be attenuated by nitric oxide derived from endothelium (Brady *et al.*, 1992a) and from the nitric oxide-donating drug, sodium nitroprusside (Brady *et al.*, 1992b). The present study was designed to examine whether superfusion of nitric oxide in solution, or the stable analogue of its intracellular second messenger, 8-bromo-guanosine-3':5'-monophosphate (8-bromo-cyclic GMP), would also reduce myocyte contractility.

Guinea-pig cardiac myocytes were isolated as described previously (Harding *et al.*, 1990) and superfused with Krebs-Henseleit buffer, containing 2mM calcium, at 32°C. The contraction amplitude and velocity of shortening of electrically-stimulated (0.5 Hz) myocytes was recorded using a videomicroscopy-length detection system and analysed by computer signal averaging. Myocyte contractility was calculated as the percentage shortening of the resting length.

Solutions of nitric oxide were prepared in HEPES buffered 2mM Ca^{2+} Tyrode's solution which had been bubbled vigorously with nitrogen gas for 4h to remove oxygen. Aliquots were withdrawn and superfused into the cell bath for 7 min. The concentration of nitric oxide in the solutions was confirmed using chemiluminesence. Superfusion with 10^{-6}M nitric oxide solution reduced myocyte contraction by $24\pm9\%$ ($n=5$, $p<0.05$). 10^{-5}M nitric oxide solution reduced contractility by $26\pm9\%$ ($n=5$, $p<0.05$). Nitrogen-gassed control solution had no effect on myocyte contraction, so the effect of the nitric oxide solution was not due to hypoxia. 8-bromo-cyclic GMP also caused a concentration-dependent reduction in myocyte contractility, with a maximal reduction in contraction amplitude of $20\pm6\%$ at 3×10^{-5}M ($n=6$, $p<0.025$).

Cardiac myocyte contractility can be attenuated by nitric oxide in aqueous solution, and by a stable analogue of the intracellular messenger of nitric oxide, cGMP. These findings support our previous work, and provide further evidence for a role of nitric oxide in the modulation of myocardial contraction.

AJBB is a Medical Research Council Clinical Training Fellow.

Brady, A.J.B., Warren, J.B., Poole-Wilson, P.A., Williams, T.J. & Harding, S.E. (1992a) *Br. Heart J.* 68,149.
Brady, A.J.B., Warren, J.B., Poole-Wilson, P.A. & Harding, S.E. (1992b) *Br. J. Pharmacol.* 105,96P.
Harding, S.E., O'Gara, P., Jones, S.M., Brown, L.A., Vescovo,G. & Poole-Wilson, P.A. (1990) *Cardioscience* 1,49-53.

2P INHIBITION OF BASAL BUT NOT ACETYLCHOLINE-STIMULATED PRODUCTION OF NITRIC OXIDE IN RAT AORTA BY N^G-MONOMETHYL L-ARGININE

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The endothelium-derived relaxing factor has been identified as nitric oxide or a related compound and is now recognised to be produced by a family of nitric oxide synthase isoenzymes in a large number of different cell types. In addition to producing nitric oxide in response to agonists such as acetylcholine, the endothelial cells of rat aorta produce nitric oxide spontaneously (Martin et al., 1986). We wished to determine if basal and acetylcholine-stimulated production of nitric oxide were equally sensitive to the blocking actions of N^G-monomethyl L-arginine (L-NMMA) and N^G-nitro L-arginine (L-NOARG), two inhibitors of nitric oxide synthase.

Rat aortic rings (2.5 mm long) were suspended in tissue baths containing Krebs solution at 37 °C for isometric tension recording. Endothelial cells were removed from some rings by gentle abrasion. Basal production of nitric oxide was determined indirectly by assessing endothelium-dependent depression of phenylephrine (PE)-induced tone and agonist-stimulated production was determined by assessing the relaxation induced by acetylcholine (0.01-3 μM).

Endothelium-containing rings of rat aorta were less sensitive to the contractile actions of PE than endothelium-denuded rings: the threshold concentration and maximum response were 10 nM and 0.84 ± 0.07 g for endothelium-containing rings, respectively, but were 1 nM and 1.42 ± 0.12 g, respectively, for endothelium-denuded rings. Prior treatment with L-NMMA (0.1-1 mM) or L-NOARG (0.01-1 mM) enhanced the sensitivity of endothelium-containing but not endothelium-denuded rings to PE (0.1 nM-100 μM). The ability of L-NMMA and L-NOARG to enhance the sensitivity of endothelium-containing rings to PE was prevented by pretreatment with L-arginine (1-10 mM). Following induction of sub-maximal tone with PE, treatment with L-NOARG (0.01-0.1 mM) inhibited acetylcholine (0.01-3 μM)-induced relaxation by a maximum of 54 ± 5%. In contrast, treatment with L-NMMA (0.1-3 mM) had no effect on acetylcholine-induced relaxation. Furthermore, prior treatment with either L-NMMA (0.1-1 mM) or L-arginine (0.1-10 mM) inhibited the ability of L-NOARG to block acetylcholine-induced relaxation.

The ability of L-NOARG to enhance sensitivity to PE and block acetylcholine-induced relaxation in endothelium-containing rings is consistent with inhibition of basal and stimulated production of nitric oxide. The ability of L-NMMA to enhance sensitivity to PE in endothelium-containing rings and its inability to affect acetylcholine-induced relaxation suggest a selective blockade of basal but not stimulated production of nitric oxide. The additional ability of L-NMMA to act like L-arginine and protect acetylcholine-induced relaxation from blockade by L-NOARG suggests a complex interaction with the nitric oxide synthase responsible for agonist-stimulated production of nitric oxide. It is likely that the nitric oxide synthases responsible for basal and stimulated production of nitric oxide in rat aorta are different.

This work was supported by the British Heart Foundation and the Medical Research Funds of the University of Glasgow.

Martin, W., Furchgott, R.F., Villani, G.M. & Jothianandan. D. (1986). J. Pharmacol. Exp. Ther. **237,** 529-538.

3P ACETYLCHOLINE-INDUCED RELAXATION IN THE RAT ISOLATED MESENTERIC ARTERY: RELATIVE IMPORTANCE OF SMOOTH MUSCLE HYPERPOLARIZATION

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Cholinomimetics can stimulate endothelium-dependent smooth muscle relaxation and hyperpolarization in isolated arteries. Smooth muscle hyperpolarization appears to reflect the release of a factor distinct from EDRF (nitric oxide), which has been termed endothelium-derived hyperpolarizing factor (EDHF; Taylor & Weston, 1988). In the rat small mesenteric artery, both the hyperpolarization and relaxation which are induced in response to acetylcholine persist in the presence of the nitric oxide synthase inhibitor nitro-arginine, suggesting a major contribution from EDHF rather than EDRF in this artery (Garland & McPherson, 1992). However, an alternative explanation may be that the apparent resistance to nitro-arginine may be a function of the efficacy with which acetylcholine liberates nitric oxide (Martin *et al.*, 1992)

In the present study, we have investigated the possibility that both EDHF and nitric oxide contribute to smooth muscle relaxation in the rat small mesenteric artery. Segments of mesenteric artery were mounted in a myograph under a normalized tension (Mulvany & Halpern, 1977) and changes in tension and membrane potential recorded simultaneously. The diameters at 100 mmHg varied between 286-360μm. In the presence of noradrenaline (0.75-3μM), to contract and depolarize the smooth muscle cells, acetylcholine stimulated a pronounced increase in membrane potential, from around - 46mV to close to - 70mV. These changes in membrane potential were associated with 93% smooth muscle relaxation. In the presence of similar levels of contraction and depolarization with noradrenaline in the presence of 25mM KCl, the membrane potential changes to acetylcholine were almost totally abolished. At this time, acetylcholine still induced relaxation, but this was now significantly reduced to 45 $\pm$ 6% (mean $\pm$ s.e.mean, n=4) of the initial contraction. The relaxation obtained in the absence of a change in membrane potential was completely blocked in the presence of 100μM L-N^G-nitroarginine methyl ester (L-NAME). Smooth muscle relaxation in response to nitric oxide gas in solution (0.22 - 2.2μM) was not different in segments contracted with either noradrenaline or noradrenaline and 25mM KCl.

These data show that block of membrane hyperpolarization is associated with a marked reduction in the amount of smooth muscle relaxation which is induced by acetylcholine, and that under these conditions the relaxation is sensitive to an inhibitor of nitric oxide synthase. The results suggest that, in the rat small mesenteric artery, both EDHF and EDRF can significantly contribute to the endothelium-dependent relaxation which is induced in response to acetylcholine.

This work was supported by the Wellcome Trust.

Garland, C.J. & McPherson, G.A. (1992). Br. J. Pharmacol., 105, 429-435.
Martin, G.R., Bolofo, M.L. & Giles, H. (1992). Br. J. Pharmacol., 105, 643-652.
Mulvany, M.J. & Halpern, W. (1977). Circ. Res., 41, 19-26.
Taylor, S.G & Weston, A.H. (1988). Trends. Pharmacol. Sci., 9, 272-274.

4P REGIONAL HAEMODYNAMIC RESPONSES TO N^G,N^G-DIMETHYL-L-ARGININE (ADMA) OR N^G-MONOMETHYL-L-ARGININE (L-NMMA) IN CONSCIOUS RATS

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Recently, Vallance *et al.* (1992) reported that ADMA and L-NMMA were present in human plasma and that ADMA, like L-NMMA, had pressor effects in anaesthetised guinea pigs. Previously, we have shown that the pressor effects of L-NMMA in conscious rats were associated with regional vasoconstrictions, particularly in the mesenteric vascular bed (Gardiner *et al.*, 1990). In the present work we compared the regional haemodynamic effects of ADMA and L-NMMA in the same conscious, male, Long Evans rats (n = 8), chronically instrumented with renal, mesenteric and hindquarters pulsed Doppler flow probes and intravascular catheters. (All surgery was carried out under sodium methohexitone anaesthesia, 40-60 mg kg^{-1} i.p.). On separate experimental days, animals were randomised to receive incremental i.v. bolus doses (1-100 mg kg^{-1}) of ADMA or L-NMMA. The results, summarised in Table 1, show no differences between the effects of ADMA and L-NMMA (Wilcoxon's test).

Table 1: Cardiovascular changes (maxima) following i.v. bolus injections of ADMA or L-NMMA in the same, conscious, Long Evans rats. Values are mean $\pm$ s. e. mean (n = 8).

	1 mg kg^{-1}		10 mg kg^{-1}		100 mg kg^{-1}	
	ADMA	L-NMMA	ADMA	L-NMMA	ADMA	L-NMMA
Heart rate (beats min^{-1})	2 ± 3	2 ± 5	-24 ± 4*	-34 ± 6*	-91 ± 6*	-68 ± 7*
Mean blood pressure (mmHg)	2 ± 1	4 ± 1	10 ± 1*	17 ± 2*	42 ± 2*	46 ± 2*
Renal flow (%)	0 ± 1	-2 ± 1	-6 ± 2*	-8 ± 2*	-25 ± 4*	-22 ± 4*
Mesenteric flow (%)	-7 ± 1*	-13 ± 3*	-20 ± 2*	-26 ± 3*	-46 ± 2*	-43 ± 4*
Hindquarters flow (%)	4 ± 2	6 ± 5	-7 ± 1*	-11 ± 5	-31 ± 3*	-28 ± 5*
Renal conductance (%)	-2 ± 1	-5 ± 1*	-14 ± 2*	-21 ± 3*	-46 ± 3*	-44 ± 3*
Mesenteric conductance (%)	-9 ± 1*	-16 ± 3*	-27 ± 2*	-37 ± 3*	-62 ± 2*	-61 ± 3*
Hindquarters conductance (%)	1 ± 2	3 ± 5	-15 ± 1*	-25 ± 5*	-47 ± 2*	-48 ± 4*

* P<0.05 for change (Friedman's test).

Our findings corroborate those of Vallance *et al.* (1992), and additionally indicate that, in the presence of pathologically elevated levels of endogenous ADMA, regional blood flow, particularly that through the superior mesenteric vascular bed, might be compromised.

Gardiner, S. M., Compton, A. M., Bennett, T., Palmer, R. M. J. & Moncada, S. (1990). Hypertension, **15**, 486-492.
Vallance, P., Leone, A., Calver, A., Collier, J. & Moncada, S. (1992). Lancet, **339**, 572-575.

5P S-NITROSO-N-ACETYL-PENICILLAMINE (SNAP) ABOLISHES THE PRESSOR, BUT NOT THE VASOCONSTRICTOR, EFFECTS OF N^G,N^G-DIMETHYL-L-ARGININE (ADMA) IN CONSCIOUS RATS

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ADMA causes hypertension in association with regional vasoconstrictions in conscious rats (Gardiner *et al.*, this meeting), consistent with it being an inhibitor of nitric oxide (NO) synthase (Vallance *et al.*, 1992). We have assessed the ability of the NO donor, SNAP (Ignarro *et al.*, 1981), to reverse the haemodynamic effects of ADMA. Male, Long Evans rats were chronically instrumented with pulsed Doppler probes (to monitor changes in renal, mesenteric and hindquarters flows) and intravascular catheters. All surgery was carried out under methohexitone anaesthesia (40-60 mg kg^{-1} i.p.). On separate days animals (n = 8) received an i.v. bolus injection of ADMA (100 mg kg^{-1}) followed 5 min later by a 10 min infusion of saline, or ADMA followed 5 min later by a 10 min infusion of SNAP (300 µg kg^{-1} h^{-1}). In the presence of SNAP the pressor and bradycardic effects of ADMA were abolished, but its vasoconstrictor effects were not (Table 1).

Table 1: Cardiovascular changes in response to ADMA followed by saline or SNAP in conscious, Long Evans rats (n = 8). Values are mean ± s. e. mean.

	5 min		10 min	
	ADMA + Saline	**ADMA + SNAP**	**ADMA + Saline**	**ADMA + SNAP**
Heart rate (beats min^{-1})	-92 ± 9*	-8 ± 13†	-74 ± 5*	-7 ± 9†
Mean blood pressure (mmHg)	32 ± 2*	8 ± 3†	29 ± 2*	5 ± 2†
Renal flow (%)	-25 ± 4*	-27 ± 2*	-23 ± 4*	-25 ± 3*
Mesenteric flow (%)	-38 ± 4*	-31 ± 3*	-33 ± 5*	-25 ± 4*
Hindquarters flow (%)	-31 ± 3*	-31 ± 3*	-22 ± 3*	-27 ± 4*
Renal conductance (%)	-43 ± 3*	-32 ± 2*†	-40 ± 3*	-28 ± 3*†
Mesenteric conductance (%)	-53 ± 2*	-35 ± 4*†	-48 ± 4*	-28 ± 4*†
Hindquarters conductance (%)	-47 ± 2*	-36 ± 3*†	-39 ± 2*	-30 ± 5*†

* $P<0.05$ versus baseline (Friedman's test); † $P<0.05$ versus ADMA + saline (Wilcoxon's test).

The results indicate that abolition, by SNAP, of the pressor effects of ADMA is only partly due to reversal of its regional vasoconstrictor actions. Thus, it is likely that the major component of the antihypertensive effect of SNAP in the presence of ADMA is due to a reduction in cardiac output.

Ignarro, L. J., Lippton, H., Edwards, J. C., Barricos, W. H., Hyman, A. L. *et al.* (1981). J. Pharmacol. Exp. Ther., **218**, 739-749.
Vallance, P., Leone, A., Calver, A., Collier, J. & Moncada, S. (1990). Lancet, **339**, 572-575.

6P IMPAIRED ENDOTHELIUM-MEDIATED VASODILATATION IN AORTA OF RATS WITH VITAMIN E DEFICIENCY

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The major recognised function of vitamin E is to interrupt the free-radical chain process of lipid peroxidation. Lipid peroxidation could be a cause of endothelial cell damage and altered function in diseases in which increased lipid peroxidation has been observed. Therefore the aim of this study was to investigate the effect of vitamin E deficiency on vascular function in aortic rings from rats deprived of dietary vitamin E for period of 6 and 10 months. The animal model of vitamin E deficiency has been described in detail (Goss-Sampson et al., 1990). Briefly, Wistar rats (post-weanlings, 21-23 days old) were maintained on a vitamin E-deficient diet. Control animals were fed the same diet with the addition of α-tocopherol acetate (40 mg\Kg diet). The animals were killed by an overdose of sodium pentobarbitone and preparations isolated and set up as previously described (Rubino et al., 1992). Vascular responses were detected as changes in tension (mg) and vasodilatation was evaluated as % of noradrenaline-sustained tone. Endothelium-dependent vasodilator responses to acetylcholine (0.01-3 µM) and calcium ionophore A23187 (1-300 nM) were significantly reduced in rings from both groups of treated animals, compared to their respective controls. The maximum relaxation obtained with 3 µM acetylcholine was 79.6±1.8 % (n=9) and 36.1±12 % (n=6) in 6 months control and vitamin E-deficient rats, respectively. Similar results were obtained in 10 months control and treated animals. A23187 induced a concentration-dependent vasodilator response up to 76.6±4.4 % (n=7) in 10 months control rats and up to 25±8.3 % (n=7) in rings from vitamin E-deficient animals. The concentration-dependent vasodilatation by ATP (1-30 µM) was reduced in rings from 6 months treated rats, although a statistically significant difference between control and vitamin E-deficient rats was not reached. In contrast, endothelium-independent vasodilatation induced by increasing concentrations of sodium nitroprusside (SNP, 1-300 nM) appeared enhanced in rings from 6 months treated rats. The vasodilator responses to 3 and 10 nM SNP were significantly different between control and vitamin E-deficient rats, being respectively 20.1±4 and 45.3±5.5 in control rings (n=9) and 32.7±3.3 and 61.3±2.1 in rings from treated animals (n=7). In aortic rings from 10 months treated animals and their age-matched control vasodilator responses to SNP were not statistically different. Moreover, no difference was detected in the contractile response to increasing concentrations of noradrenaline (1-300 nM) between preparations from 6 and 10 months-treated rats and their respective controls.

In summary, in aortic rings from rats kept on a vitamin E-deficient diet for 6 and 10 months the endothelium-dependent vasodilator responses were dramatically reduced, whereas the ability of vascular smooth muscle to contract in response to noradrenaline or to relax in response to SNP was unaffected. These results indicate that vitamin E deficiency impairs the function of endothelial cells in rat aorta.

Goss-Sampsonn, M.A., Kriss, A. & Muller D.P.R. (1990). J. Neurolog. Sci. 100, 79-84.
Rubino, A., Thomann H., Henlin, J., Shilling, W. & Criscione L. (1992). Eur. J. Pharmacol. 212, 237-240.

7P NITRIC OXIDE SYNTHESIS INHIBITION AND ARTERIOVENOUS ANASTOMOSES SHUNTING IN THE PIG

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Recently, we reported that inhibition of nitric oxide (NO) synthesis by N^G-nitro-L-arginine methyl ester (L-NAME) decreased shunting through arteriovenous anastomoses (AVAs) in anaesthetized pigs (Van Gelderen et al., 1992). However, we could not exclude that this decrease was due to a reduction of cardiac output. Therefore, we studied the distribution of the carotid blood flow after intracarotid injection of L-NAME, while monitoring systemic haemodynamics. The distribution was measured by injecting radioactive microspheres in the common carotid artery. These spheres are trapped in the capillaries, but may escape through AVAs to be ultimately sieved in the lungs. Hence, blood flow to the lungs reflects AVA flow.

Two groups of anaesthetized pigs, instrumented to measure haemodynamic parameters, including carotid blood flow and its distribution, were treated i.a. with either L-NAME (0.1, 0.3 and 1.0 mg kg^{-1}; n=6) or saline (three times; n=5), followed by L-arginine (L-ARG; 100 mg kg^{-1}) in both groups.

Table 1. The effect of L-NAME and L-ARG (mg kg^{-1}) on total carotid, AVA and capillary conductance (ml.min^{-1}mm Hg^{-1}x 100). *, denotes significance vs. baseline (BL).

	L-NAME			L-ARG		Saline				L-ARG
	BL	0.1	0.3	1.0	100	BL	1	2	3	100
Total carotid	118±16	104±14	81±9*	66±5*	106±8	130±11	136±9	144±12	141±9	147±10
AVA	82±16	72±15	42±9*	29±5*	54±8*	86±4	87±7	93±4	86±7	81±5
Capillary	36±4	33±4	39±4	37±3	52±5*	44±11	48±11	51±15	55±10	66±9*

In both groups, no significant changes in systemic haemodynamics were observed; the maximum changes by L-NAME and saline were: 9.5±7% and -7±3%, respectively in mean arterial blood pressure and -20±3% and -12±4%, respectively in cardiac output. Saline treatment did not change carotid haemodynamics. However, as depicted in table 1 L-NAME reduced both total carotid and AVA conductance without changing the capillary conductance. Simultaneously, the percent difference in arteriovenous oxygen saturation increased by 92±33% ($p<.05$), whereas jugular venous NO_2-levels decreased from baseline (2.8±0.8 nmol ml^{-1}; n=6) by 34±19% ($p<.05$). The reduction in carotid conductance was fully reversed by L-ARG, but AVA conductance was only partially reversed (50±6%). L-ARG induced vasodilation as reflected by an increased capillary conductance in both L-NAME treated (50±18%; $p<.05$) and saline treated (66±18%; $p<.05$) animals. In conclusion, our results indicate that NO dilates arteriovenous anastomoses in anaesthetized pigs independently of systemic haemodynamic changes.

Van Gelderen, E.M., Den Boer M.O. & Saxena, P.R., 1992, J. Vasc. Res., **29**, 218.

8P ENDOTOXIN-INDUCED ENDOTHELIAL BARRIER DYSFUNCTION ASSESSED BY AN *IN VITRO* MODEL

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In septic shock, endotoxin (lipopolysaccharide, LPS) causes severe circulatory failure characterised by profound hypotension and vascular leakage. It is now accepted that the hypotension is mediated by massive production of nitric oxide following induction of nitric oxide synthase in the vascular wall, but the factors responsible for the associated vascular leakage are unclear. The object of this study was to determine if LPS can induce endothelial barrier dysfunction and if so investigate involvement of the L-arginine-nitric oxide system.

Endothelial barrier function was assessed using an *in vitro* model (Gudgeon & Martin, 1989) by measuring, over a 90 min incubation period, the transfer of trypan blue-labelled albumin (4%) across monolayers of bovine aortic endothelial cells (BAEC) grown on Costar Transwell membranes. Transfer was expressed as a percentage of that which would have been achieved at equilibrium.

Unstimulated transfer of albumin across BAEC was 6.9±0.8 % (n=30). Following 24 hour exposure, LPS (3-1000 ng ml^{-1}) produced a concentration-dependent increase in albumin transfer across BAEC which reached a maximum of 39.9±2.6 % (n=6). The increase in albumin transfer stimulated by LPS (30 ng ml^{-1}) was biphasic: an early transient peak of 36.7±3.9 % (n=6) occurred at 2 hours which then declined to basal levels and thereafter rose again to a second peak of 30.1±3.3 % (n=6) at 24 hours. Co-incubation with polymixin B (10 μg ml^{-1}) abolished the increase in albumin transfer stimulated by exposure to LPS (30 ng ml^{-1}) for 2 and 24 hours. The increase in albumin transfer at 2 and 24 hours was, however, unaffected by co-incubation with N^G-nitro L-arginine methyl ester (L-NAME, 500 μM) or N^G-monomethyl-L-arginine (L-NMMA, 2 mM), two inhibitors of nitric oxide synthase. Pre-treatment for up to 20 hours with dexamethasone (1 μM), an inhibitor of induction of nitric oxide synthase, was also without effect on the increase in albumin transfer stimulated by exposure to LPS (30 ng ml^{-1}) for 2 and 24 hours.

The data show that LPS can induce endothelial barrier dysfunction *in vitro* and this may account for the vascular leakage associated with septic shock. The inability of dexamethasone, L-NMMA and L-NAME to inhibit the increase in albumin transfer stimulated by LPS argues against involvement of the L-arginine-nitric oxide system.

This work was supported by the British Heart Foundation and the S.E.R.C.

Gudgeon, J.R. & Martin, W. (1989) *Br. J. Pharmacol.*, **98**, 1267-1274.

9P NIFEDIPINE INHIBITS THE INDUCTION OF NITRIC OXIDE SYNTHASE BY BACTERIAL LIPOPOLYSACCHARIDE *IN VITRO* AND *IN VIVO*

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Calcium channel antagonists including nifedipine have protective effects in circulatory shock. In endotoxin shock there is an induction of nitric oxide synthase (NOS) resulting in an enhanced formation of nitric oxide (NO) which contributes to the hypotension and vascular hyporeactivity to vasoconstrictors. Here we have investigated whether nifedipine influences NOS induction in endotoxin (lipopolysaccharide, LPS) stimulated cultured J 774.2 macrophages and rat aortic smooth muscle cells (RASMC) and in endotoxin shock.

J 774.2 macrophages or RASMC were cultured in 96-well plates to 80-90% confluence. Cells were incubated with LPS for 24 h (1 μg/ml and 50 μg/ml for J774.2 and RASMC, respectively). Nitrite accumulation was measured by the Griess reaction using a 96 well plate reader (Gross et al, 1991). Cell viability was measured using the MTT assay (Gross et al, 1991). Mean arterial blood pressure (MAP) and heart rate were measured via a carotid arterial cannula in rats anaesthetized with sodium thiopental (120 mg/kg i.p.). LPS (10 mg/kg; i.v. bolus) was administered in vehicle-treated control rats (n=8) and in rats pretreated with nifedipine (20 μg/kg 20 min prior to LPS). At 60 and 180 min after LPS, pressor responses to noradrenaline (NA, 1 μg/kg i.v.) were obtained. Calcium-dependent (constitutive) and -independent (induced) NOS activity was measured by the conversion of ^{3}H-arginine to ^{3}H-citrulline in lung homogenates (Mitchell et al, 1991).

Nitrite production amounted to 4.94±0.96 and 1.32±0.34 pmol/ml/24 h in LPS-stimulated J774.2 macrophages and RASMC, respectively (n=3). Nifedipine (10^{-7} - 10^{-5} M) inhibited the accumulation of nitrite in J774.2 macrophages stimulated with LPS, without affecting cell viability. For example, at 10^{-6} M, nifedipine inhibited nitrite production by 60±6% (n=3). In cells in which NO synthesis was already induced by LPS for 24 h and further induction was blocked by cycloheximide (1 μg/ml), nifedipine did not inhibit nitrite accumulation in the subsequent 24 h. Nifedipine also inhibited nitrite production in RASMC stimulated with LPS.

LPS caused a fall in MAP (from 122±4 to 86±7 and 61±7 mmHg, at 60 and 180 min, respectively, $p<0.01$; n=8). Pressor responses to NA were significantly reduced (to 59±6% and 54±7%, at 60 and 180 min, respectively, $p<0.01$; n=8). At 180 min there was a significant elevation of the induced NOS activity in the lung (152±6 pmol/mg/20 min, n=5). Animals pretreated with nifedipine maintained a higher blood pressure at 180 min (86±8 mmHg, n=6, $p<0.05$ when compared to control), and exhibited more pronounced pressor responses to NA (84±8%, n=6; $p<0.05$ when compared to control). The induced NOS activity was less in the lungs (115±12 pmol/mg/20 min, n=6; $p<0.05$ when compared to control). Nifedipine (10^{-8} - 10^{-5} M) did not affect induced NOS activity, when added <u>in vitro</u> to the lung homogenates. Thus, nifedipine inhibits NOS induction, and this action may contribute to the protective effect of calcium channel antagonists in shock. (This work was supported by a grant of Glaxo Group Research Ltd.)

Gross, S.S., Jaffe E.A., Levi R. & Kilbourn R.G. (1991) Biochem. Biophys. Res. Comm. 187, 823
Mitchell J.A., Sheng H., Forstermann U. & Murad F. (1991) Br. J. Pharmacol. 104, 289

10P MODULATION OF ADJUVANT ARTHRITIS BY THE L-ARGININE:NO PATHWAY

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We have investigated the possible role of the L-arginine:NO pathway in adjuvant arthritis using L-arginine and N^{G}-nitro-L-arginine methyl ester (L-NAME). Adjuvant arthritis was induced in Lewis rats (160-180 g) by intradermal injection of 0.3 mg heat-killed M. tuberculosis in mineral oil into the right hind paw. Test compounds were dissolved in the drinking water (L-arginine: 30 mg ml^{-1}; L-NAME: 1 mg ml^{-1}). Paw volumes were measured each week using a plethysmometer. Rats drinking L-arginine showed an exacerbation of the arthropathy thoughout the time course of the development of the arthritis (35 days) while in animals receiving L-NAME the process was greatly suppressed. The combined treatment of L-arginine (30 mg ml^{-1}) with L-NAME (1 mg ml^{-1}) did not modify the arthritis. Animals in which the lesions were enhanced by L-arginine gained less weight than the controls and those treated with L-NAME gained significantly more. The results (mean ± s.e.m.; n=8) on day 21 (peak response) are shown in the table where * represents $p < 0.05$ versus control (Student's t-test).

<u>Foot volume increase (secondary lesion) and body weight gain 21 days after adjuvant challenge</u>

	Control	L-Arg	L-NAME	L-Arg + L-NAME
Foot volume increase (ml)	0.52 ± 0.03	0.75 ± 0.04*	0.34 ± 0.02*	0.57 ± 0.05
Weight gain (g)	51 ± 3	40 ± 3*	63 ± 4*	55 ± 3

The proliferation of T lymphocytes collected from the rat spleen on day 21 was also investigated. T lymphocytes were challenged with M. tuberculosis (5 μg ml^{-1}) and then incubated with ^{3}H-thymidine (Gillis et al., 1978). The average incorporation of ^{3}H-thymidine (cpm per 10^{5} cells) by T lymphocytes from control rats (36,523 ± 904, n=3) was greatly increased in T cells from the L-arginine-treated group (47,320 ± 1120, n=3, $P < 0.05$) and markedly reduced in the L-NAME-treated group (25,176 ± 672, n=3, $P < 0.05$).

D-arginine (30 mg ml^{-1}), N^{G}-nitro-D-arginine methyl ester (D-NAME; 1 mg ml^{-1}) and L-lysine, an amino acid not involved in the generation of NO (30 mg ml^{-1}), were without effect on the development of arthritis and body weight gain (data not shown). These results suggest that endogenous NO modulates adjuvant arthritis, possibly by regulating T lymphocyte activation, which is known to play a major role in the pathogenesis of this condition (Rosenthale and Capetola, 1982).

Gillis, S., Ferm, M.M., Ou, W. and Smith, K.A. (1978). J. Immunol., 120, 2027-2032.
Rosenthale, M.E. and Capetola, R.J. (1982). Fed. Proc. 41, 2577-2582.

11P MURINE LEUKOCYTES LOSE LIPOCORTIN 1 BINDING CAPACITY DURING ACUTE INFLAMMATION

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Specific and saturable binding sites for lipocortin 1 (LC1) have recently been described on human circulating leukocytes (1). Given that LC1 inhibits the neutrophil migration induced by interleukin-1β (IL-1β) in a murine model (2), this study has focused on the LC1 binding capacity of murine leukocytes.

Male T.O. mice (30-32 g, Tuck, U.K.) were bled by cardiac puncture and a population of mixed leukocytes was prepared by dextran sedimentation and density gradient centrifugation. 0.5-1.0 x10^6 cells per well were seeded in 96-well plates (Falcon Primaria®, Becton Dickinson, U.K.) and incubated with or without human recombinant LC1 (0.001-1.0 μM) for 60 min at 4°C. After washing, cells were stained with the murine anti-LC1 mAb 1B (45 min at 4°C) followed by a FITC-coniugated anti-mouse IgG (Sigma, U.K.) for 30 min at 4°. Flow cytometric analysis was performed and the number of binding sites occupied per cell was calculated using standard fluorescence beads (Becton Dickinson). LC1 binding to cells obtained from a murine air-pouch in response to IL-1β (2) or from zymosan-induced murine peritonitis (3) was assessed.

Circulating murine monocytes and neutrophils bound LC1 with equivalent capacity (binding sites occupied at 1 μM, mean ± s.e.m. of 4 separate experiments, 69,590 ± 28,520 and 46,013 ± 29,978, respectively, with an estimated Kd of 9 nM and 12 nM). Negligible binding to lymphocytes was observed (< 1,000 binding sites per cell). Resident peritoneal macrophages also exhibited saturable LC1 binding: 68,161 ± 20,507 binding sites per cell at 1 μM LC1, n=7; Kd≈ 14 nM). When neutrophils were harvested from the inflammatory site (air-pouch or zymosan peritonitis) no binding was observed (<1,000 sites) even at 5 μM LC1. A loss of binding capacity was also observed on peritoneal macrophages taken 2h following treatment with zymosan (92% reduction of 0.33 μM LC1 binding, n=3).

In conclusion, murine peripheral leukocytes are able to bind LC1. However, cells harvested from inflammatory sites show dramatically reduced binding. These observations parallel the reduced LC1 binding to peripheral leukocytes taken from rheumatoid arthritis patients (1). Further studies are necessary to relate these changes to alterations in cell function.

The financial support of Ono Pharmaceutical Co. (Japan) is acknowledged. RJF is funded by an endowment from Lilly U.K. NJG is supported by the Arthritis and Rheumatism Council (U.K.).

(1) Goulding, N.J., Pan L.Y., Jefferis C.M., Rigby W. and Guyre P.M. (1992) Arthritis Rheum. **35**, 1395-1397.
(2) Perretti, M. and Flower R.J. (1993) J. Immunol. (in press).
(3) Perretti, M., Solito E. and Parente L. (1992) Agents Actions **35**, 71-78.

12P LOCALLY-INJECTED E-TYPE PROSTAGLANDINS ENHANCE OEDEMA FORMATION AND NEUTROPHIL ACCUMULATION BUT SUPPRESS EOSINOPHIL ACCUMULATION IN GUINEA-PIG SKIN

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Prostaglandins interact with other mediators to potentiate the inflammatory response (Williams & Morley, 1973). In rabbit skin, prostaglandins (PGE_1, PGE_2, PGI_2) potentiate local oedema formation and neutrophil infiltration induced by injection of permeability increasing agents and neutrophil chemoattractants (Wedmore & Williams 1981; Issekutz, 1981); effects that have been attributed to vasodilator activity (Wedmore and Williams, 1981). In this study, we have investigated the effects of locally injected prostaglandins on the eosinophil accumulation and oedema formation induced by different mediators in guinea pig skin.

The accumulation of ^{111}In-labelled eosinophils and ^{125}I-albumin leakage in response to intradermal injection (i.d.) of inflammatory agents or in passive cutaneous anaphylaxis (PCA) reactions were measured in the guinea pig skin as previously described (Faccioli et *al*, 1991). Eosinophil accumulation was expressed as ^{111}In-labelled eosinophils per skin site and oedema as μl of plasma per skin site. The accumulation of ^{111}In-neutrophils was measured in comparison.

Results showed that i.d. injection of zymozan-activated plasma (ZAP, 10% dilution in saline), a source of C5a des-arg, caused a local accumulation of eosinophils that was inhibited in the presence of PGE_1 ($3x10^{-10}$moles/site) (2433 ± 328 without PGE_1 and 1466 ± 156 with PGE_1, mean ± sem, n=9). In contrast, in the same skin sites, oedema formation induced by ZAP was potentiated (29.2 ± 3.5μl without PGE_1, 38.9 ± 4.3μl with PGE_1). A range of different doses of PGE_1 was able to potentiate oedema formation (from 10^{-11} to $3x10^{-9}$moles/site) and inhibit eosinophil accumulation (from $3x10^{-11}$ to $3x10^{-9}$moles/site) in response to ZAP. In contrast to the effects of PGE_1 on eosinophil accumulation, ^{111}In-neutrophil accumulation in response to ZAP was significantly increased by this prostaglandin. The inflammatory response induced by Compound 48/80 (3 to 30μg/site), PAF (10^{10} to 10^{-9} moles/site) and the PCA reaction (0.01 to 1.0 μg of antigen/site) was also modulated in a similar manner by local PGE_1, i.e., oedema formation was potentiated but eosinophil accumulation was inhibited. Prostaglandin E_2 ($3x10^{-10}$moles/site) also inhibited ZAP-induced eosinophil accumulation but did not alter PCA-induced responses (1.0 μg of antigen/site). Iloprost (10^{-9}moles/site) potentiated oedema formation in response to ZAP or bradykinin (10^{-10}moles/site) but it did not alter ZAP-induced eosinophil accumulation. Isoprenaline (10^{-8} to 10^{-9} moles/site) inhibited eosinophil influx induced by ZAP, PCA (1.0 μg of antigen/site) and PAF (10^{-9} moles/site) but had little effect on oedema formation.

Inhibitory effects of prostaglandins on leukocyte function have been previously demonstrated *in vitro* (Khuel et *al*, 1987). In this report, we show that prostaglandins of the E series are capable of inhibiting the influx of ^{111}In-labelled eosinophils, but not ^{111}In-neutrophils, whilst potentiating oedema formation induced by different mediators. Since one of the mechanisms by which prostaglandins are thought to regulate cell function is through a G-protein linked receptor which leads to an elevation of intracellular cAMP, this elevation may be relevant for the *in vivo* inhibition of eosinophil migration. The data obtained using isoprenaline supports this view, since the ß receptor is G-protein-linked and its activation leads to an increase in intracellular cAMP. The precise cellular target for PGE or isoprenaline is presently unknown.

We are grateful to the National Asthma Campaign and Sandoz, Basel for support.

Wedmore, C.V. & Williams, T.J. (1981) *Nature* 289, 646-650.
Issekutz, A.C. (1981) *Laboratory Investigation* 45(3), 234-240.
Faccioli L.H., Nourshargh, S, Moqbel, R. et al *Immunology* 73, 157-163.
Khuel, F.A., Zanetti, M.E., Soderman, D.D. et *al Am. Rev. Resp. Dis.* 136, 210-217.
Williams, T.J. & Morley, J. (1973) *Nature* 246, 215-217.

13P INHIBITORY EFFECTS OF THE PAF RECEPTOR ANTAGONIST WEB 2086 ON ANTIGEN-INDUCED RESPONSES IN EQUINE ALLERGIC SKIN DISEASE

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Sweet itch is a pruritic skin disease of horses hypersensitive to biting flies. Intradermal injection of PAF or antigen causes wheal formation and the accumulation of neutrophils and eosinophils in the skin of sweet itch cases (Foster et al., 1992). Similar findings have been obtained in human atopic patients (Henocq & Vargaftig, 1988). Studies with PAF receptor antagonists have suggested that PAF may play a role in wheal formation during the late cutaneous response to antigen in human atopic subjects (Roberts et al., 1988) and in plasma protein extravasation associated with allergic inflammation in the skin of rabbits (Rossi et al., 1992). In the present study the effects of i.v. administration of a PAF receptor antagonist (WEB 2086) on the vascular and cellular responses to antigen and PAF have been examined in the skin of sweet itch horses.

Wheal volumes were calculated from measurements of wheal diameter and skinfold thickness taken 30 min after i.d. injection of PAF (0.1 μg/site) or 1 and 8 h after injection of a *Culicoides* antigen extract (0.5 μg/site) in 6 allergic horses. Biopsies were also obtained 2 h after injection of PAF (1 μg/site) and antigen (5 μg/site) for quantification of inflammatory cell numbers in the dermis. One h after i.v. infusion of WEB 2086 (3 $mgkg^{-1}$), further injections of PAF and antigen were administered, and the effects of the antagonist on wheal formation and cell accumulation established.

Antigen-induced wheals were significantly reduced by pre-treatment with WEB 2086 (63 ± 19 vs $*17\pm23\mu l$ at 1h and 45 ± 15 vs $**13\pm13\mu l$ at 8h; mean $\pm$ s.e. mean; *= $p<0.05$; **=$p<0.01$, paired Student's t test). WEB 2086 also inhibited antigen-induced neutrophil accumulation by 59% (79 ± 33 vs 32 ± 14 neutrophils/4mm^2 dermis), although the reduction in cell number was not statistically significant. No effect on eosinophil accumulation was observed (107 ± 16 vs 98 ± 20 eosinophils/4mm^2 dermis). WEB 2086 significantly inhibited PAF-induced wheal formation (158 ± 20 vs $**4\pm7\mu l$) and reduced both neutrophil and eosinophil accumulation (138 ± 62 vs 24 ± 15 neutrophils/4mm^2 dermis and 5 ± 2 vs 0 ± 1 eosinophils/4mm^2 dermis).

These results suggest that the actions of PAF contribute to both the early and late phase cutaneous vascular responses to antigen in horses with the allergic skin disease sweet itch. Furthermore, PAF appears to play a role in antigen-induced neutrophil, but not eosinophil, accumulation.

We are grateful to the Home of Rest for Horses for financial support.

Foster, A.P., Cunningham, F.M. & Lees, P. (1992). Clin. Exp. Allergy, **22**, 129.
Henocq, E. & Vargaftig, B.B. (1988). J. Allergy Clin. Immunol., **81**, 691-696.
Roberts, N.M., Page, C.P., Chung, K.F. & Barnes, P.J. (1988). J. Allergy Clin. Immunol., **82**, 236-242.
Rossi, A.G., Norman, K.E., Donigi-Gale, D., Shoupe, T.S., Edwards, R. & Williams, T.J. (1992). Br. J. Pharmacol., **107**, 44-49.

14P DIFFERING PROFILES OF INHIBITION OF VARIOUS SECRETORY PHOSPHOLIPASE A_2 ENZYMES BY 'CLASSIC' INHIBITORS AND A NOVEL PLANT-DERIVED LABDANE

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Extracellular 14-kDa "secretory" $sPLA_2$ enzymes form a major class of phospholipase A_2, implicated in rheumatoid synovial tissue damage, and are potentially important therapeutic targets, although most known inhibitors show limited potency and selectivity. We have studied some well-recognised PLA_2 inhibitors and other compounds on three extracellular $sPLA_2$ enzymes in order to determine whether heterogeneity exists in inhibitor profile.

Recombinant human synovial $sPLA_2$ (synovial PLA_2) was donated by ICI Pharmaceuticals; Naja naja venom $sPLA_2$ was from Sigma (venom PLA_2); $sPLA_2$ in rat inflammatory exudate (peritoneal PLA_2) was from the peritoneal lavage fluid of Wistar rats injected 15h previously with 10ml 6% oyster glycogen. Assay of the 3 $sPLA_2$ enzymes was performed using *E.coli* membranes labelled with ^{3}H-oleic acid(1).

Four inhibitors were tested against the 3 $sPLA_2$s. They varied in their potencies and selectivity, as shown in the Table. Mepacrine and PBPB (p-bromophenacylbromide) are "classic" inhibitors of relatively low potency which inhibited venom and peritoneal PLA_2 but were ineffective against the synovial enzyme. Indomethacin, a potent NSAID cyclo-oxygenase inhibitor, surprisingly inhibited synovial and peritoneal PLA_2s selectively with higher potency than "classic" inhibitors. Labdane F2 is one of several anti-inflammatory principles of unknown mechanism isolated from the Spanish folk remedy herb *Sideritis javalambrensis* (2); this compound, unlike some close congeners obtained from the same plant, potently inhibited the mammalian $sPLA_2$ enzymes but was inactive against venom PLA_2. This action may contribute to its anti-inflammatory profile.

Approximate IC_{50} values for phospholipase A_2 inhibitors (μM)

	PBPB	Mepacrine	Indomethacin	Labdane F2
Venom PLA_2	70	250	>1000	>1000
Synovial PLA_2	>1000	>1000	35	7
Peritoneal PLA_2	80	400	28	13

In conclusion, selective profiles of inhibition of various $sPLA_2$ enzymes have been obtained. This suggests that inhibitor development programmes must be tailored to the exact enzyme in question, and that parallel screening of multiple enzyme types is desirable if specificity of action is required.

Supported in part by a SERC-CASE studentship awarded to IBL (ICI Pharmaceuticals).

1. Franson R, Dobrow R et al. (1978) J.Lipid Res. 19: 18-23.
2. Alcaraz MJ, Jimenez MJ et al. (1989) J. Nat. Prod. 52: 1088-91.

15P IONOPHORE-INDUCED CALCIUM TOXICITY IN RAT PERITONEAL LEUKOCYTES: SUPPRESSION OF LEUKOTRIENE GENERATION BUT THROMBOXANE IS SPARED

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Although moderate concentrations of calcium ionophores can activate the respiratory burst and secretion of granular enzymes in peritoneal leukocytes, we found recently that high concentrations induce much smaller responses (Charalambous & Hoult, 1992). This was attributed to a calcium-induced toxic response ('calcium overload') because (a) there was leakage of cytosolic LDH and (b) protection was afforded either by chelation of extracellular calcium (EDTA) or by reducing the extracellular calcium concentration. We have now investigated whether calcium overload toxicity is reflected in any change in the ability of the leukocytes to generate eicosanoids. Rat peritoneal leukocytes are an especially useful model for studying the mechanisms for controlling arachidonate metabolism as they express both the 5-lipoxygenase and cyclo-oxygenase pathways of arachidonate metabolism, yielding useful amounts of LTB_4 and TXB_2, respectively (Moroney et al., 1990).

Mixed peritoneal leukocytes were prepared as before, and were resuspended at 5.0×10^6 cells/ml in HBSS containing 1.26 mM Ca^{2+} and/or EDTA, and then incubated for 10 min with stimulants. Reactions were terminated by pelleting the cells (10 min, 4°C, 400g) and the amounts of LTB_4 and TXB_2 were measured by radioimmunoassay without extraction.

In normal Ca^{2+} medium (1.26 mM), calcium ionophore A23187 induced a dose-dependent release of both LTB_4 and TXB_2 in the range 10^{-8}M to 10^{-6}M, eg at 10^{-6}M A23187 TXB_2 - 26.0 ± 1.3, LTB_4 - 22.8 ± 1.0 (values as ng/5.0 x 10^6 cells/10 min, n=6). However, there was a reduction of 59.6 ± 1.5% in the amount of LTB_4 generated at 10^{-5}M A23187 compared to 10^{-6}M, implying that 5-lipoxygenase is impaired under conditions shown previously to be associated with 'calcium overload toxicity'. No such effect on TXB_2 generation was evident: yields at 10^{-5}M and 10^{-6}M A23187 were not significantly different. Thus the cyclo-oxygenase pathway appears to be unaffected under these conditions. Inclusion of 1.26 mM EDTA in the HBSS (as well as 1.26 mM Ca^{2+}) caused a small reduction in eicosanoid yield in the range 10^{-8}M to 10^{-6}M A23187; however, at 10^{-5}M A23187 the chelator was protective: the depressed LTB_4 yield was partially restored towards the level observed at 10^{-6}M (by 67.5% in experiment 1 and 71.3% in experiment 2). This is consistent with the reduction in yield at high ionophore being due to calcium overload toxicity, rather than being due to a non-specific damaging action of the ionophore.

We conclude that calcium overload toxicity in leukocytes causes not only impairment of the respiratory burst and granular enzyme secretion, together with some LDH leakage, but also a selective impairment of the lipoxygenase pathway of arachidonate metabolism. This must be at a level distal to phospholipase A_2 activation, as cyclo-oxygenase products are unaffected. High intracellular calcium concentrations may thus impair rather than facilitate mechanisms for translocation of 5-lipoxygenase and the function of the 5-lipoxygenase activating protein.

KC was supported by a MRC studentship.

Charalambous K, Hoult JRS. (1992) Br J Pharmacol, Communication *in press* (September meeting).
Moroney MA, Forder RA, Carey F, Hoult JRS. (1990) Br J Pharmacol 101: 128-132.

16P PROSTACYCLIN ANALOGUES POTENTIATE CONTRACTION OF THE GUINEA-PIG VAS DEFERENS TO ELECTRICAL FIELD STIMULATION

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Prostaglandin E_2 inhibits transmitter release from sympathetic nerve endings in the guinea-pig vas deferens and hence reduces contractile responses to electrical field stimulation (EFS). The system is highly sensitive to PGE_2 (IC_{50} ~ 2 nM) and activation of EP_3-receptors is involved. We have reported (Lawrence *et al.*, 1992) that the prostacyclin analogues iloprost and carbacyclin are weak EP_3 agonists on the vas deferens, with cicaprost being very weak. However slight potentiation of the maximal EFS response was seen with cicaprost (5 - 100 nM), occasionally with iloprost, but not with carbacyclin. In view of the high selectivity of action of cicaprost (Dong *et al.*, 1986), it appeared that an IP-receptor might be present on either the smooth muscle cells or the sympathetic nerve endings of the vas deferens.

In order to enhance the potentiating effect, the vas deferens was stimulated submaximally (35 - 45 volts, 1 msec, 1 pulse every 30 s) to give responses between 15 and 25% of maximum EFS. Under these conditions cicaprost, TEI-9063 (17α,20-dimethyl-$\Delta^{6,6a}$-6a-carba PGI_1) (Negishi *et al.*, 1991) and iloprost induced threshold effects between 1 and 2 nM and gave EC_{200} values (doubling of twitch size) of 5.4, 3.2 and 9.7 nM and maximal responses of 313, 303 and 232 % of control respectively (n = 8 - 16). The lower maximum response to iloprost was accompanied by reversal to inhibition of EFS above 70 nM. PGE_1 always inhibited EFS (IC_{50} = 1.5 nM). Cicaprost at 100 nM did not affect contractile responses of the vas deferens to noradrenaline. The results suggest that an IP-receptor is situated pre-synaptically and that its activation leads to enhancement of transmitter release.

Further experiments showed that cicaprost at 20 nM opposed the inhibitory effect of sulprostone, a potent (IC_{50} = 0.15 nM) and reasonably selective EP_3 agonist (Coleman *et al.*, 1987). The dose ratio was 2.5 at the 50% maximal EFS response level. Similar effects were seen with 100 nM cicaprost. It appears likely that a state of functional antagonism can operate with respect to transmitter release in the vas deferens and that the EP_3 agonist potencies of PGI analogues are being underestimated to some extent.

Coleman, R.A., Kennedy, I. & Sheldrick, R.L.G. (1987) *Br. J. Pharmacol.* **91**, 323P.
Dong, Y.J., Jones, R.L. & Wilson, N.H. (1986) *Br. J. Pharmacol.* **87**, 97-107.
Lawrence, R.A., Jones, R.L. & Wilson, N.H. (1992) *Br. J. Pharmacol.* **105**, 271-278.
Negishi, M., Hashimoto, H., Yatsunami, K., Kurozumi, S. & Ichikawa, A. (1991) *Prostaglandins* **42**, 225-237.

17P BRADYKININ-SENSITIVE NEURONS IN RAT DORSAL ROOT GANGLION *IN VITRO* IDENTIFIED BY UPTAKE OF COBALT IONS

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Bradykinin produces a receptor-mediated activation of sensory neurones (Burgess et al., 1989). In order to identify the subpopulation of neurones expressing bradykinin receptors we have used a novel method of cobalt-uptake into activated cells (Winter, 1987). Dorsal root ganglia (L_3-L_5) were dissected from 14-day-old rats under Enflurane anaesthesia and incubated in arteficial cerebrospinal fluid buffered with 25 mM Na-HEPES, containing 1.0 mM cobalt chloride and 300 nM tetrodotoxin (TTX). The preparation was stimulated by 500 nM bradykinin, or 50 mM KCl for 20 minutes.. Histochemical methods were used to visualise the intracellular accumulation of cobalt (Szekely and Gallyas, 1978). Marked cells were counted and characterised in camera lucida reconstructions from serial sections.

Bradykinin induced cobalt-uptake in 9.6±0.54% of dorsal root ganglion cells (DRG), a significantly larger number than stained in vehicle control tissue (1.8±0.7; $p<0.005$). The longest diameter of the soma of stained cells was significantly smaller than that of the non-marked population (23.2±0.85 μm and 25.5±0.43 μm, respectively; $p<0.0001$). Non neural cells were not marked by bradykinin exposure. The selective B2 receptor antagonist HOE-140 (5.0 μM) completely blocked the bradykinin-induced staining (2.16±0.02% of cells) and the B1 agonist [des-Arg^9]-bradykinin (500 nM) did not induce any significant change in comparison to control. In the presence of the non-selective excitatory amino acid receptor antagonist kynurenic acid the number of cells activated by bradykinin did not change (9.8±1.5%). Superfusion of KCl to the dorsal root ganglia resulted in a small, non-significant increase in the number of marked cells (2.8±0.89%).

These data provide direct evidence that a subpopulation of small diameter DRG cells are directly excited by bradykinin via B2 receptor activation. The failure of kynurenic acid to affect the number of cells activated by bradykinin excludes the possibility that cobalt staining resulted from indirect activation of neurons by glutamate release from glial and/or satellite cells. Finally, KCl induced depolarization did not result in cobalt-staining suggesting that cobalt-influx did not occur through voltage-dependent calcium-channels. Most likely ligand-gated Ca-channels are the major route for cobalt-influx.

Burgess, G. M., Mullaney, I., McNeill, M., Dunn, P. M. and Rang, H. P. (1989) Neuroscience 9, 3314-3325.
Winter, J. (1987) Neurosci. Lett. 80, 134-140.
Szekely, G. and Gallyas, F. (1975 Acta biol. Acad. Sci. hung. 26, 175-188

18P INTERLEUKIN 1-β (IL-1β), BUT NOT TUMOUR NECROSIS FACTOR-α (TNFα), CAN INDUCE BRADYKININ B_1 RECEPTOR-MEDIATED THERMAL HYPERALGESIA IN THE RAT

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Recently we have shown that in an ultra-violet (UV) irradiation-induced model of persistent inflammatory hyperalgesia in rats bradykinin (Bk) B1 receptors appear to play a major role in the maintenance of the hyperalgesia (Perkins *et al* 1992). It has also been shown that certain inflammatory mediators such as IL–1β but not others (eg TNFα) can increase responses to desArg^{9}Bk *in vitro* (deBlois *et al*, 1988). We have, therefore, investigated whether these agents can affect the expression of desArg9Bk-induced hyperalgesia *in vivo*.

One hind paw of female Sprague Dawley rats (100g) were either exposed to UVA light (90s, intensity maximum 365 nm, 69 mwatt/cm^2) or received an intra-plantar injection (50ul) of human recombinant IL-1β or TNFα. The withdrawal threshold to a focused beam of radiant heat applied to the underside of each hind paw was measured 24 and/or 48 hr after treatment.

The withdrawal latency of the ipsilateral paw 24 hr. after UV exposure fell by 22% from 22±1s (mean ± s.e.m., n=4) to 15.9±0.7s (n=8). There was no significant reduction in latency in the contra-lateral paw. desArg9Bk at this time point (1-100 nmole/kg iv) produced a dose dependent reduction in withdrawal latency in both the ipsi-lateral paw (50±4% reduction from contol at 100nmole/kg) and the contra-lateral paw (19.5±3% at 100 nmole/kg) This was reversed by co-administration of the B1 antagonist, desArg9Leu8Bk (200nmole/kg iv).

Intra-plantar injection of IL-1β (1-100 IU) produced a reduction of 30-40% in withdrawal latency 24 hr later although this was not dose-related. There was complete recovery from the hyperalgesia by 48hr and no contra-lateral hyperalgesia. desArg9Bk (10nmole/kg iv) injected 48hr after IL-1β induced hyperalgesia when measured 60min later. There was a 43%±11 reduction in latency after 1 IU, 31%±7 after 10 and 49%±6 after 100 IU of IL-1β. In addition, desArg9Bk (10nmole/kg iv) induced hyperalgesia in the contra-lateral paw following these IL-1β injections into the ipsi-lateral paw with a reduction of up to 31%±6 (100 IU IL-1β), n=4, in withdrawal latency. The desArg9BK-induced hyperalgesia was completely reversed in both paws by co-injection of desArg9Leu8BK (200nmole/kg iv).

TNFα (100 & 500 IU intra-plantar) neither reduced withdrawal latency in either paw nor did desArg9Bk have any effect 48hr following TNFα injection.

These data show that IL-1β, but not TNFα, can induce a bradykinin B1 receptor-mediated hyperalgesia similar to that seen after a non-specific inflammatory insult and is consistent with the induction of the B1 receptor by IL-1β but not TNFα *in vitro* (deBlois *et al*, 1988).

Perkins, M.N., Campbell, E.A., Davis A. & Dray A. (1992) Br. J. Pharmacol. In press
deBlois, D., Bouthillier, J. & Marceau, F., (1988). Br. J. Pharmacol., 93, 969-977

19P INTERLEUKIN 1-β, BUT NOT TUMOUR NECROSIS FACTOR-α, CAN INDUCE desArg9BRADYKININ-MEDIATED MECHANICAL HYPERALGESIA IN THE RAT

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Recently we have reported that in a Freund's adjuvant-induced model of persistent inflammatory hyperalgesia in rats, a B1 but not a B2 receptor antagonist was able to reverse the accompanying mechanical hyperalgesia (Perkins et al 1992) and we now show that in a less severe version of this model a B1 agonist is strongly hyperalgesic. Various inflammatory agents have been demonstrated to induce B1 responses in vitro (deBlois 1988) and we have investigated whether two of these agents, Interleukin-1β (IL-1β) and Tumour Necrosis Factor (TNFα) one of which (IL-1β) increases desArg9Bk responses in vitro and one (TNFα) which is inactive in this regard can affect the expression of desArg9BK-induced hyperalgesia in vivo.

Hyperalgesia to mechanical stimulation was induced by injecting female Sprague Dawley rats with 10μl of Freund's complete adjuvant intra-articularly into one knee under enflurane anaesthesia. On the third day (D3), the load (g) that the animals would tolerate on the injected leg was reduced to 84±4g (mean ± sem, n=8) compared with the uninjected leg (98±3g), indicating hyperalgesia. Drugs were given either intravenously or intra-articularly (50μl) on D3 under light enflurane anaesthesia.

desArg9Bk injected intra-articularly on D3 (50-1000pmoles) produced a dose-dependent reduction in the load tolerated by the injected leg falling to 27±3g (n=8, $p<0.05$, ANOVA followed by Dunnett's) at 1nmole and the reduction lasting for up to 5hr. This hyperalgesia was reversed by desArg9Leu8Bk given either intra-articularly (0.5nmole) or intravenously (1nmole/kg).

IL-1β (1-100 IU) injected intra-articularly into naive joints induced a dose-dependent reduction in load tolerated, falling to 49±5% (n=8, $p<0.05$) of control values at 100 IU by 6hr after injection but this had recovered by 30hr after injection. On D1 desArg9Bk (500pmole) given intra-articularly induced hyperalgesia in animals that had previously received 1IU of IL-1β, but not boiled IL-1β or saline, with a reduction of 36±4% (n=8, $p<0.05$) in the tolerated load when measured 60min after injection. desArg9Bk (1nmole) induced no hyperalgesia when given into joints of naive rats.

TNFα injected intra-articularly into naive joints (100I U) had no effect on the load tolerated by the injected leg. Intra-articular desArg9Bk (0.5nmole) induced no hyperalgesia in animals that had been treated with TNFα for up to 90min after injection of desArg9Bk.

These data show that bradykinin B1 receptor-mediated mechanical hyperalgesia can be induced by both a non-specific inflammatory insult and by IL-1β but not by TNFα in the rat . This is consistent with data *in vitro* showing induction of desArg9Bk responses by IL-1β but not by TNFα and with a role for the bradykinin B1 receptor in inflammatory hyperalgesia.

Perkins, M.N., Campbell, E.A., Davis A. & Dray A. (1992) Br. J. Pharmacol. In press

deBlois, D., Bouthillier, J. & Marceau, F., (1988). Br. J. Pharmacol., 93, 969-977

20P SUPERSENSITIVITY TO (±)BACLOFEN IN AN ANIMAL MODEL OF CHRONIC NEUROPATHIC, BUT NOT CHRONIC INFLAMMATORY, HYPERALGESIA

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Hyperalgesia to mechanical stimuli and hypersensitivity to (±)baclofen develops in an animal model of mono-neuropathy (Smith et al., 1992). The hypersensitivity to (±)baclofen may reflect transynaptic degeneration of GABAergic dorsal horn interneurones and may be a common feature of chronic hyperalgesia. We have compared the sensitivity to (±)baclofen in animal models of chronic neuropathy and inflammation.

In all studies, Glaxo-bred random hooded rats (male, 100-150g) were used. For mono-neuropathy, animals were anaesthetised with isofluorane (2% in 2 l min^{-1} NO_2, 1 l min^{-1} O_2), the sciatic nerve of the right, hind limb exposed and four ligatures (4.0 Chromic gut) loosely tied. The wound was closed, and the animals were allowed to recover for 15 to 20 days before being used in experiments. For sham-operation the nerve was exposed but not ligated (Bennett & Xie, 1988). To induce chronic inflammation, animals were anaesthetised and received unilateral, intraplantar injection of 100μl Freund's complete adjuvant (1mg ml^{-1}, Sigma, UK); animals were used in drug studies 12 to 15 days post-innoculation. Paw withdrawal thresholds (g) were determined prior to, and 30min following subcutaneous (s.c.) administration of drugs, using an Algesymeter (Ugo-Basile; 32g sec^{-1}, 500g max). Diazepam (0.75-2.5mg kg^{-1}, s.c.) was administered in acidified 0.9% saline solution and (±)baclofen (5-40 mg kg^{-1}, s.c.) was given as a suspension (10% gum acacia, 0.9% saline). For comparison between models, the dose of drug required to increase withdrawal thresholds to 120g above vehicle-treated controls ($ED_{\Delta 120}$, mg kg^{-1}, s.c.) was calculated and the mean presented in the table below, with 95% confidence limits.

Eleven days following ligation, but not sham operation, a persistent and significant ($p<0.01$, paired t-test) reduction in the control withdrawal threshold of the ipsilateral (118±4g, n=32) but not contralateral limb (163±5g, n=10) was seen. Similarly, 15 days following induction of chronic inflammation, a persistent and significant ($p<0.01$) reduction in the withdrawal threshold of the ipsilateral (173±13g, n=10) but not the contralateral (264±11g, n=10) limb had developed. Administration of (±)baclofen significantly ($p<0.05$; ANOVA, Dunnett's test) and dose-dependently increased the withdrawal threshold of mono-neuropathic, sham-operated, chronically inflamed and untreated animals compared with their respective vehicle-treated controls. The $ED_{\Delta 120}$ of ipsilateral and contralateral limbs of mono-neuropathic animals was respectively, six and two fold less than that of sham-operated controls, suggesting a bilateral supersensitivity to (±)baclofen had developed. In chronically inflamed animals the $ED_{\Delta 120}$ of ipsilateral and contralateral limbs did not differ from that of untreated animals. Diazepam was without significant effect on any group.

$ED_{\Delta 120}$ (mg kg^{-1}) for (±)baclofen treatment.

	Mono-neuropathic	Sham-operated	Chronically Inflamed	Untreated
Ipsilateral	1.8(0.2-3.6)	10.7(6.2-14.1)	12.2(11.4-13.0)	11.9(11.3-12.4)
Contralateral	4.5(2.1-6.7)	8.9(4.0-12.6)	11.2(10.4-12.0)	12.0(11.4-12.5)

We have shown that a $GABA_B$ agonist, but not an agonist at the $GABA_A$, benzodiazepine receptor site is antinociceptive in models of chronic hyperalgesia. Our results suggest supersensitivity to $GABA_B$ agonists develops in models of chronic neuropathy, but not chronic inflammation.

Smith, G.D., Hayes, A.G. & Birch, P.J. (1992) Neurosci. Letts., **Suppl. 42**, S25.

Bennet,G.J. & Xie,Y.-K.(1988) Pain, **33**, 87-107

21P EFFECT OF (-) BACLOFEN ON SUBSTANCE P-LIKE IMMUNOREACTIVITY RELEASE FROM RAT SPINAL CORD

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(-)Baclofen, the $GABA_B$ receptor agonist, exhibits antinociceptive and muscle-relaxant effects in mammals (Cutting & Jordan,1975).These effects which stem,at least in part, from an action within the spinal cord (Wilson & Yaksh,1978) can be prevented by the $GABA_B$ antagonist CGP 35348 (Malcangio et al.,1991). $GABA_B$ receptors in the spinal cord are localized on primary afferent terminals (Price et al.,1984) which contain substance P (SP) and this neuropeptide has been implicated in the transmission of nociceptive information (Yaksh et al.,1980). In the present study the effects of baclofen and GABA on SP release from the spinal cord were evaluated. Horizontal dorsal horn slices (Vibratome ~ 300 μm) sectioned complete with attached dorsal rootlets were mounted in a 3-compartment bath. The slice in the central compartment was perfused at 22 °C with Krebs' solution (1ml min^{-1}) containing bovine serum albumin (1 mg ml^{-1}), phosphoramidon 1 μM, captopril 100 μM, bacitracin 20 μg ml^{-1} and dithiothreitol 6 μM. Dorsal roots were laid across two pairs of bipolar electrodes in the two lateral compartments and immersed in mineral oil. Eight minute fractions were collected for determining SP-like immunoreactivity (SP-LI) by radioimmunoassay (Amersham Int.). In each experiment the basal level of SP-LI was determined over 24 min and the dorsal roots were then stimulated (20 V,0.5 ms,1 Hz) for 8 minute. Evoked release was calculated as percent of the mean basal outflow determined from three samples prior to stimulation. In control slices release was 182 ± 11% (n=8) (mean basal level 2.01±0.15 fmol ml^{-1}). (-)Baclofen completely prevented electrically evoked release of SP-LI at 100 μM (IC_{50} 0.8±0.2 μM; n=19). The effect of baclofen was stereospecific since the (+) isomer produced only 43 ± 10% inhibition at 500 μM (n=4). GABA mimicked (-)baclofen producing 81.4 ± 12 % inhibition at 300 μM (n=3) in the presence of nipecotic acid 100 μM. The $GABA_B$ antagonists CGP 35348 and CGP 36742 (3-aminopropyl (n-butyl) phosphinic acid) (100 μM) blocked the effect of (-)baclofen (10 μM) on the evoked release of SP-LI. CGP 36742 (100 μM) completely antagonized the inhibition of SP-LI release induced by GABA 300 μM.
In conclusion, activation of $GABA_B$ receptors in the spinal cord inhibits evoked SP-LI release which might contribute to the antinociceptive and muscle-relaxant effects of baclofen.

CGP 35348 and CGP 36742 were a gift from CIBA-GEIGY, Basel.

Cutting, D.A. & Jordan, C.C. (1975) *Br. J. Pharmacol.* **54**, 171-179.
Malcangio,M.,Ghelardini,C.,Giotti,A.,Malmberg-Aiello,P. and Bartolini,A. (1991) *Br. J. Pharmacol.* **103,** 1303-1308.
Price, G.W., Wilkin, G.P., Turnbull, M.J. & N.G. Bowery (1984) *Nature* **307,** 71-74.
Wilson,P.R. & Yaksh, T.L. (1978) *Eur. J. Pharmacol.* **51**, 323-330.
Yaksh, T.L., Jessel,T.M.,Gamse,R.,Mudge,A.W. and Seeman,S.E. (1980) *Nature* **386**, 155-157.

22P NMDA AND TACHYKININ RECEPTOR-MEDIATED CONTRIBUTIONS TO THE C-FIBRE-EVOKED RESPONSE IN THE NEONATAL RAT SPINAL CORD *IN VITRO* ARE ENHANCED FOLLOWING PERIPHERAL INFLAMMATION

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Excitatory Amino acid (EAA) and tachykinin receptor activation contribute to the generation of prolonged C-fiber evoked synaptic activity in the neonatal rat hemisected spinal cord (Thompson et al.1993). This has been further examined following the establishment of a UV-induced inflammatory hyperalgesia.

Synaptic activity was recorded extracellularly as a ventral root potential (VRP) with glass suction electrodes from a lumbar ventral root (L4 or L5) following single shock electrical stimulation of the ipsilateral dorsal root. Hemisected spinal cords were prepared as previously described (Thompson et al.1993) either from control 12 day old rat pups or from pups in which both a mechanical and thermal hyperalgesia of the left hindpaw had been induced following UV irradiation.

In untreated animals, high intensity electrical stimulation of the dorsal root (500μA,500μs), which activated C/group IV afferent fibers, evoked a prolonged VRP (8.91s duration). In the presence of the NMDA receptor antagonist D-AP5 (20μM) the area of the prolonged VRP was reduced to 78.2±3.4 % (n=9) of the control value. The area of the prolonged VRP was not significantly reduced (90.0±6.5%, n=8, of control) following superfusion with the NK1 antagonist CP-96,345 (500nM) (Snider et al.,1991) but was significantly reduced to 63.3±7.0% (n=6) of control following application of the NK2 antagonist MEN10376 (100nM) (Maggi et al.,1991). In animals in which a peripheral UV-induced hyperalgesia was present (1st day following treatment) no significant difference in the area or amplitude of the C-fiber evoked VRP was apparent compared to naive animals. However the area of the C-fiber evoked prologed VRP was further reduced to 64.1±3.0% (n=4) of control following superfusion with D-AP5. In the presence of the NK1 receptor antagonist CP-96,345, the area of the prolonged VRP was now significantly reduced to 60.2±4.0% (n=4) of control, the NK2 receptor antagonist MEN10376 produced a reduction in area equivalent to that produced in control animals (63.9±11.7% (n=3).

These experiments indicate that under normal circumstances NK2 and NMDA receptor activation but not NK1 receptor activation make a significant contribution to the acute nociceptive reflex in the neonatal rat spinal cord *in vitro*. However under conditions of peripheral hyperalgesia there is an expression of a novel NK1 receptor mediated component and an enhanced NMDA receptor mediated component to the acute nociceptive reflex.

References.
Maggi et al., *J.Pharmacol.Exp.Ther.*257. 1172-1178 (1991)
Snider et al., *Science* 251. 435-439 (1991)
Thompson S.W.N. et al., *J. Physiol.*, 459. 464P (1993)

23P EFFECTS OF NEUROKININS ON EXCITATORY AMINO ACID-INDUCED ACTIVATION IN THE NEONATAL RAT SPINAL CORD *IN VITRO*

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Activation of NMDA and neurokinin (NK) receptors occurs in the spinal dorsal horn following stimulation of nociceptive C-fibres (De Koninck and Henry, 1991; Schaible *et al.*, 1991). Furthermore the combined administration of NK and NMDA receptor antagonists results in an enhanced inhibitory effect suggesting synergism between these antagonists (Wiesenfeld-Hallin *et al.*, 1991). Here we have further studied the interactions between NKs and excitatory amino acids in the neonatal rat spinal cord.
Experiments were made *in vitro* on the isolated tail and spinal cord obtained from 1 day old rats. The tail and spinal cord were separately perfused at 24°C with an oxygenated physiological salt solution of the following composition (mM): NaCl 138.6; KCl 3.35; $CaCl_2$ 1.2; $MgCl_2$ 1.16; $NaHCO_3$ 21; NaH_2PO_4 0.58; glucose 10. Activation of spinal mechanisms was measured by recording spinal ventral root (VR; L_4) depolarization with an extracellular glass microelectrode. Drugs were superfused to the spinal cord. Amplitudes of the depolarizations were measured, averaged and and expressed in percentage of control. Data are presented as the mean±S.E.M. and, where necessary, statistical comparisons were made by using a paired Student's t-test.
Submaximal VR-responses were evoked by 10s applications of NMDA (5-10μM) and kainate (1-5 μM) to the spinal cord. NMDA responses were significantly enhanced (29%; n=12; $p<0.002$) in the presence of subthreshold concentrations of NK-A (1-10 nM; 10 min) but not by SP (1-5 nM; n=5). The metabolically stabilised SP-methylester (1-5 nM) enhanced the NMDA response slightly (17%; n=6; $p<0.01$). In the presence of a cocktail of endopeptidase inhibitors (captopril, thiorphan and bestatin, each 1.0 μM) subthreshold doses of SP significantly enhanced NMDA responses (40% ; n=3; $p<0.001$).
The possibility that the enhancement of NMDA effects was mediated via protein kinase C activation was tested using the inhibitor staurosporine (1.0 μM). While VR depolarization evoked by bradykinin (10 nM) administered to the spinal cord was blocked by staurosporine the enhancement of NMDA responses by NK-A was not altered.
These data suggest that NK2 receptor activation selectively enhances NMDA-evoked depolarization of the spinal cord. In circumstances where an excess of SP can occur in the cord NK1 receptors may also produce a similar effect. Finally protein kinase C activation does not play a role in the potentiation of NMDA responses by NK agonists.

De Koninck, Y. and Henry, J.L. (1991) Proc. Natl. Acad. Sci. USA. 88, 11344-11348.
Schaible, H.-G., Grubb, B.D., Neugebauer, V. and Oppmann, M. (1991) Eur. J. Neurosci. 3, 981-991.
Wiesenfeld-Hallin, Z., Xu, X.-J. and Dalsgaard, C.J. (1991) Soc. Neurosci. 291:8.

24P AFFINITY OF THE NK_2 ANTAGONIST SR 48968 AT NK_3-TACHYKININ AND μ-OPIOID RECEPTORS

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SR 48968, the first non-peptide antagonist of the NK_2 tachykinin receptor, has high affinity in binding (rat duodenum K_i 0.5nM) and functional (e.g. rabbit pulmonary artery pA_2 10.5) tests (Advenier *et al.*, 1992). High selectivity is also reported, e.g at NK_3 sites in rat cortex, or NK_3 receptors in rat portal vein SR 48968 was without significant activity (Advenier *et al.*, 1992).

We have tested SR 48968 in a number of assays for tachykinin receptors, and have confirmed its high affinity at the NK_2 receptor. Thus we observed competitive antagonism of responses to [β-Ala^8]NKA(4-10) in rat duodenum (pA_2 9.0 (8.3-9.7)) and guinea-pig trachea (pA_2 9.5 (8.7-10.7)), and a powerful displacement of binding ([^{125}I]-NKA 0.1nM, IC_{50} 7.1nM (4.2-11.9) in hamster urinary bladder.

In tests for the selectivity of SR 48968 we confirmed the lack of affinity at NK_1 sites in guinea-pig cortex ([^{125}I]-BH-SP 0.1nM, $IC_{50} > 10\mu M$) and at NK_3 sites in rat cortex ([^{3}H]-senktide 2.0nM, $IC_{50} > 10\mu M$). When the binding at NK_3 sites was tested in guinea-pig cortex however, appreciable affinity was observed (IC_{50} 330nM (184-532)). The species-dependent effect of SR 48968 at NK_3 receptors was found in functional tests where we confirmed the lack of affinity in the rat portal vein, but observed non-competitive antagonism of the response to senktide in the guinea-pig ileum with SR 48968 0.1-10μM.

Since the response to NK_3 receptor activation in the ileum is due to acetylcholine release, it was important to check the specificity of the block by SR 48968. We found no evidence for an interaction at the muscarinic receptor, with the response to carbachol being largely unaffected, however the cholinergic motor response to electrical field stimulation was inhibited (approximate ID_{50} 3μM). We attribute this action of SR 48968 to efficacy at the μ-opioid receptor since the effect of the compound was blocked by naloxone 1μM, or the μ-selective antagonist CTOP at 1μM. The affinity of SR 48968 at the μ-opioid receptor in guinea-pig brain was confirmed in binding assays (IC_{50} against [^{3}H]-D-Ala^2, Me-Phe^4, Gly-ol^5]enkephalin 165nM (150-175), IC_{50} against [^{3}H]-CI-977 $> 1\mu M$). When we re-examined the interaction between SR 48968 and senktide at the NK_3 receptor in the guinea-pig ileum, now in the presence of naloxone 1μM, the antagonism appeared competitive (apparent pK_B 7.6 (6.9-8.0)).

In conclusion we have confirmed the high affinity and selectivity of SR 48968 as an antagonist of the NK_2 receptor, but have found evidence for appreciable affinity at the NK_3 receptor in the guinea-pig, and efficacy at the μ-opioid receptor in this species.

Advenier, C., Emonds-Alt, X., Vilain, P., Goulaouic, P., Proietto, V., Van Broeck, D., Naline, E., Neliat, G., Le Fur, G. & Breliore, J.C. (1992). Br. J. Pharmacol., 105, 77P.

25P CHARACTERISATION OF CCK_B RECEPTORS IN GH3 CELLS

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Many studies have described the effects of CCK-A receptor activation in cell lines, but studies of the CCK-B receptor are less well documented. In the present study we have characterised the GH3 rat pituitary cell line and demonstrated the presence of CCK-B receptors using radioligand binding and calcium mobilisation studies.

Radioligand binding was performed using 50pM [^{125}I] Bolton Hunter CCK-8s in 20mM HEPES buffer pH 6.5, containing 150mM NaCl, 5mM $MgCl_2$, 1mM EGTA and 0.025% bacitracin (Chang and Lotti, 1986). Cells were grown to 75% confluence in DMEM, Hams F10 + 15% horse serum and limited to passage numbers <19. Binding to whole cells (1g to 90ml dilution) was for 90min at room temperature, with specific binding defined by 1μM CCK-8s. Where indicated cells were lysed with 5mM HEPES pH 6.5. Calcium mobilisation was measured using the calcium sensitive fluorescent dye fura-2 (Lambert and Nahorski, 1990) on a Perkin Elmer LS50 fluorimeter fitted with the Intracellular Biochemistry software.

[^{125}I] BH CCK-8s bound dose dependently and saturably to GH3 cells (Kd 85±4 pM, Bmax 23±6 fmol/mg protein, n=3). In the presence of 100nM L-365,260 little specific binding remained (12±3 % at 100pM). Binding was dose-dependently inhibited by CCK-8s IC_{50} 2.4nM (1.3;4.3, n=4), pentagastrin IC_{50} 45nM (34;61, n=3), L-365,260 IC_{50} 25nM (17;37, n=4) and devazepide IC_{50} 820nM, (630;1100, n=4), where numbers are geometric means (low and high errors of the mean). Competition curves were consistent with a single population of CCK-B receptors in this cell line. Binding of the radioligand to a lysed cell preparation was inhibited by 10μM GTP-gamma-S (66±3 %, n=3) and 10μM GppNHp (55±3 %, n=3). 10μM AppNHp control produced minimal effects (16±5 %, n=3). Addition of CCK-8s (0.1nM to 1μM) and the CCK-B selective agonist pentagastrin (0.1nM to 100μM) dose-dependently increased intracellular calcium mobilisation in fura-2 loaded GH3 cells. Resting calcium at the beginning of experiments was 158±5nM. A maximal dose of CCK-8s (1μM) increased prestimulated intracellular calcium levels by 2.85±0.16 fold and the maximum dose of pentagastrin (100μM) produced a similar maximal increase of 2.77±0.06 fold. The response to a submaximal dose of the CCK-B agonist pentagastrin (0.1μM) was dose-dependently blocked by the selective CCK-B antagonist L-365,260 (1-1000nM).

These results demonstrate that CCK receptors on GH3 cells are predominantly of the CCK-B type and the effects seen with GTP analogues suggest that this receptor is G-protein linked. Intracellular calcium mobilisation has been shown to be a functional consequence of CCK-B receptor activation using the selective CCK-B agonist pentagastrin. These results suggest that the GH3 cell line may be a useful model to study the coupling of CCK-B receptors in a pituitary cell line.

Chang R.S.L. and Lotti V.J. (1986) Proc. Natl. Acad. Sci., **83** 4923-4926.
Lambert D.G. and Nahorski S.R. (1990) Biochem. J., **265** 555-562.

26P EFFECTS OF CHOLECYSTOKININ TETRAPEPTIDE (CCK-4) AND THE CCK_B ANTAGONIST LY-288513 IN A PUTATIVE MODEL OF PANIC IN THE MOUSE

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Exposure to the calls of the Tawny Owl (Strix aluco) induces highly protean defensive behaviour in mice. This, in combination with an arena allowing access to a small strategically defensible "burrow" has led to the development of a model that has been shown to be sensitive to the actions of clinically effective antipanic agents such as alprazolam and imipramine but not to a wide range of other compounds (Hendrie and Neill, 1992). Recently it has been reported that i.v. injection of CCK-4 induces panic attacks in volunteers and in patients with panic disorder (Bradwejn et al., 1990). The panic attacks induced by CCK-4 in patients were dose-dependently blocked by the selective CCK_B antagonist L-365,260 (Bradwejn et al., 1992). Therefore, in the present study, we examined the effects of CCK-4 and a novel CCK antagonist LY-288513 (Howbert et al., 1992) in this behavioural model.

Male DBA/2 mice were injected with drug or vehicle (i.p.), individually introduced into the test arena and exposed to 3 min of tape recorded human voice followed by 2 min of birdsong. Time spent in the burrow was then recorded for the next 5 min.

CCK-4 (μg/kg)	Time in Burrow	LY-288513 (μg/kg)	Time in Burrow	
0.5	62 (8)	0.1	139 (23)	Data are expressed as % of
5.0	40 (15)*	1.0	179 (16)*	vehicle control (median values
50.0	51 (6)*	10.0	212 (16)*	with upper octiles in parentheses).
		100.0	138 (15)	Control values were 40-60s.
				* $p < 0.05$ vs. vehicle.

These data demonstrate a decrease in time spent in the burrow induced by CCK-4, indicating increased "panic-like" escape-orientated behaviour. The selective CCK_B antagonist LY-288513 induced the opposite reaction and increased time in the burrow. Previous studies showed that the selective CCK_B antagonist L-365,260 displayed a similar profile in this model whereas the selective CCK_A antagonist devazepide was inactive (Hendrie and Neill, 1992). Therefore, in view of data demonstrating CCK-4 to have panicogenic properties and a CCK_B antagonist to have antipanic effects in humans (Bradwejn et al., 1990, 1992), it can be concluded that the present model may have predictive validity for the detection of novel antipanic agents.

Bradwejn, J. Koszycki, D. & Meterissian, G. (1990) Can. J. Psychiat. **35**, 83-85.
Bradwejn, J., Koszycki, D., Couetoux du Tertre, A., van Megen, H., den Boer, J. Westenberg, H., Karkanias, J.K., Haigh, J. (1992) Soc. Neurosci. Abstr. Vol. **18**, P.763.
Hendrie, C.A. & Neill, J.C. (1992) In: *Multiple Cholecystokinin Receptors in the CNS* ed C.T. Dourish et al., pp 132-142. Oxford : Oxford University Press.
Howbert, J.J., Lobb, K.L., Brown, R.F., Reel, J.K., Neel, D.A., Mason, N.R., Mendelsohn, L.G., Hodgkiss, J.P. and Kelly, J.S. (1992) In : *Multiple Cholecystokinin Receptors in the CNS* ed C.T. Dourish, et al., pp 28-37. Oxford : Oxford University Press.

27P CHOLECYSTOKININ INCREASES C-*fos* EXPRESSION IN THE RAT NUCLEUS TRACTUS SOLITARIUS VIA CCK_A RECEPTORS

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It has been shown that systemic administration of cholesystokinin (CCK) causes an increase in Fos protein in the nucleus tractus solitarius (NTS) and area postrema of the rat (Luckman, 1992). The receptor subtype involved in this response has been investigated in the present study, by the use of selective CCK_A and CCK_B receptor antagonists.

CCK-8 sulphated (50µg/kg) was injected i.p. into male Wistar rats and animals sacrificed at times ranging from 30 min to 16 h. Antagonists were administered 30 (CI-988 1mg/kg) or 40 min (devazepide 0.1mg/kg) prior to CCK and in these experiments animals sacrificed after 30 min. The brains were rapidly frozen in isopentane at -35°C and 10µm sections cut on a cryostat. The sections were thaw-mounted onto sterile poly-L-lysine coated slides, fixed in 4% paraformaldehyde and dehydrated in ethanol. Hybridisation was overnight at 42 °C with 3000 cpm per µl of [^{35}S]-oligonucleotide probe complementary to mRNA for c-*fos*, c-*jun* or *jun* B. The slides were washed at room temperature for 30 min in 1X saline sodium citrate (SSC) followed by 30 min at 60°C in 1XSSC. The sections were dried and apposed to Betamax film (Amersham) or coated with LM1 emulsion (Amersham). The image obtained was quantified using an Image Analysis System and expressed as either % change over saline-injected controls, or as % of the maximum CCK response. Duplicate measurements were taken from each animal.

Basal expression of c-*fos* and *jun* B mRNA was very low while c-*jun* was more abundant. Administration of CCK caused a rapid increase in c-*fos* expression which peaked 30 min after injection and declined relatively slowly (455±100% above saline-injected controls at 30min; 389±136 % at 1h and 291±87% at 2h and basal levels at 6h n=5 animals). In contrast to the effect of CCK on c-*fos*, c-*jun* and *jun* B were unaffected by the peptide. The stimulation of c-*fos* expression in the NTS by CCK was attenuated by pretreatment with the CCK_A receptor antagonist, devazepide, (31±18 % max CCK response, n=5 animals; $p<0.05$ from CCK-stimulated) and unaffected by the CCK_B receptor antagonist, CI-988 (145±57% max CCK response, n=5 animals; NS). This dose of CI-988 has been shown to be active in models of anxiety in the rat (Hughes *et al.*, 1990).
In addition, pentagastrin, a selective CCK_B receptor agonist failed to mimic the CCK-induced increase in c-*fos* mRNA (CCK, 926±190 %control; pentagastrin 0.5 mg/kg 130±13 %, 2mg/kg 114±22 %).

The results support the contention that peripherally administered CCK can increase immediate early gene expression in the NTS and suggest that this is mediated by a CCK_A receptor.

Hughes, J.,Boden, P., Costall, B., Domeney, A., Kelly, E., Horwell, D., Hunter J.C., Pinnock, R.D. & Woodruff, G.N. (1990) Proc. Natl. Acad. Sci., 87, 6728-6732.
Luckman, S.M. (1992) J. Neuroendocrinol., 4, 149-152.

28P DISTRIBUTION OF THREE PUTATIVE SOMATOSTATIN (SRIF) RECEPTOR mRNA'S IN RAT BRAIN BY *IN SITU* HYBRIDISATION

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At least three cDNA's, named SSTR1-3, coding for SRIF receptors have been cloned from mouse, rat or human libraries (Yamada et al, 1992; Kluxen et al, 1992; Yasuda et al, 1992). The distribution of messenger RNA (mRNA) of these putative SRIF receptors was studied in adult rat brain using in situ hybridization histochemistry. Three oligonucleotide (40-43 mers) probes derived from regions of the 3rd intracellular loop, C and N terminus of the cDNA coding for each SSTR-1 (mouse gene), SSTR-2 (rat gene) and SSTR-3 (mouse gene) receptors were used and the data analysed by autoradiography.

Highest levels of SSTR-1 mRNA were found in lateral septal nucleus, entorhinal and frontal cortex, and cortical layers V-VI. Moderate signals were detected in taenia tecta, cortical layer IV, dentate gyrus and medial amygdaloid nucleus. Most other brain regions including superficial cortical layers, hippocampus, basal ganglia, locus coeruleus and cerebellum showed comparatively low hybridisation signals. High densities of SSTR-2 hybridisation signals were found in inferior/superior colliculus, cerebral cortical layer IV, dentate gyrus, medial habenular nucleus, taenia tecta and subiculum. Intermediate levels were detected in entorhinal and frontal cortex, superficial grey layer of the superior colliculus, all regions of Ammon's horn (hippocampus), and in median mammilary nucleus. Moderate signals were found in primary olfactory cortex, basal ganglia and deep cortical layers. Other regions such as locus coeruleus, nucleus accumbens, cortical layers I-III and cerebellum showed very low signals. High levels of SSTR-3 hybridisation signals were detected in the olfactory bulb and granular layer of the cerebellum. Intermediate densities were found in primary olfactory cortex, medial habenular nucleus, locus coeruleus, CA3, CA2 and CA4 fields of Ammon's horn, lateral habenular nucleus, islands of Calleja and mammilothalamic tract. Moderate signals were localised in frontal and entorhinal cortex, nucleus accumbens, CA1 field, inferior colliculus, taenia tecta, dentate gyrus, accumbens nucleus, basal ganglia and subiculum.

The localisation of mRNA for SSTR-1 and SSTR-2 receptors is at least in part, compatible with that of the receptors described previously. The wide and differential brain distribution of the three SRIF mRNA's suggests that the gene products (SRIF receptors) in rat brain are not restricted to neuroendocrine functions.

Kluxen, F.W., Bruns, C. & Lübbert, H. (1992) Proc. Natl. Acad. Sci. USA, **89**, 4618-4622.
Yamada, Y., Post, S.R., Wang, K., Tager, H.S., Bell, G.L. & Seino, S. (1992) Proc. Natl. Acad. Sci. USA, **89**, 251-255.
Yasuda, K., Rens-Domiano, S., Breder, C.D., Law, S.F., Saper, C.B., Reisine, T. & Bell, G.I. (1992) J. Biol. Chem., **267**, 20422-20428.

29P LACK OF SIGNIFICANT SELECTIVITY OF "BENZYL 4-DAPine" BETWEEN FOUR MUSCARINIC RECEPTOR SUBTYPES: BINDING AND FUNCTIONAL STUDIES

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Barlow et al. (1992) reported that benzyl-4-diphenyl acetoxy piperidine (benzyl 4-DAPine) was about 100-fold more potent as an antagonist of muscarinic effects in guinea-pig ileum (contraction: M_3 receptors) compared to guinea-pig atria (negative chronotropy or inotropy: M_2 receptors). We have estimated the affinity of benzyl 4-DAPine in radioligand binding experiments on cloned human muscarinic receptors Hm1-4 expressed in CHO cells (Buckley et al., 1989) and for M_1-M_3 binding sites in rat tissues (Table 1), and apparent pK_B values from agonist dose-ratios in the presence of benzyl 4-DAPine (1μM in 1:1000 DMSO) in functional assays (at 30°C; with carbachol or (±)-muscarine as agonist) in rat atria (rate) and ileum (contraction).

Table 1. benzyl 4-DAPine pK_B values (± s.e. mean) at four muscarinic receptor subtypes

	Binding				Functional	
m1/M_1	rat cerebral cortex:	6.99 ± 0.10 (4)	Hm1:	7.35 ± 0.10 (3)		
m2/M_2	rat heart:	6.98 ± 0.03 (8)	Hm2:	6.91 ± 0.11 (3)	atria:	6.7 ± 0.1 (8)
m3/M_3	rat lacrimal gland:	7.80 ± 0.04 (4)	Hm3:	7.34 ± 0.09 (3)	ileum:	7.0 ± 0.1 (9)
m4			Hm4:	7.14 ± 0.11 (3)		

Benzyl 4-DAPine had about 3-fold selectivity between Hm2 versus Hm3 or Hm1 receptors, but did not distinguish Hm2 from Hm4 receptors; it was about 6-fold more potent at M_3 binding sites than at M_1 or M_2 sites. The compound did not discriminate between rat atria M_2 and ileum M_3 receptors in functional experiments (Table 1). The discrepancy between our data and those of Barlow et al. (1992) does not seem to be due to a species difference, as binding pK_B values in rat (Table 1) and guinea-pig heart (6.95 ± 0.09, n=3) were similar. We conclude that benzyl 4-DAPine may not be a useful tool for discriminating muscarinic receptor subtypes and suggest that some of the variability encountered may be due to its poor water solubility.

Barlow, R.B., Bond, S., Holdup, D.W., Howard, J.A., McQueen, D.S., Veale, M.A., Smith, T.W., Stephenson, G.F. & Batsanov, A.S. (1992) Br. J. Pharmacol. 106, 40P.
Buckley, N.J., Bonner, T.I., Buckley, C.M. & Brann, M.R. (1989) Mol. Pharmacol., 35, 469-476.

We are grateful to Dr R.B. Barlow for supplies of benzyl 4-DAPine.

30P INCREASED DENSITY OF GUANINE NUCLEOTIDE-BINDING (G) PROTEINS IN THE FRONTAL CORTEX OF HEROIN ADDICTS

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The binding of morphine to μ-opioid receptors promotes the inhibition of adenylyl cyclase activity through pertussis toxin-sensitive guanine nucleotide-binding (G) proteins (Gi/o) (Kurose *et al.*, 1983). Preclinical studies suggest that opiates exert some of their chronic effects (tolerance, dependence and withdrawal reaction) through G proteins (see Manji, 1992). The aim of this study was to quantitate directly in the human brain the density of specific G proteins during heroin addiction. Specimens of human brain (frontal cortex-Brodmann's area 9) were obtained from 20 subjects who had died by opiate overdose or violent causes. The group of heroin addicts, with known history of opiate abuse, consisted of 12 subjects (10 men, 2 women, age: 25± 4 years, postmortem delay: 38 ± 4 h). The control group consisted of 8 subjects (8 men, age: 25 ± 3 years, postmortem delay : 36 ± 3 h) without a history of drug abuse. G protein subunits (Gαi$_{1/2}$, Gαi$_3$, Gαo, Gαs and Gβ) were separated by gel electrophoresis (SDS-PAGE) and quantitated by immunoblotting with specific antisera (AS/7, EC/2, GC/2, RM/1 and SW/1), as previously described (Young *et al.*, 1991) with slight modifications. Postmortem delays up to 48 h did not affect the quantitation of G proteins. In heroin addicts the density of specific G proteins was increased in the frontal cortex as compared with that in control subjects (Table 1). The increases of Gαi$_{1/2}$, Gαo and Gβ in the brain of addicts, paralleled those observed in the cerebral cortex of rats after repeated treatment with morphine (10 to 100 mg/kg i.p. for 5 days). In contrast, Gαi$_3$ immunoreactivity remained unchanged (Table 1). These increased G proteins are coupled to μ-opioid receptors and, hence, the observed higher cellular content after morphine/heroin might compensate the decrease in receptor function caused by desensitization and/or down-regulation during sustained agonist exposure. The rise in Gαs observed in rats only after acute (2 h) treatment with morphine suggests that the increase in Gαs immunoreactivity in the brain of human addicts could be the cellular response to an acute dose of heroin, rather than to chronic exposure to the drug.

Table 1 G proteins levels in the frontal cortex (% Control)

	Gαi$_{1/2}$	Gαi$_3$	Gαo	Gαs	Gβ
Heroin addicts	119±5**	102±9	126±7**	125±4**	125±8*
Rats (morphine)					
Chronic	132±9*	104±3	129±4*	97±7	121±7*
Acute	107±10	121±5*	112±10	127±1*	95±12

* At least P<0.05; ** at least P<0.001 (t-test)

The changes in the density of G proteins in the brain of heroin addicts could help to explain partially the molecular processes of opiate tolerance, dependence and withdrawal syndrome. The alterations detected in other inhibitory receptors, such as the α_2 adrenoceptor (Ulibarri *et al.*, 1987), during morphine dependence may also be related to the observed changes in common and specific G proteins (Gαi$_{1/2}$) coupled to μ-opioid receptors and α_2 adrenoceptors.

Kurose, H., Katada, T., Amano, T. & Ui, M. (1983) J. Biol. Chem. **258**, 4870-4875.
Manji, H.K. (1992) Am. J. Psychiatry **149**, 746-760.
Ulibarri, I., García-Sevilla, J.A. & Ugedo, L. (1987) Naunyn-Schmiedeberg's Arch. Pharmacol. **336**, 530-537.
Young, L.T., Li, P.P., Kish, S.J., Siu, K.P.& Warsh, J.J. (1991) Brain Res. **553**, 323-326.

Supported by DGICYT (Project PM91-0069) and CITRAN (Project 6/1992).

31P THE TURNOVER OF Gsα MAY INVOLVE ADP-RIBOSYLTRANSFERASE ACTIVITY IN NG108-15 CELLS

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The coupling of cell surface receptors to their effector enzymes or ion channels frequently involves the activity of G proteins. G proteins contain GTPase catalytic activity, and they are characteristically substrates for prokaryotic mono(ADP-ribosyl)transferases. There is now compelling evidence for eukaryotic mono(ADP-ribosyl)transferases, which also facilitate hydrolysis of NAD^+ and the formation of mono(ADP-ribosyl)-adducts with proteins. Their substrates also include G protein α-subunits, and ADP-ribosylation of Gsα in NG108-15 neuroblastoma x glioma hybrid cells is accompanied by a reduction in the level of Gsα and reduced capacity of iloprost to activate adenylate cyclase (Donnelly et al., 1992).

Exposure of NG108-15 cells to 10 μM iloprost for 17 h is followed by reduced capacity of iloprost or NaF to activate adenylate cyclase (Kelly et al., 1990). The effect is mediated by loss of both prostacyclin (PGI_2) receptors and Gsα from the plasma membrane (measured as cholera toxin substrate or by immunoblotting). When the cells were exposed to 50 mM nicotinamide [an inhibitor of mono(ADP-ribosyl)transferase], the loss of Gsα (and reduced sensitivity to NaF) that accompanies iloprost exposure was restored to control levels. However, the loss of PGI_2 responsiveness (and PGI_2 receptor) was unaffected.

These results contrast with experiments performed in human platelets which also show loss of PGI_2 receptors, NaF sensitivity and cholera toxin substrate after exposure to iloprost (Edwards et al., 1987). Paradoxically however there was no reduction in the abundance of Gsα immunoreactivity. In platelets, there was no restoration by nicotinamide of iloprost-dependent loss of NaF sensitivity, and nicotinamide appeared to restore the iloprost-dependent loss of cholera toxin substrate. Further experiments revealed that treatment of NG108-15 cells with 50 mM nicotinamide increased the abundance of mRNA coding Gsα, and the parallel increase in Gsα immunoreactivity was inhibited by 20 μg ml^{-1} cycloheximide.

We conclude that the capacity of nicotinamide to restore Gsα abundance and activity in NG108-15 cells is most probably dependent on *de novo* synthesis of Gsα, and that an ADP-ribosylated product may serve as a natural repressor of Gsα expression. The alternative possibility (that ADP-ribosylation of Gsα mediates loss of Gsα from the membrane of NG108-15 cells) seems improbable since Gsα is not similarly lost from platelet membranes.

Donnelly, L.E., Boyd, R.S. & MacDermot, J. (1992) Biochem. J. (in press).
Kelly, E., Keen, M., Nobbs, P. & MacDermot, J. (1990) Br. J. Pharmacol. 99, 309-316.
Edwards, R.J., MacDermot, J. & Wilkins, A.J. (1987) Br. J. Pharmacol. 90, 501-510.

32P MELATONIN AND S-20098 MEDIATED RESETTING OF RAT SUPRACHIASMATIC CIRCADIAN CLOCK NEURONES *IN VITRO*

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The nocturnal secretion of pineal melatonin is regulated by daily and seasonal variations in daylength is mediated via visual inputs to the suprachiasmatic nuclei (SCN) which function as a circadian clock in mammals. In turn melatonin regulates physiological processes, e.g. re-entrainment of rhythmicity in desynchronised animals and seasonal reproductive status, in part at least through feedback to the SCN clock (Mason & Rusak, 1990). SCN neurones *in vitro* exhibit a circadian rhythm in discharge activity which peaks during the subjective light phase of the circadian cycle (Mason, 1991). This peak in discharge activity may be recorded over successive days and reset by pharmacological challenge, e.g. it is advanced by melatonin treatment during a restricted circadian phase (McArthur *et al*, 1991). In the present study the effects of the new melatonin receptor agonist S-20098 (N[2-(7-methoxy napht-1-yl) ethyl] acetamide) (Yous *et al*, 1992) were compared with those obtained with melatonin or 2-iodomelatonin on resetting the circadian peak in neuronal discharge activity in the SCN.

Male Lister rats were kept in 12h light:12h dark lighting regimen with lights-on during circadian time (CT)00.00-12.00. Extracellular recordings were made over 2-3 successive circadian cycles from spontaneously active SCN neurones in a hypothalamic slice preparation (Mason, 1991) using a similar sampling procedure as McArthur *et al* (1991). Melatonin, 2-iodomelatonin, S-20098 (all at a concentration of 10^{-8}M) or vehicle were delivered in the aCSF superfusate for 1 hour between CT08.00-12.00, corresponding to a period within the "temporal window" of sensitivity found to exogenously applied melatonin in the SCN (Mason & Brooks, 1988). Melatonin (n=5) or 2-iodomelatonin (n=2) advanced the SCN discharge rate rhythm between 0.5-4.0 hr only when administered between CT08.00-12.00h. A similar circadian time-dependent advance (1.2-3.5 hr) in the SCN discharge rate rhythm was produced by S-20098 (n=4). A one hour superfusion of vehicle alone (n=5) was without effect. The present neurophysiological observations indicate that S-20098 mimicks the time-dependent effects of melatonin in its ability to reset the suprachiasmatic circadian pacemaker *in vitro*.

Mason, R. (1991) *Neurosci. Lett.* **123**: 160-163.
Mason, R. & Brooks, A. (1988) *Neurosci. Lett.* **95**: 296-301.
Mason, R. & Rusak, B. (1990) *Brain Res.* **533**: 15-19.
McArthur, A.J., Gillette, M.U. & Prosser, R.A. (1991) *Brain Res.* **565**: 158-161.
Yous, S., Andrieux, J., Howell, H.E., Morgan, P.J., Renard, P., Pfeiffer, B., Leisieur, D. & Guardiola-Lemaitre, B. (1992) *J. Med. Chem.* **35**: 1484-1486.

33P EFFECTS OF LEVCROMAKALIM AND NIFEDIPINE ON NORADRENALINE- AND KCl-INDUCED INCREASES IN TENSION AND INTRACELLULAR CALCIUM IN RAT ISOLATED RESISTANCE VESSELS

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In large blood vessels such as the rabbit aorta, cromakalim inhibits noradrenaline (NA)-induced tone, an effect not shared by nifedipine and which is associated with the inhibition of the refilling of NA-sensitive Ca-stores (Bray *et al.*, 1991). Cromakalim fully relaxes intermediate size vessels (coronary arteries) precontracted with 30mM KCl but only slightly reduces intracellular calcium concentration ($[Ca^{2+}_i]$) as determined by fura-2 epifluorescence (Yanagisawa *et al.* 1990). In the present study the ability of levcromakalim and nifedipine to relax precontracted small mesenteric resistance vessels (MRVs) was compared with their ability to reduce the increase in $[Ca^{2+}_i]$ induced by NA and KCl.

MRVs removed from male Sprague-Dawley rats were loaded with fura-2 AM (5μM) at 4°C for 2h. $[Ca^{2+}_i]$ changes were determined by the change in fluorescence of the tissue excited at alternating 340/380nm wavelengths. A maximal fluorescence response was defined by the change associated with perfusing the tissue with 90mM K+-rich physiological salt solution (PSS). Tissues were perfused with either 10μM NA or 30mM K+-rich PSS and then exposed to increasing concentrations of levcromakalim or nifedipine using a cumulative protocol. Tension studies were performed by mounting MRVs on tungsten wires in a small vessel myograph. Tissues were precontracted with 10μM NA or 30mM K+-rich PSS and relaxed with increasing concentrations of either levcromakalim or nifedipine.

Exposure of the tissue to 30mM K+-rich PSS or 10μM NA evoked a sustained increase in fluorescence signal (mean increases were 70 ± 5.2% and 67 ± 5.3%, respectively, of the 90mM K+-induced plateau: mean ± s.e.mean n=8). Nifedipine (10nM-10μM) produced a full reduction of the increase in fluorescence signal induced by both 30mM K+-rich PSS and 10μM NA. In comparison, levcromakalim (10nM-10μM) reduced the increase in fluorescence signal induced by 10μM NA but had no effect on that generated by 30mM KCl. Application of 10μM NA to MRVs produced a sustained increase in tension which was relaxed by both levcromakalim and nifedipine. 30mM K+-rich PSS produced a smaller increase in tension compared to NA and this was also relaxed by increasing concentrations of levcromakalim and nifedipine .

In contrast to the KCl-induced contractions, those evoked by NA were relaxed by nifedipine and levcromakalim with a parallel reduction in $[Ca^{2+}_i]$. This suggests that the increase in $[Ca^{2+}_i]$ induced by 30mM K+-rich PSS is greater than that required to activate the contractile proteins. Both levcromakalim and nifedipine inhibited the NA-induced increase in tension and $[Ca^{2+}_i]$. This indicates that in MRVs the predominant relaxant effect of levcromakalim is exerted via hyperpolarisation-induced inhibition of voltage-operated Ca-channels.

Supported by an SERC-CASE Award with SmithKline Beecham (I. G.) and by SmithKline Beecham (D.C.).

Bray, K. M., Weston, A. H., Duty, S., Newgreen, D. T., Longmore, J., Edwards, G. & Brown, T. J. (1991) *Br. J. Pharmacol.* 102, 337-344

Yanagisawa, T., Teshigawara, T. & Taira, N. (1990) *Br. J. Pharmacol.* 101, 157-165.

34P THE EFFECTS OF POTASSIUM CHANNEL OPENERS ON THE HUMAN MYOMETRIUM BEFORE AND AFTER THE ONSET OF LABOUR

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Potassium channel openers (KCOs) have been shown to act as uterine relaxants in the rat, in vitro and in vivo (Piper et al 1990). We have therefore investigated the effect of potassium channel openers, and the antagonism of their action by sulphonylureas, on human myometrium in pregnancy, before and after the onset of parturition. Non-labouring and labouring myometrial tissue were obtained from elective and emergency Caesarean sections respectively (with Local District Ethical Committee approval). Uterine strips were mounted for isometric recording under 2g tension in Krebs Henseleit physiological solution maintained at 37°C, gassed with 95%O_2/ 5%CO_2 and equilibrated for 2h. Phasic spasms were then induced with 0.5nM oxytocin for a 60 min period. Cromakalim (0.1,1.0 and 10mM) or pinacidil (0.1,1.0 and 10mM) were added in a cumulative manner to the bath at 30-min intervals and their relaxant effect assessed by expressing the integrated tension as a percentage of that before drug addition. In a further experiment, glibenclamide (0.3,1.0 and 10 mM) or vehicle, or tolbutamide(10,100 and 500mM) or vehicle were added to the bath before the concentration effect curve to the agent was repeated. The effect of both KCOs was also assessed against KCL (10-80mM) induced contractions. Both KCOs are potent relaxants of human myometrium. The pD_2 values for cromakalim for non-labouring and labouring myometrium are 6.57 ± 0.09 (n=10) and 6.42 ± 0.06 (n=6). The values for pinacidil are 6.13 ± 0.13 (n=7) and 6.13 ± 0.12 (n=9). Addition of glibenclamide resulted in a rightward shift of the concentration effect curve for both relaxants and Schild analysis yielded pA_2 values of 6.45 ± 0.12 (n=10) and 6.42 ± 0.06 (n=6) for it's interaction with cromakalim in non-labouring and labouring myometrium and similarly 6.78 ± 0.32 (n=7) and 6.59 ± 0.31 (n=9) for pinacidil. The relaxant effect was not antagonized by tolbutamide in the concentration range tested; the mean pD_2 value was unchanged. Finally, both cromakalim and pinacidil inhibited contractions induced by KCl concentrations less than, but not greater than 40mM.

In conclusion, KCOs have a relaxant effect on human myometrium, before and after the onset of labour, which may be due, at least in part, to activation of ATP-sensitive potassium channels (K_{ATP}). KCOs therefore have potential for tocolysis.

Piper, I., Marshall E., Downing S.J., Hollingsworth M. & Sadrei H. (1990) Br. J. Pharmacol. 101, 901-907.

Supported by Cambridge Health Authority, Glaxo & MRC.

35P Mg^{2+}-DEPENDENT INHIBITION OF K_{ATP} CHANNEL ACTIVITY BY SULPHONYLUREAS IN CRI G1 INSULIN SECRETING CELLS

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The hypoglycaemic sulphonylureas, tolbutamide and glibenclamide, have been shown to inhibit directly the ATP sensitive potassium (K_{ATP}) channel present in insulin secreting cells (Sturgess et al., 1985; Ashcroft & Ashcroft, 1990). Recently it has been shown that the potency of tolbutamide and the non-sulphonylurea moiety of glibenclamide, meglitinide, is enhanced in isolated patches by the presence of cytosolic MgADP (Schwanstecher et al., 1992). In this study we have used isolated inside-out patches obtained from the CRI G1 cell line in order to investigate the effect of intracellular Mg^{2+} on the ability of the sulphonylureas tolbutamide and glibenclamide, and the non-sulphonylurea meglitinide, to modulate K_{ATP} channel activity.

In symmetrical 140mM KCl with a internal bath solution containing 0.65mM Mg^{2+}, tolbutamide (0.1mM) produced a 96.1 ± 2.9% (n=7) and a 95.1 ± 4.2% (n=7) reduction in K_{ATP} channel activity at depolarised and hyperpolarised potentials respectively. In contrast, when the internal bath solution contained no added Mg^{2+} and was supplemented with 10mM EDTA there was a statistically significant ($P<0.01$) reduction in the inhibitory effect of tolbutamide to 29.2 ± 3.9% (n=5) and 34.5 ±3.3% (n=5) at depolarised and hyperpolarised potentials respectively. A significant reduction in inhibitory effect ($P<0.01$) was also seen for glibenclamide (1μM) and meglitinide (10μM) but was not observed for ATP or the barbiturate, thiopentone (0.3mM).

When the intracellular Mg^{2+} was replaced with 0.65mM Mn^{2+}, a statistically significant reduction ($P<0.05$) in the inhibitory effectiveness of tolbutamide (0.1mM) was again observed, although this was not as marked as that seen in the absence of either Mg^{2+} or Mn^{2+}. In experiments where the pipette solution (i.e. extracellular solution) was exchanged for one containing no Mg^{2+}, the normal channel inhibitory effect of tolbutamide was observed provided that the intracellular bath solution contained Mg^{2+}.

These observations lead us to infer that the K_{ATP} channel inhibitory properties of the sulphonylureas are dependent upon the presence of intracellular Mg^{2+}.

K.L. is a Wellcome prize student.

Ashcroft, S.J.H., Ashcroft, F.M, (1990) Cellular Signalling 2, 197-214
Schwanstecher, C., Dickel, C., Panten, U. (1992) Mol. Pharm. 41(3), 480-486
Sturgess, N.C., Ashford, M.L.J., Cook, D.L., Hales, C.N. (1985) Lancet ii, 474-475

36P THE EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON VASCULAR REACTIVITY IN PERFUSED RAT MESENTERIC ARTERIES

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Altered vasoreactivity may underlie the increased risk of peripheral microvascular disease associated with diabetes mellitus. However, previous studies investigating the effect of diabetes on vasoreactivity using both 'ring' preparations and intralumenally perfused arteries have been inconclusive (White and Carrier, 1990). The aim of this study was to investigate the effect of untreated streptozotocin(STZ)-induced diabetes of 2,3 and 4 weeks duration on vasoreactivity of the mesentery to noradrenaline (NA), vasopressin (AVP) and potassium chloride (KCl).
Diabetes was induced in male Wistar rats by intravenous administration of STZ (70 mg/kg). Following STZ treatment, mean (±SEM) body weight of diabetic rats was significantly lower than age-matched non-diabetic rats at all three time points (240±7g and 374±9g respectively, $p<0.0001$). Mean plasma glucose concentration (39.6±2.2 mM and 9.4±0.4 mM , $p<0.0001$) and glycated haemoglobin assayed by boronic acid affinity chromatography (10.8±0.4% and 1.9±0.1%, $p<0.0001$) were significantly higher in the diabetic group. Mesenteric arteries were isolated from diabetic and non-diabetic rats under anaesthesia and intralumenally perfused in parallel according to the method of McGregor (1965) as modified by Douglas and Hiley (1990). Dose response curves for NA (0.2-20uM), AVP (0.45-24.7nM) and KCl (25-125mM) were performed sequentially with 30 min equilibration periods between each stimulus.
Basal perfusion pressures in diabetic and non-diabetic rats were 26±2 mmHg and 25±1 mmHg respectively. A decrease in ED50 for NA was observed in diabetic rats which was significant ($p<0.05$) after 3 and 4 weeks of diabetes (Table 1). Maximal response above basal(MAX) to NA was unaffected. AVP responsiveness was not affected by diabetes. MAX and ED50 for KCl were significantly decreased after 2 and 4 weeks of diabetes.

Table 1. Pharmacological Parameters

Group (n)	Duration of diabetes (weeks)	Vasoactive Agent: Noradrenaline MAX (mmHg)	Noradrenaline ED50 (uM)	Vasopressin MAX (mmHg)	Vasopressin ED50 (nM)	Potassium Chloride MAX (mmHg)	Potassium Chloride ED50 (mM)
STZ-diabetic (6)	2	98 ± 6	1.7 ± 0.5	77 ± 9	2.8 ± 0.6	59 ± 6*	53 ± 3*
non-diabetic (6)	-	98 ± 2 6	2.9 ± 0.8	80 ± 10	2.6 ± 0.3	91 ± 9	77 ± 4
STZ-diabetic (6)	3	97 ± 1	1.5 ± 0.3*	69 ± 6	1.8 ± 0.5	84 ± 5[a]	67 ± 4[a]
non-diabetic (6)	-	100 ± 1	3.3 ± 0.7	72 ± 9	2.5 ± 0.3	90 ± 13	68 ± 3
STZ-diabetic (5)	4	88 ± 4	1.4 ± 0.2*	72 ± 6	3.2 ± 0.6	66 ± 6*	49 ± 2*
non-diabetic (5)	-	104 ±10	3.5 ± 0.7	79 ± 4	3.3 ± 0.6	89 ± 7	69 ± 4

*- Significantly different from age matched controls ($p<0.05$)
a- Significantly different from rats with diabetes of 2 and 4 weeks duration ($p<0.05$)

The selective increase in noradrenaline sensitivity observed in STZ-diabetic rats supports a role for altered noradrenergic reactivity in diabetes which could be important in the pathogenesis of diabetic microangiopathy.

The financial support of the Scottish Office Home and Health Department is gratefully acknowledged.
Douglas, S.A. and Hiley, R. (1990) Br. J. Pharmacol. 101, 81-88.
McGregor, D.D. (1965) J. Physiol. 177, 21-30.
White, R.E. and Carrier, G.O. (1990) J. Pharmacol. Exp. Ther. 253, 1057-1062.

37P REDUCED mRNA FOR NEUROTROPHIC FACTORS AND GAP-43 IN PERIPHERAL NERVE AND TARGET TISSUE OF DIABETIC RATS: EFFECTS OF BRAIN-DERIVED NEUROTROPHIC FACTOR

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Experimental diabetes in rats is associated with reduced levels of a range of axoplasmic proteins; this may be explained by a diabetes-induced impairment of neurotrophic support. In sensory neurones gene expression of the axoplasmic growth associated protein, GAP-43, is regulated by nerve growth factor (NGF) (Federoff *et al.*, 1988). In diabetes the levels of NGF available to the sensory neurone might be lowered leading to a down-regulation of GAP-43 gene expression. This study was designed to test whether administration of a neurotrophic factor could reverse diabetes-induced deficits in GAP-43 mRNA and/or neurotrophic factor mRNA levels in sciatic nerve, muscle and foot skin, using brain-derived neurotrophic factor (BDNF), because it is a member of the NGF family of growth factors and exhibits many of the physiological properties of NGF. Male Wistar rats (starting weight 310-360g) were allotted at random to 4 groups. Two groups were made diabetic with streptozotocin (50 mg/kg i.p.) for 4 weeks, with one diabetic and one control group treated with BDNF (0.9 mg/rat s.c. three times per week). Treatment with BDNF had no effect on final body weight, plasma glucose levels, or intake of water and food in control or diabetic rats. BDNF was also without effect on the consequences of hyperglycaemia in sciatic nerve - content of glucose and its metabolites, sorbitol, and fructose were all elevated in diabetic rats irrespective of treatment. Total RNA was isolated from tissues as described by Chomzynski and Saachi (1987) and subjected to Northern transfer. A 450base truncated NGF sense transcript was added at the start of the isolation procedure permitting quantification of mRNA and total RNA yields. Northern blots were hybridised using ^{32}P-labelled cDNAs for NGF, CNTF, BDNF and GAP-43. Hybrids were detected by autoradiography and quantified using image analysis. In sciatic nerve of diabetic rats NGF mRNA levels (pg/g tissue wet wt $\pm$ 1SD; n=4 except where stated) were reduced (51 $\pm$ 17.06; n=3) compared with control (124.5 $\pm$ 9.75). Treatment of diabetic rats with BDNF reversed the deficit in NGF mRNA levels - BDNF-treated control (113.1 $\pm$ 27) compared with BDNF-treated diabetic (123.9 $\pm$ 18.1). CNTF mRNA levels were reduced by 25% in diabetic sciatic nerve, BDNF treatment succeeded in preventing this deficit. NGF mRNA levels in foot skin were unaffected by diabetes or BDNF treatment. The levels of NGF mRNA in soleus muscle were decreased in diabetic (26.0 $\pm$ 7.0) compared with control (53.3 $\pm$ 25); BDNF treatment had no effect. The reduced neurotrophic factor mRNA levels in sciatic nerve and soleus muscle coincided with deficient GAP-43 mRNA levels (arbitrary linear units $\pm$ 1SD; n=4) in dorsal root ganglia (L_4 + L_5) - levels in diabetic ganglia (0.62 $\pm$ 0.11) compared with those in control ganglia (1.0 $\pm$ 0.16). BDNF treatment prevented this deficit (BDNF-treated controls - 0.86 $\pm$ 0.23 vs BDNF-treated diabetic - 0.84 $\pm$ 0.23). These findings implicate reduced trophic support in neuronal diabetic neuropathy and indicate that treatment with an exogenous neurotrophic factor may prevent the deficits.

Chomczynski, P. & Sacchi, N. (1987) Anal. Biochem. 162, 156-159.
Federoff, H.J., Grabczyk, E. & Fishman, M.C. (1988) J. Biol. Chem. 263, 19290-19295.

38P ESSENTIAL FATTY ACID TREATMENT PREVENTS NERVE ISCHAEMIA AND ASSOCIATED CONDUCTION ANOMALIES IN EXPERIMENTAL DIABETES

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Peripheral nerves of diabetic rats and patients exhibit exaggerated resistance to hypoxic conduction failure; this develops in association with endoneurial ischaemia (Cameron *et al.*, 1991). This study was designed to confirm the existence of nerve ischaemia using laser Doppler flux monitoring in diabetic rats and to examine its association with exaggerated resistance to abrupt anoxia by examining the effect of supplementation with essential fatty acids on the two phenomena. Diabetes was induced with streptozotocin (50 or 65 mg/kg i.p.) and control and diabetic sub-groups maintained on a diet containing 5% evening primrose oil (EPO). As a control for the enhanced lipid intake, the other two sub-groups were given a diet enriched with 5% coconut oil (CO). Five or six weeks later rats were either anaesthetised (chloral hydrate 400-500 mg/kg i.p.) for blood flux measurements or killed by a blow to the head and one sciatic nerve removed for measurement of action potential conduction *in vitro* (Calcutt *et al.*, 1991). The sciatic nerve was used for measurement of Doppler flux (NBF) with concomitant recording of arterial pressure from one carotid. EPO did not influence the weight loss or the hyperglycaemia of diabetes. NBF (mean $\pm$ SD in arbitrary units) was markedly reduced in CO-treated diabetics (177.5$\pm$43.8) compared to CO-controls (379.9$\pm$21.6); this deficit was prevented by EPO treatment in diabetics (358.8$\pm$44.3) when compared with either CO-controls or EPO-treated controls (394.3$\pm$24.3). All animals were hypotensive under the chloral hydrate anaesthetic regime (range of mean systemic arterial pressures = 70-75 mm Hg), with no differences between the groups. A parallel study examined the influence of a normotensive anaesthetic regime on NBF. In animals anaesthetised by continuous infusion of alphaxalone and alphadolone (3:1), both controls (mean arterial pressure = 111$\pm$6.9 mm Hg) and diabetics (112$\pm$6.7) were normotensive and gave NBF values similar to those of the other study (controls = 355$\pm$32; diabetics = 158$\pm$28). Compound action potential amplitude (as % initial value $\pm$ SD) after 40 min of anoxia was reduced in nerves from CO-controls (21.4 $\pm$ 9.2) compared to those from CO-diabetics (59.4 $\pm$ 12.3), confirming the exaggerated resistance to hypoxia in diabetes. Nerves from EPO-diabetics lay mid way between the two (41.1 $\pm$ 8.9). EPO treatment of non-diabetic rats rendered the nerves even less resistant to anoxia with an amplitude after 40 min of 6.2 $\pm$ 5.7 %. These observations suggest that the sciatic nerves of diabetic rats with short-term streptozotocin-diabetes are markedly ischaemic and that this ischaemia is involved in the development of increased resistance to acute anoxic/ischaemic conduction failure in diabetic nerve. The findings also promote evening primrose oil as a potential treatment to prevent nerve ischaemia.

This work was funded by Ono Pharmaceuticals, Osaka, Japan

Cameron,N.E., Cotter,M.A. & Low,P.A. (1991) Am.J.Physiol. 261, E1-E8.
Calcutt,N.A., Ettlinger,C.B., Carrington,A.L., Diemel,L.T. & Tomlinson,D.R. (1991) J.Neurol.Sci. 103, 116-123.

39P FUNCTIONAL AND STRUCTURAL PROPERTIES OF AORTAE FROM HETEROZYGOUS AND HOMOZYGOUS WATANABE HERITABLE HYPERLIPIDAEMIC RABBITS

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One in every 500 people is heterozygous (hH) for familial hypercholesterolaemia, and 1 in 1,000,000 people are homozygous (hh). Watanabe Heritable Hyperlipidaemic (WHHL) rabbits are an animal model of this disease, carrying the same genetic defect. We have examined the effect of hypercholesterolaemia on vasorelaxant properties in both hh and hH WHHL, using New Zealand White rabbits (NZW) as a normal control. Various age groups were studied including 6 and 12 months.
Aortic rings (2 mm) were suspended in an organ bath filled with Krebs bicarbonate buffer at 37°C, aerated with 95% O_2 and 5% CO_2. Rings were constricted with the EC_{50} of phenylephrine and cumulative concentration response curves to carbachol (10^{-8} M- 10^{-5} M) were obtained, followed by 10^{-4} M sodium nitroprusside (SNP). Responses to carbachol were expressed as a percentage of the phenylephrine induced contraction. The adjacent area was stained with sudan red dye which stains fatty lesions in the arterial intima, using image analysis the % area stained (% sudan) was calculated. An adjacent ring was used to prepare histological sections stained with EI/MSB. Using image analysis the % of the intimal circumference covered with lesions (% involved) and the ratio of intima to media (% I/M) were calculated. Blood was sampled for cholesterol (CHOL) measurement (CHOD-PAP) 3 monthly.
Serum cholesterol decreased with age but was significantly higher in hh at all times. In 6 and 12 month old animals, maximum relaxation was significantly impaired in both hh and hH (Table 1).

Table 1

GROUP (months)	NZW(6)	NZW(12)	hh(6)	hh(12)	hH(6)	hH(12)
CHOL (mM/l) at 3 months			20±4	21±4	3±0.7	4±2
CHOL (mM/l) at study	0.9±0.3	0.5±0.2	17±3*	9±3*	2±0.5	2±1
Max. Relaxation (%)	94±5	96±7	58±22*	20±25*	77±8*	77±29*
% Sudan	0±0	0±0	32±23*	85±26*	0±0	11±29
% Involved	0±0	0±0	28±17*	70±39*	0±0	9±25
% I/M	0±0	0±0	11±9*	79±71*	0±0	9±23
n	7	7	5	7	7	7

Values given are means ± SD. * denotes significantly different from control NZW (ANOVA, $p < 0.05$).

All rings relaxed completely when exposed to 10^{-4} M SNP. There is a linear relationship between maximum relaxation and % sudan (r= -0.917), and also between maximum relaxation and % involved (r = -0.954). Although there is a relationship between maximum relaxation and % I/M, it appears to be non-linear. Cholesterol levels at use correlate with maximum relaxation (12 month old WHHL : r = -0.754; 6 month old WHHL : r = -0.802). However there is wide scatter around the line of regression at both ages.
In conclusion, these results show that both hh and hH WHHL have impaired endothelium dependant relaxation. This loss of function correlates better with the severity of atherosclerotic lesions than serum cholesterol levels.

40P FORSKOLIN RESPONSES IN EPITHELIA OF THE CYSTIC FIBROSIS MOUSE

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Cystic fibrosis is a fatal, autosomal, recessive disease in which the CF gene encodes for a protein (CFTR, cystic fibrosis transmembrane conductance regulator) which is functionally deficient, commonly missing phenylalanine at position 508 in the 1480 amino acid protein (Riordan *et al.*, 1989). CFTR functions as an epithelial Cl^- channel in tissues absorbing and secreting inorganic ions electrogenically (for a review see Cuthbert, 1991). Recently, transgenic CF mice have been described (Snouwaert *et al.*, 1992; Colledge *et al.*, 1992; Dorin *et al.*, 1992), facilitating approaches for pharmacological and gene therapies. A disrupting genetic element was inserted into mouse embryonal cells to give heterozygote mice carrying a disrupted CFTR allele. Heterozygote carriers were bred to give CF mice (-/-) plus wild types (+/+) and carriers (+/-). F2 genotypes had the expected 1:1:2 ratio. Epithelial ion transport was measured as short circuit current in tracheal (4 mm^2) and caecal (60, 20 or 4 mm^2) epithelia. Data for +/+ and +/- mice have been pooled as no differences have been detected between these groups with the present numbers of experiments. CF (-/-) mice failed to thrive and rarely survived the neonatal period, thus fewer data are available for this group. Non-CF tracheal epithelia had a basal current (mean ± s.e.) of 20.7 ± 2.3 μA cm^{-2} (n = 12) of which 50% (11.1 ± 2.2 μA cm^{-2} n = 6) was accounted for by sodium absorption, since it was sensitive to amiloride (100 μM), applied apically. Forskolin (10 μM) increased SCC by 22.7 ± 2.2 μA cm^{-2} (n = 6) in the presence of amiloride and SCC could be further enhanced by A23187 (1 μM). The current generated by forskolin or forskolin plus A23187 was inhibited by frusemide (1 mM, applied basolaterally) by 93.2 ± 6.5%, indicating it was due to Cl^- secretion. In CF airway epithelia the basal SCC was 7.9 μA cm^{-2} and was increased by only 4.8 μA cm^{-2} by forskolin (mean of 2). Caecal epithelia showed no response to A23187 and barely discernible responses to amiloride. However forskolin increased chloride secretion by 205 ± 43.5 μA cm^{-2} (n = 14) which was inhibited by 84.0 ± 5.1% by frusemide in non-CF tissues when an epithelial area of 60 mm^2 was used. As CF mice had poor survival, methods were developed to deal with smaller areas of caecal epithelium. In non-CF tissues, forskolin-sensitive SCC and percentage inhibition by frusemide were 39.0 ± 6.9 μA cm^{-2} and 92.7 ± 9.8% respectively (n = 7) for an area of 20 mm^2 and 14.3 ± 3.5 μA cm^{-2} and 107.9 ± 23.5% respectively (n = 4) for an area of 4 mm^2. The decrease in responsiveness with decreasing area is due to the increase in ratio of circumference to area, resulting in greater edge damage. Nevertheless the response to forskolin in -/- caecae was < 1.0 μA cm^{-2} (mean of 5). Thus the -/- mouse mimics the disease state in so far as there is a failure of cAMP-mediated Cl^- secretion.

Riordan, J.R., Rommens, J.M., Kerem, B. *et al.* (1989) *Science* 245, 1066-1073.
Cuthbert, A.W. (1991) *Thorax* 46, 124-130.
Snouwaert, J.N. Brigman, K.K., Latour, A.M. *et al.* (1992) *Science* 257, 1083-1088.
Colledge, W.H., Ratcliff, R., Foster, D., Williamson, R. & Evans, M. (1992) *Lancet* 340, 680
Dorin, J.R., Dickinson, P., Alton, E.W.F.W. *et al.* (1992) *Nature* 359, 211-215.

41P PHOTODYNAMIC ACTION OF SULPHONATED ALUMINIUM PHTHALOCYANINE ON THE RELEASE OF ARACHIDONIC ACID AND PGE_2 FROM PERIFUSED RAT PANCREATIC ACINI

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Light-activated photodynamic drugs which generate singlet oxygen form an important group of compounds with therapeutic potential, especially as antitumour agents, but their precise mechanism of action is not known. Upon activation at wavelengths > 570 nm the photodynamic agent sulphonated aluminium phthalocyanine (SALPC) elicits amylase release and plasma membrane permeabilization in perifused isolated rat pancreatic acini. Amylase release precedes membrane permeabilization by some 6 min (Matthews & Ciu, 1990) suggesting that the initial release of amylase may be due to a direct stimulation by singlet oxygen of secretagogue receptors or their coupled G-proteins and effector systems including phospholipase A_2 (PLA_2). We report here the results of experiments to test the involvement of PLA_2 activation, arachidonic acid (AA) mobilization, and prostaglandin production in photon-activated SALPC action. Dispersed acini were isolated by collagenase digestion from the rat pancreas, loaded into small columns and perifused with a HEPES buffered medium at 37°C. Acini were illuminated for 10 min when required with a quartz-halogen light source ($\lambda > 570$ nm) and amylase release was measured by a spectroscopic method. In some experiments perifused acini were prelabelled with 1-^{14}C-arachidonic acid and arachidonate mobilization assessed by liquid scintillation counting of released radiolabel; in others, prostaglandin E_2 (PGE_2) production was determined by a peroxidase - conjugate immunoassay in parallel with amylase release.

Previous work (Matthews & Cui, 1990) has demonstrated an immediate release of amylase by light-activation of membrane-bound SALPC. In contrast the kinetics of AA release on activation of SALPC by light (18400 lux) are biphasic, inhibition of basal AA release from 1.0 to 0.76 ± 0.07 ($P < 0.05$, n=6) preceding an eventual stimulation to 29.4 ± 7.7 ($P < 0.001$, n=6) times the basal value. Calcium influx may activate PLA_2 following photochemical membrane permeabilization by SALPC and account for the delayed increase in AA release since the calcium ionophore A23187, 1 μM, increased AA release from perifused acini by 4.85 ± 0.6 ($P < 0.001$, n=4) times basal output. In pancreatic cells AA can also be released by sequential stimulation of phospholipase C and diacylglycerol lipase (Dixon & Hokin, 1984). SALPC may therefore evoke AA release via G protein stimulation because NaF (20 mM), which directly activates G-proteins in the plasma membrane, caused a 2.9 ± 0.09 ($P < 0.001$, n=4)-fold increase in AA release. Direct activation of PLA_2 by the reference compound mellitin, 2 μg ml^{-1}, also caused a marked output of AA, 32.6 ± 4.4 ($P < 0.001$, n=4) times the basal output. Finally, measurements of PGE_2 and amylase in the same experiments reveal that when membrane-bound SALPC is activated by light (18400 lux) peak amylase release (10.6 ± 1.3 x basal; $P < 0.001$, n=7) precedes peak PGE_2 production (5.2 ± 0.9 x basal; $P < 0.001$, n=7).

Light activation of SALPC and singlet oxygen generation may therefore elicit AA mobilization via an action on G-proteins or via PLA_2 activated either directly or by Ca^{2+} influx. Although AA release and eicosanoid production may be important in the longer term cytolytic actions of SALPC they are likely to be parallel rather than causal events in its immediate photodynamic action.

Dixon, J.F. & Hokin, L.E. (1984) *J. Biol. Chem.* 259, 14418-14425.
Matthews, E.K. & Cui, Z.J. (1990) *Biochem. Pharmacol.* 39, 1445-1457.

42P EFFECT OF THIOLS ON THE PHOTODYNAMIC ACTION OF SULPHONATED ALUMINIUM PHTHALOCYANINE ON GUINEA-PIG TAENIA COLI

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Photon activation of halogenated fluorescein derivatives causes a marked calcium-dependent contraction of smooth muscle via a Type II photodynamic action involving the localized generation of highly reactive singlet oxygen (Matthews & Mesler, 1984). In the experiments reported here the taenia coli from the guinea-pig has been used as a model smooth muscle system on which to examine the effects of a new photodynamic drug, tetra-sulphonated aluminium phthalocyanine (SALPC), in the presence and absence of thiol containing reductants. Small lengths (1 cm) of taenia coli were superfused at 32°C and muscle activity recorded isotonically in a light-sealed enclosure. All photodynamic experiments involved pretreatment of the muscle with SALPC (10^{-5} M) for 30 min, followed by washout of the drug for 30 min, and subsequent illumination with a quartz-halogen light source at 3.2 x 10^4 lux ($\lambda > 570$ nm) for a further 30 min. Relaxation responses were determined in muscle precontracted with either carbachol 5 x 10^{-5} M or KCl 23.5 mM. All muscle responses (contraction or relaxation) are expressed as a percentage of that produced by carbachol 5 x 10^{-5} M.

Light activation of SALPC-sensitized muscle elicited a triphasic response: (i) an initial brief contraction, (ii) a subsequent relaxation followed by (iii) a secondary sustained contraction. In precontracted SALPC-sensitized muscle, light activation produced a triphasic relaxation response, i.e. (i) a rapid relaxation followed by (ii) a transient contraction preceding (iii) a secondary sustained relaxation. Neither L-cysteine, 10 mM, N-acetylcysteine 10 mM, nor dithiothreitol (DTT), 5 mM, affected the initial contraction induced by photon activation of SALPC but markedly decreased the secondary sustained contraction from 92.8 ± 1.1% to 17.7 ± 5.8% ($P < 0.001$, n = 4), 95.0 ± 1.8% to 71.7 ± 7.0% ($P < 0.05$, n = 5) and 92.6 ± 0.8% to 5.9 ± 2.4% ($P < 0.001$, n = 4) of the standard carbachol (5 x 10^{-5} M) contraction, respectively. Glutathione (GSH), 10 mM, was without significant effect (n = 4).

In contrast, in precontracted muscle all four thiol reductants potentiated significantly the sustained secondary relaxation seen in response to photon activation of SALPC, with relaxation increasing in magnitude from 33.7 ± 10.2% to 68.1 ± 8.7% ($P < 0.05$, n = 5), 30.4 ± 5.6% to 73.2 ± 8.7% ($P < 0.01$, n = 4), 34.1 ± 5.2% to 67.9 ± 3.3% ($P < 0.001$, n = 5), and 48.2 ± 9.0% to 76.7 ± 5.3% ($P < 0.05$, n = 4), that of the standard carbachol (5 x 10^{-5} M) contraction for L-cysteine, 10 mM, N-acetylcysteine 10 mM, DTT, 5 mM, and GSH, 10 mM, respectively. A membrane permeant cGMP analogue, 8-PCPT-cGMP, 2 x 10^{-4} M, alone caused a triphasic relaxation response similar to that produced by light activation of an SALPC-sensitized precontracted preparation in the presence of thiol reagents. The pattern of 8-PCPT-cGMP-induced relaxation was very similar in taenia coli precontracted with either carbachol, 5 x 10^{-5} M or with KCl, 24.5 mM, making it unlikely that the relaxation response was nerve-mediated.

From these experiments it is concluded that the generation of singlet oxygen by photon-activation of bound SALPC leads to membrane permeabilization, calcium entry and muscle contraction but these effects may be opposed by a direct stimulatory action of singlet oxygen upon guanylate cyclase which is enhanced by the action of thiol reagents and mimicked by cGMP analogues.

Matthews, E.K. & Mesler, D.E. (1984). *Br. J. Pharmacol.* 83, 555-566.

43P INVOLVEMENT OF PROTEIN KINASE C IN FREQUENCY-DEPENDENT FACILITATION OF TRANSMITTER RELEASE FROM SYMPATHETIC NERVE TERMINALS IN THE GUINEA-PIG VAS DEFERENS

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Action potential evoked transmitter release from sympathetic nerve terminals occurs intermittently at the level of the individual varicosity and is monoquantal. The degree of intermittence is related to the frequency of nerve stimulation and the number of stimuli in a train i.e. frequency-dependent facilitation (see Brock & Cunnane, 1988). The biochemical mechanisms underlying facilitation remain obscure. Recently, we have demonstrated that the protein kinase C (PKC) activator, phorbol 12,13-dibutyrate (PDBu) markedly increases transmitter release from the first stimulus in a train (Wardell & Cunnane, 1991). The aim of the present study was to investigate whether PKC plays a physiological role in the transmitter release process by studying the effects of PKC activators and inhibitors on facilitation.

Excitatory junction potentials (EJPs) were recorded from the guinea-pig vas deferens using conventional intracellular recording techniques. Focal extracellular recordings of nerve terminal impulses (NTIs) and excitatory junction currents (EJCs) were also made as previously described (Brock & Cunnane, 1988). Transmitter release was therefore assessed electrophysiologically. The PKC activator PDBu (0.01 - 0.3 μM) markedly affected the pattern of facilitation of EJPs evoked by trains of 25 stimuli at 1 Hz in a concentration dependent manner. For example, 0.1 μM PDBu increased the amplitude of the first EJP in a train by 440 ± 78% (n= 6). The effect of PDBu on extracellularly recorded EJCs was similar to the effect of increasing the frequency of nerve stimulation, namely to increase the number of EJCs evoked per train of stimuli i.e. it increased the probability of release. The PKC inhibitors staurosporine and Ro-31,8220 (3 - 10 μM) when given alone caused a concentration-dependent reduction in the rate of facilitation. Alterations in the pattern of facilitation were not accompanied by detectable changes in either the NTI or the sensitivity of the postjunctional membrane. The potentiating effects of PDBu on EJPs and EJCs were antagonised by staurosporine and Ro-31,8220. When transmitter release was abolished by the calcium - entry blockers ω-conotoxin (0.1 μM) or cadmium chloride (100 μM) PDBu failed to increase transmitter release. Thus, calcium entry into nerve terminals through N-type calcium channels is obligatory for PDBu to exert its effects.

These findings suggest that PKC plays an important role in the release of transmitter from sympathetic nerve terminals in the guinea-pig vas deferens. It seems probable that calcium entering during the nerve action potential triggers the progressive activation of PKC which in turn leads to facilitation by mechanisms not yet elucidated.

Brock, J.A. & Cunnane, T.C. (1988). *J. Physiol.* **399**, 602-632
Wardell, C.F. & Cunnane, T.C. (1991). *J. Auton. Nerv. Sys.* **33**, 174-176

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44P IDENTIFICATION OF [^{3}H]αβ-METHYLENE-ATP BINDING SITES IN RAT BRAIN AND PERIPHERAL TISSUES

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Burnstock and colleagues have described the use of [^{3}H]αβ-methylene-ATP to label purine P_{2X} receptors in rat bladder (Bo & Burnstock, 1990) and vas deferens (Bo et al, 1992). In the present study we have further examined the tissue distribution of these binding sites in the rat.

Washed membranes were prepared from tissues obtained from male Sprague-Dawley rats (250-350g) using published methods (Bo et al., 1992). Binding assays were conducted in 250μl of 50mM Tris 3mM $CaCl_2$ buffer (pH 7.4) for 40 min at 4°C. The radioligand concentration was 2 nM. Incubations were terminated by vacuum filtration over 1mM ATP pre-treated GF/B filters using 10mM KH_2PO_4 buffer (4°C, pH 7.4). In all studies NSB was defined using 0.1mM βγ-methylene-ATP. All results are shown as the mean±SEM from 3-9 experiments.

In preliminary studies, specific ligand binding (>100dpm/mg tissue wet weight) was identified in vas deferens, bladder, spleen, liver, heart and brain. Only low levels of binding (<10dpm/mg tissue) were detected in rat lung or rat salivary gland. In all tissues, binding was rapid ($t_{1/2}$ 5 - 8 min) and fully reversible. In saturation studies the radioligand identified high and low affinity binding sites (see table). Heterogeneity of binding was also evident in the competition studies (using 2nM radioligand) where a range of purine receptor ligands competed for two populations of binding sites. Although the proportion of high and low affinity binding sites identified by the competing ligands varied between tissues, the affinity estimates for the competing ligands at the high affinity sites (see table) and low affinity sites (data not shown) were similar between tissues.

	Saturation Studies				Competition Studies (pIC_{50} at High Affinity Site)			
Tissue	pK_d (High)	Bmax (high)	pK_d (Low)	Bmax (low)	αβ-MeATP	ATP	2-MeS-ATP	Suramin
Vas Deferens	9.14 (0.15)	1012 (85.3)	7.15 (0.40)	7470 (3200)	8.3 (0.20)	7.2 (0.29)	7.1 (0.15)	5.3 (0.19)
Spleen	9.00 (0.03)	1140 (64.1)	7.01 (0.05)	6480 (467)	8.5 (0.29)	7.2 (0.25)	6.8 (0.13)	5.5 (0.23)
Brain	8.92 (0.14)	870 (408)	6.85 (0.02)	14000 (689)	8.2 (0.11)	7.6 (0.02)	6.2 (0.47)	5.6 (0.24)
Heart	8.94 (0.25)	219 (128)	6.80 (0.35)	13400 (9280)	8.8 (0.32)	7.4 (0.08)	6.2 (0.27)	5.7 (0.31)

Bmax is in fmoles/mg protein. 2-me-S-ATP = 2 methyl-thio-ATP. αβ-meATP = αβ-methylene-ATP.

These results confirm a previous report of high and low affinity binding sites for [^{3}H]αβ-meATP in vas deferens (Bo et al., 1992). Furthermore, this study also demonstrates that these binding sites are present in a range of other tissues in the rat, most notably in the brain. Further studies are required in these tissues to determine if the [^{3}H]αβ-meATP binding sites correspond to functional purine P_{2X} receptors.

Bo, X., & Burnstock, G. (1990). Br. J. Pharmacol., 101, 291-296
Bo, X., Simon, J., Burnstock, G., & Barnard, E.A. (1992). J. Biol. Chem., 267, 17581-17587

45P ADENOSINE A_2 RECEPTOR MODULATION OF STRIATAL ACETYLCHOLINE AND GABA RELEASE

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A canine adenosine A_2 receptor cDNA has recently been used to assess the distribution of this receptor in the rat brain. This study indicated that the receptor was expressed primarily in GABA/met-enkephalin containing neurones of the striatum (Schiffman *et al.*, 1991). However, this finding contradicts previous work where the A_2 receptor was found to be localised on the cholinergic interneurones of the striatum (Brown *et al.*, 1990). We have therefore investigated the ability of this receptor to modulate the release of both acetylcholine and GABA from nerve terminals derived from the rat striatum.

Synaptosomes were pre-loaded with radiolabelled neurotransmitter, drawn onto Whatman GF/B filters and perfused with buffer at a rate of 0.5 ml min^{-1}, maintained at 37°C and aerated with 95% O_2 + 5% CO_2. After an initial equilibration period of 36 min eluent was collected at 2 min intervals for scintillation counting. Release was evoked by a 90 s depolarising stimulus of elevated KCl, with modulation of release being assessed by changes in the ratio of the radiolabel released between two stimuli S_1 and S_2 spaced 12 min apart. Experimental controls were run in parallel where both S_1 and S_2 contained elevated KCl alone, whereas in test experiments putative modulators (agonists) were also present in the S_2 stimulation. When antagonists were used they were included in the perfusion buffer 8 min prior to, and during, S_2.

Using this system we found that the A_2 selective agonist CGS21680 inhibited [^{3}H]-GABA release evoked by 15 mM KCl, with a maximum inhibition of 29 ± 4% (n = 14) and an EC_{50} value of 5 x 10^{-11} M. In contrast CGS21680 stimulated the release of [^{3}H]-acetylcholine, with a maximum stimulation of 26 ± 5% (n = 4). Further characterization of the receptors mediating these effects yielded similar pharmacological profiles, i.e. CGS21680 > NECA > PIA, consistent with both receptors being of the A_2 subtype. The A_2 selective antagonists CP66,713 and CGS15943A, but not the A_1 selective antagonist DPCPX, abolished the CGS21680 mediated effects on both acetylcholine and GABA release suggesting the involvement of A_2 receptors (Williams & Jacobsen, 1990).

In contrast when the release of the transmitters was evoked using 30 mM KCl, CGS21680 (10^{-12} M to 10^{-7} M) had no effect on GABA release but did stimulate acetylcholine release (maximum 30 ± 7% (n = 9), EC_{50} 10^{-11} M).

We have therefore demonstrated an A_2 mediated inhibition of GABA release consistent with its localisation in GABA/met-enkephalin containing neurones. We have also shown an A_2 mediated stimulation of acetylcholine release which is consistent with cholinergic localisation of the A_2 receptor. In summary there appears to be an A_2 receptor located on both the GABA/met-enkephalin and the cholinergic containing neurones of the striatum, which suggests that there may be two different A_{2a} receptor subtypes.

Brown, S.J., James, S., Reddington, M. & Richardson, P.J. (1990) *J. Neurochem.* **55**, 31-38.
Schiffman, S.N., Jacobs, O. & Vanderhaeghen, J.J. (1991) *J. Neurochem.* **57**, 1062-1067.
Williams, M. & Jacobsen, K.A. (1990) In *Adenosine and adenosine receptors*, ed. Williams, M. pp. 17-55. New Jersey: Humana.

46P SOLUBILIZATION AND CHARACTERIZATION OF $GABA_B$ RECEPTORS FROM PIG BRAIN

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In order to understand the mechanisms by which ligands of the $GABA_B$ receptor elicit their effects at the molecular level, it is important to solubilize and purify the receptor complex. Ohmori & Kuriyama (1991) have recently described the purification of $GABA_B$ receptors from bovine brain. In an attempt to pursue this further, we are currently performing experiments with porcine brain.

Crude synaptic membranes were prepared from whole pig cerebrum (Zukin *et al.* 1974), washed extensively and preincubated with 5 mM $MgSO_4$. For solubilization of the receptors, membranes were incubated with 0.5 % CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate) and centrifuged at 100,000 x g for 60 min. $GABA_B$ binding was performed as described by Bowery *et al.* (1983). Soluble receptors were precipitated by addition of 0.15 % γ-globulin and 16 % polyethyleneglycol 8000. The maximum concentration of CHAPS tolerated in the binding assay was 0.05 %.

In preliminary experiments various detergents were tested and 0.5 % CHAPS proved to be the most favourable solubilizing 22.7 ± 4.7 (mean ± s.e.mean, n = 6) of $GABA_B$ sites present in the starting material labelled with 5 nM [^{3}H]-GABA. Effects of baclofen and CGP 35348 (P-[3-aminopropyl]-P-diethoxymethylphosphinic acid, Table 1) and Ca^{2+} and GTP sensitivity (Table 2) on $GABA_B$ binding were compared for membrane-bound and solubilized receptors.

Table 1: [^{3}H]-$GABA_B$ binding: IC_{50} values ([μM], mean ± s.d.) of curve fitted to mean data of 3 - 5 experiments

	crude memb	sol memb
(-) baclofen	0.048 ± 0.002	0.024 ± 0.004
(+) baclofen	> 30	> 30
CGP 35348	15.4 ± 1.6	25.6 ± 5.0

Table 2: Ca^{2+} and GTP sensitivity: mean ± s.e.mean values [% of specific binding] of 4 - 6 experiments

	crude memb	sol memb
no Ca^{2+} added [% of binding with 2.5 mM Ca^{2+}]	53.5 ± 4.0	43.8 ± 9.7
100 μM GTP	14.4 ± 1.6	15.3 ± 9.1

Thus, solubilized and membrane-bound $GABA_B$ receptor binding sites appear to exhibit similar characteristics.

M. F. is grateful for financial support from Roche Research Foundation and The Royal Society.

Bowery, N.G., Hill, D.R. and Hudson, A.L. (1983) *Br. J. Pharmac.* 78, 191-206
Ohmori, Y. and Kuriyama, K. (1991) *Neurochemical Research* 16, 357-362
Zukin, S.R., Young, A.B. and Snyder, S.H. (1974) *Proc. Nat. Acad. Sci. USA* 71, 4802-4807

47P IDENTIFICATION OF THE $GABA_A$ RECEPTOR α_3 SUBUNIT IN IMR-32 NEUROBLASTOMA CELLS

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IMR-32 human neuroblastoma cells contain a functional $GABA_A$ receptor, as assessed by radioligand binding and chloride flux (Anderson et al, 1991). We have now investigated the $GABA_A$ receptor subunits expressed in this cell line by immunoblotting using subunit-specific antibodies. IMR 32 cells were maintained in Eagle's medium plus: 10% heat-inactivated foetal calf serum, 1% L-glutamine, 1% non-essential amino acids and 50μgml^{-1} gentamicin. [^{35}S]-TBPS (t-butylbicyclophosphorothionate) and [^{3}H]-SR95531 (2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)pyridazinium bromide) binding used freshly prepared membranes. Western blotting used SDS gel electrophoresis under denaturing conditions in 10% polyacrylamide slab gels, affinity purified peptide antibodies and the ECL western blotting system for detection (Duggan et al, 1991).

Table 1 Analysis of $GABA_A$ receptors in IMR-32 cells and calf cortex

	Immunoblot		[^{35}S]-TBPS binding		[^{3}H]-SR-95531 binding	
	α_1 (Mr 53,000)	α_3 (Mr 58,000)	Kd(nM)	Bmax(fmolmg^{-1} prt)	Kd(nM)	Bmax(fmolmg^{-1} prt)
IMR-32	ND	++	31 ± 2	180 ± 24	43 ± 12	243 ± 30
Calf cortex	+++	++	120 ± 5	2216 ± 170	57 ± 15	475 ± 31

+ = relative intensity of signal. Values as mean ± s.e.mean (n=3). ND = Not detected.

[^{35}S]-TBPS and [^{3}H]-SR-95531 binding sites were present in IMR-32 cell membranes (Table 1). Anti-Cys α3 454-467 GABA receptor subunit antibodies recognised a major band with Mr58000 in IMR-32 membranes which was coincident with the immunoreactive species in calf cerebral cortex membranes. The signal was prevented by prior incubation with peptide (200nM). Therefore one subunit of the $GABA_A$ receptor is expressed in IMR-32 cells. This cell line may express a homogeneous population of $GABA_A$ receptors, and thus provide a useful model for studying $GABA_A$ receptor structure and regulation.

Anderson, S.M.P., Cross, A.J., De Souza, R.J. & Green, A.R. (1991) Br.J. Pharmacol. **104**, 37P.
Duggan, M.J., Pollard, S. & Stephenson, F.A. (1991) J. Biol. Chem. **266**: 24778-24784.

48P THE EFFECT OF THE PARTIAL BENZODIAZEPINE RECEPTOR AGONIST FG 8205 AND DIAZEPAM IN RODENT MODELS OF SEDATION

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Recent reports suggest that FG 8205, a partial benzodiazepine receptor agonist, induces an anxiolytic-like effect in rodents and primates in the absence of marked sedative effects (Tricklebank *et al.*, 1990). For example, in a rat conditioned emotional response test, the minimum effective dose (MED) of FG 8205 was 0.5 mg/kg, while motor deficits revealed in a rat swim test, though present at these doses, were slight. However, the testing session for the swim test was less than 60s and it is possible that during this short period, the stress-induced by the test may increase the activity of the rat and consequently mask the sedative properties of FG 8205. As a consequence the effects of FG 8205 have been compared to the full benzodiazepine receptor agonist, diazepam, in two additional tests of sedation in the rat: spontaneous locomotor activity and chain-pulling on a random interval (RI) 30s schedule for food rewards.

In order to measure locomotor activity, male hooded-Lister rats (210-250g, Bantin and Kingman, Hull, U.K., n=8 per group) were placed in perspex boxes (300 x 210 x 300 mm) equipped with photo beams, 30 min after p.o. administration of either vehicle (0.5% methocell + 0.02% Tween 80) or FG 8205 (0.3 - 30.0 mg/kg) or diazepam (0.3 - 30.0 mg/kg). Locomotor activity was determined by the total number of beam-breaks counted during the session. In the chain-pulling experiment, male hooded-Lister rats (210-250g, Bantin and Kingman, Hull, U.K., n=10 per group) maintained at 85% of their free-feeding weight were trained in operant boxes to pull a chain for food rewards which were delivered on average every 30s. On the test day the rats were placed in the operant boxes 30 min after p.o. administration of either vehicle or FG 8205 (1.0 - 30.0 mg/kg) or diazepam (1.0 - 30.0 mg/kg). Following a significant ANOVA *post hoc* Newman Keuls tests were used to test significant differences between groups.

Both FG 8205 and diazepam dose-dependently decreased locomotor activity (MED=1.0mg/kg and 3.0 mg/kg respectively). Doses of 30 mg/kg of FG 8205 and diazepam reduced beam breaks by 81% and 61% respectively (mean beam breaks/ 40 min ± s.e. mean: Vehicle=316±31, FG 8205=59±31; Vehicle= 442±33, diazepam=176±25). Similar effects were seen in the chain pulling experiment with FG 8205 which dose-dependently decreased chain-pulling rates (MED = 1.0 mg/kg; mean chain-pulls/min ± s.e. mean: Vehicle=11.7±2.1; FG 8205 (30 mg/kg)=1.7±0.1). Although diazepam also dose-dependently reduced chain-pulling rates (mean chain-pulls/min ± s.e. mean: Vehicle=15.6±2.8; diazepam(30 mg/kg)=3.8±2.1), the MED (10 mg/kg) was higher than that seen in the locomotor activity experiment (3 mg/kg).

These results suggest that when tested over an extended period anxiolytic doses of FG 8205 have sedative properties of similar magnitude to those induced by the full benzodiazepine receptor agonist diazepam.

Tricklebank, M.D., Honore, T., Iversen, S.D., Kemp, J.A., Knight, A.R., Marshall, G.R., Rupniak, N.M.J., Singh, L., Tye, S., Watjen, F. & Wong, E.H.F. (1990) *Br. J. Pharmacol.* **101**, 753-761.

49P CORRELATION BETWEEN THE POTENCIES OF DIFFERENT (BZ)ω RECEPTOR LIGANDS TO PRODUCE A ROTOROD DEFICIT AND INHIBITION OF *IN VIVO* [^{3}H]-FLUMAZENIL BINDING

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Benzodiazepines and other compounds with affinity for (Bz) ω sites have a complex pharmacology consisting of anti-convulsant, anxiolytic, muscle-relaxant and sedative actions. It is not yet clear, however, whether these different pharmacological effects are associated with activity at different subtypes of receptor. Several recently described compounds, including the imidazopyridines zolpidem and alpidem and the β-carboline, abecarnil, have been reported to have less propensity to produce muscle relaxation and ataxia and, as these drugs have also been shown to have selectivity for (Bz_1) ω_1 sites, it has been proposed that muscle relaxation may be associated with activity at (Bz_2) ω_2 sites (Benavides et al, 1988).

The present study set out to investigate this hypothesis using 14 compounds taken from several chemical classes, but which all have known affinity for (Bz) ω sites (triazolam, lorazepam, flunitrazepam, suriclone, brotizolam, alprazolam, clonazepam, midazolam, diazepam, zopiclone, zolpidem, oxoquazepam, tetrazepam and CL 218,872). Correlations were calculated between the potencies of these compounds to produce a rotarod deficit in rats (a measure of motor coordination at least partly related to muscle relaxation) and their potencies to inhibit in vivo [^{3}H]-flumazenil binding in different regions of the central nervous system (Benavides et al, 1992). It was reasoned that, as different CNS regions contain different proportions of (Bz_1) ω_1 and (Bz_2) ω_2 sites, correlations should be higher for regions containing relatively greater concentrations of (Bz_2) ω_2 sites if activity at this receptor subtype is responsible for the rotarod deficit.

All compounds produced dose-related decreases in the time rats were able to stay on the rotarod with ED_{50} values ranging from 0.34 mg/kg for triazolam to 18.8 mg/kg for CL 218,872. Potencies to produce the rotarod deficit were positively correlated with inhibition of [^{3}H]-flumazenil binding for all CNS regions. However, the correlation was higher (r_s = 0.84, $p < 0.001$) for the spinal cord, which contains a higher proportions of ω_2 (Bz_2) receptors, than for the cerebellum which contains a lower proportion of (Bz_2) ω_2 sites ($r_s = 0.72$, $p < 0.01$). These results, therefore, are consistent with the hypothesis that activity at (Bz_2) ω_2 receptors is associated with the rotarod deficit. However, interpretation of these results is complicated by the observation that doses of the different drugs which produced this deficit were associated with a wide range of levels of receptor occupation. Thus, the ED_{50} dose of flunitrazepam produced 19 % receptor occupation in the spinal cord whereas the equivalent dose of clonazepam gave rise to 78 % occupation. This indicates that the different compounds tested may have a wide range of different intrinsic activities at (Bz) ω receptors mediating their myorelaxant effect.

Benavides, J., Peny, B., Dubois, A., Perrault, G., Morel, E., Zivkovic, B. and Scatton, B. (1988). J Pharmacol Exp Ther 245, 1033-1041
Benavides, J., Peny, B., Durand, A., Arbilla, S. and Scatton, B. (1992). J. Pharmacol. Exp. Ther. 263, 884-896.

50P PRIOR PLUS-MAZE EXPERIENCE ABOLISHES ANXIOLYTIC RESPONSE TO DIAZEPAM IN THE MURINE LIGHT/DARK TEST

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A single prior undrugged exposure of rats or mice to the elevated plus-maze can abolish the anxiolytic response to benzodiazepines on retest at intervals ranging from 48h to 14 days (Lister, 1987; File et al., 1990; Rodgers et al., 1992). In the present study, we have examined whether such experience alters subsequent diazepam response in a different model of anxiety, the light/dark exploration test. Subjects were 120 group-housed adult male DBA/2 mice. On day 1, half of the subjects were exposed to the elevated plus-maze for 5 min while the remainder were merely transported to the test laboratory. On days 2-9, equal numbers of exposed and non-exposed mice received daily i.p. injections of vehicle (saline/Tween), 2.0 or 4.0 mg/kg diazepam. Thirty minutes after the final injection, mice were placed (5 min) either on the centre platform of the elevated plus-maze or in the centre of the light compartment of the light-dark box. Test sessions were recorded on videotape, and data analyzed by ANOVA.

Table: Effects of prior plus-maze exposure on response to diazepam (2-4 mg/kg) in the plus-maze (open arm measures) and light/dark (light compartment (L) measures) tests. * $P<0.05$, ** $P<0.005$ vs veh; #$P<0.05$ vs.non-exposed.

	Non-Exposed			Exposed		
	0	**2**	**4**	**0**	**2**	**4**
% open entries	24.6±3.0	42.9±2.7**	44.9±2.2**	19.3±2.0	23.0±1.6	21.5±2.2
% open time	13.3±1.4	36.4±2.4**	38.0±2.5**	7.2±1.2	10.6±1.9	10.5±4.2
% line Xs (L)	24.8±2.3	39.4±3.1*	37.1±3.4*	35.8±4.3#	42.6±4.8	36.7±4.1
% rears (L)	28.4±3.9	46.7±3.3*	38.8±3.8	42.2±3.8#	45.9±6.6	41.4±5.0
% time (L)	19.2±2.3	31.1±1.7*	30.3±2.9*	28.0±1.7#	34.4±3.2	32.1±5.6

While prior experience of the maze per se tended to reduce retest measures of open arm activity, it actually increased light compartment activity in the light/dark test. Despite this differential effect on control patterns of behaviour, the results show that such experience abolishes the anxiolytic response to diazepam in both the elevated plus-maze and light/dark tests of anxiety. We conclude that prior maze experience generally alters the influence of diazepam on responses to novel, potentially dangerous environments.

File, S.E. Mabbutt P.S. & Hitchcott, P.K. (1990). Psychopharmacology 102: 98-101.
Lister, R.G. (1987) Psychopharmacology 92: 180-185.
Rodgers, R.J., Lee, C. & Shepherd J.K. (1992) Psychopharmacology 106:102-110.

51P EFFECT OF ENTORHINAL INJECTION OF VOLKENSIN ON THE STRUCTURE AND NEUROTRANSMITTER RECEPTORS OF THE HIPPOCAMPUS

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Volkensin, a suicide transport agent, has been used previously to destroy cortico-striatal pyramidal neurones (Pangalos et al, 1991). In the present study, pyramidal neurones of the hippocampal-entorhinal pathway have been studied.

Rats were anaesthetised (Halothane/N_2O/O_2), placed in a stereotactic frame, and injections of volkensin (0.5μl; 4ng/μl) were made at 2 sites into the left posterior ventral entorhinal cortex. 14 (n=5) or 21 (n=6) days later, the animals were transcardially perfused (phosphate buffered saline (PBS) followed by 20% sucrose in PBS), the brain rapidly frozen (isopentane/dry ice at -40°C), sectioned (12μm on a cryostat) and the total area of the dorsal CA1, CA3 and dentate gyrus , assessed by Nissl staining, determined ipsi- and contra-lateral to the volkensin injection (length of cell layer at 8-12 levels within the ventral hippocampal area multiplied by the anterior-posterior distance between). Atrophy was observed, based on the area of ipsilateral compared with contralateral CA3. This was significantly reduced by 24% at 14 days, (P=0.018, Student's paired t-test) and 17% at 21 days, (P=0.014). No histological changes were observed in CA1 or dentate gyrus. In closely matched sections, binding (density and area or length) to the NMDA, m_1, kainate, $5HT_{1A}$, and $GABA_A$ receptors was determined by autoradiography (Chu et al, 1990; Ulas et al, 1990; Bowen et al, 1992) for the hippocampal fields. A ratio of either length or area of receptor binding to that of the corresponding hippocampal cell field (Nissl stained sections) for ipsi- and contralateral hippocampi was obtained. There were no significant side to side difference in this ratio for indices of NMDA, m_1, kainate or $5HT_{1A}$ for CA1 or CA3. The ipsilateral CA1+CA3 area ratio for $GABA_A$ appeared to be increased (19%, p=0.05) compared to that contralaterally, but only at 14 days. Although there was no apparent dentate atrophy, at 21 days there was reduced binding to the $5HT_{1A}$ (13%, p=0.02) and $GABA_A$ (19%, p=0.011) receptors. There were no significant changes in grey levels (reflecting density) for any receptor. The most straightforward interpretation of these results is that cell loss caused atrophy and the receptors studied (except $GABA_A$) are present on CA3 pyramidal neurones. The lack of change in grey levels suggests that the cells lost do not carry a disproportionate number of any one receptor compared to the remaining neurones. In contrast, some neocortical pyramidal cells appeared enriched with the $5HT_{1A}$ receptor (Francis et al, 1992).

Supported by The Brain Research Trust ("Miriam Marks Department"), and The Wellcome Trust.

Bowen, D.M., Francis, P.T., Pangalos, M.N. & Chessell, I.P. (1992). J. Reprod. Fert. In Press.
Chu, D.C.M., Albin, R.L., Young A.B. & Penney, J.B. (1990). Neurosci. **34** 341-357.
Francis, P.T., Pangalos, M.N., Pearson, R.C.A., Middlemiss D.N., Stratmann, G.C. & Bowen, D.M. (1992). J. Pharmacol. Exp. Therapeut. **261** 1273-1281.
Pangalos, M.N., Francis, P.T., Middlemiss, D.N., Pearson, R.C.A. & Bowen, D.M. (1991). J. Neurosci. Meth. **40** 17-29.
Ulas, J., Monaghan, D.T. & Cotman, C.W. (1990). J. Neurosci. **10** 2352-2362.

52P ACUTE AND SUBACUTE EFFECTS OF DOPAMINE AGONISTS ON NEUROPEPTIDE GENE EXPRESSION IN THE STRIATUM OF THE RAT

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The descending striatal neurons containing enkephalin and substance P are differentially regulated by the dopaminergic input from the substantia nigra (Graybiel, 1990). Lesions of the substantia nigra with 6-OHDA cause parallel elevations of D-2 receptor and enkephalin gene expression in the striatopallidal pathway, which are reversed by administration of the D-2 agonist quinpirole (Gerfen et al., 1990; Pollack and Wooten, 1992). In contrast, 6-OHDA induces a decrease of D-1 receptor and substance P mRNA levels of striatonigral neurons. These are reversed by treatment with SKF38393, a D-1 agonist (Gerfen et al., 1990). However, little is known of the regulation of striatal peptide gene expression by different dopamine receptor subtypes in normal rats.

The abundance of enkephalin and substance P mRNA in the striatum was studied after acute and subacute (twice daily, 7 days) administration of D-1 and D-2 agonists. Male Wistar rats were given subcutaneously SKF38393 (5 mg/kg), quinpirole (5 mg/kg), apomorphine (5 mg/kg), piribedil (10 mg/kg), pergolide (0.5 mg/kg) or saline. Peptide mRNA levels were assessed from the coronal brain sections (12 m) using in situ hybridization histochemistry as described previously (Young et al., 1986). After hybridization with ^{35}S-labeled oligonucleotides complementary to preproenkephalin A or preprotachykinin cDNA, sections were apposed to a film and subsequently analysed by computerized densitometry.

None of the dopamine agonists given acutely resulted in alterations of the striatal enkephalin mRNA. Repeated injections of pergolide decreased enkephalin mRNA by 23% ($p<0.01$). There was also a trend toward a lower enkephalin mRNA level after subacute administration of apomorphine ($p=0.057$). Abundance of substance P mRNA was affected following acute apomorphine (+25%, $p<0.01$) and subacute pergolide (+35%, $p<0.05$) treatment.

The expression of enkephalin and substance P mRNA in the striatum showed a significant resistance to dopaminergic manipulation in the normal rat brain. This may be a consequence of maximal endogenous dopaminergic control of peptide gene expression, which can not be further enhanced by agonist treatment.

Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma Jr, F.J. and Sibley, D.R. (1990) Science. 250, 1429-1432.
Graybiel, A.M. (1990) TINS. 13, 244-254.
Pollack, A.E. and Wooten, G.F. (1992) Mol. Brain Res. 12, 111-119.
Young III, W.S., Bonner, T. and Brann, M.R. (1986) Proc. Natl. Acad. Sci. 83, 9827-9831.

53P MODIFICATION OF GLUTAMATE AND GABA-MEDIATED SYNAPTIC TRANSMISSION BY DOPAMINE IN THE RAT ENTORHINAL CORTEX *IN VITRO*

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It has been postulated that schizophrenia may involve a decline in activity of excitatory glutamate pathways which is manifested as an apparent overactivity of an opposing dopamine (DA) inhibitory influence (e.g. Wachtel and Turski, 1990). We have recently suggested (Pralong and Jones, 1992) that such an imbalance could occur in the entorhinal cortex (EC) an area which shows severe disruption of neuronal organization in schizophrenics (Arnold *et al*, 1991). In a previous communication (Pralong and Jones, 1992) we described the direct effects of DA on neurones recorded intracellularly in a slice preparation of the rat EC. The present abstract describes preliminary observations concerning the effects of DA on synaptic responses of neurones in the EC. Slices of the EC were cut from the rat brain and maintained *in vitro* at the interface between artificial CSF (34°C) and carbogen gas. Conventional intracellular recordings were made from neurones in layer II. Synaptic responses were elicited with bipolar platinum stimulation electrodes positioned in afferent pathways. DA and other agents were applied by bath perfusion.

Synaptic responses of layer II cells were complex (see Jones and Heinemann, 1991) and exhibited fast and slow EPSPs mediated by AMPA and NMDA receptors, and fast and slow IPSPs mediated by $GABA_A$ and $GABA_B$ receptors, respectively. DA (10-250 µM) reduced the amplitude of all components of the untreated synaptic potentials but, because of the complex nature of these potentials, it was possible to quantify this effect only in the case of the fast EPSP. DA (100 µM) reduced the amplitude of this by 30±5 % (mean ± s.e.m., n=17). In the presence of the D2 antagonist, sulpiride (30 µM) the reduction of the fast EPSP was 17±6% (n=11) and when the D1 antagonist SCH23390 (30 µM) was included the reduction was 11±3% (n=9). The D1 agonist SKF38393 (30 µM) reduced the fast EPSP by 18±4% (n=9) whereas the D2 agonist produced only a 10±5% decrease (n=7). To examine the effects of DA more specifically we have begun to look at pharmacologically isolated components of the synaptic potentials. The slow EPSP was recorded in the presence of CNQX (10 µM) and bicuculline (5 µM). DA reduced this NMDA-receptor mediated EPSP by 56±4% (n=14). DA in the presence of sulpiride (30 µM) produced a 47±7% reduction (n=3) whereas when SCH23390 (30 µM) was added the reduction was only 17±2%, (n=9). Finally, examination of a monosynaptic fast IPSP recorded in the presence of CNQX (10 µM) and 2-AP5 (20 µM) showed a reduction of this potential of 31±6% (n=5) by DA. The results suggest that both D1 and D2 receptors may modulate glutamate transmission in the EC although the effect on the isolated NMDA-receptor mediated EPSP suggest a more prominent role of D1. In addition, GABAergic transmission is also modulated by DA although the receptors involved have not yet been defined.

Arnold, S.E., Hyman, B.T., Van Hoesen, G.W. and Damasio, A.G. (1991) *Arch. Gen. Psychiat.*48, 625-632
Jones, R.S.G. and Heinemann, U. (1991) in *Excitatory amino acids and synaptic transmission.* ed. Wheal, H.V. and Thomson, A.T. pp 265-285. London: Academic Press
Pralong E. and Jones, R.S.G. (1992) *Br. J. Pharmac.* (in press)
Wachtel, H. and Turski, L. (1990) *TIPS.* 13, 219-220

Supported by the Royal Society and the Wellcome Trust. EP is a Royal Society-Swiss National Science Foundation Invited Fellow.

54P BEHAVIOURAL RESPONSES TO THE PUTATIVE D_3 DOPAMINE RECEPTOR AGONIST 7-OH-DPAT IN COMPARISON WITH OTHER 'D_2-LIKE' AGONISTS

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Molecular biology has revealed families of 'D-1-like' [$D\text{-}1_A$, $D\text{-}1_B$/D-5] and 'D-2-like' [$D\text{-}2_{L/S}$, D-3, D-4] dopamine (DA) receptors (Sibley & Monsma, 1992). In the absence of selective agents able to discriminate between individual members of each family, their behavioural roles have remained unexplored. Recently, 7-OH-DPAT has been identified (Levesque et al, 1992) as a selective D-3 agonist, and we have studied its behavioural effects in comparison with other 'D-2-like' agonists: RU 24213, the putative 'autoreceptor' agonist B-HT 920 (Waddington & O'Boyle, 1989), and the preferential D-3 agonist quinelorane (LY 163502; Sokoloff et al, 1992).

Using previously described procedures (Daly & Waddington, 1992), male rats (n=8) were challenged s.c. with drugs and behavioural responses quantified using a rapid time-sampling behavioural check-list technique. At 10 µg/kg, 7-OH-DPAT reduced ($P<0.05$) the levels of spontaneous sniffing and rearing shown by vehicle controls, while at higher doses (0.1-10.0 mg/kg), 7-OH-DPAT failed to induce any stereotypy but induced ($P<0.01$) episodes of sniffing, locomotion and, weakly ($P<0.05$), chewing. In the absence of D-3-selective compounds, studies with 'D-2-like' antagonists are not informative; however, dose-response curves for the induction of sniffing and of locomotion by 7-OH-DPAT were each shifted readily to the right ($P<0.01$) by 5.0 mg/kg of the selective D-1 antagonist BW 737C, without evidence of myoclonic jerking that such pretreatment releases as a response to B-HT 920, quinelorane and RU 24213 (Daly & Waddington, 1992).

This reduction in spontaneous activity effected by low doses of 7-OH-DPAT would be consistent with a 'D-2-like' autoreceptor-mediated effect, via D-3 receptors of known presynaptic localisation and high affinity for DA (Levesque et al, 1992), but a role for inhibitory postsynaptic sites can not be excluded. At higher doses, 7-OH-DPAT induced episodes of non-stereotyped DA-ergic behaviours, the profile of which was distinct from those induced by B-HT 920, RU 24213 or quinelorane, though only 7-OH-DPAT and quinelorane induced non- stereotyped chewing; 7-OH-DPAT seems to act at a site participating in co-operative but not in oppositional 'D-1-like':'D-2-like' interactions. It can not be excluded that these behavioural responses to the putative selective D-3 agonist 7-OH-DPAT reflect activity at other 'D-2-like' receptors at higher doses, and these issues will be clarified only when selective D-3 antagonists are identified.

This work was supported by the Wellcome Trust. We thank Boehringer, Lilly, Roussel and Wellcome Foundation for gifts of drugs; 7-OH-DPAT was kindly made available under the RBI/NIMH synthesis programme.

Daly, S.A. & Waddington, J.L. (1992) Eur. J. Pharmacol. 213, 251-258.
Levesque, D., Diaz, J., Pilon, C. et al (1992) Proc. Natl. Acad. Sci. 89, 8155-8159.
Sibley, D.R. & Monsma, F.J. (1992) Trends Pharmacol. Sci. 13, 61-69.
Waddington, J.L. & O'Boyle, K.M. (1989) Pharmacol. Ther. 43, 1-52.
Sokoloff, P., Andrieux, M., Besancon, R. et al (1992) Eur. J. Pharmacol.-Mol. Pharmacol. 225, 331-337.

55P A CLOZAPINE-INSENSITIVE RESPONSE TO DOPAMINE AGONISTS IN RAT SUBSTANTIA NIGRA

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Dopamine receptors can be classified into five types, D_1 to D_5, according to molecular biological and pharmacological criteria. In vitro electrophysiological studies in the rat substantia nigra have shown that the spontaneous activity of dopaminergic neurones can be inhibited by activation of somatodendritic autoreceptors of the D_2 type (Lacey et al., 1987). Results from the present study show that the dopamine agonists quinpirole and N,N-dipropylamino-5,6-dihydroxytetralin (dpADTN) differ in their susceptibility to antagonism by D_2 antagonists and that dpADTN is not antagonised by the atypical neuroleptic clozapine.

Substantia nigra slices (350μm) from male Wistar-derived rats (100-180g) were mounted for extracellular recording and superfused with oxygenated artificial cerebrospinal fluid containing Sch 23390, phentolamine and methysergide (all 1.0μM). Dopaminergic neurones were identified as such from characteristics described by Grace and Bunney, (1983). All drugs were applied in the superfusion medium and allowed sufficient time to equilibrate with the tissue. Each pA_2 value was calculated from at least three experiments.

The spontaneous firing of the cells was concentration-dependently inhibited by dopamine (3 - 30μM) and by two D_2-selective agonists, quinpirole (0.01-0.1μM) and dpADTN, (0.03-0.3μM). The response to quinpirole was antagonised by four D_2 antagonists with pA_2 values corresponding to their D_2 binding pK_i values obtained in rat striatal membranes using ^{3}H-spiperone as ligand; table 1. In contrast, the antagonism of dpADTN by haloperidol, sulpiride and metoclopramide produced Schild plots with slopes less than unity and significantly lower pA_2 values (table 1; 95% confidence limits in brackets). Clozapine was almost inactive at 30μM causing at most a 3-fold shift of the concentration-response curve.

Table 1 Potency values for antagonism of three dopamine agonists by D_2 antagonists.

Antagonist	Values of pA_2 and Schild slope for antagonism of:						Binding affinity (pK_i)
	Quinpirole		dpADTN		Dopamine		
Haloperidol	8.9 (8.7-9.1)	1.1 (1.0-1.2)	7.6 (7.3-7.9)	0.6 (0.4-0.8)	7.8 (7.5-8.1)	1.3 (1.2-1.4)	8.9 (8.7-9.1)
(±)-Sulpiride	7.5 (7.2-7.8)	1.0 (0.9-1.1)	6.6 (6.4-6.8)	0.8 (0.6-1.0)	not tested		7.6 (7.4-7.8)
Metoclopramide	8.1 (7.9-8.3)	1.1 (1.0-1.2)	7.1 (6.9-7.3)	0.7 (0.5-0.9)	not tested		7.9 (7.7-8.1)
Clozapine	6.6 (6.3-6.9)	0.9 (1.0-1.2)	<5		not tested		6.7 (6.5-6.9)

Thus, in addition to the expected values for quinpirole there appears to be an inhibitory effect of dpADTN which is less potently antagonised by the neuroleptics tested, with clozapine being extremely weak. These antagonist potency values do not implicate D_3 or D_4 receptors. The pharmacological characterization of the response to quinpirole is consistent with an action at a single receptor whereas the response to dpADTN is more complex, one explanation being interaction with another receptor.

Grace, A.A and Bunney, B.S. (1983). Neurosci., 10, 301-315
Lacey, M.G. et al (1987). J. Physiol.(Lond.), 392, 397-416.

56P EVIDENCE FOR A CLOZAPINE-INSENSITIVE RESPONSE TO THE DOPAMINE AGONIST, N,N-DIPROPYLAMINO-5,6-DIHYDROXYTETRALIN (dpADTN), ON DOPAMINERGIC NERVE TERMINALS

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We have previously used the technique of fast cyclic voltammetry in brain slices to confirm the presence of D_2 autoreceptors on the terminals of dopaminergic neurones (Bull et al., 1991). Subsequently, we have found that the inhibition of dopamine release by the D_2 receptor agonist, dpADTN, is poorly antagonised by D_2 receptor antagonists, and particularly by the atypical antipsychotic drug, clozapine.

Dopamine release was evoked by a single, locally-applied electrical pulse every 2 min in either striatal or accumbens slices from Wistar-derived rats, and measured by fast cyclic voltammetry (Bull et al., 1990). Stable release could be recorded for several hours. Drugs were added to the superfusate, which included dopamine D_1 and α-adrenoceptor antagonists. Values presented are from three separate experiments. The dopamine D_2 receptor affinity of antagonists was also measured in competition binding assays using [^{3}H]spiperone in rat striatal membrane homogenates. These values are presented as mean pK_i of at least 3 determinations.

Quinpirole (0.01-0.1μM) and dpADTN (0.03-0.3μM) concentration-dependently inhibited the evoked release of dopamine in both striatum and nucleus accumbens. After 45 min equilibration with antagonist the response to quinpirole was antagonised in both tissues by haloperidol, sulpiride, metoclopramide and clozapine with potency values equal to those found in D_2 binding studies (table 1). However, the response to dpADTN was antagonised by haloperidol, sulpiride and metoclopramide with lower potency, and clozapine did not significantly antagonise the response at all (table 1). Schild slopes were also low.

Table 1 Potency (pA_2 value) of D_2 antagonists in striatum and nucleus accumbens in antagonising quinpirole- or dpADTN-induced inhibition of dopamine release (Values in parentheses are 95% confidence limits)

Antagonist	Striatum				Nucleus accumbens				D_2 binding
	Quinpirole		dpADTN		Quinpirole		dpADTN		
	pA_2	slope	pA_2	slope	pA_2	slope	pA_2	slope	pK_i
Haloperidol	9.1 (8.8-9.4)	1.2 (1.1-1.3)	7.6 (7.3-7.9)	0.7 (0.4-1.0)	8.8 (8.6-9.0)	1.1 (0.9-1.3)	6.7 (6.4-7.0)	0.7 (0.4-1.0)	8.9 (8.7-9.1)
(±)Sulpiride	7.6 (7.5-8.2)	0.9 (0.8-1.0)	6.4 (6.1-6.7)	0.7 (0.5-0.9)	7.6 (7.4-7.8)	1.1 (1.0-1.2)	6.4 (6.1-6.7)	0.7 (0.4-1.0)	7.6 (7.4-7.8)
Metoclopramide	7.6 (7.4-7.8)	0.8 (0.6-1.0)	6.6 (6.3-6.9)	0.6 (0.3-0.9)	7.2 (7.0-7.4)	0.9 (0.7-1.1)	6.8 (6.6-7.0)	0.6 (0.3-0.9)	7.9 (7.7-8.1)
Clozapine	7.0 (6.6-7.4)	0.9 (0.8-1.0)	<5	-	7.1 (7.0-7.2)	0.9 (0.8-1.0)	<4.5	-	6.7 (6.5-6.9)

One possible interpretation of these data is that in addition to the D_2 receptor there is also a novel type of dopamine autoreceptor on dopaminergic nerve terminals, activation of which inhibits neurotransmitter release. This putative receptor appears to be present on both mesolimbic and nigro-striatal neurones, but there is as yet no evidence to indicate whether it may also be present on postsynaptic neurones.

Bull, D.R. et al. (1990). J. Neurosci. Meth., 32, 37-44.
Bull, D.R. and Sheehan, M.J. (1991). Br. J. Pharmac., 102, 232P

57P BRADYKININ IS THE MAIN MEDIATOR OF COLLAGENASE-INDUCED OEDEMA IN THE RAT PAW

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The collagenase activity of the ß-toxin is thought to play a major role in the pathology of gas gangrene caused by *Clostridium histolyticum*, especially because it can destroy the connective tissue barrier (Hatheway, 1990). A typical symptom of gas gangrene is a pronounced oedema which rapidly spreads from the point of entry of the bacteria. We have investigated the mediators by which collagenase (EC 3.4.24.3) from *Clostridium histolyticum* causes oedema and plasma protein extravasation in the hindpaw of rats anaesthetized by pentobarbitone.

The subplantar injection of collagenase (100 µg in 100 µl saline) into the hindpaw induced paw oedema and plasma protein extravasation, which were measured by a plethysmometer and the extravasation of Evans blue, respectively. Paw oedema reached a maximal volume within 30 min and remained stable for at least 5 hours.

The following pretreatments were used to elucidate possible mediators of the collagenase-induced paw oedema: (1) the bradykinin antagonist HOE-140 [DArg^0-Hyp^3-Thi^5-DTic^7-Oic^8-bradykinin] (100 nmol kg^{-1}, s.c.), (2) capsaicin (125 mg kg^{-1}, s.c., 21 days before the experiments), (3) the substance P antagonist CP-96,345 or its inactive enantiomer CP-96,344 (both 10 µmol kg^{-1}, i.v) (Lembeck *et al.*, 1992), (4) indomethacin (10 and 30 µmol kg^{-1}, i.p.), (5) mepyramine (35 µmol kg^{-1}, i.p.) plus cimetidine (40 µmol kg^{-1}, i.p.), (6) captopril (10 and 50 µmol kg^{-1}, i.v.).

Subplantar injection of saline increased paw volume by 21 ± 1 %. HOE-140 pretreatment significantly ($P<0.01$) inhibited both the collagenase-induced paw oedema (from 54 ± 4 to 32 ± 2 % paw volume increase) and plasma protein extravasation (from 73 ± 4 to 28 ± 5 pmol E.blue mg^{-1} wet wt). The inhibition caused by HOE-140 was significantly ($P<0.05$) greater than that caused by capsaicin (from 54 ± 4 to 43 ± 4 % paw volume increase, and from 73 ± 4 to 42 ± 5 pmol E.blue mg^{-1} wet wt). The inhibitory effect of capsaicin given together with HOE-140 did not exceed that of HOE-140 alone. CP-96,345 inhibited the paw oedema (from 57 ± 3 to 40 ± 1 % paw volume increase) to a similar extent as capsaicin but did not inhibit the plasma protein extravasation. Pretreatment with HOE-140 plus CP-96,345 inhibited the paw oedema (from 57 ± 3 to 31 ± 3 % paw volume increase) to a greater extent than CP-96,345 alone, and also significantly ($P<0.001$) inhibited plasma protein extravasation (from 76 ± 6 to 32 ± 6 pmol E.blue mg^{-1} wet wt). Pretreatment with HOE-140 plus CP-96,345, however, was no more effective than HOE-140 alone. CP-96,344 (the inactive enantiomer) did not inhibit paw oedema indicating the effect of CP-96,345 to be specific. Indomethacin, mepyramine plus cimetidine, or captopril did not affect paw oedema or plasma protein extravasation.

The collagenase-induced paw oedema provides a model of oedema with pathological relevance and has the advantages of a fast onset and stability for several hours. Bradykinin appears to be the main inflammatory mediator of the collagenase-induced paw oedema and plasma protein extravasation. Part of the effect of bradykinin arises from release of substance P from sensory nerves. Captopril does not increase the effects of collagenase probably because the activity of angiotensin converting enzyme has already been abolished by peptide inhibitors produced from collagen by the collagenase (Oshima *et al.* 1979). Histamine and prostaglandins seem not to be involved.

Hatheway, C.L. (1990) *Clin. Microbiol. Rev.* 3/1, 66-98
Lembeck, F., Donnerer, J., Tsuchiya, M. & Nagahisa, A. (1992) *Br. J. Pharmacol.* 105, 527-530
Oshima, G., Shimabukuro, H. & Nagasawa, K. (1979) *Biochim. Biophys. Acta* 566, 128-137

58P FATE OF PANCREATIC ENZYMES IN CAERULEIN-INDUCED ACUTE PANCREATITIS AND EFFECTS OF THE BRADYKININ ANTAGONIST, HOE 140

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Hyperstimulation of the exocrine function of the pancreas by i.v. infusion of high doses of the cholecystokinin analogue, caerulein, in rats leads to morphological changes of the gland similar to those observed in the most frequent clinical form of acute pancreatitis. Pretreatment with the long-acting bradykinin antagonist, DArg-[Hyp^3-Thi^5-DTic^7-Oic^8]-bradykinin (HOE 140) (Lembeck *et al.*, 1991), prevents the development of hypotension and the pancreatic oedema (Griesbacher & Lembeck, 1992). This greatly facilitates the egress of activated enzymes from the pancreas, resulting in a significant augmentation of the activities of these enzymes in the blood serum (Griesbacher *et al.*, 1993). In order to clarify whether pancreatic enzymes appear in the blood after secretion into the small intestine or after direct absorption into the circulation, the activities of amylase and lipase were determined in the common pancreatic/bile duct.

Rats, anaesthetized with pentobarbitone and phenobarbitone, were pretreated with captopril (50 µmol/kg, i.p.). Caerulein (4 nmol/kg/h) was infused i.v. for a period of 120 min. HOE 140 (100 nmol/kg) was injected s.c. 30 min prior to the caerulein infusion. The pancreatic/bile fluid was collected in fractions of 10 min from the common pancreatic/bile duct close to the duodenum. In caerulein-induced acute pancreatitis, the activities of the pancreatic enzymes in the pancreatic juice were significantly ($P<0.05$) elevated only during the first 20 min (peak 352 ± 104 u/10min and 117 ± 38 u/10min, respectively). Thereafter, the enzyme activities declined sharply to levels (10–40 u/10min) that were not different from saline controls although the enzyme activities determined in the pancreatic tissue itself and in the blood serum continued to increase. Although pretreatment with HOE 140 greatly augmented the caerulein-induced rise in the activities of amylase and lipase in the blood serum (Griesbacher *et al.*, 1993), the enzyme activities in the pancreatic secretions were not elevated throughout the experiment.

The infusion of caerulein caused a transient reduction of blood glucose levels by 1.98 ± 0.18 mmol/l. HOE 140 had no effect on blood glucose on its own and did not affect the caerulein-induced reduction of blood glucose. In order to test for a possible disturbance of liver function, the activities of γ-glutamyl transpeptidase and of alanine aminotransferase were determined in the blood serum at the end of the 2 h infusion of caerulein. The enzyme activities were not different from those of control animals. Pretreatment with HOE 140 had no effect on the serum activities of these enzymes.

In summary, absorption of pancreatic enzymes into the blood during acute pancreatitis occurs via a pathway not involving the pancreatic duct. Although HOE 140 greatly augments the egress of activated pancreatic enzymes into the blood, this does not have adverse effects on the function of the endocrine pancreas and that of the liver, at least during the period of observation.

Griesbacher, T. & Lembeck, F. (1992) *Br. J. Pharmacol.* 107, 356–360.
Griesbacher, T., Tiran, B. & Lembeck, F. (1993) *Br. J. Pharmacol.*, in press.
Lembeck, F., Griesbacher, T., Eckhardt, M., Henke, S., Breipohl, G. & Knolle, J. (1991) *Br. J. Pharmacol.* 102, 297–304.

59P CHARACTERISATION OF BRADYKININ RECEPTORS IN THE ELECTRICALLY-STIMULATED RAT VAS DEFERENS

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Bradykinin (BK) has been shown to enhance both the magnitude of the electrically-evoked twitch (pre-junctional/neurogenic response) and the basal muscle tension (post-junctional/musculotropic response) in the electrically-stimulated rat vas deferens (Rifo et al., 1987). The aim of this present study was to further characterise the BK receptors mediating the above responses in the field stimulated rat vas deferens.
Segments (1.5cm from prostatic end) of vas deferens were removed from adult male Wistar rats (250-350g) and suspended between parallel platinum electrodes in a 5ml organ bath containing Krebs solution gassed with 95% O_2/5% CO_2 at 35°C. Electrical field stimulation was delivered by a stimulator, at supramaximal voltage, with a 1msec pulse duration and at a frequency of 0.33Hz. Contractile responses were measured using isometric force transducers. Cumulative concentration-response curves were constructed to BK (0.001-10μM) in the field stimulated vas deferens. These BK cumulative response curves were reproducible (both neurogenic and musculotropic components) for a further 3 times if a rest period of 30min (no electrical stimulation) was allowed between cumulative curves. In studies with BK antagonists, an equilibration period with the tissue of 20min was used between cumulative curves.
BK or B_2 agonists (Met-Lys-BK and Lys-BK) were found to enhance both the neurogenic and musculotropic responses with no difference in the order of relative activities (Met-Lys-BK>Lys-BK=BK). The B_1 agonist, des-Arg^9-BK (0.001-10μM) had no effect in this preparation. Moreover, BK concentration response curves were not affected by the B_1-antagonist, des-Arg^9-Leu^8-BK (3-30μM). The table below shows that using BK, the B_2 antagonists (I-IV) behaved as competitive surmountable antagonists (3-100μM) however the slope for antagonists (IV) for the neurogenic and musculotropic responses were significantly different. In addition, the B_2 antagonist V (10-100nM), demonstrated a different profile of antagonism: competitive surmountable antagonism for the neurogenic response and unsurmountable antagonism of the musculotropic response.

	BK antagonist	Neurogenic response		Musculotropic response	
		pA_2	slope	pA_2	slope
I:	D-Arg^0-Hyp^3-D-Phe^7-BK	6.00±0.17	1.10±0.09	6.29±0.17	0.90±0.09
II:	Thi^{5,8}-D-Phe^7-BK	4.96±0.49	1.05±0.21	5.57±0.12	0.96±0.07
III:	D-Phe^7-BK	5.56±0.06	1.23±0.08	5.45±0.11	1.19±0.14
IV:	D-Arg^0-Hyp^3-Thi^{5,8}-D-Phe^7-BK	5.79±0.07	1.26±0.06*	6.31±0.18	0.99±0.03
V:	Hoe 140 (D-Arg-Hyp^3-Thi^5-DTic^7-Oic^8)-BK	8.50±0.46	1.19±0.14	#	

Results are mean ± s.e.m. of 7-8 experiments. # caused rightward shift and depression of maximal response. * P<0.05 (paired t-test).
The above results suggest that the BK receptors mediating the neurogenic and musculotropic responses in the field stimulated rat vas deferens are of the B_2 type. The reasons for the differing profiles of antagonism between the neurogenic and musculotropic responses seen with certain B_2 antagonists requires further investigation, but receptor subtypes as proposed by Rifo et al., 1987 remains a possibility.

This work was supported by Glaxo Group Research. AURA is a SERC-GLAXO student.

Rifo, J., Pourrat, M., Vavrek, R.J., Stewart, J.M., Huidobro-Toro, J.P. (1987). Eur. J. Pharmacol., 142, 305-312.

60P CHARACTERISTICS OF THE BRADYKININ-INDUCED INCREASE IN INTRACELLULAR CALCIUM ION CONCENTRATION OF SINGLE CULTURED BOVINE TRACHEAL SMOOTH MUSCLE CELLS

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Changes in intracellular calcium ion concentration within the smooth muscle cell play an important role in the initiation and maintenance of contraction of smooth muscle from a variety of tissues. It has been suggested that bradykinin (BK) is involved in the pathogenesis of asthma (Christiansen *et al.*, 1987) and recent studies have shown that BKinduces a biphasic increase in intracellular calcium ion concentrations in human airway smooth muscle cells (Murray and Kotlikoff, 1991). However, characterisation of the receptor via which BK elicits a change in intracellular calcium ion concentration in smooth muscle cells has yet to be elucidated although it has been suggested that activation of a putative B_3 receptor subtype is responsible for the bradykinin-induced calcium ion mobilisation in guinea-pig tracheal smooth muscle cells (Farmer *et al.*, 1991). This study reports the involvement of the BK B_2 receptor in mediating a change in intracellular calcium ion concentrations of bovine tracheal smooth muscle (BTSM) cells and the complexity of evaluating the responses of individual cells which differs from that of cell population or isolated tissue studies.
Cell cultures were established from explants of fresh bovine tracheal smooth muscle as described previously (Marsh and Hill, 1992). Cells to be used for image analysis were sparsely seeded onto 22mm circular glass coverslips and grown for 72 hours in DMEM supplemented with 10% FCS and 2mM glutamine at 37oC in 10% CO_2. Cells were washed three times with a physiological salt solution containing 2mM $CaCl_2$ (PSS) then loaded with 5μM Fura-2 AM in PSS containing 10% FCS for 30 min at 37oC. Following a further three washes, coverslips were placed in a holder with 900μl PSS and maintained at 37oC by means of a heated chamber. BK (in PSS) was added in a volume of 100μl directly onto the cells and where appropriate antagonists were added 2 min prior to this agent. Changes in fluorescence were monitored using a Nikon epifluorescence microscope and calcium ion concentrations were evaluated using the 340/380 ratio method of Grynkiewitz *et al.* (1985) employed by the software of the Applied Imaging Magical image analysis system.
Bradykinin (0.1nM-10μM; n=26-45 for each concentration of BK) produced a monophasic increase in intracellular calcium ion concentration which remained slightly elevated above basal levels for several minutes in the presence of extracellular calcium. On analysis of these data, it was found that the BK-induced increase was concentration-dependent revealing a log EC_{50} of -8.7 ± 0.4. It also became apparent that not all of the cells in any one field of view were responding to a particular concentration of BK and those doing so produced different responses. Addition of the BK B_2 receptor antagonist, D-Arg-Hyp3,Thi5,8-D-Phe7-BK, 2 min prior to the agonist, produced a shift of the concentration-response curve to the right (log EC_{50} = -7.1 ±0.3 and -5.8 ± 0.2 for 1μM and 10 μM respectively) yielding a K_D of 26nM. In addition to theses observations it was also noted that a lag time occurs in some cells between the addition of BK and the increase in intracellular calcium ion concentration.
BK produces a concentration-dependent increase in intracellular calcium ion concentration in BTSM cells and individual cells display different sensitivities to this agonist. The presence of a B_2 receptor antagonist shifts the BK concentration-response curve to the right indicating an involvement of BK B_2 receptors in the calcium response of BTSM cells.
We would like to thank the AFRC for their financial support of this work.
Christiansen, S.C., Prood, D. & Cochrane, C.G. (1987). J. Clin. Invest., **79**, 188-197.
Farmer, S.G., Ensor, J.E. & Burch, R.M. (1991). Am. J. Respir. Cell Mol. Biol., **4**, 273-277.
Grynkiewitz, G., Poenie, M. & Tsien, R.Y. (1985). J. Biol. Chem., **255**, 3440-3445.
Marsh, K.A. & Hill, S.J. (1992). Br. J. Pharmacol., **107**, 443-447.
Murray, R.K. & Kotlikoff, M.I. (1991). J. Physiol., **435**, 123-144.

61P DIFFERING NEUROKININ NK_2 RECEPTOR SIGNAL TRANSDUCTION MECHANISMS IN EPIDIDYMAL AND PROSTATIC ENDS OF THE RAT VAS DEFERENS

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The NK_2 receptor, present in both ends of the vas deferens (Tousignant *et al*, 1987), mediates smooth muscle contraction via inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) (Suman-Chauhan *et al*, 1990). IP_3 can release intracellular stores of Ca^{2+} while DAG is an activator of protein kinase C (PKC). The aims of this investigation were to determine if the neurokinin A (NKA) responses in the epididymal and prostatic parts of the rat vas deferens were mediated via activation of PKC.

Vas deferens from Sprague Dawley rats (300-400g) were bisected into prostatic and epididymal portions and set up in Krebs solution at 37^0C. The prostatic part was field stimulated at 0.2Hz, 50V, 0.5ms. Calphostin C, an inhibitor of PKC (Kobayashi *et al*, 1989), was incubated with tissues for 60 min and R59022, a DAG kinase inhibitor (de Chaffey de Courcelles *et al*, 1985) for 30 min.

The epididymal part of the vas was contracted by the non cumulative addition of NKA (EC_{50} $1.13\pm0.06\times10^{-7}$, mean±s.e.m). In Ca^{2+} free Krebs containing EGTA 1.0mM, all responses to NKA were abolished. In normal Krebs, nifedipine 1×10^{-6}M reduced responses to NKA by 88±4%. After stable contractions to NKA 1×10^{-7}M and K^+ 30mM were obtained, calphostin C, 1×10^{-6}M was added. This reduced the NKA response by 88±2% while that to K^+ was increased by 19±2%. A higher dose of the PKC inhibitor, 3×10^{-6}M, abolished the response to NKA and increased the contraction to K^+ by 33±5%. In the prostatic part of the vas the potentiation of the twitch response by NKA 1×10^{-7}M was 109±7% in tissues that had been incubated with calphostin C 3×10^{-6}M compared to 106±5% in controls. In the epididymal part R59022 1×10^{-6}M increased the control contraction to NKA 1×10^{-7}M by 54±10% while responses to K^+ 30mM were decreased by 22±4%. In the prostatic end of the vas the potentiation of the twitch response by NKA 1×10^{-7}M was 86±6% in tissues that had been incubated with R59022 1×10^{-6}M compared to 87±8% in controls.

In the epididymal portion of the rat vas deferens NKA contractions were totally dependent on extracellular Ca^{2+} influx through nifedipine sensitive channels and, therefore, an IP_3 mediated release of intracellular Ca^{2+} was not involved. The NKA contraction was abolished by the PKC inhibitor and potentiated by the DAG kinase inhibitor (which prevents the breakdown of DAG) although the NKA response in the prostatic end was unaltered by these drugs. This suggests that while the NK_2 receptor mediated response in the epididymal end of the rat vas deferens was via DAG activation of PKC, a different transduction mechanism was present in the prostatic end.

de Chaffey de Courcelles,D., Roevens,P. & Van Belle,H. (1985) J. Biol. Chem. 260, 15762-15770.
Kobayashi,E., Nakano,H. et al, (1989) Biochem. Biophys. Res. Commun. 159, 548-553.
Suman-Chauhan,N., Guard,S., Williams,B.J. et al, (1990) Br. J. Pharmacol. 101, 1001-1005.
Tousignant,C., Dion,S., Drapeau,G. & Regoli, D. (1987) Neuropeptides 9, 333-343.

62P EVIDENCE THAT A SELECTIVE NEUROKININ-1 ANTAGONIST INHIBITS OEDEMA FORMATION IN GUINEA-PIG PASSIVE CUTANEOUS ANAPHYLAXIS

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Oedema formation in guinea-pig passive cutaneous anaphylaxis (PCA) has been suggested to result from the combined action of several mediators of increased microvascular permeability that include histamine, leukotriene D_4 and platelet activating factor (Weg et al., 1991). In this study we have investigated whether substance P-like peptides may also play a role, by using a recently developed neurokinin-1 (NK_1) antagonist, RP67580 (Garret et al., 1991).

Male Dunkin-Hartley guinea-pigs were anaesthetised with sodium pentobarbitone (30-50mg/kg). Oedema formation was measured by the extravascular accumulation of i.v. ^{125}I-albumin for a 30 min period after intradermal, i.d., injection of test agents in Tyrode's solution. RP67580 was co-injected i.d. with mediators and results compared with sites in the same animals which received mediator alone. For the PCA, guinea-pigs were sensitized by i.d. guinea pig anti-ovalbumin (1μg/site) and challenged 24h later with ovalbumin (1μg)

RP67580, i.d., inhibited oedema induced by substance P, neurokinin A and in the PCA (Table 1), with little effect on that induced by bradykinin and histamine.

Table 1 Effect of RP67580 (10nmol/site) on oedema formation (mean ± s.e. mean) induced by substance P (SP), neurokinin A (NKA) and bradykinin (Bk) and PCA.

	Tyrode(100μl)	SP(30pmol)	NKA(100pmol)	Bk(100pmol)	PCA	
Control	13.6±1.4	61.9±12.0	42.0±6.5	57.1±3.2	56.8±7.2	
+RP67580	13.0±1.9	37.6±5.4	19.7±2.5	69.0±8.4	39.7±6.8	
n	8	3	5	3	7	
p	ns	p<0.05	p<0.01	ns	p<0.05	(modified t-test)

The results suggest that RP67580 is a useful NK_1 antagonist for use in skin studies in that it is a selective inhibitor of oedema induced by substance P-like peptides and, unlike some earlier NK_1 antagonists, is not pro-inflammatory when injected i.d. More importantly, results suggest that substance P-like peptides may play a role in acute allergic oedema formation.

We thank Rhone Poulenc Rorer for RP67580

Garret, C., Carruette, A., Fardin, V., Moussaoui, S., et al., (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 10208-10212
Weg, V., Watson, M.L., Cordeiro, R.S.B. & Williams, T.J. (1991) Eur. J. Pharmacol. **204**, 157-163

63P HAEMODYNAMIC EFFECTS OF NEUROPEPTIDE Y AND NORADRENALINE IN ANAESTHETISED, AREFLEXIC RATS

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We have shown previously that in pentobarbitone anaesthetised, catecholamine depleted, bilaterally vagotomised rats, neuropeptide Y (NPY) elevated blood pressure by increasing total peripheral resistance (TPR) without affecting cardiac index (CI) (Haworth et al, 1992). However, it has been shown in conscious rats that NPY depressed cardiac output (Scott et al, 1990). In view of the reported potentiation of the effects of other pressor agents by NPY (Aubert et al, 1988) we have now investigated whether the combined effects of NPY and noradrenaline (NA) differ from NPY alone.

Non-recovery experiments were carried out in pentobarbitone anaesthetised, bilaterally vagotomised rats which had been catecholamine depleted with syrosingopine and dosed with histamine and 5HT antagonists as described previously (Haworth et al, 1992) except that the rats were also dosed with the selective β1-adrenoceptor antagonist CGP 20712 A (1mgkg^{-1} i.v.) to prevent NA-induced tachycardia. NA was infused (1μgkg^{-1}min^{-1} i.v.) while NPY was given as a bolus injection (4.8nmolkg^{-1} i.v.). Results are expressed as mean±s.e.mean (n=4). Mean arterial pressure (mmHg) was 90±5 in controls, 158±3 after NPY ($p<0.001$), 138±3 after NA ($p<0.001$) but only 166±4 after a combination of NPY and NA ($p<0.001$). TPR (mmHgml^{-1}min100g) was 6.51±1.02 in controls, 11.93±1.03 after NPY ($p<0.01$), 9.67±0.87 after NA (not significant) but 24.50±5.27 after NPY plus NA ($p<0.02$). Heart rate was not significantly different from control (299±8beatsmin^{-1}) after any treatment and CI was not different from control (14.7±2.1 mlmin^{-1}100g^{-1}) after NPY (13.5±1.2) or NA alone (14.5±1.1). However after a combination of NPY and NA, CI was only 7.8±1.6mlmin^{-1}100g^{-1} ($p<0.05$) explaining the observed lack of effect of NA on the pressor response to NPY. Coronary vascular conductance (μlmin^{-1}g^{-1}mmHg^{-1}) was not significantly different between controls (29.5±5.0) and rats dosed with NPY (24.0±4.1), NA (23.7±2.6) or NPY plus NA (21.6±3.3). Rats dosed with a combination of NPY and NA also showed evidence of pulmonary oedema. The lung weight (x1000)/bodyweight ratio after NPY plus NA was 6.38±0.22 compared with 4.36±0.45 in controls ($p<0.01$). After NPY plus NA P_{CO2} (mmHg) was 45.6±1.2 compared with 34.0±2.8 in controls ($p<0.01$) while P_{O2} (mmHg) was 58.2±7.4 compared with 96.6±7.1 in controls ($p<0.01$). There was no significant difference between these parameters in rats dosed with NPY or NA alone.

These results indicate that a combination of NPY and NA not only reduced cardiac output, by a mechanism not involving coronary vasoconstriction, but also induced symptoms of heart failure. The high TPR in rats dosed with NPY and NA suggests that the pressure overload due to peripheral vasoconstriction was sufficient to impair cardiac function and cause pulmonary oedema.

Aubert, J.F., Waeber, B., Rossier, B., Geering, K., Nussberger, J. & Brunner, H.R. (1988) J.Pharmacol.Exp.Ther. 246, 1088-1092.
Haworth, D., Culbert, J-A., Edmonds, A.E. & Thomson, D.S. (1992) BPS London Meeting, Sept, C100.
Scott, N.A., Michel, M.A., Boublik, J.H., Rivier, J.E., Motomura, S., Crum, R.L.,Landon, M. et al., (1990) Am.J.Physiol. 259, H174-H180.

64P THE INHIBITORY EFFECT OF SOMATOSTATIN (SS)$_{14}$, SS$_{28}$, SS$_{25}$ AND SOME CYCLIC ANALOGUES ON NEUROGENIC CONTRACTIONS IN GUINEA-PIG ILEUM AND VAS DEFERENS

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SS$_{14}$, SS$_{28}$ and SS$_{25}$ inhibit neurogenic contractions in guinea-pig ileum (McIntosh et al., 1986). We have now further characterised this action and also compared the effect of these and other cyclic peptides on neurogenically mediated contractions of guinea-pig vas deferens.
Tissues were removed from male Dunkin Hartley guinea-pigs (approx. 250g) and mounted in organ baths containing a modified Krebs solution for recording isometric tension changes (Feniuk et al., this meeting). Transmural stimulation was applied to guinea-pig ileum (0.1Hz 0.1ms) continously and vasa deferentia (5Hz 1.5s durn. 0.5ms) every 30s at supramaximal currents. Concentration-effect curves were obtained by non-cumulative addition allowing at least 10 min between doses to avoid desensitisation. Potencies of compounds were compared with that of SS$_{14}$ (Table 1). The effect of changing external Ca^{2+} concentrations and the effect of the putative SS$_{14}$-receptor blocking drug, cyclo 7-aminoheptanoyl-Phe-D-Trp-Lys-Thr[Bzl] (CPP) (Endou et al., 1989), on responses to SS$_{14}$ were also examined.

Inhibitory effects of SS$_{14}$, SS$_{25}$, SS$_{28}$ and various cyclic SS analogues on neurogenic contractions in guinea-pig ileum and rat vas deferens. Values are means± sem or geometric mean (95% confidence limits) from 4-5 experiments.

	Guinea-pig Ileum			Guinea-pig vas deferens		
	EC50 (nM)	Max % inhibition	EPMR	EC50 (nM)	Max % inhibition	EPMR
SS$_{14}$	5.2 (3.7-7.4)	68±5	1.0	83 (62-111)	84±3	1.0
SS$_{28}$	8.0 (5.3-12.1)	69±8	2.2±0.6	4.3 (2.1-8.7)	100±0	0.03±0.005
SS$_{25}$	6.7 (3.8-11.8)	65±8	1.2±0.3	16 (11-24)	100±0	0.07±0.02
D-Trp-Lys-Thr-Phe-Pro-Phe	9.7 (3.4-27.4)	54±10	2.1±0.5	22 (8-25)	65±3	0.28±0.09
D-Trp-Lys-Thr-Phe-Pro-Tyr	4.4 (3.0-6.3)	62±9	1.9±0.9	12 (3-41)	65±15	0.16±0.01
Angiopeptin	3.1 (1.1-8.8)	48±6	1.0±0.3	18 (13-25)	67±7	0.41±0.16
Octreotide	1.9 (0.8-4.6)	69±4	1.0±0.5	39 (14-105)	82±9	0.27±0.10

All compounds exhibited similar potency to SS$_{14}$ in ileum. In contrast SS$_{14}$ was significantly weaker and SS$_{28}$ and SS$_{25}$ were much more potent than SS$_{14}$ in the vas deferens (Table 1). CPP (1μM) had no effect on the inhibitory action of SS$_{14}$ and SS$_{28}$ in ileum or vas deferens (agonist concentration ratios 1.11±0.47; 2. 52±1.43; 1.16±0.13 and 0.92±0.12) respectively.
In keeping with a potential prejunctional mechanism of action the inhibitory effect of SS$_{14}$ was inversely related to changes in external Ca^{2+} concentration and SS$_{14}$ had little effect on contractile responses to carbachol or α-β-methylene ATP in ileum and vas deferens respectively.
It is possible that the differences in agonist potencies may reflect the presence of different SS receptors in these tissues. However, a potential differential influence of amino peptidases on the potencies of these peptides must be considered (see Feniuk et al., this meeting).

McIntosh, C.H.S., Bakich, V., Kwok, N. and Brown, J.C. (1986) Can. J. Physiol. Pharmac., 64, 303-306.
Endou, M., Hattori, Y., Nakaya, H. and Kanno, M. (1989) J. Pharm. Exp. Ther., 250, 726-733.
Feniuk, W., Humphrey, P.P.A., Jarvie E. and Roberts, A. This meeting.

65P POSITIVE INOTROPIC EFFECTS OF ANGIOTENSIN II IN THE PITHED RAT

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Angiotensin II (Ang II) is known to induce a positive inotropic response in isolated heart and heart muscle preparations of several species, although not in the hearts of adult rats (Baker *et al.*, 1992). In intact animals this inotropic response has so far not been clearly demonstrated. In the present study we established the cardiovascular responses to Ang II in pithed rats.

Male Wistar rats (320-350 g) were pithed and the left jugular vein and common carotid artery were cannulated for the administration of drugs and for monitoring the diastolic blood pressure (DBP), respectively. A catheter was inserted into the left ventricle via the carotid artery to measure the left ventricular pressure (LVP), dP/dt_{max} and heart rate (HR). In separate experiments aortic pressure was controlled by means of a catheter which was connected to a saline reservoir and inserted into the aortic arch via the left carotid artery.

Ang II (0.01-30 nmol·kg^{-1}) induced a dose-dependent increase in DBP, dP/dt_{max} and HR in pithed rats. The maximal responses for these parameters were 102±2 mmHg, 18073±806 mmHg·sec^{-1} and 107±11 beats·min^{-1}, respectively. Compared with those of noradrenaline (125±3 mmHg, 16500±924 mmHg·sec^{-1} and 106±12 beats·min^{-1}, respectively), the responses to Ang II were not significantly different except for the rise in DBP for which noradrenaline caused a 23 mmHg ($n=6$, $p<0.05$) stronger increase than Ang II. After pretreatment with propranolol (1 mg·kg^{-1} i.v.), the positive chronotropic effect of Ang II was abolished. Ang II elevated DBP and dP/dt_{max} with maximal increases of 96±2 mmHg and 10798±656 mmHg·sec^{-1},respectively. Although vasopressin elevated the DBP to the same maximal value (94±4 mmHg, $n=6$) as Ang II, it produced only a small increase in dP/dt_{max} (5164±554 mmHg·sec^{-1}) which may be merely due to the increase of the afterload. When the aortic pressure was maintained at 80 mmHg and the chronotropic response was blocked by propranolol, Ang II still produced a dose-dependent increase in dP/dt_{max} (6610±365 mmHg·sec^{-1}, $ED_{50}=0.17$ nmol·kg^{-1}, $n=5$). Losartan (3 mg·kg^{-1} i.v.) but not PD123177 (100 mg·kg^{-1}) caused parallel rightward shifts of the dose-response curve of Ang II without altering the maximal response (6350±451 mmHg·sec^{-1}, $ED_{50}=5.6$ nmol·kg^{-1}, $n=5$).

The results indicate that in pithed rat Ang II produces a rise in contractile force similar to that caused by noradrenaline and a pressor response which is similar to that of vasopressin. The Ang II-induced positive inotropic response is a direct one, which occurs independently of the ß-adrenergic system. The response is probably mediated by AT_1-receptors.

Baker, K.M., Booz, G.W. & Dostal, D.E. (1992) *Annu. Rev. Physiol.* **54**, 227-241

66P TYROSINE KINASE PATHWAYS AND THE CONTRACTILE ACTIONS OF ANGIOTENSIN II AND EPIDERMAL GROWTH FACTOR-UROGASTRONE IN CORONARY ARTERIAL STRIPS

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We have previously documented the ability of epidermal growth factor-urogastrone (EGF-URO) to elicit a contractile response in porcine coronary arterial (PCA) strips *via* a mechanism that involves the generation of contractile cyclooxygenase products, so as to be blocked by indomethacin (INDO, 3 µM) (Gan and Hollenberg, 1990). In more recent work, we have observed that the contractile actions of angiotensin-II (A-II) in the PCA preparation are also blocked by INDO, so as to suggest a signal transduction pathway that might be in common with the tyrosine kinase (PYK) pathway(s) activated by EGF-URO. To explore this possibility, we decided to test the hypothesis that a PYK pathway might be involved in the contractile action of A-II. To this end, we examined the ability of two PYK inhibitors, genistein (GS) and tyrphostin (TP) to inhibit the contractile actions of A-II and EGF-URO, and we have assessed the presence of tyrosine kinase enzyme activity in the PCA tissue: (a) by Western blot analysis (WBA) of A-II-treated tissues, using a monoclonal anti-PY antibody (αPY) and (b) by chromatographic isolation of $p60^{src}$-related PYK activity, using a newly developed assay specific for src-related PYK's (Cheng et al., 1991). Both GS and TP (≤ 40 µM) selectively blocked contractions caused by A-II and EGF-URO in the PCA without appreciably affecting contractions caused by KCl. A-II-treatment led to an increase in the intensity of a number of αPY-staining bands, upon WBA; the intensity of these bands was reduced by treating tissues with GS, prior to A-II stimulation. In both membranes and cytosolic fractions from the PCA tissue, we detected src-related PYK activity that could be inhibited by GS and TP. The PYK activity in membrane extracts could be separated into two peaks by sequential chromatography on hydroxylapatite and mono-Q columns. Protein in both peaks exhibited cross-reactivity with an antibody directed at a domain in common with a number of $p60^{src}$-related PYK's. We conclude that A-II can cause its contractile effects in PCA tissue by a signal that, in part, involves a PYK pathway. Our work also establishes, in the PCA tissue, the presence of src-related PYK activity that may play a role in the regulation of tissue contractility by agonists such as A-II.

(Supported by grants from the Canadian MRC and the Alberta Heart Foundation).

Gan, B.S. & Hollenberg, M.D. (1990) *J. Pharmacol. Exp. Ther.* 252, 1277-1282.
Cheng, H.-C., Nishio, H., Hatase, O, Ralph, S. & Wang, J.H. (1992) *J. Biol. Chem.* 267, 9248-9256.

67P A PHARMACODYNAMIC STUDY OF SR 47436, A NON-PEPTIDE AT_1 RECEPTOR ANTAGONIST, ON BLOOD PRESSURE IN PRIMATES.

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(introduced by P.R. Saxena)

SR 47436 is a potent and specific AT_1 receptor antagonist (Nisato *et al.*, 1992; Cazubon *et al.*, unpublished) which effectively lowers arterial blood pressure and inhibits the hypertensive responses to angiotensin II (AII) in the conscious monkey. As sufficiently long duration of action is the ultimate requisite for the development of this class of antihypertensive agents, in the present investigation we have followed the time course of the hypotensive effect and the drug levels of SR 47436 in the plasma (using HPLC) after a single oral dose of 10 mg/kg in conscious cynomolgus monkeys. At the same time the degree of inhibition of blood pressure responses induced by exogenous AII (100 ng/kg, i.v. bolus) was also studied.

Table 1 Time course of the effect of SR 47436 (10 mg/kg oral) and placebo on mean arterial blood pressure (MAP; mmHg) and hypertensive response to AII (AIIR; mmHg) in conscious cynomolgus monkeys (mean±s.e.mean; n=5 each).

	Time (hr)	0	0.25	0.5	1	3	6	9	28
MAP	Placebo	108±4	108±4	108±4	108±4	104±4	102±4	103±4	106±5
MAP	SR 47436	105±3	94±3*	91±3*	86±6*	87±3*	88±2*	92±2*	93±3*
AIIR	Placebo	35±2	-	36±2	39±2	36±2	38±2	35±2	35±2
AIIR	SR47436	36±2	-	12±2*	12±6*	16±4*	14±2*	14±1*	20±2*

0, Baseline; *, $P < 0.05$ vs baseline; Dunnett's test following significant 2-way ANOVA with repeated measures.

In the animals treated with SR 47436 (n=5), baseline mean arterial pressure and heart rate averaged 105±3 mmHg and 195±6 b/min versus 108±4 mmHg and 186±4 b/min in the placebo-treated (control) group (n=5). Though SR 47436 did not significantly affect heart rate (maximal change -8% from control values), the drug lowered MAP. The onset of the hypotensive effect (-11 mmHg) was already noticeable 15 min postdose and a maximal decrease in MAP (-20 mmHg) was observed from 1 to 6 hr postdose (Table 1). Even 28 hr postdose a small decrease in MAP (12 mmHg) was still evident. The time course of the hypotensive activity of SR 47436 seemed to correlate with the inhibition of the pressor response to AII, which was about 60% from 0.5 to 9 hr postdose and was still 48% at 28 hr (Table 1). Mean plasma level of SR 47436 was maximal (1.85 mg/l) after 0.5 hr and it was still 0.43 and 0.19 mg/l at 9 and 28 hr, respectively. The mean plasma half-life of SR 47436 was 20 hr. No significant changes were observed in the control group throughout the study.

In conclusion, our results show that SR 47436 is an effective and long-lasting (at least 24 hr) hypotensive agent in monkeys.

Nisato, D., Cazaubon, C., Lacour, C., Gougat, J., Guiraudou, P., Bernhart, C., Perreaut, P., Brelière, J.C. & Le Fur, G. (1992). *Br. J. Pharmacol.*, **105**, 84P.

68P SB203186, A POTENT 5-HT_4 RECEPTOR ANTAGONIST, IN PORCINE SINOATRIAL NODE AND HUMAN AND PORCINE ATRIUM

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SB203186, piperidinoethyl-indole-3-carboxylate hydrochloride, has been shown to be a potent and selective $5HT_4$ receptor antagonist (pK_B = 8.3) of 5-hydroxytryptamine (5HT)-induced tachycardia in piglet isolated right atrium (Kaumann *et al.*,1992). We have now determined the affinities of SB203186 for $5HT_4$ receptors of human right atrial appendage and piglet left atrium which mediate positive inotropic effects of 5HT and compared them with those published for tropisetron (Kaumann *et al.*, 1990 & 1991). We have also compared the blocking potencies of SB203186 and tropisetron against 5HT-induced tachycardia in anaesthetised Yucatan pigs and 2-5 day old Camborough hybrid piglets.

Methods for the *in vitro* studies were as described by Kaumann *et al.* (1990 & 1991). Briefly, strips of human right and piglet left atrium were suspended isometrically in modified Krebs solution at 37°C and paced (0.5Hz). Single concentration-inotropic-effect curves to 5-HT in the presence of (μM) cocaine (6), (±)-propranolol (0.4), ascorbate (200), and also, in piglet, rolipram (20) were compared in the absence and presence of various concentrations of SB203186. For the *in vivo* studies, dose-response curves to 5HT-induced tachycardia were obtained following section of the vagosympathetic trunk and in the presence of (±)-propranolol, 1 $mg.kg^{-1}$ i.v. Five dose-response curves were highly reproducible in control animals and comparisons have been made following increasing doses of SB203186 (i.v. and i.d.) or a single dose of tropisetron (i.v.). The time-course of antagonism by SB203186 both i.v. and i.d. was studied by its influence on the tachycardic response to 5HT, 10 $\mu g.kg^{-1}$ i.v., repeated at intervals over a period of 3h in anaesthetised piglets.

Table 1: Comparison of the blocking potencies of SB203186 and tropisetron (numbers of patients or pigs are shown in parentheses).

	Human atrium	Piglet atrium			Yucatan pig (4)	Camborough piglet (6)
	pK_B (mean±s.e.mean)	pK_B (mean±s.e.mean)		Dose ($mg.kg^{-1}$)	Dose-ratio	Dose-ratio
SB203186	8.7 ± 0.1 (9)	8.3 ± 0.1 (4)	SB203186	0.1	3.2	2.6
Schild slope	1.18 ± 0.18	1.16 ± 0.12		0.3	7.9	6.3
				1	23	14
				3	50	not determined
Tropisetron	6.7 ± 0.1 (6)	6.7 ± 0.1 (11)	Tropisetron	1.7	2.1	not determined
Schild slope	0.98 ± 0.11	1.02 ± 0.10				

In anaesthetised piglets (n=3), SB203186, 0.1 $mg.kg^{-1}$ i.v. prevented the 5HT-induced tachycardia within 5 min of the injection. Blockade faded with a $t_{1/2}$ of 30 min and disappeared at 2 h. SB203186, 0.3 $mg.kg^{-1}$ administered i.d. caused blockade which was maximal at 10 min and faded with a $t_{1/2}$ of 2 h. We conclude that SB203186 is a potent competitive antagonist of porcine and human $5HT_4$ receptors. Its potency is 40 to 100 times higher *in vitro*, and at least 20 times more potent *in vivo* than that of tropisetron. Its blocking kinetics *in vivo* suggest good absorption and duration of action. SB203186 is therefore a useful tool to further characterise $5HT_4$ receptors. We are grateful to the surgeons of Papworth Hospital for the supply of atrial appendages.

Kaumann, A.J., Sanders, L., Brown, A.M., Murray, K.J. & Brown, M.J. (1990) Br. J. Pharmacol. 100, 879- 885.
Kaumann, A.J., Brown, A.M. & Raval, P. (1991) Br. J. Pharmacol. 102, 98P
Kaumann, A.J., Medhurst, A., Boyland, P., Vimal, M. & Young, R.C. (1992) 2nd International Symposium on Serotonin, Houston. 44P

69P 5-HT-INDUCED RELAXATION OF SUMATRIPTAN PRECONTRACTED BEAGLE CORONARY ARTERY

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5-Hydroxytryptamine (5-HT)$_1$-like receptors mediate contraction of beagle isolated coronary arteries although 5-HT, but not sumatriptan, also produced relaxation (Parsons et al. 1992). We have, therefore, further investigated contractile and relaxant 5-HT receptors in this preparation.

Coronary arteries (endothelium removed) were set as described previously (Parsons et al . 1992). Concentration-effect curves to 5-HT, ergonovine or α-methyl-5-HT (to investigate 5-HT$_2$ receptor populations) were determined in the absence or presence of either ketanserin (ket)(1 μM) or ket (1 μM) and methiothepin (meth) (200 nM). The relaxant effects of 5-HT, 5-carboxamidotryptamine (5-CT), α-methyl-5-HT, ergonovine and sodium nitroprusside (SNP) were also studied on elevated tone induced by sumatriptan (10 μM), to saturate contractile 5-HT$_1$-like receptors.

Table 1: Effects of 5-HT receptor agonists on dog coronary artery. Data from n ≥ 3 rings, from ≥ 2 dogs (*p < 0.05 from control)

Basal tone: -log EC_{50} (mean ± s.e.mean) values for **contraction**. Intrinsic activity relative to 5-HT between parentheses.

	no antagonist	**ket (1 μM)**	**meth (200 nM) + ket (1 μM)**
5-HT	7.40 ± 0.06 (1.0)	7.16 ± 0.07* (0.81)	4.69 ± 0.11* (1.11)
α-methyl 5-HT	5.91 ± 0.05 (0.91)	5.65 ± 0.05* (0.70)	< 4.49* (> 0.2)
ergonovine	7.45 ± 0.08 (0.78)	7.04 ± 0.05* (0.70)	6.09 ± 0.06* (0.51)

Relaxation of 10 μM sumatriptan contraction: -log IC_{50} (50% reversal of tone) (mean ± s.e.mean) (% reversal of precontraction)

	SNP	**5-CT**	**5-HT**	**α-methyl-5-HT**	**ergonovine**
-log IC_{50}	8.38 ± 0.07 (139)	6.46 ± 0.09 (69.8)	4.42 ± 0.26 (74.4)	contraction/relaxation	≤ 4.6 (≥ 52)

5-HT, ergonovine and α-methyl-5-HT produced contraction at low concentrations. Concentrations > 2 μM for 5-HT and ≥ 20 μM for α-methyl-5-HT or ergonovine produced relaxation. On elevated tone to sumatriptan (10 μM), 5-HT, 5-CT, ergonovine and SNP all produced relaxation whereas α-methyl-5-HT produced an initial further contraction (29 ± 8.7% 2 nM - 20 μM) followed by relaxation (≥ 20 μM).

The small effect of ket shows only a minor role for 5-HT$_2$ receptors in this preparation. Sumatriptan may be used as a spasmogen to saturate contractile receptors to allow expression of relaxant effects to 5-HT. The high potency of 5-CT compared to 5-HT, ergonovine and α-methyl-5-HT may show the presence of smooth muscle 5-HT$_1$ receptors distinct from the 5-HT$_{1C}$ and 5-HT$_{1E}$ receptor subtypes.

Parsons, A.A., Stutchbury, C., Raval, P. & Kaumann, A.J. (1992) Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 592-596

70P ROLE OF PROTEIN KINASE C AND DIGLYCERIDE IN LTB$_4$-INDUCED ACTIVATION OF THE RESPIRATORY BURST OXIDASE IN GUINEA-PIG EOSINOPHILS

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The signal transduction mechanisms involved in activation of eosinophils are poorly understood. We have investigated the roles of protein kinase C (PKC) and diglyceride (DG) in activation of the respiratory burst oxidase in guinea pig eosinophils in response to LTB_4, and have examined potential sources of DG.

Eosinophils were harvested from the peritoneal cavity of human serum-treated male Dunkin-Hartley guinea pigs, and purified on discontinuous Percoll gradients. Cells (purity >90%) were resuspended in Hepes-buffered physiological salt solution. Measurements of H_2O_2 production, inositol(1,4,5)trisphosphate (IP_3) accumulation and phospholipase D (PLD) activity were made using previously described methods (Dent et al., 1991; Challis et al., 1990; Randall et al., 1990).

LTB_4 caused a concentration-dependent stimulation of H_2O_2 production with an EC_{50} of 28.0±4.7 nM (n=4). Stimulation with 100 nM LTB_4 was inhibited by U 75 302, a LTB_4 receptor antagonist, with an IC_{50} of 386±69 nM (n=4). The phorbol ester 4β-phorbol 12,13-dibutyrate (4ß-PDBu) evoked a concentration-dependent increase in H_2O_2 with an EC_{50} of 21.6±2.1 nM (n=4). R 59 022 (10 μM), a DG kinase inhibitor, caused a 2.65-fold leftward shift in the LTB_4 concentration-response curve (EC_{50}=10.5±1.6 nM; n=4), but did not affect the 4ß-PDBu response. Ro 31-8220 (10 μM), a PKC inhibitor, inhibited the rate of H_2O_2 generation stimulated by 30 nM LTB_4 by 49.9±4.3% (n=4), but increased the amount of H_2O_2 from 2.62±0.17 to 5.28±0.57 nmol/10^6 cells (n=4). Ro 31-8220 (10 μM) inhibited the response to 4ß-PDBu (1 μM) by 91.8±2.4% (n=4).

To investigate potential sources of DG we measured IP_3 accumulation, an index of phosphoinositidase C (PIC) activity, and PLD activity. LTB_4 stimulated a concentration-dependent increase in IP_3 mass with an EC_{50} of 200±50 nM (n=4). LTB_4 (1 μM) did not activate PLD. In contrast, 4ß-PDBu activated PLD in a concentration-dependent manner with an EC_{50} of 206±49 nM (n=4).

The results of this study suggest that DG and PKC are involved in activation of the respiratory burst oxidase in guinea pig eosinophils. Given that LTB_4 increased IP_3 mass but did not stimulate PLD, it appears that the major source of DG is derived predominantly from membrane phospholipids following the activation of PIC. The finding that Ro 31-8220 increased the amount of H_2O_2 generated in response to LTB_4 suggests that PKC also has an inhibitory action, at the level of PIC.

Supported by the Wellcome Trust. We are grateful to Dr. L. Garland of Wellcome Research Laboratories for invaluable assistance and for the gift of [^{3}H]butanol, and to Roche Products Ltd. for the gift of Ro 31-8220.

Challis, R.A., Chilvers, E.R., Willcocks, A.L. and Nahorski, S.R. (1990) Biochem. J. **265**, 421-427.
Dent, G., Giembycz, M.A., Rabe, K.F. and Barnes, P.J. (1991) Br. J. Pharmacol. **103**, 1339-1346.
Randall, R.W., Bonser, R.W., Thompson, N.T. and Garland, L.G. (1990) FEBS Lett. **264**, 87-90.

71P INHIBITION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE BY IBUDILAST: COMPARISON WITH ROLIPRAM

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Ibudilast is an anti-asthma drug of uncertain mode of action (Kawasaki *et al.*, 1992). We have investigated its inhibitory actions against partially-purified cyclic nucleotide PDE isoenzymes I, II, III, IV and V from pig aorta, guinea-pig eosinophils or human trachea and compared its activity with rolipram. PDE isozymes were partially purified from the 100,000 g cytosolic fraction of human trachea or pig aorta by DEAE chromatography. For preparation of eosinophil PDE IV, cells were harvested from the peritoneal cavity of horse-serum treated (0.5 ml, i.p., biweekly) guinea-pigs and centrifuged on discontinuous metrizamide gradients. Eosinophils (100 - 200 x 10^6) were homogenised in isotonic buffer containing a cocktail of protease inhibitors. The homogenate was centrifuged (105,000 g, 30 min) and the pellet resuspended in homogenization buffer. Membrane-bound PDE IV was solubilised with deoxycholate (0.5 %) and NaCl (100 mM). Vanadate/glutathione complex (V/GSH) was prepared and PDE activity measured (Souness *et al.*, 1985). We have also compared the abilities of ibudilast and rolipram to inhibit PDE IV in intact eosinophils by measuring their effects on cAMP accumulation. cAMP was extracted with 5 % trichloroacetic acid and quantified by RIA (NEN).

PDE isoenzyme	*source*	*n*	*ibudilast* (IC50 μM)	*rolipram* (IC50 μM)
I	pig aorta	2	55	>100
III	pig aorta	2	38	>100
IV	pig aorta	2	12	3
V	pig aorta	2	12	>100
IV	guinea pig eosinophil:			
	(membrane bound)	3-5	0.87 ± 0.37	0.2 ± 0.04
	(solubilized)	3-10	0.11 ± 0.05	0.012 ±0.003
	(V/GSH-treated)	3	0.11 ± 0.02	0.02 ± 0.01

In human trachea (n = 2) ibudilast inhibited PDEs II, IV and V with similar potencies (IC_{50}s: 12, 7, 10 μM). Rolipram was not active against PDE II or V (IC_{50}s: >100 μM) being selective for PDE IV (IC_{50}: 3 μM). Like rolipram, solubilization or V/GSH treatment increased the inhibitory potency of ibudilast on eosinophil PDE suggesting a similar interaction of these compounds with the enzyme. Both ibudilast and rolipram enhanced isoprenaline-induced cAMP accumulation in intact eosinophils (EC_{50}s: 0.33 ± 0.06 μM, n = 4; 0.20 ± 0.004 μM, n = 3). These results show ibudilast to be a potent, though non-selective PDE inhibitor.

Kawasaki, A., Hoshino, K., Osaki, R., Mizushima, Y. & Yano, S. (1992) J. Asthma **29**, 245-252.
Souness, J.E., Thompson, W.J. & Strada, S.J. (1985) J. Cyclic Nucleotide Protein Phosphorylation Res. **10**, 383-396.

72P CLASSIFICATION OF THE PROSTAGLANDIN EP-RECEPTOR LOCATED ON THE RAT ISOLATED TRACHEA

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Using Prostaglandin E_2 (PGE_2) and related analogues we have attempted to classify the prostaglandin EP- receptor mediating smooth muscle relaxation of the bethanechol (BCh) contracted rat trachea.

Tracheal ring preparations from male Sprague-Dawley rats (300-500g) were prepared for isometric force recordings in Kreb's solution containing indomethacin (2.8μM) and GR32191B (1μM), maintained at 37°C and gassed with 5% CO_2 in oxygen. Preparations were contracted with 5μM BCh (≈ BCh [A_{50}]) and cumulative concentration-effect (E/[A]) curves to PGE_2 were constructed. Preparations were washed and after 60 min an E/[A] curve to one of the following test agonists was constructed: 11-deoxy-PGE_1, 16,16-dimethyl PGE_2, Misoprostol, AH13205 and Sulprostone. Data were fitted using a logistic function from which potency estimates were made.

Table 1. Equi-effective molar ratio (PGE_2 = 1)

Preparation	11-deoxy-PGE_1	16,16-dimethyl-PGE_2	Misoprostol	AH13205	Sulprostone
Rat trachea (RTr)	2.4 [1.3-4.4]	2.2 [1.3-6.9]	16.1 [12.2-21.2]	>1500	Inactive
Rabbit jugular vein (RbJV)	2.1†	2.1†	8.3†	-	>3000†
Cat trachea (CTr)	13§	20¶	3.7¶	29‡	> 10,000‡

Published data: † Lawrence & Jones, 1992. § Dong *et al*, 1986. ¶ Coleman *et al*, 1988. ‡ Nials *et al*, 1991.
Rat trachea: n ≥ 7 determinations [95% C.L.]. p[A_{50}] for PGE_2 - 7.84 ± 0.07.

The response to PGE_2 was not shifted by the EP_1 receptor antagonist AH6809 (5μM) and the EP_1/EP_3 receptor agonist Sulprostone was inactive. These results and the low potency of the EP_2 receptor agonist AH13205 suggest that the receptor located on the RTr is similar to the RbJV but different from the CTr, and not of the EP_1, EP_2 or EP_3 receptor subtypes. Recently Louttit *et al* have tentatively proposed the existence of an EP_4 receptor subtype on porcine vasculature on which AH13205 and Sulprostone show very low potency and AH23848B is a selective antagonist. On the grounds of potency order information, the prostaglandin EP-receptor on the RTr is atypical and may be of the EP_4 receptor subtype however definitive classification will require the use of AH23848B.

Coleman, R.A., Humphray, J.M., Sheldrick, R.L.G. & White, B.P. (1988) *Br. J. Pharmacol.*, **95**, 724P.
Dong, Y.J., Jones, R.L. & Wilson, N.H. (1986) *Br. J. Pharmacol.*, **87**, 97-107.
Lawrence, R.A. & Jones, R.L. (1992) *Br. J. Pharmacol.*, **105**, 817-824.
Louttit, J.B., Head, S.A. & Coleman, R.A. (1992) 8th International Conference on Prostaglandins and Related Compounds, 257P.
Nials, A.T., Coleman, R.A., Hartley, D. & Sheldrick, R.L.G. (1991) *Br. J. Pharmacol.*, **102**, 24P.

73P EFFECTS OF IBUDILAST ON AIRWAYS SMOOTH MUSCLE CONTRACTION *IN VITRO* AND *IN VIVO*: COMPARISON WITH ROLIPRAM

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Ibudilast is an anti-asthma drug which has been claimed to be a selective, competitive antagonist at LTD_4 receptors in guinea-pig airway smooth muscle (Ohashi *et al.*, 1986). More recently however, Etoh *et al.* (1990) demonstrated that ibudilast does not displace ^3H-LTD_4 from guinea-pig lung membrane suggesting an alternative mechanism of action. Since ibudilast has been claimed to inhibit cAMP phosphodiesterase (PDE) in guinea-pig lung (Nishino *et al.*, 1983) we have compared the airway smooth muscle relaxant properties of ibudilast with those of the selective PDE type IV inhibitor rolipram *in vitro* and *in vivo*. Cumulative concentration-response curves to either ibudilast or rolipram were constructed in guinea-pig isolated tracheal strips precontracted with methacholine, histamine or LTD_4 (EC_{30}, 30 % maximum spasmogen contractile response). The bronchodilator properties of ibudilast and rolipram were assessed *in vivo* in the anaesthetized, ventilated guinea-pig. Spasmogens were administered (i.v.) at 6 min intervals and the dose titrated to give a 60-70 % maximum increase in pulmonary inflation pressure (PIP). After two stable responses to spasmogen, ibudilast or rolipram were administered intratracheally as a dry powder 1 min prior to the next dose. Each animal received only a single dose of ibudilast or rolipram. EC_{50} and ED_{50} (concentrations producing 50 % inhibition of spasmogen-induced contraction *in vitro* and *in vivo*) values for ibudilast and rolipram are shown in Table 1.

treatment	*n*	*ibudilast*		*rolipram*	
		EC_{50} (nM)	*ED_{50} (μg)*	*EC_{50} (nM)*	*ED_{50} (μg)*
methacholine	3 - 6	1950 ± 400	82	99 ± 9	36
histamine	3 - 7	180 ± 70	108	34 ± 17	22
LTD_4	3 - 7	120 ± 50	26	26 ± 8	< 2

Ibudilast, whilst less potent than rolipram, produced significant reversal of contractile responses *in vitro* and *in vivo* independent of the spasmogen used. This suggests that rather than being a LTD_4 antagonist, ibudilast possesses airway smooth muscle relaxant properties similar to rolipram suggesting activity as a functional antagonist perhaps associated with inhibition of airway smooth muscle PDE.

Etoh, S., Ohashi, M., Baba, A., Iwata, H. (1990). Br. J. Pharmacol., 100, 564-568.
Nishino, K., Ohkubo, H., Ohashi, M., Hara, S., Kito, J., Irikura, T. (1983). Jpn. J. Pharmacol., 33, 267-278.
Ohashi, M., Kanai, R., Nishino, K., Sato. T., Takayanagi, I. (1986). Prostaglandins, 32, 875-888.

74P PHORBOL ESTER (PMA) CHALLENGE OF TRACHEAL SMOOTH MUSCLE CELLS AND ITS EFFECT UPON ADENYLYL CYCLASE, G_S AND INTRACELLULAR CYCLIC AMP

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We have demonstrated that the recombinant α sub-unit of G_S is a substrate for a protein kinase C-catalysed phosphorylation *in vitro* (Pyne *et al.*, 1992a) and have therefore now assessed if activators of protein kinase C can modulate adenylyl cyclase regulation in intact cells. Primary cultures were obtained according to the method of Panettieri *et al.* (1989). 24 hours prior to experimentation, cells were placed in DME containing 1% FCS and 1% DHS. Cells were challenged with PMA (10^{-11}-10^{-5}M) for 10 minutes. Incubations were stopped by addition of either ice-cold 0.25M-sucrose, 10mM-Tris/HCL, pH 7.4, 1mM-EDTA for adenylyl cyclase assay or ethanol for cyclic AMP measurement. Results are expressed as mean ± SD (student t-test).

PMA (10^{-7}M, 10 min) challenge enhanced basal and GppNHp-stimulated adenylyl cyclase activity in subsequently prepared membranes (specific activity, control 26.7 ± 3.15, GppNHp (10^{-4}M) 143.3 ± 5.6, PMA challenge 49.8 ± 1.85, GppNHp (10^{-4}M) 322.7 ± 6.7pmol/min/mg, n=3, $p < 0.005$ for control vs PMA). Whilst pertussis toxin (0.1μg/ml) pre-treatment (18h) also provoked a similar enhanced response (control 51.7 ± 2.7, GppNHp (10^{-4}M) 251.3 ± 2.1pmol/min/mg, n=3, $p<0.001$) the PMA challenge of pertussis toxin-treated cells resulted in an enhanced basal and GppNHp-stimulated response that was larger in magnitude than either agent alone (control 62.9 ± 2, GppNHp (10^{-4}M) 391 ± 4.7pmol/min/mg, n=3, $p<0.005$ for comparison with corresponding control and GppNHp-treated in pertussis toxin-treated cells). The PMA (10^{-7}M) challenge also enhanced GppNHp-stimulated cholera toxin-catalysed ADP-ribosylation of G_S, suggesting that PMA induces conformational changes in G_S that apparently allow it to become a more favourable substrate for cholera toxin. The PMA (10^{-7}M) challenge of cholera toxin-treated cells (18h, 1μg/ml) resulted in elevated intracellular cyclic AMP accumulation (C_o/C_x per 2.5 x 10^5 cells, cholera toxin-treated 2.8 ± 1, PMA 10^{-6}M challenge 10.77 ± 0.05, PMA 10^{-7}M 9.74 ± 0.322, n=3, $p<0.005$ comparison to cholera toxin-treated alone). This was a concentration-dependent event (10^{-11}-10^{-5}M). We were unable to detect changes in cAMP with PMA in non-cholera toxin-treated cells.
We suggest that PMA, possibly via protein kinase C can positively modulate pathways regulating adenylyl cyclase regulation. This may involve modulation of adenylyl cyclase itself, G_s and G_i function. This is in contrast to the action of methacholine which triggers a receptor-mediated modulation of G_S via an unidentified mechanism, leading to negative regulation (Pyne *et al.* 1992b).

We wish to acknowledge the National Asthma Campaign, Scottish Home and Health Department

Panettiera, R.A. , Murray.R.K., De Palo, L.R.Yadvish, P. A. & Kotlikoff, M.I. (1989) Am. J. Physiol. 256, C329-C335.
Pyne, N.J., Freissmuth, M. & Palmer, S. (1992a) Biochem. J. 285, 333-338.
Pyne, N. J., Grady, M., Shehnaz, D., Stevens, P., Pyne, S. & Rodger, I.W. (1992b) Brit. J. Pharmacol. (in press).

75P THE SELECTIVE PROTEIN KINASE C INHIBITOR, Ro31-8425, INHIBITS ADHESION OF HUMAN NEUTROPHILS STIMULATED BY PHORBOL DIBUTYRATE, BUT NOT BY C5a

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The aim of this study was to investigate the involvement of protein kinase C (PKC) and $[Ca^{2+}]_i$ in stimulated adhesion of human neutrophils. Neutrophils were coloaded with acridine orange (or BCECF) and fura-2 to measure adhesion and $[Ca^{2+}]_i$ respectively (Davies *et al*, 1990). Effects of C5a, ionomycin and phorbol 12,13-dibutyrate (PDBu) on adhesion to plastic and on $[Ca^{2+}]_i$ were measured in the same preparations of neutrophils. In the presence of extracellular Ca^{2+}, C5a stimulated adhesion (EC_{50} = 0.6nM) and increases in $[Ca^{2+}]_i$ (EC_{50} = 0.03nM). The Ca^{2+} ionophore, ionomycin, stimulated increases in both adhesion and $[Ca^{2+}]_i$ with similar dose-dependencies (EC_{50} = 100nM). Maximum adhesion was similar in response to both ionomycin and C5a. When $[Ca^{2+}]_i$ was plotted against adhesion in response to both C5a and ionomycin, a minimum $[Ca^{2+}]_i$ of ~300nM was found to be required for both ionomycin and C5a to promote adhesion. Stimulation with increasing concentrations of ionomycin caused further increases in $[Ca^{2+}]_i$ which correlated with increasing adhesion. With C5a, further dose-dependent increases in adhesion were not associated with any further increase in $[Ca^{2+}]_i$, suggesting an additional stimulatory component. The phorbol ester, PDBu, which activates directly PKC and did not increase $[Ca^{2+}]_i$ in neutrophils, stimulated adhesion (EC_{50} = 0.5nM). PDBu-stimulated adhesion was dose-dependently inhibited by a selective PKC inhibitor Ro31-8425 (IC_{50} = 0.5μM) while C5a-stimulated adhesion was unaffected by Ro31-8425 (at 10μM). However, a non-selective protein kinase inhibitor, staurosporine (100nM) inhibited both C5a- and PDBu-stimulated adhesion; this suggests that protein kinases other than PKC are involved in C5a-stimulated adhesion. C5a, ionomycin and PDBu also stimulated similar dose-dependent adhesion of human neutrophils to human endothelial cells. A spontaneously transformed human umbilical endothelial cell line, which expresses ICAM-1 but not E-selectin, was used. Adhesion was blocked by an anti-CD18 monoclonal antibody which suggests that it is mediated by β2 integrins (Mac-1 and LFA-1) binding to ICAM-1. As with adhesion to plastic, the response to PDBu was inhibited by Ro31-8425 while the response to C5a was unaffected, and the responses to both C5a and PDBu were inhibited by staurosporine. Similarly both C5a and PDBu upregulated neutrophil cell surface expression of Mac-1 (analysed by FACS), and Ro31-8425 inhibited the response to PDBu but not to C5a. These results suggest that C5a-stimulated adhesion of neutrophils is dependent on a threshold of $[Ca^{2+}]_i$ and that other signalling pathways are involved which are not dependent on PKC. However, direct activation of PKC by phorbol esters can also promote adhesion. By contrast, Ro31-8425 did inhibit superoxide production by neutrophils when stimulated either with receptor agonists or direct activators of PKC (Muid *et al*, 1991).

Davies, M.P.A., Hallam, T.J. & Merritt, J.E. (1990) Biochem. J. **267**, 13-16.
Muid, R.E., Dale, M.M., Davies, P.D., Elliott, L.H., Hill, C.H., Kumar, H., Lawton, G., Twomey, B.M., Wadsworth, J., Wilkinson, S.E. & Nixon, J.S. (1991) FEBS Lett. **293**, 169-172.

76P CHARACTERIZATION OF PMA- AND BRADYKININ-STIMULATED PHOSPHATIDYLCHOLINE HYDROLYSIS IN PRIMARY CULTURES OF TRACHEAL SMOOTH MUSCLE CELLS

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(introduced by B. Furman)

Sustained airway smooth muscle contraction can be evoked by activation of protein kinase C (PKC) using phorbol myristate acetate (PMA) (Park & Rasmussen, 1985). The endogenous activator of PKC, *sn*-1,2-diacylglycerol (DAG) can be derived from both inositol lipids and phosphatidylcholine (PtdCho) (Billah & Anthes, 1990). We have used primary cultures of guinea pig tracheal smooth muscle to determine the routes of formation of agonist-stimulated PtdCho-derived DAG, i.e. by phospholipases (PL) C and D.

Primary cultures of guinea pig tracheal smooth muscle (Panettieri *et al.*, 1989; passage 3, at 14-18 days from isolation) were incubated with [^{3}H]palmitate (2 μCi/ml) in DME/1% FCS/1% DHS for 24 h to selectively label PtdCho. Cells were washed and pre-incubated in Krebs-Hensleit buffer containing 1% (w/v) bovine serum albumin for 30 min prior to stimulation with bradykinin (BK) or phorbol myristate acetate (PMA). Lipid extracts were prepared and [^{3}H]-labelled metabolites resolved by tlc. PLD activity was determined by the accumulation of [^{3}H]phosphatidylbutanol ([^{3}H]PtdBut) in the presence of butan-1-ol (Pai *et al.*, 1988).

Both BK (1 μM) and PMA (100 nM) stimulated PLD activity as indicated by the accumulation of [^{3}H]PtdBut (control 650±100, BK 1800±50*, PMA 3600±250*, dpm, n=3). The response was blocked by inhibition of PKC using staurosporine (BK, by 86±11%$^+$, PMA, by 88±11%$^+$, n=3) and in cells that had been pre-treated with PMA for 24 h to down-regulate PKC (BK, by 88±11%$^+$, PMA, by 87±6%$^+$, n=3). Thus, activation of PLD by BK and PMA appears to be mediated by PKC. In contrast, PMA was unable to elicit the production of [^{3}H]DAG whereas BK induced the accumulation of [^{3}H]DAG (control 1812±77, BK 2928±116*, PMA 2053±114†, n=3) which was partially inhibited by pre-treatment of the cells with butan-1-ol, suggesting the involvement of PLD in the [^{3}H]DAG response. However, BK-stimulated [^{3}H]DAG production persisted in protein kinase C down-regulated cells (3032±160*, n=3). Therefore, BK appears to be able to activate both a PLC and PLD directed against PtdCho whereas PMA is able to activate PLD alone. Since one of the consequences of PKC activation by PMA is the stimulation of a PLD activity, its immediate product, phosphatidate, should be considered as a second messenger in the process of sustained contraction of airway smooth muscle.

$p<0.0005$ *vs control, $^+$vs untreated cells, †no significant difference vs control; Student's t-test.

Funded by The Wellcome Trust.

Billah, M.M & Anthes, J.C. (1990) Biochem. J. 269, 281-291.
Pai J-K., Siegel, M.I., Egan, R.W. & Billah, M.M. (1988) Biochem. Biophys. Res. Comm. 150, 355-364.
Panettieri R.A., Murray, R.K., De Palo, L.R., Yadvish, P.A. & Kotlikoff, M.I. (1989) Am. J. Physiol. 256, C329-C335.
Park, S. & Rasmussen, H. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 8835-8839.

77P EFFECTS OF (±)-SALBUTAMOL UPON ALLERGIC BRONCHOSPASM AND AIRWAY HYPERREACTIVITY IN THE GUINEA-PIG

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Protracted infusion of (±)-salbutamol exaggerates allergic airway hyperreactivity in the guinea-pig (Morley et al., 1991). In this species, the degree of allergic hyperreactivity varies with the spasmogen used for detection of this phenomenon (Morley et al., 1992); hence, the spectrum of changed responsivity due to (±)-salbutamol has been defined.

Ovalbumin (OA) (10 μg) in $Al(OH)_3$ (10 mg) was co-injected with B. pertussis (0 and 14 days) in the guinea-pig and antiserum (21 days) was injected intravenously (1 ml/animal); one week later, responsivity to spasmogens was defined before, and after, infusion of OA (32 or 56 μg/kg) for 1 hr, measuring resistance in cm H_2O/l/sec (R_L) and compliance in ml/cm H_2O (C_{dyn}) electronically. Following exposure to (±)-salbutamol (1 mg/kg/day, subcutaneously for 6 days), infusion of OA increased R_L (pre 86.8±1.4, post 128.2±6.6) (n=95) to a significantly ($p<0.001$) greater extent than in vehicle-treated animals (R_L pre 94.6±2.0, post 102.8±2.8) (n=80). Thus, infusion of OA in vehicle-treated animals increased R_L by 9% and C_{dyn} by 1 %, whereas infusion of OA in animals previously exposed to (±)-salbutamol, increased R_L by 48% and decreased C_{dyn} by 34 %. In animals exposed to OA after infusion of (±)-salbutamol for 6 days, changed responsivity of the airways, expressed as the ratio (post/pre) of $\log_{10}$ dose-effect slopes from a common origin was: leukotriene C_4 (LTC_4)(9.6), histamine (Hist) (6.3), leukotriene E_4 (LTE_4) (5.0), serotonin (5-HT) (3.9), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (2.4), acetylcholine (ACh) (2.3) and bradykinin (BK) (1.7); values which differed, in magnitude and rank order, from vehicle-treated animals: LTC_4 (5.5), Hist (3.2), $PGF_{2\alpha}$ (3.1), BK (2.7), LTE_4 (2.5), 5-HT (1.3) and ACh (1.1) (n=10 or 25 in each group). Within two hours of commencing infusion of OA, lungs were lavaged. In animals exposed to (±)-salbutamol, numbers ($x10^6$) of macrophages (9.1±0.8 v 15.3±1.6) and eosinophils (1.1±0.2 v 2.1±0.4), but not lymphocytes (0.3±0.1 v 0.3± 0.1) or neutrophils (1.2±0.3 v 0.5±0.4) were decreased significantly ($p<0.05$) by comparison with vehicle-treated animals. Of animals exposed to (±)salbutamol, 38/235 were maximally responsive to spasmogen prior to, and 40/235 died during or following infusion of OA. Of animals exposed to vehicle, 2/166 died on or following exposure to OA. These 80 animals were excluded. It is concluded that exacerbation of airway reactivity by (±)-salbutamol is not associated with an influx of leukocytes into the airway lumen and differs from exacerbation airway reactivity induced by antigen (Morley et al., 1992) or endotoxin (Chapman et al., 1992).

Chapman, I.D., Hoshiko, K., Mazzoni, L. and Morley, J. (1992) This meeting
Morley, J., Hoshiko, K., Chapman, I.D. and Mazzoni, L. (1992) J. Physiol. 446, 117P.
Morley, J., Chapman, I.D., Foster, A., Hoshiko, K. and Mazzoni, L. (1991) Br. J. Pharmacol. 104, 295P.

78P PAF-INDUCED HYPERREACTIVITY OF THE HUMAN NASAL AIRWAY

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Nasal hyperreactivity is a clinical feature of allergic rhinitis (Andersson *et al.* 1987). Allergen exposure can lead to the release of a variety of mediators including platelet-activating factor (PAF; 1-O-Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine), which has inflammatory activities compatible with it being a mediator of hyperreactivity of the lower airways (Page *et al.* 1984). We have investigated the effect of inhaled PAF on hyperreactivity of the human nasal airway in normal subjects.

Subjects were challenged with either 200μg histamine or 100μg bradykinin administered to each nostril by aerosol. These doses cause sub-maximal increases in nasal airway resistance. Nasal blockage was assessed objectively by measuring nasal airways resistance (NAR), using active posterior rhinomanometry. The response to each challenge was quantified as the maximal increase in NAR during the post-challenge measurements. Results were expressed as % increase above the vehicle control value. Subjects were then given 60μg PAF to each nostril, and repeat histamine and bradykinin challenges were performed at 2,6 and 24 hours post-PAF. In addition, some subjects underwent a nasal lavage with 5ml of sterile saline following PAF treatment, and the eosinophil cationic protein (ECP) concentration of the recovered lavage fluid was determined by radioimmunoassay. In another study, dose-response curves to histamine (50-400μg to each nostril) were established in 12 subjects. Each was then given either 30μg or 60μg PAF to each nostril, 3 times during the day and repeat histamine challenges were performed 24 hours following the last dose of PAF, and in some subjects, 48 hours and 7 days later.

Following PAF inhalation, no increase in nasal airway resistance was measured in any of the subjects. No reaction was obtained by vehicle alone. All subjects sneezed and experienced nasal itching and slight nasal blockage after histamine challenge. However, histamine challenge at 2,6 and 24 hours following PAF-pretreatment resulted in increases in NAR of 162%, 215% and 148% respectively, whereas the response to histamine with vehicle pretreatment was 79% (n=4).Thus NAR was elevated at each repeat challenge following PAF treatment and was also longer lasting than the response when only vehicle pretreatment was used. The maximum hyperreactivity was seen at 6 hours, when the % increase in NAR following histamine challenge was significantly larger than that obtained before PAF treatment ($P<0.05$). The maximal increase in ECP concentration of the nasal lavage fluid, which was 8.3μg/l compared with 2.9μg/l before PAF treatment ($P<0.05$), occurred at this time. Nasal blockage following histamine challenge at 48 hours and 7 days post-PAF was not significantly different from that without PAF pretreatment. Subjects challenged with bradykinin experienced nasal blockage only, which was augmented and longer lasting following PAF-pretreatment, with a maximal hyperreactivity occuring at 6 hours when NAR increased by 92% following bradykinin challenge as compared with an increase of 27% pre-PAF treatment (n=5, $P<0.01$).

It can be concluded that PAF causes a non-specific nasal airway hyperreactivity,which is greatest at 6 hours, and which is accompanied by an increase in ECP concentrations of nasal lavage fluid which is maximal at the time of greatest hyperreactivity.

Andersson M *et al.* (1987) Allergy 42, 631-637.
Page CP *et al.* (1984) TIPS 5, 105-197.

79P CHARACTERISATION OF HISTAMINE H_1-RECEPTORS ON HeLa CELLS

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HeLa cells may be a useful model system on which to study histamine H_1-receptor activation of phosphoinositidase C, without the potential complication of histamine-stimulated Ca^{2+} entry (Arias-Montaño & Young, 1992), which appears to play an important role in histamine-induced inositol phosphate formation in mammalian brain (Alexander *et al.*, 1990). However, the dissociation constants for antagonist binding to H_1-receptors on HeLa cells, determined from inhibition of [^{3}H]-mepyramine binding, have been reported to be much higher than those in brain tissues (Raymond *et al.*, 1991), raising the possibility that these cells possess a different subtype of the H_1-receptor. We have therefore set out to characterise histamine H_1-receptors on HeLa cells in more detail.

HeLa cells were cultured and labelled with [^{3}H]-inositol as described previously (Bristow *et al.*, 1991). Incubations with agonists in the presence of 30 mM Li^+ were for 30 min and [^{3}H]-inositol phosphates were separated by anion-exchange chromatography. Under the conditions employed inositol monophosphate ([^{3}H]-IP_1) is much the major phosphate present. Inhibition of [^{3}H]-mepyramine binding to HeLa cell membranes was measured as described by Treherne *et al.* (1991).

Histamine produced an 8-fold stimulation of basal [^{3}H]-IP_1 levels. Both N^{α}-methyl- and N^{α},N^{α}-dimethylhistamine gave the same maximal stimulation as histamine, but betahistine was a partial agonist (39% of histamine maximum). The EC_{50}s of the agonists in HeLa cells were closely similar to those in guinea-pig cerebellum, although in the latter tissue N^{α},N^{α}-dimethylhistamine was a partial agonist (Carswell & Young, 1986). The K_d values for mepyramine and N-methyl-4-methyldiphenhydramine determined from inhibition of histamine-induced [^{3}H]-IP_1 formation (3.4 and 6.7 nM, respectively) were in good agreement with those obtained from inhibition of the binding of 2 nM [^{3}H]-mepyramine (4.7 and 6.0 nM). The latter values and those for (+)-chlorpheniramine and temelastine were similar to values reported from measurements of inhibition of [^{3}H]-mepyramine binding in rat and human cerebral cortex.

Histamine H_1-receptors present on HeLa cells thus appear to have properties similar to those of H_1-receptors in mammalian brain. The much higher K_d values observed in the earlier study (Raymond *et al.*, 1991) probably represent a secondary binding site detected in the presence of a high concentration of [^{3}H]-mepyramine and of a very high concentration of the antagonist used to define non-specific binding.

Alexander, S.P., Hill, S.J. & Kendall, D.A. (1990) *Biochem. Pharmacol.* **40**, 1793-1799.
Arias-Montaño, J.A. & Young, J.M. (1992) *Br. J. Pharmacol.* **105**, 23P.
Bristow, D.R., Arias-Montaño, J.A. & Young, J.M. (1991) *Br. J. Pharmacol.* 104, 677-684.
Carswell, H. & Young, J.M. (1986) *Br. J. Pharmacol.* **89**, 809-817.
Raymond, J.W., Alberst, F.J., Middleton, J.P., Lefkowitz, R.J., Caron, M.G., Obeid, L.M. and Dennis, V.W. (1991) *J. Biol. Chem.* **266**, 372-379.
Treherne, J.M., Stern, J.S., Flack, W.J. & Young, J.M. (1991) *Br. J. Pharmacol.* 104, 1745-1751.

80P EFFECTS OF (R)-α-METHYLHISTAMINE AND SCOPOLAMINE ON WATER MAZE PERFORMANCE IN THE RAT

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The histamine H_3-autoreceptor has been proposed to control the synthesis and release of central histamine through a negative feedback mechanism (Arrang et al., 1983) and muscarinic heteroreceptors on histaminergic nerve terminals also regulate histamine release (Gulat-Marnay et al., 1989). Evidence exists for a histaminergic dysfunction in brains of Alzheimer disease patients (Mazurkiewicz-Kwilecki et al., 1989), suggesting a possible role for histamine in cognition. The present study examined the effect of chronic treatment with the selective H_3 agonist, (R)-α-methylhistamine ((R)-α-MeHA), and the muscarinic antagonist, scopolamine, on spatial learning and memory in the rat using a water maze task. Male Hooded Lister rats (230-320g) were trained on a water maze (Morris, 1981). Rats were tested over 12 trials (2 trials per day for 6 days), each lasting 180s and all rats remained on the platform for 20s. Two hours before testing, three paired groups of rats (n=9 per group) were injected, i.p., with either saline (SAL, 1 ml/kg) or (R)-α-MeHA (R_1, 6.3 mg/kg; R_2, 10 mg/kg). Each pair of groups (SAL, R_1 and R_2) was then injected, i.p., 100 min later with either SAL or scopolamine (SCOP, 0.5 mg/kg). 24 hr after the last training trial, animals were tested for retention of spatial memory (transfer test, 60s) and visual acuity (raised platform test). Maze acquisition, transfer and raised platform test data was analysed using analysis of variance (ANOVA).

Analysis of training trials indicated that SAL/SCOP rats were significantly (ANOVA, $F=11.3$, $p<0.01$) impaired in their performance compared to SAL/SAL controls whereas R_1/SAL, R_2/SAL and R_2/SCOP rats were not. R_1/SCOP rats exhibited increased escape latencies compared to SAL/SAL controls although this effect was not significant (ANOVA, $F=3.3$, $p=0.09$). Comparison of R_1/SCOP and R_2/SCOP groups with SAL/SCOP indicated that pretreatment with R_2 reversed a SAL/SCOP-induced deficit (ANOVA, $F=6.0$, $p<0.05$), but that with R_1 did not. In the post-training transfer test of memory retention (transfer latencies (% of total test time), mean ± SEM), R_2/SAL (23.2 ± 1) treatment resulted in enhanced spatial retention ($p<0.05$), whereas R_1/SAL (14.4 ± 2.0) was without effect in comparison to SAL/SAL (17.4 ± 1.5) controls. A memory impairment was observed after SAL/SCOP (9.1 ± 1.4, $p<0.001$) and R_1/SCOP (11.8 ± 1.5, $p<0.05$) treatment. Moreover, pretreatment with R_2 (14.0 ± 1.9) resulted in a reversal of the SCOP-induced deficit ($p<0.05$). None of the treatments resulted in impaired visual acuity. This study confirms previous evidence that the muscarinic antagonist, scopolamine, induces a learning deficit in the water maze (Hunter et al., 1986) and furthermore, suggests a dose-related reversal of this deficit by the selective H_3 agonist (R)-α-MeHA. These results indicate a possible functional H_3-receptor interaction with central cholinergic systems in rodent spatial learning and memory.

C.P.S. Smith is a SERC CASE student in conjunction with SmithKline Beecham.

Arrang, J.M., Garbarg, M. & Schwartz, J.C. (1983). Nature, **302**, 832-837.
Gulat-Marnay, C., Lafitte, A., Arrang, J.M. & Schwartz, J.C. (1989). J. Neurochem. **52**, 248-254.
Hunter, A.J., Roberts, F. & Tutty, C.A. (1986). Br. J. Pharmacol. **87**, 41P.
Mazurkiewicz-Kwilecki, IM. & Nsonwah S. (1989). Can. J. Physiol. Pharmacol. **67**, 75-78.
Morris, R.G.M. (1981). Learn. Motiv. **12**, 239-260.

81P EXCITATORY AMINO ACID ANTAGONISTS AND INHIBITORS OF GLUTAMATE RELEASE AS ANTICONVULSANTS IN THE GENETICALLY EPILEPSY-PRONE RAT

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Reproducible clonic seizures may be induced in genetically epilepsy-prone (GEP) rats upon exposure to a sound stimulus (100-110 dB, 60s) (Faingold, 1988). Compounds which reduce the excitatory effects of glutamate were compared with diazepam, carbamazepine, and phenytoin for anticonvulsant efficacy and impairment of locomotor performance using a rotarod (9cm diameter, 12 r.p.m., 60s) in GEP rats: Competitive NMDA, D(-)CPPene, CGP 37849 and its 1-ethyl ester CGP 39551; Non-competitive NMDA, MK801 (dizocilpine) H· maleate; Non-NMDA, NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzoquinoxaline·Li) and GYKI 52466 (1-(4-amino-phenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine·HCl); NMDA glycine site partial agonist ACPC (1-aminocyclopropanecarboxylate). Glutamate release blockers, BW 1003C87 (5(2,3,5-trichlorophenyl)-2,4-diaminopyrimidine ethane·HSO_3) and lamotrigine (3,5-diamino-6-(2,3- dichlorophenyl)-1,2,4-triazine) isethionate.
In separate experiments animals (250-500g) received vehicle or test compounds (i.p.) 1h after control tests and were retested at the times after administration detailed in table 1. ED_{50} values (µmole/kg) refer to the dose of compound corresponding to 50% of the number of animals protected from clonic seizure or for a 50% impairment of locomotor performance.

Table 1 ED50 values (µmole/kg, i.p.) for antagonism of clonic seizures and for ataxia

Drug	(m.w.)	Anticonvulsant effect						Locomotor impairment						
		15′	30′	1h	2h	4h	8h	7.5′	15′	30′	1h	2h	4h	8h
D(-)CPPene	(250)	--	--	2.7	--	1.9	>2.4	--	--	13	10	5	10	>48
CGP 37849	(209)	--	--	2.0	--	2.0	>2.4	--	--	25	22	24	59	>59
CGP 39551	(237)	--	--	>42	--	21	18	--	--	143	105	55	63	101
MK801	(337)	0.5	--	0.03	0.01	0.004	0.01	--	0.2	--	0.2	0.1	0.2	0.2
ACPC	(101)	>1484	1053	1286	--	>1484	--	--	--	--	>1484	--	>1484	--
NBQX	(342)	50	40	79	--	>79	--	--	328	459	459	--	>459	--
GYKI 52466	(330)	24	26	30	--	>61	--	--	26	49	>61	--	--	--
BW 1003C87	(400)	--	7.9	2.2	3.8	6.0	NDD	--	--	41	45	60	124	--
Lamotrigine	(383)	--	NDD	37	7.3	4.8	NDD	209	188	95	103	121	178	NDD
Diazepam	(285)	0.8	0.5	1.9	3.1	NDD	--	6.2	9.8	10	13	>18	--	--
Carbamazepine	(236)	--	254	168	98	132	330	258	135	159	169	207	224	NDD
Phenytoin	(274)	NDD	NDD	52	31	45	NDD	--	489	421	320	310	394	493

--: Not tested, NDD: Not dose-dependent data. Glutamate antagonists and release inhibitors have similar anticonvulsant potency in this rodent model of reflex epilepsy.

Faingold, C.L. (1988) *Gen. Pharmac.* **19**, 331-338

82P INTERACTION BETWEEN CYCLOTHIAZIDE AND 2,3-BENZODIAZEPINE ON NON-N-METHYL-D-ASPARTATE RECEPTORS IN RAT NEOCORTEX *IN VITRO* AND SPINAL CORD *IN VIVO*

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Earlier work from our group suggests that low concentrations of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate depolarise cortical slices by pharmacologically distinct receptors whereas these two agonists share similar receptors on spinal neurones in vivo (see Lodge et al., 1991). As part of our attempts to investigate this difference, we have examined the actions of cyclothiazide, reported to reduce AMPA receptor desensitization (Patneau et al., 1992), on both wedge-shaped slices of rat cerebral cortex using a grease seal technique and on spinal neurones in pentobarbitone-anaesthetised rats using the technique of microiontophoresis.

On cortical wedges, cyclothiazide 1-100uM potentiated the action of <5uM concentrations of AMPA (but not those of kainate or N-methyl-D-aspartate) with an ED50 of about 7.5uM. Both the 2,3-benzo-diazepine, GYKI 53655 3.16uM, and 6-nitro,7-sulphamoylbenzo[F]quinoxalinedione (NBQX), 1uM, produced a larger rightward shift of the concentration-response curve to AMPA (dose-ratio 8-10) than of that to kainate (DR: <2). Cyclothiazide, 10uM, reversed the AMPA antagonism by GYKI 53655 but not that by NBQX. All the above effects were rapidly reversible.

On spinal neurones in vivo, GYKI 53655, locally ejected (3-10nA; 4mM in 200mM NaCl; n=9) and intravenously administered (5mg/kg; n= 9) reduced the actions of AMPA and kainate in parallel (75-100%) with only minor effects on those of NMDA, as reported previously for GYKI 52466 and NBQX. Cyclothiazide (10nA; 10mM in 200mM NaCl) reversibly enhanced the actions of both AMPA and kainate, effects of low ejecting currents of both agonists being the most affected (n=4). In preliminary experiments on spinal neurones, cyclothiazide reduced the antagonistic effects of GYKI 53655 to a greater extent than those of NBQX.

Our results are consistent with the presence of an allosteric site on the AMPA receptor-channel complex at which 2,3-benzodiazepines and cyclothiazide interact to modulate the agonist activation.

Lodge, D., Jones, M.G., and Palmer, A.J.. (1992) Canad. J. Physiol. Pharmacol. **69**, 1123-1128
Patneau, D.K., Vyclicky, Jr.,L. and Mayer, M.L. (1992) Neurosci. Soc. Abs. **18**, 115.6.

83P ANIRACETAM REVERSES THE ANTICONVULSANT ACTION OF THE NON-NMDA ANTAGONIST, GYKI 52466, IN DBA/2 MICE

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Selective, AMPA(α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)/kainate antagonists, such as GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine.HCl) and NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline) have recently been shown to have potent anticonvulsant activity in several primate and rodent seizure models, including that of sound-induced seizures in the audiogenic DBA/2 mouse (Chapman *et al.*, 1991; Smith *et al.*, 1991).
Aniracetam (1-p-anisoyl-2-pyrrolidinone) potentiates responses to AMPA, but not to kainate and NMDA (Ito *et al.*, 1990). Administration of aniracetam (12.5 to 100 nmol, i.c.v.) to DBA/2 mice has no convulsant effect on its own, but reverses the anticonvulsant protection of GYKI 52466 (60 μmol; i.p.; 15 min) in a dose-dependant manner. Pretreatment with aniracetam (50 nmol; i.c.v.; 15 min before drugs) decreases the anticonvulsant efficacy of GYKI 52466 by 7 fold. The ED_{50} values (μmol/kg; lower and upper confidence limits, 95% probability) for protection against sound-induced clonic seizures in DBA/2 mice in the absence and presence of aniracetam pretreatment for GYKI 52466 are shown in Table 1. The anticonvulsant efficacy of NBQX is also reduced by aniracetam pretreatment, but to a lesser extent (2 fold, see Table 1). The anticonvulsant efficacy of the competitive NMDA antagonist, D(-)CPPene, in contrast, is not affected by aniracetam pretreatment. Thus, aniracetam selectively modulates antagonist action at the non-NMDA receptor.

Table 1 Effect of aniracetam on the anticonvulsant efficacies of 2 non-NMDA antagonists and one competitive NMDA antagonist against clonic seizures in DBA/2 mice.

	ED_{50} (μmol/kg, i.p., clonic phase) alone		+ aniracetam (50 nmol, i.c.v.)		Ratio
GYKI 52466 (15 min)	20.1	(11.9 - 33.9)	142	(91.7 - 219)	7.1
NBQX (15 min)	39.7	(33.8 - 46.7)	85.6	(63.9 - 115)	2.2
D(-)CPPene (30 min)	1.64	(1.48 - 1.84)	1.72	(1.08 - 2.8)	1.0

Chapman, A.G., Smith, S.E., & Meldrum, B.S. (1991) Epilepsy Res. 9, 92-96.
Ito, I., Tanabe, S., Kohda, A. & Sugiyama, H. (1990) J. Physiol. (Lond) 424, 533-543.
Smith, S.E., Dürmüller, N., & Meldrum, B.S. (1991) Eur. J. Pharmacol. 201, 179-183.

84P DEVELOPMENTAL SHIFT IN THE PHARMACOLOGICAL CHARACTERISTICS OF [^{3}H]IFENPRODIL BINDING TO THE NMDA RECEPTOR IN THE RAT CEREBELLUM

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The anti-ischaemic activity of ifenprodil has been attributed to its antagonism at the N-methyl-D-aspartate (NMDA) receptor that appears directed towards the polyamine modulatory site of this receptor complex. In agreement with this hypothesis, we have previously demonstrated that polyamine-sensitive [^{3}H]ifenprodil binding in the rat cerebral cortex identifies a site within the NMDA receptor. In the present study, we have characterized the polyamine-sensitive [^{3}H]ifenprodil binding site in the rat cerebellum.

The binding of [^{3}H]ifenprodil (31 Ci/mmol) to membranes of the cerebellum from the Sprague-Dawley rat (IFFA CREDO, France) was studied at an incubation temperature of 4°C and a radioligand concentration of 2 nM in 5 mM Tris-HCl buffer (pH 7.4) (Schoemaker, 1992). Data are presented as the means ± SEM of at least 3 experiments.

[^{3}H]Ifenprodil binding to membranes from the 8 day-old rat (K_d=27 ± 11 nM, B_{max}=0.80 ± 0.30 pmol/mg protein) is fully inhibited by spermine (IC_{50}=5.9 ± 0.3 μM) and is partially inhibited by the competitive NMDA antagonist CGP37849 (IC_{50}=1.5 ± 0.4 μM) to 57 ± 2% of controls. As opposed to data obtained in the rat cortex, the glycine antagonist 5,7-dichlorokynurenate fails to affect binding in concentrations up to 10 μM. During development, the K_d and B_{max} of [^{3}H]ifenprodil binding increased to 175 ± 20 nM and 3.3 ± 0.2 fmol/mg protein, respectively, in the adult cerebellum while the IC_{50} for spermine increases to 28 ± 5 μM. The major change in these parameters occurs in the third postnatal week. [^{3}H]Ifenprodil binding to membranes from the adult cerebellum is no longer affected by CGP37849, a modification in its pharmacological characteristics that mainly occurs in the fourth postnatal week.

Ifenprodil partially inhibits non-equilibrium [^{3}H]MK801 binding in the 8 day-old rat with high affinity (IC_{50}=16 ± 9 nM), but only shows a low affinity inhibition (IC_{50}=810 ± 140 nM) in the adult. Similarly, spermine stimulates [^{3}H]MK801 binding in the 8 day-old cerebellum to a maximum of 175 ± 11% at 30 μM, but monophasically inhibits binding in the adult.

Thus, high affinity, polyamine-sensitive [^{3}H]ifenprodil binding sites are present in the cerebellum of the immature rat and are allosterically coupled to the glutamate recognition site of the NMDA receptor. During postnatal development, the pharmacological characteristics of the [^{3}H]ifenprodil binding site change and its allosteric coupling to the glutamate recognition site is lost. In parallel, as reflected by [^{3}H]MK801 binding, the NMDA receptor disengages from the modulatory effects mediated through the polyamine site of the receptor complex. These observations add a further dimension to the complexity of the NMDA receptor and its subtypes.

Schoemaker, H. (1992) Brit. J. Pharmacol. **108**, 17P

85P EFFECT OF NMDA ON 1S,3R-ACPD-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN NEONATAL RAT CEREBRAL CORTEX SLICES

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Glutamate exerts its excitatory neurotransmitter actions in brain through a complex array of receptor subtypes which can be broadly divided into ionotropic and metabotropic classes (Mayer & Miller, 1990). In the present study we demonstrate that NMDA-receptor activation potentiates phosphoinositide responses to the metabotropic receptor-selective agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (ACPD) in neonatal rat cerebral cortex slices.

Slices (350 x 350 μm) were prepared from 7-8 day old rats of either sex. Slices were pre-incubated in Krebs-Henseleit buffer (KHB) for 60 min. Packed slices (25μl) were transferred to 250 μl KHB and incubated for 60 min with [^{3}H]inositol (0.5 μCi/vial). Following addition of LiCl (5 mM final concn.), slices were challenged with appropriate additions of ACPD and/or NMDA for 15 min. Incubations were terminated with 1 M trichloroacetic acid and following diethylether extraction, supernatants were applied to Dowex-1 (Cl^-) columns to resolve the total [^{3}H]inositol phosphate ([^{3}H]InsPx) fraction. Ins(1,4,5)P_3 mass determinations were made in parallel experiments, omitting the [^{3}H]inositol labelling step.

ACPD caused a [^{3}H]InsPx accumulation which was linear with time for at least 15 min. The stimulation was concentration-dependent (EC_{50} 18 + 4 μM), with a maximal stimulation at 300 μM ACPD of 12.8 + 0.9 fold-over-basal. NMDA caused a small increase in [^{3}H]InsPx accumulation (1.7 + 0.1 fold for 10 μM NMDA) and greatly potentiated the [^{3}H]InsPx accumulation evoked by ACPD. The [^{3}H]InsPx response to 300 μM ACPD being 1.9 + 0.1 fold greater in the presence of 10 μM NMDA. The presence of 10 μM NMDA also caused a significant 3-4 fold decrease in the EC_{50} for ACPD-stimulated [^{3}H]InsPx accumulation. Significant enhancements of the [^{3}H]InsPx response to 300 μM ACPD were observed at concentrations of NMDA up to 300 μM, with the greatest effect being observed at 10-30 μM. In the presence of 10 μM ACPD, the potentiation of the [^{3}H]InsPx response by 10 μM NMDA was completely and concentration-dependently (IC_{50} 80 + 10 nM) reversed by the non-competitive NMDA-receptor antagonist MK-801. ACPD (300 μM) evoked a rapid, transient increase in Ins(1,4,5)P_3 accumulation (basal 23.5 + 1.0; 15 s stim. 145.6 + 7.9 pmol/mg protein) which decreased to only 1.8 fold-over-basal by 10 min after agonist addition. The ACPD-stimulated Ins(1,4,5)P_3 mass accumulation was significantly enhanced at 1, 5 and 10 min (by 1.6 - 2.4 fold) in the presence of 10 μM NMDA.

We conclude that NMDA-receptor activation can have an important modulatory influence on inositol phospholipid hydrolysis and Ins(1,4,5)P_3 turnover following metabotropic-receptor stimulation. Furthermore, the fact that NMDA-/metabotropic receptor 'crosstalk' can occur at the level of Ins(1,4,5)P_3 may have important implications for the regulation of neuronal Ca^{2+} homeostasis, and adaptive responses such as long-term potentiation (Collingridge & Singer, 1990).

Collingridge, G.L. and Singer, W. (1990) Trend Pharmacol. Sci. 11, 290-296.
Mayer, M.L. and Miller, R.J. (1990) Trends Pharmacol. Sci. 11, 254-260.

86P AGONIST AND ANTAGONIST ACTIONS OF PHENYLGLYCINE DERIVATIVES AT DEPOLARIZATION-MEDIATING (1S,3R)-ACPD RECEPTORS IN NEONATAL RAT MOTONEURONES

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It was recently reported that (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3H-PG) selectively antagonized (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (ACPD)-induced depolarizations of neonatal rat motoneurones (Jones *et al*, 1992) and excitation of rat thalamic neurones (Eaton *et al*, 1992a). The same substance also selectively depressed the synaptic excitation of thalamic cells evoked by noxious stimuli (Eaton *et al* 1992b). We now report the actions of a series of related hydroxy and/or carboxy substituted phenylglycines on depolarizations induced in neonatal rat motoneurones by N-methyl-D-aspartate (NMDA), (S)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and (1S,3R)-ACPD.

(R)- and (S)-3-hydroxyphenylglycine (3H-PG), (R)- and (S)-4-carboxyphenylglycine (4C-PG), (R)- and (S)-4C3H-PG and (RS)-α-methyl-4-carboxyphenylglycine (αM4C-PG) were prepared in our laboratory. They were tested on the hemisected isolated spinal cord from neonatal (1 to 5 day-old) rats. Recordings were made from ventral roots and the standard medium contained tetrodotoxin (10^{-7} M).

(R)-3H-PG showed no activity against any of the agonist-induced depolarizations. (R)-4C-PG and (R)-4C3H-PG (5-50 μM) were moderately potent and selective NMDA antagonists; equipotent molar concentration ratios (EPMRs) relative to D-2-amino-5-phosphonopentanoate (D-AP5) were 13.4 $\pm$ 1.2 (n = 3) and 6.1 $\pm$ 1.4 (n = 3), respectively. At 1 mM, both the latter PGs showed weak-moderate AMPA antagonist activity but were inactive against ACPD-induced depolarizations.

(S)-3H-PG (100-300 μM) produced a small 'desensitizing' depolarization which decayed over a period of 3-15 min, similar to that produced at lower concentrations by (1S,3R)-ACPD (5-50 μM) (Jones *et al*, 1992). (S)-3H-PG (50-300 μM) reduced or abolished responses to (1S,3R)-ACPD. Such antagonism appeared to be competitive at the lower but not the higher (S)-3H-PG concentrations, and may have involved an element of 'cross-desensitization' with ACPD-induced responses. (S)-4C-PG (200-500 μM) and (RS)-αM4C-PG (100-500 μM) competitively antagonized (1S,3R)-ACPD-induced depolarizations with apparent K_D values of 208$\pm$15 (n = 4) and 341$\pm$30 (n = 5), respectively. L-2-Amino-3-phosphonopropionate (L-AP3; 1 mM), an uncompetitive antagonist at phosphoinositide (PI)-linked ACPD receptors, had no action on ACPD- or (S)-3H-PG-induced depolarizations. No depressant effects on NMDA- or AMPA-induced depolarizations were produced by any of the (S)-PG derivatives or by (RS)-αM4C-PG.

These results extend to (S)-3H-PG, (S)-4C-PG and (RS)-αM4C-PG the selective antagonist properties at ACPD depolarizing receptors previously reported for (S)-4C3H-PG (Jones *et al*,1992). (S)-4C-PG and (RS)-αM4C-PG are also competitive antagonists of ACPD-stimulated phosphoinositide hydrolysis in rat cerebral cortex (Porter *et al*, 1993), which suggests that PI-linked metabotropic receptors underly the depolarizing effect of ACPD on neonatal rat motoneurones. (S)-3H-PG and (S)-4C3H-PG, which are low-efficacy agonists at ACPD-stimulated PI-linked receptors (Jones *et al*, 1992 and unpublished observations), may act as partial agonists at ACPD receptors mediating depolarization in neonatal rat motoneurones. However, the lack of antagonist action of L-AP3 at these latter receptors remains to be explained.

Jones,P.St.J.L.,Porter,R.H.P.,Birse,E.F.,Pook,P.C-K.,Sunter,D.C.,Udvarhelyi,P.M.,Wharton,B.,Roberts,P.J. & Watkins,J.C. (1992) *Br.J.Pharmacol.* **106** 47P.
Eaton,S.A.,Salt,T.E.,Udvarhelyi,P.M.,Wharton,B. & Watkins,J.C. (1992a) *Br.J.Pharmacol.* **106** 48P.
Eaton,S.A.,Salt,T.E.,Sunter,D.C.,Udvarhelyi,P.M. & Watkins,J.C. (1992b) *Br.J.Pharmac.* **107** 244P.
Porter,R.H.P.,Roberts,P.J.,Jane,D.E.,Sunter,D.C. & Watkins,J.C. (1993) this meeting.

87P DIFFERENTIAL ACTIONS OF THE METABOTROPIC GLUTAMATE RECEPTOR ANTAGONISTS 4C-PG AND αM4C-PG AT L-AP4-LIKE RECEPTORS IN NEONATAL RAT SPINAL CORD

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A range of substances, including L-2-amino-4-phosphonobutyrate (L-AP4), (1S,3R)- and (1S,3S-)1-aminocyclopentane-1,3-dicarboxylate (ACPD) and both (S)-4-carboxy-3-hydroxy- and (S)-3-carboxy-4-hydroxyphenylglycine (4C3H-PG and 3C4H-PG, respectively) depress monosynaptic excitation of neonatal rat motoneurones by a presumed presynaptic action (Pook *et al*, 1992; Birse *et al*, 1992). Similar effects of L-AP4 and 1S,3R-ACPD in hippocampus have been linked to the activity of metabotropic receptors (Baskys and Malenka, 1991). We report here that two competitive antagonists at phosphoinositide (PI)-linked metabotropic receptors (Porter *et al*, 1993) have opposite actions at L-AP4-like receptors in neonatal rat motoneurones.
Dorsal root-evoked ventral root potentials (DR-VRPs) were recorded from hemisected spinal cords of 1-5 day old rats bathed in a medium containing 1 mM Mg^{2+} and 50 μM D-2-amino-5-phosphonopentanoate (D-AP5). (S)-4-Carboxyphenylglycine (4C-PG; 200-1000 μM) slightly depressed the monosynaptic response when added to the superfusion medium alone, and, when co-administered with L-AP4 (7.5-12.5 μM), 1S,3R-ACPD (5-10 μM) or (S)-3C4H-PG (150 μM) potentiated the depressant effects on the monosynaptic response produced by each of these substances. In contrast, (RS)-α-methyl-4-carboxyphenylglycine (αM4C-PG; 100-1000 μM) alone had no effect on the DR-VRP, but antagonized the depressant effect of all these substances as well as those produced by 1S,3R-ACPD (5 μM) or (S)-4C3H-PG (100 μM). Results obtained with L-AP4 and 1S,3S-ACPD are shown in the Table.

	% Depression of DR-VRP			
	Control	+(S)-4C-PG (500 μM)	Control	+(RS)-αM4C-PG (1 mM)
L-AP4	33±4(3)	86±1(3)	55±4(5)	37±4(5)
(1S,3S)-ACPD	45±12(3)	65±9(3)	62±4(5)	30±5(5)

Since (S)-4C-PG and (RS)-αM4C-PG have similar actions as competitive antagonists of ACPD-stimulated PI hydrolysis in cerebral cortex (Porter *et al*, 1993), the present results support previous conclusions (Pook *et al*, 1992) that the receptors mediating L-AP4-like synaptic depressant effects in neonatal rat motoneurones differ from PI-linked metabotropic glutamate receptors in cortex. αM4C-PG is the first antagonist to be reported for L-AP4-like depressant effects. Preliminary observations suggest, however, that both the potentiating effects of (S)-4C-PG and the antagonist effects of (RS)-αM4C-PG vary quantitively according to the synaptic depressant agonist used, raising the possibility that more than a single type of receptor mediates these effects.
Acknowledgements Supported by the Medical Research Council and US PHS 25640.

Pook,P.C-K.,Sunter,D.C.,Udvarhelyi,P.M. & Watkins,J.C. (1992) *Exp.Physiol.* **77** 529-532.
Birse,E.F.,Mewett,K.N.,Pook,P.C-K.,Tyrell,K.,Udvarhelyi,P.M.,Wharton,B. & Watkins,J.C. (1992) *J.Physiol.(Lond.)* **452** 185P.
Baskys & Malenka,R.C. (1991) *J.Physiol.(Lond.)* **444** 687-701.
Porter,R.H.P.,Roberts,P.J.,Jane,D.E.,Sunter,D.C. & Watkins,J.C. (1993) this meeting.

88P ACTIONS OF THE METABOTROPIC GLUTAMATE RECEPTOR ANTAGONIST (RS)-α-METHYL-4-CARBOXYPHENYLGLYCINE ON RESPONSES OF THALAMIC NEURONES TO ACPD

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We have previously shown that the excitatory action of the putative metabotropic glutamate receptor agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (ACPD) on thalamic neurones *in vivo* can be antagonised by (S)-4-carboxy-3-hydroxyphenylglycine and (S)-4-carboxyphenylglycine (Eaton *et.al.*, 1992). Unfortunately, these compounds have a degree of partial agonist action on ACPD-stimulated inositol phosphate hydrolysis. We have therefore tested the actions of the novel antagonist (RS)-α-methyl-4-carboxyphenylglycine (αM4C-PG), which is a competitive antagonist of cortical ACPD-stimulated inositol phosphate hydrolysis (Porter *et.al.*, 1993), on thalamic neurones.

Extracellular action potential recordings and iontophoretic drug applications were made with multibarrel glass electrodes in urethane-anaesthetised rats (Salt & Eaton, 1991). Responses of 13 ventrobasal thalamus neurones to ACPD, NMDA and either kainate or AMPA, applied iontophoretically, were challenged with concurrent iontophoretic ejections of αM4C-PG (164±11nA, 50mM pH 8 solution). This antagonist reduced the excitatory responses to ACPD to 25%±4.8% (mean ± s.e.m., n=13) of their control values while responses to NMDA and AMPA were reduced to a much lesser extent (94%±8.3% and 82%±7.2% of control values, respectively). Responses to noxious sensory stimulation (tail immersion at 52°C for 15-40s) were reduced to 30%±7.5% (n=4) of control values by αM4C-PG, when ejected with ACPD-selective iontophoretic currents. These findings show that αM4C-PG, an antagonist at ACPD-stimulated metabotropic receptors, can act as a selective antagonist of ACPD-evoked excitations in the thalamus. Furthermore, this antagonist appears to be useful in the study of possible synaptic roles for ACPD-activated receptors.

Supported by the Medical Research Council and U.S. Public Health Service (NSS26540).

Eaton, S.A., Salt, T.E., Sunter, D.C. Udvarhelyi, P.M. & Watkins, J.C. (1992) Brit. J. Pharmacol. Proceedings, **In Press.**
Porter, R.H.P., Roberts, P.J., Jane, D.E, Sunter D.C. & Watkins, J.C. (1993) Brit. Pharm. Soc. Proc., **This Meeting.**
Salt, T.E. & Eaton, S.A. (1991) Eur. J. Neuroscience **3**, 1104-1111.

89P (S)-4-CARBOXY-3-HYDROXYPHENYLGLYCINE (4C3H-PG) AND (RS)-4-CARBOXYPHENYLGLYCINE (4C-PG) DISTINGUISH MULTIPLE METABOTROPIC GLUTAMATE RECEPTOR RESPONSES IN THE NTS

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Monosynaptic evoked currents mediated by α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and A-type γ-aminobutyric acid ($GABA_A$) receptors are reversibly inhibited in the rat NTS by the application of the metabotropic glutamate receptor agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (ACPD) (Glaum & Miller, 1992a). (1S,3R)-ACPD mimics the kynurenate-insensitive actions of glutamate on cardiovascular reflexes *in vivo* (Pawloski-Dahm & Gordon, 1992). Recently, we have demonstrated that (1S,3R)-ACPD produces effects in the NTS *via* inhibition of glutamate release and modulation of AMPA and $GABA_A$ receptor sensitivity, as well as producing a direct excitatory effect on NTS neurones (Glaum & Miller, 1992b). We have now characterised the ability of (S)-4C3H-PG and (RS)-4C-PG, two recently reported metabotropic glutamate receptor antagonists (Roberts *et al*, 1992), to inhibit (1S,3R)-ACPD effects in the NTS.

Whole cell patch/slice voltage clamp recordings from 56 neurones in 18-48 day old rat tranverse brainstem slices were made as previously described (Glaum & Miller 1992a). Postsynaptic currents were evoked by stimulation in the region of the *tractus solitarius*. EPSCs were evoked in the presence of bicuculline (10 μM) and D-2-amino-5-phosphonopentanoate (D-AP5; 50 μM). IPSCs were evoked in the presence of D-AP5 and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM). AMPA was pressure ejected from a visually-guided pipette in the presence of bicuculline/D-AP5 supplemented with 1 μM tetrodotoxin (TTX). The $GABA_A$ agonist muscimol was pressure ejected in the presence of D-AP5/DNQX and, in some instances, TTX.

(1S,3R)-ACPD (10-100 μM) produced a dose-dependent decrease in monosynaptic EPSCs and IPSCs. 4C3H-PG (200 μM) or (RS)-4C-PG (500 μM) significantly reduced the inhibition of EPSCs and IPSCs by (1S,3R)-ACPD. Similarly, the (1S,3R)-ACPD-mediated increase in the AMPA (I_{AMPA}) and decrease in the muscimol (I_{MUSC}) evoked currents were antagonized by 4C3H-PG and 4C-PG. Both compounds exhibited weak agonist effects, suggesting that they behave as partial agonists with low efficacy. In contrast, the direct inward current produced by (1S,3R)-ACPD (I_{ACPD}) was not blocked by either antagonist.

Treatment (μM)	EPSC	IPSC	I_{AMPA}	I_{MUSC}	I_{ACPD}**
1S,3R-ACPD, 25	51.7±6.7	34.0±3.4	152.9±14.6	68.8±1.4	8.18±2.0
+ 4C3H-PG, 200	80.6±5.3*	83.0±0.3*	122.5±1.7*	93.6±1.4*	5.8±2.5
+ 4C-PG, 500	79.8±7.4*	62.5±6.5*	116.3±3.6*	92.3±5.1*	11.3±5.9

values shown are % control (mean±s.e.m) or **pA, $3 \leq n \leq 10$, *$p < 0.05$ *vs* control

The present data suggest that at least two distinct (1S,3R)-ACPD receptor effects mediated by (1S,3R)-ACPD in the NTS can be distinguished by 4C3H-PG and 4C-PG. The pharmacology of the presynaptic glutamate autoreceptor underlying the reduction in the EPSC and the postsynaptic (1S,3R)-ACPD receptor mediating the modulatory effects on I_{AMPA} and I_{MUSC} remains to be elucidated.

Glaum,S.R. & Miller,R.J. (1992a) *J.Neurosci.* **12** 2251-2258.
Glaum,S.R. & Miller,R.J. (1992b) *J.Neurosci.* (in press).
Pawloski-Dahm,C. & Gordon,F.J. (1992) *Am.J.Physiol.* **363** 1611-1615.
Roberts,P.J.,Birse,E.F.,Eaton,S.A.,Jones,P.L.St.J.,Pook,P.C-K.,Porter,R.H.P.,Udvarhelyi,P.M.,Wharton,B.,Salt,T.E. & Watkins,J.C. (1992) *Soc.for Neurosci.* **18** 648.

90P CEREBROPROTECTION BY A PYRIMIDINE DERIVATIVE, BW1003C87, IN A MODEL OF FOREBRAIN ISCHAEMIA IN THE RAT

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Antagonists of glutamate receptors have been shown to decrease neuronal damage in rodent models of cerebral ischaemia. In the present study we have used an inhibitor of glutamate (GLU) release, BW 1003C87 (5(2,3,5-trichlorophenyl) -2,4- diaminopyrimidine ethane HSO3) in a model of transient severe forebrain ischaemia (4-vessel occlusion) in the rat. We have investigated the effect of the drug on amino acid (AA) release and on neuronal damage induced by ischaemia. Adult male Wistar rats were prepared for 4-vessel occlusion (Pulsinelli and Brierley, 1979) under chloral hydrate anaesthesia, by cauterisation of the vertebral arteries and isolation of the common carotid arteries. At the same time, a transverse microdialysis fibre was inserted through the dorsal hippocampus or through the striatum. The following day, the fibre was perfused with a phosphate buffer solution and ischaemia was induced by carotid occlusion for 20 min in awake rats. Extracellular AA concentration was measured using HPLC analysis coupled with fluorescent detection. In a separate experiment rats were submitted to ischaemia and perfusion-fixed 3 days post-ischaemia. Lesions were assessed in the hippocampus and in the striatum using a 6-point scale (0: 0-10% cell loss, 1: 10-30%, 2: 30-50%, 3:50-70%, 4: 70-90%, 5: 90-100%). 20 min ischaemia led to an extracellular accumulation of GLU, aspartate, taurine and GABA in both hippocampus and striatum. BW1003C87, 10 and 20mg/kg i.p. 20 min prior to ischaemia significantly reduced the extracellular accumulation of all the AA in hippocampus and striatum during ischaemia and early the reperfusion. BW1003C87, 10 and 20mg/kg given i.p. 20 min prior to ischaemia and 4h post-ischaemia significantly reduced the hippocampal CA1 (65 and 74% protection respectively) and the striatal (59 and 53% protection) lesions. Delayed administration of BW1003C87, 20mg/kg i.p. given post-ischaemia either at 0 and +4h or at +2h and +6h significantly reduced CA1 lesions (85 and 76% protection respectively) whereas no significant protection was seen in the striatum. These data indicate that BW1003C87 is strongly able to inhibit the extracellular accumulation of the AA during a 4-vessel occlusion. The drug is also a potent cerebroprotector in this model in which NMDA receptor antagonists fail to protect CA1. However the effect of BW1003C87 on ischaemic GLU release can not explain the neuroprotection in CA1 since the delayed administration (+2h and +6h) is still highly protective. Protection could however be mediated by an action on sodium channel as previously described (Meldrum et al., 1992).

Pulsinelli, W.A. & Brierley, J.B. (1979) Stroke 10, 267-272.
Meldrum, B.S., Swan, J. H., Leach, M.L., Millan, M.H., Gwinn, R., Kadota, K., Graham, S.H., Chen, J. & Simon, R.P. (1992) Brain Res. 593, 1-6.

91P NEUROPROTECTIVE EFFECT OF ENADOLINE IN THE ACUTE RAT MODEL OF FOCAL ISCHAEMIA

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In addition to playing a role in antinociception, the opioid system has been implicated in post-ischaemic neurodegeneration. Recent studies suggest that stimulation by opioid agonists, including enadoline (CI-977), selective for the κ-type, can ameliorate neuronal degeneration Hayward *et al.*, 1992; Kusomoto *et al.*, 1992). We have further investigated this ability of enadoline to afford neuroprotection in the rat model of acute focal ischaemia.

In isoflurane anaesthetized Sprague-Dawley rats (325-425g), focal ischaemia was induced by permanent occlusion of the left middle cerebral artery (MCAO) via a subtemporal approach (Tamaru *et al.*, 1981). Animals were sacrificed 4h later. Throughout the pre- and post-ischaemic period key physiological parameters were monitored (Table 1). Enadoline at doses of 0.1, 0.3, 1.0mg/kg (n=8) was administered subcutaneously 30min prior to ischaemia. Animals were perfusion fixed and brains removed, multiple sections were taken throughout the cerebrum and stained with haemotoxylin-eosin or with a combination of cresyl violet and luxol fast blue. Areas and the volumes of ischaemic damage were assessed using an image analyser at 9 preselected coronal planes.

Enadoline produced a dose-dependent reduction in the volume of ischaemic damage which was most marked at 1.0mg/kg (saline-treated controls $144.2 \pm 12.7 mm^3$; enadoline-treated $99.1 \pm 11.4 mm^3$, $P < 0.01$ t-test with Bonferroni correction).

Table 1: Physiological variables (mean ± s.e.mean) measured in saline and enadoline (1mg/kg) treated animals.

Treatment Group	Time post MCAO (h)	MABP (mmHg)	Temp (°C)	PO_2 (mmHg)	PCO_2 (mmHg)	pH	Glucose (mmol/l)
Saline	1	95±4	36.9±0.1	130±4	38.0±0.8	7.45±0.01	9.8±0.5
Enadoline (1mg/kg)	1	95±3	37.0±0.1	144±3	35.2±1.0	7.49±0.01	9.1±0.1
Saline	4	88±3	36.9±0.1	129±4	35.4±0.4	7.45±0.01	8.4±0.2
Enadoline (1mg/kg)	4	93±5	37.0±0.1	141±4	36.0±0.5	7.46±0.01	8.9±0.3

These results indicate that enadoline is an effective anti-ischaemic agent in the rat model of acute focal ischaemia, without altering important physiological parameters that influence the degree of ischaemic damage.

Hayward, N.J., McKnight, A.T. & Woodruff, G.N. (1992). Mol. Neuropharmacol., **2**, 221-227.
Kusumoto, K., Mackay, K.B. & McCulloch, J. (1992). Brain Res., **576**, 149-151.
Tamura, A., Graham, D.I., McCulloch, J. & Teasdale, G.M. (1981). J. Cereb Blood Flow Metab., **1**, 53-60.

92P AGONIST-INDUCED CHANGES OF A_1 AND A_2 ADENOSINE RECEPTOR FUNCTION IN RAT BRAIN SLICES

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During brain ischemia and hypoxia, adenosine (ado) levels are massively increased due to both the rapid depletion of metabolic ATP and to the excessive ischemia-associated glutamatergic neuroexcitation. The so-formed ado is believed to counteract ischemia-associated neuronal death, mostly via activation of the A1 ado receptor subtype. It is however known for a variety of receptors that prolonged agonist exposure can result in agonist-induced receptor desensitization with consequent losses of receptor function. The prolonged and massive exposure of brain ado receptors to the endogenous ligand during ischemia might therefore result in changes of receptor sensitivity leading to a weakening of ado neuroprotective activity. To address this issue, we have studied agonist-induced changes of ado receptors in rat brain slices obtained from either rat striatum or cortex. Slices were exposed to ado analogues for selected time periods (15-60 min) and repeatedly washed after agonist removal. Ado receptor function in control and agonist-exposed slices was evaluated by measuring coupling with the cAMP-producing system in striatum (i.e., A1 and A2 receptor-mediated inhibition and stimulation of adenylate cyclase activity, respectively) and with membrane inositol phosphates (InsPs) formation in cortex (i.e., A1 receptor-mediated inhibition of histamine-stimulated InsPs production, Paoletti et al., 1992). In rat striatum, exposure to either Cyclo-Pentyl-Ado (CPA) or N-Ethyl-Carboxamido-Ado (NECA) resulted in a rapid, time-, and dose-dependent desensitization of A1 receptors, as shown by a gradual and statistically significant loss of A1-mediated inhibition of adenylate cyclase activity. For example, after pre-exposure to 10 μM CPA for 15, 30 and 60 min, maximal A1 receptor mediated inhibition of adenylate cyclase (% of basal activity set to 100%) was, respectively, 93±3 ($P<0.05$), 110±5($P<0.01$) and 115±5 ($P<0.01$), with respect to 73±4 in control slices. Such a rapid agonist-induced loss of A1 receptor function was also confirmed in rat cortex. After a 15 min pre-exposure of slices to either 0.1, 1 or 10 μM CPA, A1 receptor mediated inhibition of InsPs formation (% of maximal histamine stimulation set to 100%) was 81±4 ($P=0.05$), 107±5 ($P<0.02$) and 105±4 ($P<0.02$) with respect to 68±3 in control slices. A2 receptors seemed resistant to agonist-induced desensitization, at least for the exposure periods utilized in this study. In striatum, the loss of A1 receptor function was accompanied by a concomitant increase of A2 receptor-mediated stimulation of adenylate cyclase activity, suggesting an "unmasking" of A2 stimulatory receptors as a consequence of the loss of inhibitory receptors (Abbracchio et al., 1992). These results therefore indicate that the over-exposure to ado analogues can result in a functional unbalance between A1 and A2 ado receptors, which might be at the bases of changes of ado neuroprotective activity in brain pathological conditions characterized by an increased release of the nucleoside.

Abbracchio, M.P., Fogliatto G, Paoletti, A.M., Rovati, G.E. & Cattabeni, F. (1992) Eur. J. Pharmacol. (Mol. Pharmacol. Sect.), in press
Paoletti, A.M., Balduini, W., Cattabeni, F., Abbracchio, M.P. (1992) Neurosci. Res. Commun. 11, 19-26.

93P ENHANCEMENT OF ETHANOL INTAKE IN WISTAR RATS FOLLOWING PRETREATMENT WITH LOW DOSES OF 8-OH DPAT AND GEPIRONE USING A LIMITED ACCESS PARADIGM

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Preclinical and clinical evidence supports a role of enhanced central 5-HT function in the attenuation of voluntary alcohol intake (Sellers *et al,* 1992). It has been reported that 8-OH DPAT (DPAT) and gepirone (GEP), agonists at the 5-HT_{1A} receptor, reduce ethanol (EtOH) intake in rats (Svensson *et al,* 1989, Knapp *et al,* 1992). As 5-HT_{1A} agonists reduce 5-HT transmission, particularly at low doses, these findings are contrary to what one may predict. This may be due to the doses examined, which induce aspects of the 5-HT syndrome and which may therefore be incompatible with EtOH drinking. The aim of the present study was to investigate the effects of DPAT and GEP on EtOH and water intake at doses previously shown to enhance behaviours reflective of reduced 5-HT transmission.

Following a 42 day acquisition phase, male Wistar rats had access to a 12% EtOH solution and water (n=20, E/W) or water alone (n=5, W) for 40 mins each day in individual "drinking" cages. They were then returned to group housing, where food and water was available *ad libitum.* Once stable fluid intakes were attained, rats were randomly allocated to one of four treatment sequences and, using a within-subjects design, the effect of pretreatment with vehicle (V), 31, 62 and 125 µg/kg s.c. DPAT on EtOH and water intake was assessed. Following a washout period, the effect of GEP (0.3, 1 and 3 mg/kg s.c.) and V pretreatment was similarly examined. A final phase assessed the effects of DPAT at a higher (250 µg/kg) and lower (6 µg/kg) dose.

E/W rats typically exhibited a high stable EtOH preference (86±4%); the amount of EtOH consumed ranged from 0.17-0.93 g/kg between rats. W rats consumed 2.5±0.7 ml. DPAT at 31 ans 62 µg/kg enhanced EtOH intake (e.g. V = 0.45±0.04 g/kg, 62 µg/kg = 0.70±0.08 $p<0.050$); however, at 125 µg/kg EtOH intake was unaffected. 62 and 125 µg/kg DPAT markedly reduced water intake in the W group (V = 2.5±0.7ml, 62 µg/kg = 0.6±0.2, 125 µg/kg = 0.3±0.1 $p<0.05$). At this higher dose most rats exhibited flattened body posture and lower lip retraction. GEP did not reduce water intake in W group and no change in intake of either fluid was recorded for the E/W group. However, when rats were subdivided as high (0.76±0.04 g/kg, n=8) or low (0.35±0.04 g/kg, n=8) EtOH drinkers, marked changes were observed. Thus,l and 3 mg/kg GEP enhanced EtOH intake in the low drinkers (0.65±0.14 & 0.62±0.12 g/kg respectively, $p<0.05$) whilst intake was attenuated following 3 mg/kg dose in high drinkers (0.44±0.05 $p<0.05$). Water intake in both subsets was not altered. In a final study, 6 µg/kg DPAT did not affect intakes in either the E/W or W group. 250 µg/kg DPAT markedly attenuated fluid intake (>50%) in the E/W and W rats, and induced marked 5-HT syndrome.

Low doses of DPAT and GEP enhanced EtOH intake in low EtOH drinking rats and thus support a role of 5-HT in the modulation of EtOH intake. Suppression in EtOH intake observed at higher doses was comparable with those reported in other studies. However, as these observations were not confined to EtOH intake, this may suggest that these effects are non-specific.

Knapp, D.J., Benjamin, D. & Pohorecky, L.A. (1992) Drug Dev. Res. 26, 319-341
Sellers, E.M., Higgins, G.A. & Sobell, M.B. (1992) TiPS 13, 69-75
Svensson, L., Engel, J & Hård, E. (1989) Alcohol 6, 17-21

94P STEREOSPECIFIC BLOCKADE OF 8-OH-DPAT-INDUCED HYPOTHERMIA IN RODENTS BY THE SELECTIVE 5-HT_{1A} RECEPTOR ANTAGONIST, WAY 100135

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The characterisation of 5-HT_{1A} receptor function has been hampered by a lack of selective 5-HT_{1A} receptor antagonists. For example, a number of putative 5-HT_{1A} antagonists (eg. NAN-190 and BMY 7378), which block behavioural responses mediated by 5-HT_{1A} receptors, behave as partial agonists at somatodendritic 5-HT_{1A} autoreceptors (VanderMaelen et al., 1987; Lum et al., 1990). WAY100135 [N-tert-butyl 3-4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide dihydrochloride] has recently been shown to block both postsynaptic and presynaptic (somatodendritic) 5-HT_{1A} receptor mediated responses and to have no intrinsic agonist action (Fletcher et al., 1991; Routledge et al., 1992). Here we report the effects of WAY100135 and its (+)-and (-)-enantiomers on 8-OH-DPAT-induced hypothermia in the mouse and the rat.

Groups of 8 female albino Tuck T/O mice (24-35g) and male Sprague-Dawley rats (175-215g; Charles River) were used. The antagonists or saline-vehicle were administered s.c. or i.p. 20 min before the s.c. injection of a challenge dose of 8-OH-DPAT (0.5 mg/kg for mice; 0.25 mg/kg for rats). Rectal (mice) or oesophageal (rats) body temperatures were recorded immediately before each drug injection, and at 15 and 30 min after 8-OH-DPAT. Hypothermic responses (mean ± s.e.m.) to 8-OH-DPAT in saline-pretreated animals, measured as the maximum decrease in body temperature recorded in this latter period, ranged from -2.3 ± 0.4 to -3.6 ± 0.5°C for mice and from -1.7 ± 0.2 to -2.1 ± 0.2°C for rats. WAY100135 and its (+)-enantiomer (1-10 mg/kg s.c.) dose-dependently attenuated 8-OH-DPAT-induced hypothermia in both the mouse and the rat, with minimum effective doses ($P<0.05$; ANOVA/t-test) of 3 and 1 mg/kg s.c. respectively in both species. ID_{50} values (doses to inhibit hypothermia by 50% (with 95% confidence limits)) for WAY100135 and the (+)-enantiomer were 2.5 (1.5-4.1) and 1.6 (1.0-2.6) mg/kg s.c. respectively in the mouse, and 3.0 (1.7-5.3) and 1.3 (0.9-1.8) mg/kg s.c. respectively in the rat. In comparison, the (-)-enantiomer of WAY100135, at doses of 3-30 mg/kg s.c., had no significant effect on the hypothermic response to 8-OH-DPAT in either species. Neither WAY100135 nor its enantiomers had any intrinsic effects on body temperature at any of the doses tested. In contrast, the putative 5-HT_{1A} antagonist, NAN-190 (1.0-3.0 mg/kg i.p.), induced a significant and dose-related hypothermia (0.7-2.0°C) in mice, an effect which was additive to the hypothermia (3.0-3.6°C) induced by 8-OH-DPAT, i.e. NAN-190 did not attenuate 8-OH-DPAT-hypothermia in the mouse. In rats, NAN-190 significantly decreased body temperature (by 0.8°C) at a dose of 1.0 mg/kg i.p. but dose-dependently attenuated 8-OH-DPAT-induced hypothermia over the dose-range 0.1-1.0 mg/kg, with an ID_{50} value of ~0.8 mg/kg i.p. These results with NAN-190 confirm those of a previous study (Moser, 1991). In additional experiments, WAY100135 (3-30 mg/kg s.c.), did not significantly modify the hypothermia induced by either the dopamine receptor agonist, apomorphine (0.5 mg/kg s.c.), or the α_2-adrenoceptor agonist, UK-14,304 (0.5 mg/kg s.c.). The present data are in agreement with previous reports showing that WAY100135 is a selective and silent antagonist at central 5-HT_{1A} receptors and further confirm that this activity resides predominantly in the (+)-enantiomer.

Fletcher, A., Bill, D.J., Bill, S.J., Brammer, N.T., Cliffe, I.A., Forster, E.A., Reilly, Y. & Lloyd, G.K. (1991) Soc. Neurosci. Abstr., **17**, 92.
Lum, J.T., Hoffman, W.E. & Piercy, M.F. (1990). Soc. Neurosci. Abstr., **16**, 1034.
Moser, P.C. (1991). Eur. J. Pharmacol., **193**, 165-172.
Routledge, C., Gurling, J., Forster, E.A., Wright, I., Fletcher, A. & Dourish, C.T. (1992). Br. J. Pharmacol., **107**, 5P.
VanderMaelen, C.P. Braselton, J.P. & Gellbach, G. (1987). Soc. Neurosci. Abstr., **13**, 1649.

95P LOCALIZATION OF 5-$HT_{1D\alpha}$ AND 5-HT_{1B} RECEPTOR MESSENGER RNA IN RAT BRAIN AND TRIGEMINAL GANGLIA

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The recent cloning of the genes encoding the rat 5-HT_{1B} and rat 5-$HT_{1D\alpha}$ receptor (the species equivalents of the human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors, respectively, Voigt et al., 1991, Hamblin et al., 1992) allows localization of both 5-HT_{1B} and 5-$HT_{1D\alpha}$ messenger RNA (mRNA) in rat using in situ hybridization histochemistry (for methodology see Bruinvels et al., 1992a). Specific oligonucleotides were synthesized (both 45 bases complementary to the mRNA encoding the putative third intracellular loop) in order to compare the distribution of 5-HT_{1B} and 5-$HT_{1D\alpha}$ mRNA in rat brain and to study the possible presence of the mRNAs in the rat trigeminal ganglia. In these ganglia both mRNAs were detected in the large ganglion cell bodies, where both hybridization signals were equal in intensity. In rat brain the 5-HT_{1B} mRNA hybridization signal was stronger and more abundant than the signal obtained with the 5-$HT_{1D\alpha}$ mRNA probe. The regions where both 5-HT_{1B} and 5-$HT_{1D\alpha}$ mRNA were observed include: olfactory tubercle and primary olfactory cortex, frontal cortex, caudate-putamen, nucleus accumbens, superior colliculus, central grey and dorsal raphé nucleus. Regions where solely 5-HT_{1B} mRNA was detected include: CA1 field of the hippocampus, anterior olfactory nucleus, Purkinje cells of the cerebellum in addition to some thalamic nuclei and the hypothalamic arcuate nucleus.

Comparing these data to the distribution of 5-HT_{1B} binding sites in rat brain, which we presented previously (Bruinvels et al., 1992b), few regions (such as caudate-putamen and subthalamic nucleus) were found to contain both mRNA and receptor proteins. The absence of 5-HT_{1B} mRNA in globus pallidus and substantia nigra, subiculum and deep cerebellar nuclei, but its presence in caudate-putamen, CA1 field of the hippocampus, Purkinje cells and dorsal raphé nucleus, suggests that these 5-HT_{1B} receptors are transported to the nerve terminals where they could function as presynaptic auto- and heteroreceptors. Whether this is also the case for the 5-HT_{1D} site, where the regional distribution of mRNA and binding sites correspond neither, remains to be examined with lesion studies. Since no 5-HT_{1B} or 5-HT_{1D} binding sites were detected on the rat trigeminal ganglia, it can be postulated that both 5-HT_{1B} and 5-HT_{1D} receptors are transported to the terminals of the trigeminovascular fibres, where both receptors could function as release-modulating heteroreceptors.

Bruinvels, A.T., Landwehrmeyer, B., Moskowitz, M.A. and Hoyer, D. (1992a). Eur. J. Pharmacol., Mol. Pharmacol. Sect. **227**, 357-359.
Bruinvels, A.T., Palacios, J.M. and Hoyer, D. (1992b). Br. J. Pharmacol. **107**, 6P.
Hamblin, M.W., McGuffin, R.W., Metcalf, M.A., Dorsa, D.M. and Merchant, K.M. (1992). Mol. Cell. Neurosci. in press.
Voigt, M.M., Laurie, D.J., Seeburg, P.H. and Bach, A. (1991). EMBO J. **10**, 4017-4023.

96P WHICH 5-HT RECEPTORS ARE INVOLVED IN THE MODULATION OF 5-HT SYNTHESIS BY THE 5-HT UPTAKE BLOCKER, CITALOPRAM?

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Acute administration of the 5-HT uptake blocker, citalopram (10 mg/kg po), decreases the in vivo synthesis of 5-HT in rat cortex by 50%, as determined by the measurement of the accumulation of 5-HTP after inhibition of aromatic amino acid decarboxylase with m-hydroxybenzylhydrazine (NSD 1015) (Moret & Briley, 1992). The aim of the present work was to study through which 5-HT receptors citalopram exerts its effect on 5-HT synthesis.

Rats were administered drugs ip 30 min (45 min for antagonists) before the administration of NSD 1015 (100 mg/kg ip). Thirty minutes later the animals were sacrificed, the fronto-parietal cortex isolated, homogenized and centrifuged and the 5-HTP content of the supernatant analysed by HPLC with electrochemical detection.

The 5-HT_{1A} agonist, 8-OH-DPAT, and the relatively selective 5-HT_{1B} agonist, CGS 12066B, inhibited 5-HT synthesis with ID_{30} values of 0.03 and 2.2 mg/kg respectively. The 5-HT_{1A}/5-HT_2 antagonist, spiperone, alone at 10 - 100 mg/kg, had no effect on basal 5-HT synthesis. At 30 mg/kg it partially reversed the inhibitory effect of citalopram (10 mg/kg) from 54 % inhibition to 45 % ($p= 0.05$), that of 8-OH-DPAT (0.1 mg/kg) from 52 % to 23 % ($p< 0.001$) and that of CGS 12066B from 46% to 28% ($p< 0.005$). Neither the 5-HT_2 agonist, DOI (10 mg/kg), or antagonist, ritanserine (30 mg/kg), had any effect alone. In addition, ritanserine (30 mg/kg) did not modify the inhibitory effect of citalopram (10 mg/kg). Methiothepin was the only compound to increase the basal synthesis of 5-HT (50% increase at 12 mg/kg). At 3 mg/kg, a dose inactive on the basal rate of 5-HT synthesis, methiothepin was devoid of effect on the citalopram-induced inhibition of 5-HT synthesis.

These results suggest that the effect of citalopram on 5-HT synthesis is partially but not totally mediated through 5-HT_{1A} receptors. The reversal of citalopram-induced inhibition of 5-HT synthesis by the 5-HT_{1A} antagonist was less than that induced by 8-OH-DPAT. In addition methiothepin stimulated 5-HT synthesis, an effect reminiscent of its increase of 5-HT release in vitro (Moret & Briley, 1986) which is mediated through blockade of 5-HT_{1B} autoreceptors (Engel et al., 1986) in rat brain. These two elements suggest that 5-HT_{1B} autoreceptors may also be involved.

Engel,G., Göthert,M., Hoyer,D., Schlicker,E. & Hillenbrand,K. (1986) Naunyn-Schmiedeberg Arch Pharmacol **332**, 1-7
Moret,C. & Briley,M. (1986) Neuropharmacology **25**, 169-174
Moret,C. & Briley,M. (1992) Neuropharmacology **31**, 679-684

97P EFFECT OF CHRONIC INTRACEREBROVENTRICULAR 5-HT_{1C} RECEPTOR ANTISERUM INFUSION ON THE 5-HT BEHAVIOURAL SYNDROME AND 5-HT TURNOVER

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Although 5-hydroxytryptamine$_{1C}$ (5-HT_{1C}) receptors have been implicated in behaviours such as hypolocomotion (Kennett & Curzon, 1990) the lack of specific antagonists that discriminate between 5-HT_{1C} and 5-HT_2 receptors complicates 5-HT_{1C} functional studies. By raising a polyclonal antiserum against the N-terminal ten amino acids of the rat 5-HT_{1C} receptor protein, we have developed a radioimmunoasay (RIA) which identifies a protein whose distribution correlates with 5-HT_{1C} binding sites in rat brain (Sharma *et al.*,1992). The present study now reports the effect of chronic intracerebroventricular (i.c.v.) infusion of this antiserum on the 5-HT agonist-induced behavioural syndrome and 5-HT turnover in selected rat CNS regions to further examine 5-HT_{1C} function.

Guide cannulae were implanted above the left lateral ventrical in male Wistar rats using sodium methohexitone (60 mg kg^{-1} i.p) anaesthesia. Polydipsia induced by angiotensin II (20ng, 2µl, d 7) was used to confirm the i.c.v. cannula position, before continuous infusion (d 9 to 24) of either a non-immune or the 5-HT_{1C} receptor antiserum (n=7 each, diluted 1:10 in 0.154M saline at 0.5 µl h^{-1}) using osmotic mini-pumps. Back muscle contractions (BMC) and wet-dog shakes were counted and reciprocal forepaw treading, flat body posture and lateral head weaving scored (rated 0 to 3, once min^{-1} for 20 min) separately after a submaximal dose (2.5 mg kg^{-1} i.p.) of the non-selective 5-HT agonist, 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT), before (d 8) and during (d 21) antiserum infusion. On d 24 selected CNS regions were taken to measure 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by HPLC with electrochemical detection and thyrotrophin-releasing hormone (TRH; co-existing with 5-HT in bulbospinal raphe neurones) by RIA.

5-HT_{1C} antiserum infusion significantly increased ($P<0.05$, Student's t-test) 5-MeODMT-induced BMC (69 ± 10 mean ± s.e.mean in 30 min) compared with those observed pre-infusion (33 ± 7), while this behaviour was unaltered by control serum (55 ± 8 and 42 ± 15, respectively). In contrast, neither 5-HT_{1C} nor control antisera altered the flat body posture (45 and 53 before and during 5-HT_{1C} infusion, respectively), lateral head weaving (15 and 14) or reciprocal forepaw treading (18 and 20) evoked by 5-MeODMT. 5-HT and 5-HIAA levels and their ratio (an index of turnover) in the brainstem and the thoracic and cervical spinal cord were comparable in both antiserum groups. Following 5-HT $_{1C}$ antiserum infusion there was no significant difference in TRH levels in the brainstem (being 35.5 ± 4.3 compared with 48.1 ± 4.6 pg mg $protein^{-1}$, mean ± s.e.mean, in controls) nor in any other CNS region.

BMC was the only component of the 5-HT behavioural syndrome affected by the 5-HT_{1C} antiserum. The observed augmentation of BMC following antiserum infusion suggests a negative influence of 5-HT_{1C} receptors on this behaviour. Although intrathecal TRH potently enhances 5-HT-induced BMC (Fone *et al.*, 1991), the lack of effect of the 5-HT_{1C} antiserum on spinal TRH levels implies that the observed enhancement of BMC is not due to alteration of endogenous TRH. 5-HT turnover was unaffected in any CNS region, as expected from the presumed postsynaptic location of 5-HT_{1C} receptors.

We acknowledge the SERC (A.S.) for financial support.

Fone, K.C.F., Robinson, A.J. & Marsden, C.A. (1991) *Br. J. Pharmacol.*, 104, 1547-1555.
Kennett , G. & Curzon, G. (1990) *Trends Pharmacol. Sci.* 11, 181-182.
Sharma, A., Landon, M. & Fone, K.C.F. (1992) *Br. J. Pharmacol.*, 105, 219P.

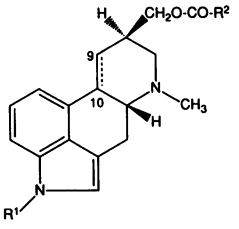
98P SOME LYSERGOL-RELATED ERGOTS ACTIVATE 5-HT_{1C} RECEPTORS

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Ergotamine and dihydroergotamine are potent 5-HT_{1C} receptor agonists (Brown et al., 1991). Pertz and Eich (1992) recently described a series of O-acylated derivatives of lysergol and dihydrolysergol which are potent 5-HT_2 receptor antagonists. Since 5-HT_{1C} and 5-HT_2 receptors are similar, we studied whether these ergots can stimulate 5-HT_{1C}-receptor-linked phospholipase C in piglet choroid plexus and determined their 5-HT_{1C} receptor binding affinities. Methods were as described (Brown et al., 1991).

Table 1 Effects of 5-HT, lysergol and derivatives on inositol phosphate accumulation and [^{3}H]-mesulergine binding.

	R^1	R^2	pEC_{50}	I.A.[a]	log(CR)[b]	pKp^c	pK_i[d]	pK_B (5-HT_2)[e]
5-HT	-	-	7.7	1.0	1.8	-	8.1	-
Lysergol	H	-	7.0	0.4	1.8	7.4	7.7	-
PE01	H	R,S-1-MePr	7.2	0.24	2.0	7.0	n.m.	8.4
PE02	iPr	R,S-1-MePr	-	n.a.	-	-	8.3	8.3
PE03	H	S-1-MePr	7.1	0.20	1.8	7.0	7.9	8.3
PE04	iPr	S-1-MePr	-	n.a.	-	-	8.9	8.1
PE05	H	1-EtPr	6.7	0.25	1.8	6.9	8.4	8.1
PE06	iPr	1-EtPr	-	n.a.	-	-	8.4	8.0



[a] intrinsic activity relative to 5-HT; [b] concentration ratio for antagonism by 10^{-7} M mesulergine; [c] -log(K_p/M), calculated from antagonism of the 5-HT response by the ergot; [d] -log(K_i/M), from inhibition of [^{3}H]-mesulergine binding; [e] from blockade of 5-HT_2 receptor-mediated responses in rat tail artery (Pertz & Eich, 1992); n.a., no measurable activity; n.m., not measured.

Lysergol and its R^1-unsubstituted derivatives were partial agonists for inositol phosphate accumulation (I.A. of 0.2-0.4, Table 1) while derivatives of dihydrolysergol had marginal activity (I.A. of 0.02-0.06, not shown). The antagonism by mesulergine is consistent with 5-HT_{1C} receptor interaction. These compounds are therefore further ergots with 5-HT_{1C} receptor agonist activity. However, they lack activity at 5-HT_2 receptors in rat tail artery, despite having similar affinity (Pertz and Eich, 1992), suggesting that they have different abilities to cause conformational change at the two receptor types.

We thank Drs Pertz and Eich for their generous gift of samples of the derivatives of lysergol and dihydrolysergol.

Brown, A.M., Patch, T.L. & Kaumann, A.J. (1991). *Br. J. Pharmacol.* **104**, 45-48.
Pertz, H. & Eich, E. (1992). *Naunyn Schmiedeberg's Arch. Pharmacol.* **345**, 394-401.

99P GR85548: A POTENT SELECTIVE AGONIST FOR THE INTRACRANIAL VASCULAR 5-HT$_1$ RECEPTOR

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The actions of GR85548 (N-methyl-3-(1-methyl-4-piperidinyl)-1H-indole-5-ethanesulphonamide) have been examined in a range of isolated tissue preparations and compared with those of sumatriptan, a 5-HT$_1$ receptor agonist, which is effective in the acute treatment of migraine (Humphrey et al., 1988; Ferrari et al., 1991). Isolated ring preparations of dog basilar and middle cerebral arteries (MCA) and piglet vena cava (PVC) and strip preparations of dog saphenous vein (DSV) (5-HT$_1$) and rabbit aorta (5-HT$_2$) were prepared for measurement of isometric tension as described previously (Humphrey et al.,1988; Connor et al.,1989). 5-HT$_4$ receptor activity was assessed in guinea-pig isolated colon (Elswood et al, 1991). 5-HT$_{1A}$, 5-HT$_{1D}$ and 5-HT$_3$ binding was carried out using [^{3}H]-8-OH-DPAT, [^{3}H]-5-HT (in the presence of mesulergine 0.1μM and BMY 7378 0.2μM; Yocca et al.,1987) and [^{3}H]-GR65630 (Kilpatrick et al.,1989) respectively, as the ligand in rat hippocampal, guinea-pig striatal and rat entorhinal cortex membranes, respectively. GR85548 caused concentration-dependent contractions of dog basilar, MCA, and DSV, being approximately equipotent with 5-HT (Table 1). GR85548-induced contractions of dog basilar artery and DSV were resistant to blockade by ketanserin (1μM) or ondansetron (1μM) but were antagonised by methiothepin (0.1 μM; pK_B = 8.5±0.1 and 8.3±0.1 respectively, n=4). GR85548-induced contractions in DSV were resistant to blockade by a wide range of specific receptor antagonists, including phentolamine (1μM), propranolol (1μM), mepyramine (1μM) and indomethacin (2.8μM). In addition, like 5-HT, GR85548 inhibited neurogenically mediated contractions of DSV (Table 1). GR85548 had high affinity for 5-HT$_{1D}$ binding sites (pK_i=8.1±0.1,n=3), about 10-fold lower affinity for 5-HT$_{1A}$ binding sites (pIC_{50}=7.1±0.1,n=3) and weak affinity for 5-HT$_3$ binding sites (pK_i=5.9). GR85548 (up to 10μM) had no agonist or antagonist activity at 5-HT$_2$ receptors, was devoid of relaxant effects via 5-HT$_1$ receptors in PVC precontracted with α-methyl 5-HT, and had no contractile activity at 5-HT$_4$ receptors.

Table 1. Effects of GR85548 and sumatriptan in 5-HT$_1$ receptor containing preparations. Values are means (95% CL) or ±s.e.m., n≥3

Isolated tissue	Response	Equipotent molar ratio (5-HT=1)		EC_{50} (nM)	Max response (5-HT=100%)
		GR85548	Sumatriptan	GR85548	GR85548
Dog Basilar Artery	Contraction	1.5(0.6-4.1)	4.9(1.9-12.7)	110(60-180)	105±8
Dog MCA	Contraction	1.7(0.8-4.2)	4.5(2.6-7.6)	68(47-98)	114±13
Dog Saphenous Vein	Contraction	1.1(0.8-1.5)	4.6(2.5-8.2)	51(27-97)	103±6
Dog Saphenous Vein	Inhibition of neurogenic contraction	0.5(0.1-3.4)	4.2(3.1-5.6)	10(7-20)	117±24

These results demonstrate that GR85548 has a similar pharmacological profile of action *in vitro* to sumatriptan but is about 4 fold more potent. Thus GR85548 is a potent, selective agonist for the 5-HT$_1$ receptor mediating vasoconstriction and inhibition of neuronally mediated contractions. The ability of GR85548 to contract isolated intracranial blood vessels suggests that this compound, like sumatriptan, will be an effective anti-migraine agent in man.

Connor HE, Feniuk W and Humphrey PPA (1989) Br. J. Pharmacol.,96,379-387.
Elswood CJ, Bunce KT and Humphrey PPA (1991) Eur. J. Pharmacol., 196, 149-155.
Ferrari MD, the Subcutaneous Sumatriptan Int. Study Group (1991) N.Engl.J.Med., 325, 316-321
Humphrey PPA, Feniuk W, Perren MJ, Connor HE, Oxford AW, Coates IH and Butina D (1988) Br.J. Pharmacol.,94,1123-1132
Kilpatrick GJ, Jones BJ and Tyers MB (1989) Nature, 330, 746-748.
Yocca FD, Hyslop DK, Smith DW and Maayani S (1987) Eur. J. Pharmacol., 137, 293-294.

100P EFFECTS OF WAY 100 135, 8-OH DPAT AND SUMATRIPTAN ON 5-HT RELEASE EVOKED BY A SINGLE ELECTRICAL PULSE IN SLICES OF GUINEA-PIG DORSAL RAPHÉ NUCLEUS

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Previous studies have demonstrated that the 5-HT$_{1A}$ receptor agonist 8-OH DPAT inhibits electrically evoked release of 5-HT from the dorsal raphé nucleus (DRN) of the guinea-pig (O'Connor *et al.*, 1992). The present study confirms and extends these observations in the guinea-pig, using the selective 5-HT$_{1A}$ receptor antagonist (±)WAY 100 135 (Fletcher *et al.*, 1991). A possible role for 5-HT$_{1D}$ receptors in the guinea-pig DRN has also been investigated using the 5-HT$_{1D}$ receptor agonist sumatriptan.

Release of 5-HT was monitored by fast cyclic voltammetry (O'Connor and Kruk, 1991a) in guinea-pig brain slices containing the DRN. Slices (350μm thick) were perfused with oxygenated ACSF at 32° C, at a rate of 1.2ml min^{-1}, and a carbon fibre microelectrode positioned in the mediodorsal region of the DRN. The experiments used either single pulse stimulation (0.1ms; 20V) applied every 2 min, or 5 pulse stimulation at 1 or 100Hz, every 5min, using a bipolar tungsten stimulating electrode.

The electrochemical signal obtained in response to stimulation had an oxidation peak potential of approximately 610mV and two reduction potentials, at 0 and -600 mV. Stable release could be measured for more than 6h, this being approximately 3±0.1nM (n=9) for a single pulse and 13±4nM (n=3) for 5 pulses at 100Hz. The selective 5-HT reuptake inhibitor citalopram (1μM) caused an 82±2% increase in the height of the signal, and a 145±13% increase in the time for the signal to return to half of its maximum height ($t_{1/2}$; n=3). However, the selective noradrenaline or dopamine reuptake inhibitors oxaprotiline (1μM) or GBR12909 (1μM), respectively, or the monoamine oxidase inhibitor nialamide (1μM), did not enhance the electrochemical signal (n=3). The electrochemical signal was reversibly abolished in the presence of Ca^{2+}-free ACSF, or tetrodotoxin. These electrochemical and pharmacological studies indicate that the released substance was 5-HT.

Perfusion of the slice with 8-OH DPAT resulted in a dose-related inhibition of the 5-HT released by a single pulse electrical stimulation (IC_{50}=42±2nM;n=9). This inhibition was dose-dependently antagonised by (±)WAY 100 135 (60-300nM) with a mean pK_B value of 7.7 (95% conf. limits 7.5-7.8; n=8). (±)WAY 100 135 (300nM) alone had no effect on single pulse release (n=3), or on release evoked by 5 pulses at 100Hz (n=3), indicating that there is no serotonergic tone at the 5-HT$_{1A}$ receptors in the DRN slice using these stimulation conditions. However, (±)WAY 100 135 (300nM) increased 5-HT release by 46±7% (area under the curve; n=3) to 5 pulses at 1Hz, a stimulation paradigm where the released 5-HT feeds back onto the autoreceptors (O'Connor and Kruk, 1991b). Sumatriptan, 1-100nM, also produced a dose-related inhibition of 5-HT release (IC_{50}=30±2nM, n=6) which was not antagonised by (±)WAY 100 135 (300nM;n=3).

Thus, 5-HT release in the guinea-pig DRN can be reproducibly evoked by a single pulse of electrical stimulation, and measured by fast cyclic voltammetry. This release may be modified by agents that are selective for 5-HT$_{1A}$ or 5-HT$_{1D}$ receptors, consistent with a modulatory autoreceptor role for these receptor subtypes in the guinea-pig DRN.

Fletcher, A., Bill, D.J., Bill, S.J., Brammer, N.T., Cliffe, I.A., Forster, E.A., Reilly, Y., Lloyd, G. K. (1991). Soc. Neurosci. Abstr. 17 (1):39.3.
O'Connor, J.J., Kruk, Z.L. (1991a). J. Neurosci. Methods 38:25-33.
O'Connor, J.J., Kruk, Z.L. (1991b). Brain Research 568:123-130.
O'Connor, J. J., Trout, S. J., Kruk, Z. L. (1992). Br. J. Pharmacol. (In Press).

101P MULTIPLE 5-HT TERMINAL AUTORECEPTORS IN GUINEA-PIG CEREBRAL CORTEX DEMONSTRATED BY *IN VITRO* [^{3}H]5-HT RELEASE

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Release of neuronal 5-HT is under the control of terminal autoinhibition. Recently Wilkinson & Middlemiss (1992) demonstrated differing pA_2 values for the autoreceptor antagonist methiothepin depending on the autoreceptor agonist used. This suggests that in the guinea-pig hippocampus there may be two 5-HT autoreceptors. We now report further evidence in support of this hypothesis from detailed agonist studies in guinea-pig cortex.

Male guinea-pigs (350-600g) were sacrificed by cervical dislocation, decapitated and cerebral cortices removed. Tissue was cross chopped on a MacIlwain chopper and incubated in [^{3}H]5-HT (100nM) for 15mins at 37°C in the presence of pargyline (10 µM). Slices were then placed in a Brandel 2000 Superfusion System and perfused with Krebs solution. Tissue [^{3}H]5-HT release was stimulated by a Brandel stimulator delivering constant current square wave pulses. Slices were subjected to 3 periods of stimulation; a pre-collection period ,S1 and S2 periods. The stimulation parameters were 20mA amplitude, 2msec pulse width, 1Hz frequency, 2 mins duration. Drugs were perfused during the S2 period ; agonists were added 8mins and antagonists 28mins prior to the S2 stimulation. Following measurement of radioactivity the S2/S1 % fractional release was calculated and data expressed as a % of control release.

5-HT and sumatriptan inhibited [3H]5-HT release in a biphasic manner. 5-HT had a high affinity compenent (pEC_{50}=8.69±0.27 , E_{max}=42%±16%) and a low affinity component (pEC_{50}=7.37±0.01 , E_{max}=39%±11%). At 5-HT concentrations higher than 100nM, homoexchange occured; whether there is further inhibition of release above this concentration is difficult to determine. Sumatriptan also displayed different affinities for these two sites (pEC_{50}=7.32±0.07, and 6.15±0.07 ; E_{max}=27%±1% and 34%±1% respectively). However 40% of control release was unaffected by up to 10µM sumatriptan. The concentration response curve to 5-carboxamidotryptamine (5-CT) was monophasic (pEC_{50}=8.64±0.2 ; E_{max}=82%±0.2%) up to 30nM above which there was some evidence for an additional component (pEC_{50}=7.24±0.20) which virtually abolished [^{3}H]5-HT release. In the presence of methiothepin (30nM) the monophasic portion of the 5-CT curve became biphasic which suggests that methiothepin is also discriminating between the two sites which have similar affinity for 5-CT.

In conclusion, we propose that there may be up to three 5-HT autoreceptors which display differential affinities for 5-HT, 5-CT, sumatriptan and methiothepin. How these receptors correlate with the various components of 5-$HT_{1D/1E}$ binding and the multiplicity of 5-HT1D and 5-HT1E clones remains to be determined.

Wilkinson LO & Middlemiss DN , Naunyn-Schmiedeberg's Arch Pharmacol (1992) 345:696-699

102P BINDING PROFILE OF THE 5-HT_{1C} AND 5-HT_2 RECEPTORS IN HUMAN BRAIN: COMPARISON WITH CORRESPONDING RECEPTORS IN THE PIG AND RAT

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The 5-HT_{1C} and 5-HT_2 receptors form one of the sub-families of 5-HT receptors, as defined by base sequence homology (Peroutka, 1992) and similarity in biochemical mechanism, namely activation of phospholipase C (Julius et al., 1990). In the present study we have investigated the binding characteristics of the 5-HT_2 receptor in human frontal cortex and the 5-HT_{1C} receptor in human choroid plexus in order to compare the ligand binding profile of these receptors both with each other and with published data from other species.

Human brain tissue (n=4) was dissected at post-mortem from non-neurological cases within 24-48h of death and stored for up to 4 weeks at -70°C. After thawing, frontal cortex (Brodman area 11) was homogenised in 10 volumes 5mM Tris/EDTA then centrifuged at 1000g. Membranes from this supernatant were washed by centifugation at 30 000g at 4°C and finally resuspended in incubation buffer (50mM Tris, 5mM $MgCl_2$, 1mM EGTA, pH 7.4) at a final tissue density of approximately 1mg/ml protein. Membranes of choroid plexus, taken from the lining of the lateral ventricles, were prepared in similar fashion and resuspended in incubation medium at approximately 0.3mg/ml protein. Binding with [^{3}H]ketanserin or [^{3}H]mesulergine was carried out for 60min at 37°C and terminated by filtration through Whatman GF-C filters. Non-specific binding was defined by the addition of 1µM mianserin for both radioligands.

Specific binding of [^{3}H]mesulergine to membranes of human choroid plexus was saturable (Bmax=897 ± 226 fmol/mg protein) and of high affinity (Kd=0.83nM, pKd=9.09 ± 0.04 M). Inhibition by serotonergic antagonists identified the anticipated relative potency, mesulergine>mianserin>ketanserin>spiperone. The affinity for 5-HT (pKi=6.74) was low and was not altered by addition of 10µM GppNHp. Comparison with ligand affinities at the 5-HT_{1C} receptor in pig choroid plexus (Hoyer et al., 1985) revealed a highly significant correlation (r^2=0.98, n=8, p<0.001). Specific binding of [^{3}H]ketanserin to membranes of human frontal cortex also demonstrated saturability (Bmax=125 ± 12 fmol/mg protein, Kd=0.69 nM, pKd=9.19 ± 0.09 M). The order of potency of compounds to inhibit [^{3}H]ketanserin binding differed from that at the 5-HT_{1C} receptor, with spiperone=ketanserin>mianserin>mesulergine. The affinity for 5-HT (pKi=5.50) was lower than that at the 5-HT_{1C} receptor and was also not altered by addition of 10µM GppNHp. Comparison with ligand affinities at the 5-HT_2 receptor in rat frontal cortex (Hoyer et al., 1985) did not demonstrate a significant correlation (r^2=0.10, n=10, p=0.4). Comparison of ligand affinities for the human 5-HT_{1C} and 5-HT_2 receptors indicated a weak correlation (r^2=0.42, n=19, p<0.01) which became non-significant when comparison was restricted to the antagonist ligands (r^2=0.24, n=14, p=0.1). Mesulergine and spiperone proved to be the most selective ligands for the 5-HT_{1C} and 5-HT_2 receptors respectively.

We conclude that despite the similarities noted above, the ligand binding profiles of the 5-HT_{1C} and 5-HT_2 receptors in human brain are quite distinct. Furthermore, whilst the 5-HT_{1C} receptor in pig choroid plexus is a good model of that in the human, the affinity of ligands for the 5-HT_2 receptor in rat frontal cortex may differ markedly from that in the human.

Hoyer, D., Engel, G. & Kalkman, H.O. (1985) Eur. J. Pharmacol. 118, 13-23
Julius, D., Huang, K.N., Livelli, T.J., Axel, R. & Jessel, T.M. (1990) Proc. Nat. Acad. Sci. U.S.A. 87, 928-932
Peroutka, S.J. (1992) Neuropharmacol. 31, 609-613

103P CLONING AND FUNCTIONAL EXPRESSION OF A 5-HT$_3$ RECEPTOR cDNA FROM N1E-115 NEUROBLASTOMA CELLS

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5-Hydroxytryptamine$_3$ (5-HT$_3$) receptors are ligand-gated, cation selective ion channels mediating membrane depolarization and neuronal excitation (Peters *et al.*, 1992). The mouse neuroblastoma cell line, N1E-115, expresses a relatively high density of 5-HT$_3$ receptors (Lummis *et al.*, 1990). Total RNA isolated from this cell line was used to synthesize a full length 5-HT$_3$ receptor cDNA using the polymerase chain reaction (PCR). Single stranded oligonucleotide primers were designed to the previously published 5-HT$_3$ R-A sequence (Maricq *et al.*, 1991). First strand cDNA's were synthesized from the N1E-115 RNA as a template for a 30 cycle PCR (conditions of the reaction being: denaturing temperature 95°C for 2 min; annealing at 65°C for 1 min; extension at 72°C for 4 min). The resulting 2 kilobase cDNA hybridized strongly with the α^{32}P labelled 5-HT$_3$ R-A clone in Southern blot analysis.

The amino acid sequence predicted from the cDNA is 98% homologous with that reported for the 5-HT$_3$ R-A, the most striking difference being the deletion of 6 amino acid residues in the large cytoplasmic loop between the putative M3 and M4 domains. The nucleotide sequence coding for this region is consistent with the deletion resulting from an alternative splicing mechanism. Utilizing specific primers flanking the position of the deletion, PCR analysis of total RNA isolated from either N1E-115 or NCB-20 hybridoma cells indicated the presence of both the 'short' and 'long' (Maricq *et al.*, 1991) form of the 5-HT$_3$ R-A in these cells.

In stage VI *Xenopus laevis* oocytes injected with *in vitro* transcripts of the short form of the 5-HT$_3$ R-A, bath applied 5-HT (0.3 - 100μM) evoked a concentration dependent inward current response with a mean amplitude,for 3μM 5-HT, of -130.7 $\pm$ 20.2 nA (mean $\pm$ s.e.; n = 63) at a holding potential of -30mV. Such currents were mimicked by the 5-HT$_3$ receptor agonists m-chlorophenylbiguanide (0.3-30μM), 1-phenylbiguanide (10μM - 1mM) and weakly, by 2-methyl-5-HT (3 - 300μM). Currents induced by 3μM 5-HT were insensitive to methysergide (1μM; n = 2) and ketanserin (1μM; n = 2). By contrast, the selective 5-HT$_3$ receptor antagonists ondansetron and granisetron produced a concentration dependent and fully reversible block of the response, with IC_{50} values of 1.22 $\pm$ 0.18 nM (n = 4) and 92.0 $\pm$ 7.8pM (n = 3) respectively. Additionally, the non-selective antagonists (+)-tubocurarine, metoclopramide and cocaine blocked reversibly with IC_{50} values of 1.49 $\pm$ 0.15 nM (n = 4), 72.0 $\pm$ 13.7 nM (n = 4) and 2.3 $\pm$ 0.2 μM (n = 4) respectively.

We thank the SERC and SmithKline Beecham for financial support and Dr. T. Blackburn for helpful discussion.

Lummis, S.C.R., Kilpatrick, G.J. and Martin, I.L. (1990) Eur. J. Pharmacol., *189*, 223-227.
Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M. and Julius, D. (1991) Science, *254*, 432-437.
Peters, J.A., Malone, H.M. and Lambert, J.J. (1992) Trends Pharmacol. Sci., *13*, 391-397.

104P CHARACTERISATION OF A RADIOLABELLED 5-HT$_3$ RECEPTOR AGONIST, [^{3}H]m-CHLOROPHENYLBIGUANIDE

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5-HT$_3$ receptors have been studied using a wide variety of antagonists, but there are only a few specific agonists available. The most potent 5-HT$_3$ agonist identified to date is m-chlorophenylbiguanide (mCPBG), and in the present study we characterise the binding of [^{3}H]mCPBG in N1E-115 neuroblastoma cell membranes. We have previously demonstrated these cells possess high levels of 5-HT$_3$ receptors as measured by [^{3}H]GR65630 binding, and physiological studies have shown that mCPBG acts as a 5-HT$_3$ receptor agonist (Lummis et al.,1990; Sepúlveda et al.,1991).

Saturation binding experiments with [^{3}H]mCPBG (26Ci/mmol; NEN) at 0°C reveal two high affinity sites in N1E-115 neuroblastoma cell membranes when analysed using the computer programmes EBDA and LIGAND: K_d =0.0056 $\pm$ 0.0044 and 1.44 $\pm$ 0.32nM, B_{max} =1.51 $\pm$ 1.05 and 332.5 $\pm$ 41.7 fmol/mg protein respectively (mean $\pm$ SE, n=3). The EC_{50} for the mCPBG-induced response in N1E-115 neuroblastoma cells is 1.4μM (Sepúlveda et al.,1991); thus the high affinity [^{3}H]mCPBG binding sites may represent high affinity desensitised states of the receptor.

The affinities of a range of 5-HT$_3$ selective antagonists against [^{3}H]mCPBG binding (0.5nM) show the expected characteristics of a 5-HT$_3$ receptor (order of potency: granisetron > quipazine > ondansetron > MDL72222 > 5-HT > 2-methyl 5-HT). Specific [^{3}H]mCPBG binding is also decreased in the presence of physiological concentrations of Mg^{2+} and Ca^{2+}, as has been previously reported in physiological studies (Peters et al.,1988). The availability of [^{3}H]mCPBG may therefore facilitate studies on 5-HT$_3$ receptor agonist interactions.

We would like to thank S. Hurt (NEN) and G.J. Kilpatrick (Glaxo Group Research) for [^{3}H]mCPBG, and Glaxo Group Research for financial support. SCRL is a Royal Society University Research Fellow.

Lummis, S.C.R, Kilpatrick, G.J. & Martin, I.L.(1990) *Eur. J. Pharmacol.* 189, 223-227.
Peters, J.A., Hales, T.G. & Lambert, J.J. (1988). *Eur. J. Pharmacol.* 151, 491-495.
Sepúlveda, M.-I., Lummis, S.C.R. & Martin, I.L. (1991) *Br. J. Pharmacol.* 104, 536-540.

105P EFFECT OF 5-HT$_3$ RECEPTOR ANTAGONISTS ON THE DISCRIMINATIVE AND REINFORCING PROPERTIES OF OPIOIDS

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Previous research has indicated that 5-HT$_3$ receptor antagonists (e.g. MDL72222, ondansetron and ICS205-930) may prevent the acquisition of a conditioned place preference to morphine using training doses of 1 mg/kg (Carboni et al., 1989) and 1.5 mg/kg (Higgins et al., 1992). Because this observation suggests that 5-HT$_3$ antagonists block an aspect of opioid reward, there is a need to extend these findings to other related models to determine more fully if these drugs represent useful therapies for the treatment of opioid abuse. In the present studies, we have investigated the effects of 5-HT$_3$ antagonists in models of intravenous heroin self-administration and morphine drug discrimination.

Male Wistar rats (300-400g) were used throughout. For the drug discrimination studies, rats were trained to discriminate morphine from saline using a standard 2-lever operant procedure using food as the reinforcer (available under FR10 schedule). Two separate groups were run using morphine training doses of 1.5 mg/kg sc (n=12; a comparable stimulus to previous place conditioning studies) and 3 mg/kg sc (n=8). Experimental sessions were conducted 6 days/week and for discrimination testing both levers were active. Heroin self-administration was examined in separate groups of animals implanted with exteriorised jugular catheters. During 60 min daily sessions run 7 days/week, the rats were trained over the course of 3-4 weeks to respond for heroin (0.03 mg/kg body wt./infusion) made available under an FR5 schedule of reinforcement. Drug testing commenced once stable daily rates of responding were established.

In the drug discrimination studies, virtually all animals showed reliable control to both morphine doses by 65 training sessions. This morphine cue was completely blocked by naloxone (0.01-0.25 mg/kg sc) but not ondansetron (0.001-1 mg/kg sc), MDL72222 (0.1-3 mg/kg sc) or ICS205-930 (0.001-1 mg/kg ip), except for a mild attenuation (-30%) seen at the 0.01 mg/kg doses of ondansetron and ICS205-930 in the 1.5 mg/kg morphine group. Heroin self-administration was reliably increased by naloxone pre-treatment (e.g. vehicle: 10.5 ± 1.0 infusions/1h, naloxone 0.25 mg/kg: 21.5 ± 2.9 infusions/1h; n=6; $p<0.05$) and by halving the heroin infusion dose. Both ondansetron (0.01-1 mg/kg) and MDL72222 (0.1-3 mg/kg) pre-treatment failed to influence the pattern of heroin self-administration. It is concluded that 5-HT$_3$ antagonists may only show a limited spectrum of activity across various models of opioid abuse.

Carboni, E., Acquas, E., Leone, P., Di Chiara, G. (1989) Psychopharmacol. **97**, 175-178.
Higgins, G.A., Joharchi, N., Nguyen, P., Sellers, E.M. (1992) Psychopharmacol. **106**, 315-320.

106P DEVELOPMENT OF A RADIOLIGAND BINDING ASSAY FOR THE 5-HT$_4$ RECEPTOR: USE OF A NOVEL ANTAGONIST

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5-Hydroxytryptamine (5-HT$_4$) receptors have been identified both centrally and peripherally using a variety of functional assays (Bockaert et al., 1992). However, the lack of selective antagonists with high affinity has prevented the full characterization of this receptor and the development of a receptor binding assay. We report on a novel, selective and high affinity 5-HT$_4$ receptor antagonist, GR113808 ([1-[2-[methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate. In functional preparations containing 5-HT$_4$ receptors, the guinea-pig proximal colon (Elswood, Bunce and Humphrey, 1991) and the rat lower oesophagus (Reeves, Bunce and Humphrey, 1991), GR113808 (0.001μM-0.1μM) behaved as an antagonist producing concentration-dependent rightward displacements of the 5-HT concentration-response curve. In the guinea-pig colon the maximum response to 5-HT was depressed by 30±5% by 0.1μM GR113808. pA_2 values of 9.2±0.2 and 9.5±0.3 with Schild slopes of 1.1 (0.8-1.3) and 0.7 (0.4-1.0) (not significantly different from unity) were obtained in the colon and oesophagus respectively. A range of binding assays revealed that GR113808 was at least 3000-fold selective for the 5-HT$_4$ receptor relative to other receptor types investigated.

Radioligand binding studies with ^{3}H-GR113808 and homogenates of guinea-pig striatum, following a method similar to that used for 5-HT$_3$ receptor binding (Kilpatrick, Jones and Tyers, 1987), revealed a single binding site of high affinity (K_d=0.2nM, Bmax=93fmol/mg protein). Specific binding was saturable, reversible and displayed rapid kinetics (association and dissociation being complete within 3 min). Specific ^{3}H-GR113808 binding was inhibited by a range of 5-HT$_4$ receptor agonists and antagonists (Table 1). Specific binding was unaffected by compounds with no functional activity at this receptor. Thus, ^{3}H-GR113808 represents a useful tool for investigating the 5-HT$_4$ receptor.

Table 1

Compound	pK_i	Hill number	Compound	pK_i	Hill number
5-HT	7.3±0.1	0.77±0.06	ICS205-930	7.2	0.81
5-Methoxytryptamine	6.5±0.1	0.65±0.04	SDZ205-557	8.2	1.01
R,S-Zacopride	6.8±0.1	0.77±0.04	GR113808	9.5	0.94
Renzapride	7.0±0.1	0.84±0.01			

Preliminary autoradiographic studies with ^{3}H-GR113808 in guinea-pig and rat brain indicated a discrete localization with highest levels of specific binding being present in the striatum, olfactory tubercle, nucleus accumbens, globus pallidus and substantia nigra.

Bockaert, J., Fozard, J.R., Dumuis, A. and Clarke, D.E. (1992). Trends in Pharmacol. Sci., 13, 141-145.
Elswood, C.J., Bunce, K.T. and Humphrey, P.P.A. (1991). Eur. J. Pharmacol., 196, 149-155.
Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1987). Nature, 330, 746-748.
Reeves, J.J., Bunce, K.T. and Humphrey, P.P.A. (1991). Br. J. Pharmacol., 103, 1067-1072.

107P ACTIVATION OF 5-HT RECEPTORS LOCATED IN THE FOREBRAIN CAUSES SYMPATHOEXCITATION IN ANAESTHETIZED CATS: A ROLE FOR 5-HT_2 AND/OR 5-HT_{1C} RECEPTORS

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In anaesthetized rats it has been demonstrated that the rise in blood pressure caused by i.c.v. 5-hydroxytryptamine (5-HT) is mediated by activation of 5-HT_{1A} receptors which cause sympathoexcitation and 5-HT_2 and/or 5-HT_{1C} receptors which release vasopressin (Anderson et al., 1992). In anaesthetized cats i.c.v. 5-HT has also been reported to produce a rise in blood pressure (Coote et al., 1987). Hence, the present investigation was carried out to determine the nature of the 5-HT receptors mediating this pressor effect of i.c.v. 5-HT in anaesthetized cats.

Cats, anaesthetized with α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (6 mg kg^{-1}), were artificially ventilated after neuromuscular blockade with vecuronium bromide (200 μg kg^{-1}). Simultaneous recordings were made of renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activities, blood pressure, heart rate and femoral arterial conductance (FAC). Cumulative doses of 5-HT (10-160 nmol kg^{-1}; n=5), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; 80-320 nmol kg^{-1}; n=4) and 5-carboxamidotryptamine (5-CT; 2.5-40 nmol kg^{-1}; n=4) were given by microinjection (20 μl over 1 min) into the lateral ventricle. Drugs were restricted to the lateral and third ventricles by cannulation of the Aqueduct of Sylvius. To prevent the activation of peripheral 5-HT_2 receptors following i.c.v. administration of DOI, the peripherally acting 5-HT_2/5-HT_{1C} antagonist BW501C67 (Fuller et al.,1986; 1 mg kg^{-1}) was administered i.v. 15 min before DOI. In 3 separate animals, cinanserin (265 nmol kg^{-1}) was given i.c.v. 5 min before 5-HT.

5-HT, DOI and 5-CT caused maximum increases in mean BP of 16 ± 3, 22 ± 2 and 12 ± 4 mmHg, respectively associated with a tachycardia of 28 ± 5, 24 ± 5 and 18 ± 5 beats min^{-1} and a decrease in FAC of 11 ± 4, 28 ± 4 and 12 ± 4 ml $mmHg^{-1}$ min^{-1} x 10^{-3}, respectively. These changes were also associated with varying degrees of sympathoexcitation. 5-HT and DOI increased CNA (94 ± 24 % and 137 ± 33 %) and SNA (16 ± 5 % and 50 ± 15 %) without effect on RNA. 5-CT increased CNA by 99 ± 27 %, SNA by 57 ± 11 % and RNA by 42 ± 18 %. Pretreatment with cinanserin blocked the effects of 5-HT. Cinanserin alone caused a small reduction in blood pressure and heart rate associated with an increase in FAC and no change in sympathetic nerve activity.

These results demonstrate that activation of forebrain 5-HT_2 and/or 5-HT_{1C} receptors causes sympathoexcitation. However, these results also suggest that 5-HT_1 receptors are involved in this sympathoexcitatory pathway.

I.K.A is in receipt of a SERC CASE Studentship. Mr. S. Wilkinson's technical assistance is acknowledged.

Anderson, I.K., Martin, G.R. & Ramage, A.G. (1992) Br.J. Pharmacol. 107, 1020-1028
Coote, J.H., Dalton, D.W., Fenuik, W., & Humphrey, P.P.A. (1987) Neurpharmacol. 26, 147-154
Fuller, R.W., Kurz, K.D., Mason, N.R. & Cohen, M.L. (1986) Eur. J. Pharmacol. 125, 71-77

108P ENDOTHELIN-INDUCED CONTRACTION OF GUINEA-PIG AORTA AND PIG CORONARY ARTERY: EVIDENCE FOR MULTIPLE RECEPTORS

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Both endothelin ET_A and ET_B receptors can mediate contraction of blood vessels (Ihara et al., 1992; Moreland et al., 1992). In the present study, the endothelin receptors mediating contraction of guinea-pig aorta and pig coronary artery were characterized, using endothelin-1, endothelin-3, sarafotoxin S6b, the ET_B receptor agonists sarafotoxin S6c (Williams et al., 1991) and [$Ala^{1,3,11,15}$]endothelin-1 (4Ala-ET-1; Saeki et al., 1991) and the ET_A receptor antagonist cyclo-(D-Trp-D-Asp-Pro-D-Val-Leu) (BQ-123, from Neosystem Laboratoire, Strasbourg, F; Ihara et al., 1992).

Rings (2-3 mm long) were prepared from guinea-pig aortas (male albino, 300-350g) and from pig left circumflex coronary arteries (from the local abattoir). The endothelium was removed mechanically. Rings were suspended in a modified Krebs bicarbonate solution gassed with 95% O_2 and 5% CO_2, at 37°C, under a tension of 1g (aorta) or 4g (coronary artery). Isometric contractions were recorded. One cumulative concentration-response curve was obtained in each ring. Antagonists were present 30 min before the addition of agonist and control rings were run in parallel.

Table 1. Effects of endothelin analogues. E_{max} are given as means ± s.e.mean, EC_{50} as geometric means with 95 % confidence intervals from n experiments.

Analogue	Guinea-pig aorta			Pig coronary artery		
	E_{max} (g)	EC_{50} (nM)	n	E_{max} (g)	EC_{50} (nM)	n
Endothelin-1	2.09 ± 0.14	5.0 (3.9- 6.5)	28	11.7 ± 0.4	12.6 (10.5- 15.1)	87
Sarafotoxin S6b	2.68 ± 0.22	39.8 (26.9-58.9)	10	14.5 ± 1.7	14.8 (11.0- 20.0)	15
Endothelin-3	≥ 0.7	≥ 500	10	3.9 ± 0.7[a]	9.6 (4.7 - 19.5)	19
				6.7 ± 0.9[b]	320 (200 -540)	
Sarafotoxin S6c	no effect	up to 3 µM	6	4.8 ± 0.5	1.5 (0.9 - 2.4)	20
4Ala-ET-1	no effect	up to 1 µM	4	5.0 ± 0.5	41.7 (29.5- 58.9)	10

[a] first phase [b] second phase of biphasic response

In guinea-pig aorta, endothelin-3 was either without effect or did not reach a maximal effect at 3 µM. Neither sarafotoxin S6c (3 µM) nor 4Ala-ET-1 (1 µM) antagonized the effects of endothelin-1. BQ-123 (0.1 to 1 µM) behaved as a competitive antagonist of endothelin-1 (pA_2 7.4±0.1, slope 0.91±0.17, n=4). In pig coronary artery, the concentration-response curve to endothelin-3 was biphasic. BQ-123 (0.1 to 1 µM) did not affect the concentration-response curve to endothelin-1. BQ-123 (10 µM) shifted this curve to the right, but rendered it biphasic. Preincubation with sarafotoxin S6c (0.3 µM), used as an antagonist, abolished the contraction to 4Ala-ET-1 and the first phases of the concentration-response curves to endothelin-3 and, in the presence of 10 µM BQ-123, to endothelin-1.

The data confirm the involvement of multiple endothelin receptors in the contraction of blood vessels. The pharmacological features of the receptor in guinea-pig aorta are consistent with an ET_A receptor. In pig coronary artery, at least two endothelin receptors appear to be present. One of these is likely to be the ET_B receptor; the other has neither the characteristics of the ET_A nor of the ET_B receptor.

Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida S., Kimura S., Fukami T., Ishikawa K., Nishikibe M. & Yano, M. (1992). Life Sci. 50, 247-255.
Moreland, S., McMullen, D.M., Delaney C.L., Lee, V.G. & Hunt J.T. (1992). Biochem. Biophys. Res. Comm. 184, 100-106.
Saeki, T., Ihara, M., Fukuroda, T., Yamagiwa, M. & Yano, M. (1991). Biochem. Biophys. Res. Commun. 179, 286-292.
Williams, D.L.Jr., Jones, K.L., Pettibone, D.J., Lis, E.V. & Clineschmidt, B.V. (1991). Biochem. Biophys. Res. Commun. 175, 556-561.

109P ENDOTHELIN-1 STIMULATES PROTEIN KINASE C ACTIVITY AND PROTEIN TYROSINE PHOSPHORYLATION IN RAT-1 FIBROBLASTS

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Endothelin-1 (ET-1) has been shown to be a mitogen for both smooth muscle cells and fibroblasts in culture. This process is associated with the hydrolysis of membrane phospholipids and the subsequent activation of protein kinase C (PKC) (McNulty *et al.* 1990). However, recently it has been shown that ET-1 may also stimulate the tyrosine phosphorylation of a number of proteins implicated in the regulation of cell division (Force *et al.* 1991). Since it is unclear as to whether tyrosine phosphorylation is dependent on prior protein kinase C activation, we have examined the relationship between ET-1 stimulated PKC activity and tyrosine phosphorylation in rat-1 fibroblasts, a cell line in which ET-1 is a complete mitogen.

In all experiments cells were grown on 6 well plates and quiesced in serum free conditions for 40hrs. Protein kinase C activity was measured by the method of Heasly and Johnson (1989) using the peptide PKC 19-31 (Ser^{25}) as a substrate for PKC mediated phosphorylation. Tyrosine phosphorylation was assessed in whole cell lysates by SDS-PAGE followed by Western blotting using a polyclonal anti-phosphotyrosine antibody.

ET-1 (100 nM) stimulated an approximate 2 fold increase in peptide pseudosubstrate phosphorylation in rat-1 fibroblasts which peaked between 2 and 5 min of stimulation (2 min: basal = 1115 ± 121 cpm/sample, ET-1 stimulated = 2236 ± 237 cpm/sample, n=3). The protein kinase C activator TPA (100nM) also stimulated peptide pseudosubstrate phosphorylation and to a greater extent than that observed for ET-1 (10 min: basal = 1144 ± 231, TPA stimulated = 3551 ± 365, n=3). However, in cells pretreated with TPA for 48 hrs or preincubated for 10 min in the presence of the PKC inhibitor Ro-318220 (Davis *et al.* 1989) both ET-1 and TPA-stimulated pseudosubstrate phosphorylation was abolished (control cells: basal = 738 ± 120, TPA = 4790 ± 674, ET-1 = 1846 ± 262, 10μM Ro-318220 for 10 min: basal = 544 ± 81,TPA = 709 ± 53, ET-1 = 665 ± 28, n=3). ET-1 also stimulated a dose dependent increase in the tyrosine phosphorylation of at least 4 major proteins, one of 80kDa and 3 of approximately 120kDa. The response was biphasic reaching a peak at 2 min, then declining to near basal values at 10 min before increasing once more between 10-60 min. PMA also stimulated the tyrosine phosphorylation of the same set of proteins but to a much smaller extent than ET-1. However, preincubation with 10μM Ro-318220 signifcantly reduced tyrosine phosphorylation in response to ET-1.

These results suggest that in rat-1 fibroblasts, ET-1 stimulates tyrosine phosphorylation in both a PKC-dependent and -independent manner. It is likely that the activation of both protein kinase C and tyrosine kinase pathways is involved in regulating cell divison in response to the peptide. We wish to thank Dr G Lawton (Roche Products Ltd, Welwyn Garden City, Herts) for the gift of Ro-318220.

This work was funded by grants from the Medical Research Council and the Wellcome Trust.

MacNulty, E.E., Plevin, R. & Wakelam, M.J.O. (1990) Biochem. J. 272, 761-766.
Force, T., Kymakis, J.M., Avruch, J. & Bonventre, J.V. (1991) J. Biol. Chem. 266, 6650-6656.
Heasley, L. & Johnson, G. (1989) J. Biol.Chem. 264, 8646-8652.
Davis, P., Hill, CH., Leech, E., Lawton, G., Nixon, T.S., Sedgewick, A.D., Wadsworth, S.E., Wesmacott, D. & Wilkinson, S.E. (1989) FEBS, Letts 259, 61-63.

110P A NEW NON-SELECTIVE ENDOTHELIN ANTAGONIST BLOCKS THE VASOCONSTRICTION INDUCED BY ENDOTHELIN-1 IN THE RAT KIDNEY, BOTH *IN VIVO* AND *IN VITRO*

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The ET_A receptor antagonist, BQ-123 (Ihara *et al.*, 1992), blocks the pressor but not the depressor or renal vasoconstrictor effects of endothelin 1 (ET-1) in the anaesthetised rat (Cristol *et al.*, 1992). We have investigated the effects of BQ-123 and a novel endothelin receptor antagonist, PD145065 (Cody *et al.*, 1992), on the renal vasoconstriction induced by ET-1 in the isolated perfused kidney of the rat. In addition, the effect of PD145065 has been studied in anaesthetised rats in vivo.

Male Wistar rats (200-350g) were anaesthetised with pentobarbitone sodium (60 mg/kg, i.p.). The right renal artery was cannulated, the kidney removed, perfused with Krebs' buffer at constant flow (10 ml/min) and perfusion pressure recorded. After equilibration for 30 min, antagonist (10 μM) or vehicle was infused for 3 min prior to cumulative ET-1 infusions (range 10^{-12} to $6x10^{-10}$ M) and for the duration of the experiment. In the isolated perfused kidney, BQ-123 and PD145065 attenuated the rise in perfusion pressure induced by ET-1 ($3x10^{-10}$ M) by 57 ± 9% (n=6) and 96 ± 2% (n=4), respectively. In other experiments, rats were anaesthetised with thiopentone sodium (120 mg/kg, i.p.). The right femoral vein was cannulated for infusion of saline (1.5 ml/h), the jugular vein for administration of drugs, and the carotid artery for measurement of mean arterial blood pressure (MAP). An ultrasonic flow probe was placed around the left renal artery to measure renal blood flow (RBF). After a 30 min stabilisation period, PD145065 (5 μmol/kg) or vehicle control (0.1 % BSA in 0.9% saline) was given as a bolus i.v injection 3 min prior to ET-1. Four ET-1 injections (see table) were given at hourly intervals. The changes in renal blood flow and renal vascular resistance (RVR) 3 min after injection of ET-1 in vivo are shown (mean ± sem, n=6 for each group; *p<0.05; unpaired, two-tailed t-test) in the table:

	VEHICLE			PD145065		
ET-1 (nmol/kg)	Δ MAP (mm Hg)	Δ RBF (%)	Δ RVR (mm Hg ml^{-1} min)	Δ MAP (mm Hg)	Δ RBF (%)	Δ RVR (mm Hg ml^{-1} min)
0.1	7 ± 1.1	-16 ± 2.2	4 ± 0.6	2.5 ± 1.2*	-1.4 ± 1.0*	0.6 ± 0.2*
0.25	5 ± 2.2	-27 ± 2.5	7 ± 0.7	2.5 ± 1.7	-5.8 ± 1.6*	1.3 ± 0.4*
0.5	18 ± 2.8	-59 ± 6.7	46 ± 19	12 ± 2.5	-9.8 ± 2.3*	3.1 ± 0.6*
1.0	23 ± 3.1	-88 ± 4.3	424 ± 158	14 ± 2.2*	-22 ± 1.4*	7.9 ± 1.4*

Thus, in vitro, the renal vasoconstrictor effects of ET-1 were completely blocked by the ET_A/ET_B antagonist, PD145065 (Cody *et al.*, 1992), but only partially attenuated by the selective ET_A-receptor antagonist, BQ-123. In vivo, PD145065 strongly inhibited the increase in RVR induced by ET-1. Hence, in the rat kidney, vasoconstriction induced by ET-1 is mediated by both ET_A and ET_B receptors.

We thank Parke-Davis Pharmaceutical Research for funding this work and the Medicinal Chemistry Department for providing BQ-123 and PD145065, (Ac-(D-Bhg-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp), (D-Bhg = 5H-dibenzyl[a,d]cyclo-heptene-10,11-dihydro-glycine).

Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukami, T. *et al.* (1992) Life Sci. **50**, 247-255.
Cristol, J.P., Warner, T.D., Thiemermann, C. & Vane, J.R. (1992).Br.J.Pharmacol.**107**, 278P.
Cody, W.L., Doherty, A.M., He, J.X., Topliss, S., Haleen, S., *et al.* (1992) 22nd European Peptide Symposium, Abs 274.

111P EVIDENCE THAT PHOSPHOLIPASE D CAN REGULATE ENDOTHELIN-STIMULATED PHOSPHOLIPASE C ACTIVITY IN SK-N-MC CELLS

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Activation of phospholipase D (PLD) is known to be a widespread consequence of stimulation of phospholipase C (PLC)-linked receptors. However, the significance of PLD in cell signalling events is unclear. We have previously reported (Wilkes & Boarder, 1991) that endothelin-1 (ET-1) interacts with the human neuroblastoma derived SK-N-MC cell-line at ET_A receptors linked to PLC. Here we show that ET-1 also elicits a PLD response in these cells, and provide evidence that the PLD response, in turn, contributes to the regulation of the agonist-stimulated PLC activity.

SK-N-MC cells were grown close to confluence in 24-well plates. PLD responses were determined in cells labelled for 24 h with $^{32}P_i$ (0.25 MBq/well), pre-incubated with butanol (50 mM) for 10 min followed by a 3 min incubation in the absence or presence of ET-1. The phosphatidylbutanol (PBut) product, unique to PLD activity, was isolated by thin-layer chromatography and [^{32}P]PBut quantified. Mass assay of Ins(1,4,5)P_3 was carried out as described previously (Challiss et al., 1988).

ET-1 concentration-dependently stimulated the accumulation of [^{32}P]PBut, with a maximal stimulation of 288 ± 45% being observed at 100 nM ET-1. The presence of butanol diverts the PLD product away from phosphatidic acid, and subsequent diacylglycerol formation, to the stable product PBut. ET-1 (100 nM) caused a sustained increase in Ins(1,4,5)P_3 mass accumulation (basal 2.6 ± 0.3; +ET-1 (5 min) 7.2 ± 0.9 pmol/well (n=5)). In the presence of the protein kinase C (PKC) inhibitor Ro 31-8220 (compound 3 in Davis et al., 1989) there was a large increase in the response to ET-1 throughout the 10 min experimental time-course (+ET-1 + 10 μM Ro 31-8220 (5 min) 21.2 ± 0.5 pmol/well (n=3)). Moreover, in the presence of butanol the Ins(1,4,5)P_3 response to ET-1 was also significantly increased (+ET-1 + 50 mM butanol (5 min) 14.5 ± 0.8 pmol/well (n=3)). Thus, 5 min after ET-1 challenge butanol caused a potentiation of the Ins(1,4,5)P_3 response which was 47 ± 3% of that evoked by Ro 31-8220.

These data suggest that the ET-1 stimulation of PLC is profoundly depressed by the influence of PKC in these cells, and this modulation can be relieved by inhibition of PKC by Ro 31-8220. Furthermore, by diverting the PLD product away from phosphatidic acid/diacylglycerol formation, butanol can also releive this inhibitory influence. These results provide the first evidence that agonist-stimulated PLC activity may itself be regulated by products of PLD activation.

This work was supported by the Medical Research Council and the Wellcome Trust.

Challiss, R.A.J., Batty, I.H. & Nahorski, S.R. (1988) Biochem. Biophys. Res. Commun. **157**, 684-691
Davis, P.D., Hill, C.H., Keech, E., Lawton, G., Nixon, J.S., Sedgwick, A.D., Wadsworth, J., Westmacott, D. and Wilkinson, S.E. (1989) FEBS Lett. **259**, 61-63
Wilkes, L.C. & Boarder, M.R. (1991) Br. J. Pharmacol. **104**, 750-754

112P ENDOTHELIN RECEPTORS IN HUMAN CARDIAC TISSUE: A QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS

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In the present experiments we have investigated endothelin receptors in human atrial and ventricular tissue and the human coronary artery using quantitative receptor autoradiography. Tissue was obtained from organ donors, whose heart valves were used for homograft transplantation purposes. Displacement of [^{125}I]Sf6b (Sarafotoxin 6b) (30 pM)- and [^{125}I]ET-1 (30 pM)-labeled binding sites was studied using ET-1, the ET_A receptor selective ligand BQ-123 (cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]) and the ET_B receptor selective ligand 4Ala-ET-1 ([Ala^{1,3,11,15}]ET-1) (Nakamichi et al., 1992). Displacement curves were analyzed for every individual segment (n=7-14 in 2 experiments) and a fitting procedure for the optimal fit (biphasic or monophasic) was performed using computerized nonlinear regression (Grafit).

Specific binding of [^{125}I]Sf6b and [^{125}I]ET-1 in ventricular and atrial muscle was homogeneously distributed over the tissue and specific binding was more dense in atrium and coronary artery (relative optical density (r.o.d.): 0.14±0.01 and 0.16±0.01, respectively) than in ventricular muscle (0.10±0.01 r.o.d.). In the coronary artery, binding was especially dense in the media, in a thin adventitial layer and between media and the intimal hyperproliferated tissue.

Table 1. pIC_{50} (-Log IC_{50}) for the monophasic displacement of ET-1 (pIC_{50}), and in case of biphasic displacement a high (pIC_{50} high) and low (pIC_{50} low) affinity phase of 4Ala-ET-1 and BQ-123. *: monophasic displacement. N.D.: Not detected: plateau was reached.

		Ventricle		Atrium		Coronary Artery	
		[^{125}I]ET-1	[^{125}I]Sf6b	[^{125}I]ET-1	[^{125}I]Sf6b	[^{125}I]ET-1	[^{125}I]Sf6b
ET-1	pIC_{50}	10.16±0.08	9.49±0.08	9.61±0.04	8.95±0.07	9.72±0.02	9.35±0.04
4Ala-ET-1	pIC_{50} high	9.59±0.34	9.48±0.16	9.05±0.33	9.36±0.09	6.40±0.12*	8.40±0.14
	pIC_{50} low	6.05±0.20	6.54±0.12	5.79±0.17	6.91±0.05		6.99±0.09
BQ-123	pIC_{50} high	7.04±0.24	7.49±0.10	6.67±0.12	7.45±0.40	6.84±0.08*	9.03±0.25
	pIC_{50} low	N.D.	N.D.	N.D.	N.D.		7.24±0.14

ET-1 diplaced [^{125}I]Sf6b and [^{125}I]ET-1 monophasically in atrium, ventricle and coronary artery. 4Ala-ET-1 and BQ-123 displaced [^{125}I]ET-1 and [^{125}I]Sf6b labeled sites biphasically in atrial and ventricular muscle. In the human coronary artery 4Ala-ET-1 and BQ-123 displaced [^{125}I]ET-1 labeled sites monophasically in 6 and 7 segments respectively, out of 7 individually analyzed tissue segments. 4Ala-ET-1 and BQ-123 displaced [^{125}I]Sf6b labeled sites biphasically in 9 segments each, out of 14 individually analyzed tissue segments (Table 1). These data indicate that: (1) both [^{125}I]ET-1 and [^{125}I]Sf6b label putative ET_A and ET_B binding sites in human ventricular and atrial muscles as shown previously by Davenport et al. (1992); (2) [^{125}I]ET-1 labels a similar ET_A binding site in the human coronary artery, but (3) in the coronary artery, [^{125}I]Sf6b may also label a non-ET_A, non-ET_B binding site with relatively high affinity for both BQ-123 and 4Ala-ET1. *W.A.B. is supported by the Netherlands Heart Foundation, grant 89.252.

Nakamichi, K., Ihara, M., Kobayashi, M., Saeki, T., Ishikawa, K & Yano, M. (1992) Biochem.Biophys.Res.Commun. **182**,144-150.
Davenport, A.P., Molenaar, P. & Kuc, R.E. (1992) Br. J. Pharmacol. **107**, 304P.

113P PARADOXICAL INTERACTIONS BETWEEN ENDOTHELIN-1 AND CALCITONIN GENE-RELATED PEPTIDE IN THE RAT GASTRIC MICROCIRCULATION

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Local intra-arterial infusion of the vasoconstrictor peptide, endothelin-1 (ET-1; Yanagishawa et al, 1988) induces rat gastric mucosal injury (Whittle & Esplugues, 1988). This mucosal damage is inhibited by concurrent local infusion of the vasodilator sensory neuropeptide, calcitonin gene-related peptide (CGRP), which may reflect interactions between these mediators on microvascular tone (Whittle & Lopez-Belmonte, 1991). In the present study, therefore, the effects of ET-1 and CGRP on gastric mucosal blood have now been determined in the anaesthetised rat using laser Doppler flowmetry (LDF).

Rats (240g) were anaesthetised with pentobarbitone, the stomach exposed and the left gastric artery cannulated for the local administration of the agents (Whittle & Esplugues, 1988), with haemorrhagic injury in the mucosa being assessed macroscopically 20 min later. The laser Doppler probe (Perimed PF3) was placed against the surface of the corpus mucosa via a plastic cannula inserted through the forestomach (Tepperman & Whittle 1991). Local infusion of ET-1 (100pmol kg^{-1} min^{-1} i.a. for 10 min in 0.1% bovine serum albumin) induced haemorrhagic mucosal damage involving 95±3% of total mucosal area (n=9, mean±s.e.m; $P<0.001$). This dose of ET-1 had a biphasic action on LDF, with a transient initial increase in LDF (maximal of 59±8% of basal) lasting 2-3 min being followed by a sustained fall in LDF (-31±12% of basal, n=9; $P<0.01$). By contrast, lower doses of ET-1 (5-20 pmol kg^{-1} min^{-1} i.a.) caused only a sustained hyperaemic response, with ET-1 (10pmol kg^{-1} min^{-1}) increasing LDF by 59±14% of basal (n=8) in a dose inducing damage to 54±6% of the mucosa (n=8; $P<0.01$). Local infusion of rat α–CGRP (50pmol kg^{-1} min^{-1} i.a.) alone increased LDF by 32±4% of basal (n=8; $P<0.001$). However, concurrent infusion of CGRP reduced ($P<0.001$) the hyperaemic response to ET-1 (5pmol kg^{-1} min^{-1}) from 111±13% (n=15) to 25±3% of basal (n=8), while damage was decreased from 34±3% to 14±4% of total area ($P<0.05$).

These findings indicate that local vasoconstriction in the microcirculation could contribute to the mechanisms underlying the gastric injury induced by high doses of ET-1. By contrast, lower doses of ET-1 that caused significant mucosal haemorrhage, induced only a sustained hyperaemic response, indicating an injurious action independent of local vasoconstriction. This response could reflect the local release of vasodilator mediators such as prostacyclin or nitric oxide. Moreover, the finding that CGRP reduced both damage and the increase in LDF induced by ET-1 could suggest that this hyperaemic response was secondary to the initiation of mucosal damage, while the protective action of CGRP in this model is therefore unlikely to be the consequence of local vasodilatation. The mechanisms by which intravascular ET-1 can induce such mucosal damage may involve a direct disruptive action on the microvascular endothelium, an effect attenuated by CGRP.

Tepperman, B.L. & Whittle, B.J.R. (1991). Br. J. Pharmacol., 102, 95-100.
Whittle, B.J.R. & Esplugues, J.V. (1988). Br. J. Pharmacol., 95, 1011-1013.
Whittle, B.J.R. & Lopez-Belmonte, J. (1991). Br. J. Pharmacol., 102, 950-954
Yanagisawa, M., Kurihara, H., Kimura, S., Tombe, Y., Kobayashi, M., Yazaki, Y., Goto, K. & Masaki, T. (1988). Nature, 323, 411-415.

114P CHANGES IN INOSITOL PHOSPHATE-CYTOSOLIC CALCIUM-VASOREACTIVITY COUPLING WITH HYPERTENSION

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In vascular smooth muscle, essential hypertension is characterized by hyper-reactivity to vasoactive agonists, and by abnormalities in calcium handling and the phosphoinositide signalling system (Guild et al., 1992). In order to verify this hypothesis, we investigated the effects of noradrenaline (NOR) and endothelin-1 (ET) upon vasoreactivity, cytosolic calcium $[Ca^{2+}]_{cyt}$ and the accumulation of total inositol phosphates (IPs) in tail arteries from 3 month old SHR and WKY.
Tail arteries were removed under anesthesia. One centimeter of the vessel was inserted into a spectrofluorimeter cuvette, and perfused at a constant flow rate of 2 ml/min. Contractions induced by NOR (1-10 μM; 1 min) or by ET (1 μM; 1 min) were expressed as changes in the basal perfusion pressure (PP, mm Hg). Simultaneously, using the fluorescent dye fura2, changes in $[Ca^{2+}]_{cyt}$ were monitored as percentage changes in the fluorescent ratio $R_{340/380}$. In other segments of tail artery loaded with $[^3H]$-inositol, total IPs (cpm/mg tissue) were extracted from the vessel by anion exchange chromatography on Dowex anion exchange column. We measured basal and NOR (0.1-10 μM; 30 min) or ET (0.0001-1 μM; 30 min) - induced IPs accumulation.
SHR tail arteries showed an increase in basal $[Ca^{2+}]_{cyt}$ (96.8 ± 6.9 nM vs 62.5 ± 8.8 nM, $P < 0.05$, SHR vs WKY) with no change in the basal perfusion pressure (35.8 ± 3.4 mm Hg vs 40.5 ± 3.8 mm Hg, SHR vs WKY) or basal IPs accumulation (139.4 ± 45.9 cpm/mg vs 132.2 ± 29.3 cpm/mg, SHR vs WKY). When stimulated with NOR, SHR tail arteries showed hyper-reactivity (table 1) associated with an increase in calcium mobilization. ET - evoked contraction and calcium mobilization were similar in SHR and WKY rats.

Table 1: Contractions and calcium mobilizations induced by NOR and ET in the perfused rat tail artery.

	SHR		WKY	
	PP, mm Hg	% changes $R_{340/380}$	PP, mm Hg	% changes $R_{340/380}$
NOR 1 μM	37.3 ± 8.6*	26.7 ± 3.9	15.9 ± 4.5	19.8 ± 2.9
NOR 3 μM	140.5 ± 19.7*	37.8 ± 3.9*	48.6 ± 11.4	25.3 ± 3.5
NOR 10 μM	255.6 ± 26.7*	54.8 ± 6.1*	109.1 ± 22.0	39.1 ± 4.9
ET 1 μM	70.9 ± 14.1	18.7 ± 2.3	51.4 ± 11.2	17.4 ± 2.7

Results are expressed as mean ± SEM; * = $P < 0.05$ (Student's t-test) vs WKY, n = 11 per group.

Total IPs accumulation was similar in SHR and WKY after agonist (ET or NOR) stimulation. In both hypertensive and normotensive rats, for a given calcium mobilization, higher levels of IPs were observed after ET than NOR stimulation (1 μM) (WKY: 715.6 ± 162.2 cpm/mg vs 276.9 ± 56.6 cpm/mg, $P < 0.05$, ET vs NOR stimulation).
In conclusion, changes in contractility associated with modifications in the calcium handling between SHR and WKY suggested that alterations in the signal - transduction system occur with hypertension. The different effects of ET and NOR could be related to interactions with other signalling pathways.

Guild, S.B., Jenkinson, S. & Muir, T.C. (1992), Br. J. Pharmacol., 106, 859-864.

115P RECEPTOR AND TISSUE DEPENDENT EFFECTS OF ABANOQUIL (UK52046)

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Abanoquil (UK52046, Greengrass et al., 1991) is an antidysrhythmic agent which, in radioligand binding studies, has been shown to possess a high affinity for α_1-adrenoceptors, particularly the α_{1A}-adrenoceptor subtype. The present study examines the effects of abanoquil on the α_1-adrenoceptor-mediated responses of the rat left atrium, vas deferens, spleen and aorta.

Tissues were set up under 1g tension in Krebs-bicarbonate solution at 37°C. Left atria were paced at 1Hz. Single concentration-response curves to phenylephrine were obtained in the absence or presence of abanoquil following a 60 min contact time. Experiments were performed in the presence of cocaine (10μM), corticosterone (10μM) and propranolol (1μM).

Abanoquil acted as a non-competitive α_1-adrenoceptor antagonist on the vas deferens and spleen, but the vas deferens was more sensitive to this action, abanoquil reducing responses from 1.89 $\pm$ 0.21g to 1.31 $\pm$ 0.12g at only 1nM ($P=0.01$). On the spleen, abanoquil concentrations greater than 10nM were required to significantly reduce responses, 30nM abanoquil reducing the mean maximum response from 0.33 $\pm$ 0.03g to 0.21 $\pm$ 0.02 ($P<0.01$, Student's t-test).

On rat left atria and aorta, abanoquil (1-10nM) acted as a competitive antagonist, producing rightward shifts of phenylephrine concentration-response curves without altering maximum responses. A mean pK_B value of 10.1 $\pm$ 0.1 ($n=10$) was obtained on left atria which was significantly greater ($P<0.05$) than the value of 9.3 $\pm$ 0.1 ($n=20$) obtained on rat aorta. Schild plots for both tissues were linear with slopes of 1.01 $\pm$ 0.08 for atria and 0.87 $\pm$ 0.11 for aorta.

These results demonstrate that abanoquil acts as a competitive α_1-adrenoceptor antagonist on rat cardiac muscle and has a higher affinity for the α_1-adrenoceptors of the rat left atrium than those of the rat aorta. On smooth muscle, abanoquil can act as a purely competitive α_1-adrenoceptor antagonist or have non-competitive actions depending on the tissue being examined.

AHJ is a British Heart Foundation student.

Greengrass, P.M., Russell, M.J. & Wyllie, M.G. (1991) Br. J. Pharmacol.,104, 321P.

116P MECHANISM OF ADENOSINE FORMATION AND RELEASE FROM ADULT RAT VENTRICULAR MYOCYTES

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Adenosine may be produced from extracellular AMP by ecto-5'-nucleotidase. Alternatively, it may be produced intracellularly as a result of imbalance between energy demand and supply (Newby et al., 1990). ATP degradation is accompanied by a rise in intracellular concentration of AMP followed by adenosine production, which is probably due to the action of the IMP-selective or the AMP-selective cytosolic 5'-nucleotidase enzyme. Adenosine then exits out of the cell via the nucleoside transporter to act on extracellular receptors. Adenosine 5'-(α,β-methylene) diphosphate (AOPCP), an inhibitor of ecto-5'-nucleotidase decreased adenosine release by 80% in metabolically poisoned adult rat myocytes (Bukoski and Sparks, 1986). However, a nucleoside transport inhibitor also reduced adenosine release by 60%. These results are difficult to explain since the ectoenzyme is inaccessible to cytoplasmic nucleotides and furthermore the cytosolic enzymes are not inhibited by AOPCP. We have reassessed the site of adenosine formation in adherent adult rat myocytes. We have also examined the effect of 5'-deoxy-5'-isobutylthioadenosine (IBTA), an inhibitor of the IMP-selective cytosolic enzyme which may be used to test its role in intact cells (Skladanowski et al., 1989).

All experiments were done in duplicate ($n=3$). Cells were preincubated (60 min) with inhibitors of adenosine deaminase (2'-deoxycoformycin, 30 μM) and adenosine kinase (5-iodotubercidin, 10 μM) to minimize further metabolism of adenosine, with in addition, IBTA (3 mM) or dipyridamole (10 μM) or inhibitory antibodies to ecto 5'-nucleotidase. The cells were then rinsed three times with Krebs-buffer and ATP catabolism was induced by addition of Krebs-buffer containing 30 mM-2deoxyglucose, 2 μg/ml oligomycin and in addition IBTA (3 mM) or dipyridamole (10 μM). Incubations were terminated 10-60 min later and samples were assayed by hplc. 2-Deoxyglucose with oligomycin induced a 70$\pm$13% fall in ATP concentration in 30 min. Adenosine accumulation accounted for 50$\pm$18% of the fall in ATP concentration. A concentration of anti-(rat liver ecto-5'-nucleotidase) serum which inhibited the ecto-enzyme by 88 $\pm$ 7%, did not inhibit adenosine accumulation. Furthermore, dipyridamole, an inhibitor of the symmetric nucleoside transporter, decreased by 78$\pm$5% the appearance of the newly formed adenosine in the medium showing that adenosine is produced intracellularly in these cells in response to oxidative metabolism. IBTA inhibited by 87$\pm$4% the appearance of adenosine in the medium, but inhibited total adenosine accumulation by adult myocytes to the same extent as dipyridamole after 30 min. IBTA, like dipyridamole (69$\pm$6%), inhibited incorporation of extracellular adenosine (10 μM) into cellular nucleotides by 61$\pm$5%. We conclude that IBTA acts primarily to inhibit adenosine release from myocytes. The lack of additional effect of IBTA compared to dipyridamole, on adenosine formation rates imply that the IMP-selective cytosolic 5'-nucleotidase plays a minor role in this tissue.

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Bukoski, R.D. & Sparks, H.V. (1986) J. Mol. Cell. Cardiol. 18, 595-605.
Newby, A.C., Worku, Y., Meghji, P., Nakazawa, M. & Skladanowski, A.C. (1990) NIPS 5, 67-70.
Skladanowski, A.C., Sala, S.B. & Newby, A.C. (1989) Biochem. J. 262, 203-208.

117P IMPACT OF ENALAPRIL ON MYOCARDIAL β-ADRENOCEPTORS IN PACING-INDUCED HEART FAILURE

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Rapid right ventricular pacing (250 beats/min) in the dog reliably produces congestive heart failure (CHF) associated with a sequential rise in plasma noradrenaline (NA) and elevated plasma renin activity (PRA; Armstrong et al., 1986). In addition, in this model of CHF, like the clinical syndrome there is a decline in myocardial ß-adrenoceptor density (ßAR; Larosa et al., 1992). Converting enzyme inhibitors have proven useful in the management of patients with CHF and may function, in part, by modulating sympathetic transmission. We therefore undertook a study to evaluate the impact of chronic enalapril treatment (5 mg bid) or placebo treatment on myocardial ßAR-density and affinity, NA and PRA in dogs paced to peak CHF.

Treatment was initiated by blinded random assignment of dogs to enalapril (n=5) or placebo (n=6) 1 wk following the onset of pacing, at which time the animals exhibited mild cardiomegaly. Each group was paced to a conventional biologic end point (Armstrong et al., 1986) and sacrificed. In the enalapril treated group severe CHF occurred in 33±3 (s.e.mean) days compared to 24±3 days for the placebo treated group. Therefore in 4 unpaced dogs, enalapril was given for 26 days and 5 further dogs acted as placebo controls and were similarly unpaced. Blood samples, in all 4 groups, for NA and PRA were taken immediately prior to sacrifice. Following sacrifice, a small segment (1g) of left ventricle was homogenized and frozen for subsequent assay for ßAR density and affinity.

The results (mean ± s.e.m.) are shown in table 1 for ßAR (Bmax; fmol/g), affinity (K_D;pM), NA (nM) and PRA (ng/ml/h) where * represents $P < 0.05$ vs placebo CHF and nd = not determined.

Group	Bmax	K_D	NA	PRA
Control	2784±122*	21±6	1.7±0.5*	nd
Enalapril, unpaced	3390±380*	65±18*	0.7±0.1*	1.2±0.2
Enalapril, CHF	1919±170*	70±15*	6.4±1.1	21.1±2.5*
Placebo, CHF	1180±100	12±2	5.9±1.4	2.9±1.0

These data suggest that enalapril produces an increase in K_D in both groups (unpaced and CHF) reflecting desensitization of ßAR. There is a trend, in CHF, for enalapril to increase Bmax towards control, whereas enalapril does not affect Bmax in the unpaced group. These differences in ßAR occur independent of changes in NA and PRA suggesting that enalapril does not directly modulate NA turnover. It is noteworthy that Bmax for the enalapril CHF group is similar to that following 1 wk of pacing (Larosa et al., 1992) and thus we may speculate that enalapril treatment reduces detrimental symptoms by preventing rather than reversing the decreased ßAR density.

Supported by the Heart and Stroke Foundation of Ontario.
Armstrong, P.W., Stopps, T.P., Ford, S.E. & deBold, A.J.(1986) Circulation 74, 1075-1084.
Larosa, G., Seeman, P., Armstrong, P.W. & Forster, C. (1992) Cardiovasc. Res. In press

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118P EFFECT OF THE Na/H EXCHANGE INHIBITOR ETHYL-ISOPROPYL-AMILORIDE (EIPA) DURING ISCHAEMIA AND REPERFUSION

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Na/H exchange may play an important role during ischemia/reperfusion and in particular during the first minutes of reperfusion, where it exchanges intracellular protons generated during ischemia for extracellular sodium ions. In such a way, Na/H exchange can contribute to the sodium load observed during reperfusion (Frelin et al., 1988).
Isolated guinea pig hearts (Langendorff preparation) were perfused at 37°C with Tyrode solution (1.8 mM $CaCl_2$) at constant flow. The left ventricular pressure (LVP) was monitored by a latex balloon in the left ventricle. In the first set of experiments, ion sensitive mini-electrodes (pH and K^+) were inserted into the wall of the left ventricle. Hearts were made ischemic for 1 minute and reperfused for 30 min. The first ischemia was used as a reference for the following 5 ischemias each separated by 30 min of reperfusion. EIPA was given cumulatively with change of drug concentration 20 min before each ischemia. Besides the change in the signal of the ion selective electrodes (E_{K+} and E_{H+}), the area under the LVP curve ($area_{LVP}$) during 1 min of ischemia was monitored. The $area_{LVP}$ during all ischemias was expressed as % of the normoxic period preceeding the first ischemia. In a second set of experiments, hearts were pretreated with different concentrations of EIPA and made ischemic for 60 min by reducing the flow to 100 µl/min. The hearts were then reperfused for 60 min and cellular ions were determined by atomic absorption spectrometry. Additionally, the incidence of terminal fibrillations was determined at the end of reperfusion.
Ischemia: EIPA reduced the $area_{LVP}$ during 1 min of ischemia in the dose range between 1 and 3 x 10^{-6} M from 37 ± 4% of normoxic values in the absence of compound to 15 ± 3% at 3 x 10^{-6} M EIPA. The same doses of EIPA diminished the increase in E_{K+} and E_{H+}. Since the $area_{LVP}$ is considered as index of mechanical work produced by the heart, the parallell influence of EIPA on the $area_{LVP}$ and on the extracellular $[H^+]$ and $[K^+]$ suggests that the latter may primarily be related to savings in mechanical energy expenditure during ischemia.
Reperfusion: EIPA dose dependently (range: 10^{-7} to 10^{-6} M) reduced the incidence of terminal fibrillations from 100% in untreated reperfused ischemic hearts to 11% in the presence of 10^{-6} M EIPA. Untreated reperfused hearts showed a marked loss of K^+ down to 51 ± 7 mmol/kg cell, a substantial gain of Na^+ to 51 ± 6 mmol/kg cell and of Ca^{2+} to 3.1 ± 0.7 mmol/kg cell. 10^{-6} M EIPA significantly improved cellular ion contents (K^+ = 79 ± 15; Na^+ = 15 ± 7; Ca^{2+} = 1.4 ± 0.6 mmol/kg cell).
Conclusion: Higher concentrations of EIPA (10^{-6} M upwards) diminished the appearance of extracellular protons and potassium ions. However, this effect may well be related to savings in energy expenditure as evidenced by the concomittant reduction of the $area_{LVP}$. However, already 10^{-6} M EIPA nearly abolished the incidence of postischemic fibrillations. This is accompanied by a clear cut improvement of the homeostasis of Na^+, K^+ and Ca^{2+} in reperfused cells. The latter results suggest that EIPA may exert its cytoprotective effect primarily during reperfusion and to a lesser extent during the preceeding ischemia.
Ref.: Frelin et al. (1988) in "Na^+/H^+ Exchange", Grinstein ed., p 155-165, CRC Press, Boca Raton Fl, USA

119P THE EFFECTS OF ZAPRINAST ON ISCHAEMIA-INDUCED ARRHYTHMIAS AND CARDIAC CYCLIC NUCLEOTIDES

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To investigate the possible role of cyclic GMP in arrhythmogenesis, we have examined the effects of zaprinast, which is reported to have preferential inhibitory activity on the cyclic GMP-specific PDE (Souness *et al.*, 1989). Male Wistar rats (250 to 340 g, $n=12$ per group) were anaesthetized with sodium pentobarbitone 60 mg kg^{-1} i.p. Carotid arterial blood pressure and a Lead I ECG were recorded. A femoral vein was cannulated for drug administration and the trachea was cannulated for artificial ventilation. After performing a left thoracotomy, a fine silk ligature was placed around the left coronary artery close to its origin. Ten min after drug or vehicle administration, the coronary artery was occluded and arrhythmias monitored for 25 min. In separate groups ($n=5$) of sham-operated rats, 15 min after drug administration, the hearts were freeze-clamped with Wollenberger tongs pre-chilled in liquid nitrogen and then kept frozen at -70°C. Cardiac cyclic nucleotides were measured as described by Kane *et al.*, 1985.

Table 1. The effects of zaprinast on the number of ventricular premature beats (VPBs), ventricular fibrillation (VF), mortality, and cyclic nucleotides.

	VPBs (n)	VF	Mortality	cyclic GMP	cyclic AMP
		%	%	pmol g^{-1}	pmol g^{-1}
Control	1869 ± 160 (6)	83	50	19 ± 1	518 ± 21
Zaprinast 0.3 mg kg^{-1}	1031 ± 416 (3)	100	75	24 ± 4	806 ± 48*
Zaprinast 1 mg kg^{-1}	504 ± 83* (3)	75	75	15 ± 3	757 ± 40*
Zaprinast 3 mg kg^{-1}	1055 ± 368 (7)	92	42	27 ± 1*	925 ± 56*

The middle dose of zaprinast appeared to reduce the number of VPBs in survivors, but there were no significant differences between the incidences of either VF or mortality in any of the drug groups compared with the controls. Although cardiac cyclic GMP concentrations were increased by the highest dose of zaprinast, all three doses of this drug caused significant increases in cyclic AMP. These results indicate that, at the doses used here, zaprinast does not selectively increase cardiac cyclic GMP. We recommend that caution is exercised in the interpretation of results obtained with zaprinast unless *both* cyclic GMP and cyclic AMP are measured. Further studies with other agents are required to elucidate the possible role of cyclic GMP in arrhythmogenesis.

This work was supported by the British Heart Foundation.

Kane, K.A., Morcillo-Sanchez, E.J., Parratt, J.R., Rodger, I.W. & Shahid, M. (1985) *Br. J. Pharmacol.*, 84, 139-145.
Souness, J.E., Brazdil, R., Diocee, B.K. & Jordan, R. (1989) *Br. J. Pharmacol.*, 98, 725-734.

120P DEPRESSED INOTROPIC RESPONSE TO B-RECEPTOR AGONISTS IN HYPERTROPHIED HEARTS FROM RATS WITH INDUCED AORTIC STENOSIS

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Objective: To study whether the impaired inotropic response to ß-adrenoceptor stimulation found in hypertensive hearts is caused by cardiac hypertrophy or by other factors.

Design and Methods: Cardiac hypertrophy was induced in normotensive rats by stenosing the abdominal aorta (ASR) at an age of 5 weeks. The rats were than kept for 15 and 27 weeks, respectively. The functional responses to the ß-adrenoceptor agonists isoprenaline ($ß_1+ß_2$), dobutamine ($ß_1>ß_2$), terbutaline ($ß_2>ß_1$) and salbutamol ($ß_2>ß_1$) were measured in isolated, paced (5Hz) hearts and compared with those of age matched "sham" operated control rats. The data are represented as % of maximal increase. Hearts from SHR were studied as well.

Results: Although in AS rats the blood pressure increase was lower than in SHR, the cardiac hypertrophy was far more pronounced. The inotropic response (dp/dt_{max}) to all ß-adrenoceptor agonists was significantly weaker in the hypertrophied hearts from ASR at 18-20 weeks of age than in those obtained from age-matched controls ($p<0.05$) and decreased even more in hearts taken from ASR of 32-34 weeks of age, as did the activity of the post-receptor system, tested with forskolin and dbc-AMP.

Maximal inotropic response in % (E_{max})

	18-20 weeks of age			32-34 weeks of age		
	control	ASR		control	ASR	
isoprenaline (0.3µM)	173.1 ± 12.9	110.0 ± 8.2	*	168.5 ± 11.8	30.6 ± 4.6	*
dobutamine (3µM)	144.2 ± 10.2	80.8 ± 5.9	*	133.1 ± 13.2	34.6 ± 5.3	*
terbutaline (3µM)	91.1 ± 6.8	46.8 ± 6.0	*	82.4 ± 7.6	28.2 ± 6.2	*
salbutamol (3µM)	94.9 ± 6.9	60.0 ± 6.0	*	71.4 ± 8.6	17.1 ± 4.8	*
forskolin (30µM)	104.3 ± 9.6	33.8 ± 4.5	*	94.4 ± 8.7	35.7 ± 4.5	*
dbc-AMP (30µM)	67.4 ± 2.3	20.8 ± 4.3	*	58.3 ± 4.8	15.4 ± 2.8	*

Conclusions: These findings indicate that the impaired contractile response to ß-adrenoceptor agonism, which is initiated at the post-receptor level, is more related to the degree of cardiac hypertrophy than to elevated blood pressure.

121P ANTI-ISCHAEMIC EFFECTS OF R 56865 IN THE ISOLATED RAT WORKING HEART PREPARATION

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R 56865, N-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine, is a potent anti-ischaemic compound which is known to block Na^+-channels in particular during ischemia, hence protecting the ischaemic heart against calcium overload. In the present study R 56865 was investigated in rat working heart preparations, subjected to low flow ischemia. Isolated working hearts from male wistar rats (330-465 g) were prepared at 37°C with Tyrode solution (calcium concentration 2.23 mmol/l) and paced at a frequency of 5 Hz (pulse width: 5 ms, voltage: 10% above treshold, 2-4V) by means of platinum electrodes. Under normoxic conditions R 56865 reduced left ventricular pressure (LVP), maximal rate of pressure increase ($+dP/dt_{max}$), aortic output (AO), and cardiac output (CO) in a concentration dependent manner in the higher concentration range ($\geq 10^{-6}$M). Low flow ischemia was induced by lowering the cardiac afterload from 51.5 to 11.0 mmHg. After stabilisation, the hearts were pretreated with R 56865 for 15 min and subsequently subjected to low flow ischemia for 30 min. During the period of ischemia R 56865 was also infused but it was not applied during the reperfusion phase. Appropriate control experiments were performed with the vehicle DMSO (0.01 % v/v.). The 5 aforementioned parameters were determined after the stabilisation period and at the end of the reperfusion phase. From these data the recovery (in %) was calculated (see table 1).

Table 1: Recovery percentages (means ± s.e.mean; *significantly different from the control values $p<0.05$).

N	conc.	LVP (%)	$+dP/dt_{max}$ (%)	AO (%)	CF (%)	CO (%)
8	control	3.3 ± 1.52	0.0 ± 0.0	7.93 ± 8.0	10.4 ± 2.79	8.52 ± 3.08
6	10^{-8}M	0.0 ± 0.0	0.0 ± 0.0	2.06 ± 0.29	13.02 ± 2.05	5.87 ± 0.70
6	10^{-7}M	39.6 ± 14.9*	40.8 ± 12.1*	25.0 ± 8.92	41.8 ± 15.2*	29.9 ± 10.3*
6	3 x 10^{-7}M	68.6 ± 5.5*	70.8 ± 4.64*	44.6 ± 10.6*	81.0 ± 5.41*	59.8 ± 5.90*
6	10^{-6}M	79.6 ± 3.0*	82.1 ± 3.01*	54.7 ± 5.34*	92.7 ± 2.85*	67.2 ± 3.16*
6	3 x 10^{-6}M	37.8 ± 12.0*	33.3 ± 10.8*	8.57 ± 3.89	54.6 ± 14.2*	23.0 ± 7.49

In conclusion: 1) R 56865 already exerts its anti-ischaemic activity at a concentration of 10^{-7}M and associated with a slight reduction in cardiac contractile force by 12.9% ± 2.45, 2) the anti-ischaemic activity of R 56865 in the isolated rat working heart preparations exposed to low flow ischemia is most pronounced at a concentration of 10^{-6}M and associated with a reduction in cardiac contractile force by 25.7 % ± 3.26 and 3) R 56865 exerts its anti-ischaemic effect associated with a much weaker depressant effect on contractile force in the rat working heart than caused by classical calcium antagonists such as nifedipine.

122P DISCRIMINATION BETWEEN P_{2Y} AND NUCLEOTIDE RECEPTOR MEDIATED MOBILISATION OF INOSITOL 1,4,5-TRISPHOSPHATE IN AORTIC ENDOTHELIAL CELLS

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We have previously shown that populations of P_{2Y}-purinoceptors (responsive to ATP, 2 methylthio ATP (2MeSATP) and ADPβS) and nucleotide receptors (responsive to ATP and UTP) co-exist on bovine aortic endothelial cells (BAEC's) (Wilkinson et al., 1992). Both these receptors cause phospholipase C activation and release of inositol phosphates. Here we show that stimulation of the two receptor types generate inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) responses which differ in terms of time course and the influence of protein kinase C (PKC).

Confluent BAEC's at passage 4-9 in 24 well multi-plates were stimulated with a variety of purinergic agonists and UTP, and mass $Ins(1,4,5)P_3$ determined by the assay of Challiss et al., 1988. The action of changing the medium on the cells stimulated $Ins(1,4,5)P_3$ levels to 2.49 ± 0.27 (n=6) fold over resting basal after 5 sec, declining back to basal by 25 sec. Time courses for agonist stimulation of $Ins(1,4,5)P_3$ therefore had control time courses substracted. Stimulation with UTP (100μM) for 5 sec raised $Ins(1,4,5)P_3$ mass 2.07 ± 0.39 (n=6) fold over resting basal (control subtracted) and this elevation was maintained for a further 10 sec (1.71 ± 0.27 fold after 10 sec, 1.65 ± 0.22 fold after 15 sec) then declined to basal after 30 sec. 30μM 2MeSATP caused a stimulation of 1.97 ± 0.20 (n=6) fold over basal after 5 sec but this was not maintained and fell to 0.55 ± 0.21 fold over basal after 10 sec, then declined to control levels after 30 sec. Combined 2MeSATP and UTP stimulation gave an additive time course while ATP (100μM) stimulated $InsP_3$ to 2.24 ± 0.25 (n=6) fold over resting basal after 5 sec, then levels declined linearly, but remained above basal at 30 sec.

Dose response curves for 5 sec stimulations gave an order of agonist potencies of 2MeSATP>ADP>ADPβS>ATP> ATPγS>UTP (EC_{50} 0.17μM, 1.94μM, 3.07, 11.65, 44.66 and 95μM respectively). 5 mins pretreatment of cells with PKC activating tetradecanoyl phorbol acetate (10nM) completely inhibited stimulation by 2MeSATP, partially inhibited ATP stimulation and enhanced UTP stimulation. ADP stimulation was totally inhibited. 5 mins pretreatment of cells with the relatively selective PKC inhibitor Ro 31-8220 enhanced 2MeSATP, ADP and ATP stimulation but did not affect the response to UTP.

The results show that the profile of biochemical response to stimulation of co-expressed P_{2Y} and nucleotide receptors differs, indicating that the two populations may differentially modulate cell function. The data also indicated that ATP mediated its effect on endothelial cells by both receptor types while ADP acted via the P_{2Y} receptors alone.

This work was supported by The Wellcome Trust, M.R.C. and Fisons plc.

Challiss, R.A.J., Batty, I.H. and Nahorski, S.R. (1988) Biochem. Biophys. Res. Commun. **157**, 684-691.
Wilkinson, G.F., Purkiss, J.R. and Boarder, M.R. (1992) Brit. J. Pharmacol. (in press).

123P MECHANISM OF THE HYPOTENSIVE RESPONSE TO SELECTIVE ADENOSINE A_1 RECEPTOR AGONISTS IN THE PITHED RAT

A.M. Carruthers & J.R. Fozard, Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland

Low doses of selective adenosine A_1 receptor agonists reduce blood pressure (BP) and heart rate (HR) in the angiotensin II-supported circulation of the pithed rat; surprisingly, only the bradycardia but not the fall in BP could be reversed by 8-p-sulphophenyltheophylline (8-SPT), a broad-spectrum adenosine receptor antagonist injected intravenously (i.v.) at the end of the agonist injection sequence (Carruthers et al., 1992). In this report we provide further evidence that a significant component of the hypotensive response to nominally A_1 selective agonists in the pithed rat is not mediated by A_1 or A_{2A} receptors.

Male normotensive Sprague-Dawley rats (250-400 g) were anaesthetized, pithed and prepared for recording BP and HR as previously described (Carruthers et al., 1992). BP was maintained at 100-120 mmHg by infusions of angiotensin II, 0.2-0.5 μg/kg/min. Agonists were injected i.v. and dosing was cumulative; antagonists or vehicle were injected i.v. 5 min prior to starting the agonist dose-response sequence. Only one agonist curve was generated per animal.

Table 1: Cardiovascular effects of adenosine receptor agonists and their blockade by 8-SPT

Adenosine (Ado) analogues		Control			8-SPT-treated [b]			
		BP, ED_{30} [a] (nmol/kg)	HR, ED_{30} [a] (nmol/kg)	n	BP, ED_{30} [a] (nmol/kg)	HR, ED_{30} [a] (nmol/kg)	BP Shift [c]	n
N^6-cyclopentylado	(CPA)	11 ± 1	3.0 ± 0.4	8	62 ± 13	200	5.7	8
R-N^6-phenylisopropylado	(R-PIA)	21 ± 2	20 ± 3	4	56 ± 6	> 259	2.7	9
N^6-cyclopentyl-2-chloroado	(CCPA)	20 ± 2	11 ± 1	4	78 ± 6	> 270	3.9	3
N^6-cyclohexylado	(CHA)	29 ± 7	12 ± 2	4	101 ± 25	> 286	3.5	6
S-N^6-phenylisopylado	(S-PIA)	198 ± 19	337 ± 58	4	292 ± 35	> 778	1.5	4
2-[p-(2-carboxyethyl)phenyl-ethylamino]-5'N-ethylcarboxamidoado	(CGS 21680)	2.9 ± 0.2	> 20	4	84 ± 18	> 200	29	6

[a] dose to lower BP and HR by 30% [b] 40 mg/kg given i.v. 5 min prior to first dose of agonist [c] ratio: BP, ED_{30} (control)/BP, ED_{30} (8-SPT)

Selective A_1 receptor agonists (see Carruthers et al., 1992) reduced both BP and HR dose-dependently in pithed rats infused with angiotensin II. The A_{2A} selective agonist, CGS 21680, reduced BP but caused no change in HR. Following 8-SPT (40 mg/kg given i.v. 5 min before the first dose of agonist), the bradycardia was reduced (CPA) or abolished (the rest) whereas dose-dependent falls in BP were seen consistently with higher doses of each of the agonists (Table 1). For the selective A_1 receptor agonists the shifts in the BP dose-response curves ranged from 1.5 to 5.7-fold; that for CGS 21680 was 29-fold. The cardiovascular responses to CPA and R-PIA were modified qualitatively and quantitatively similarly by the potent and selective A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 0.2 - 0.5 mg/kg i.v.

In conclusion, two distinct mechanisms appear to contribute to the hypotensive responses to selective A_1 receptor agonists in the pithed rat: one, linked to the bradycardia and blocked by 8-SPT and DPCPX, is probably mediated by the A_1 receptor; the other occurs independently of changes in HR, is resistant to blockade by xanthine-based adenosine receptor antagonists and does not involve either A_1 or A_{2A} receptor activation.

Carruthers, A.M., Milavec, M., Gadient, F. & Fozard, J.R. (1992). Brit. J. Pharmacol. 106, 94P.

124P REDUCTION OF INFARCT SIZE BY L-NAME CAN BE PREVENTED BY PRETREATMENT WITH AN ADENOSINE ANTAGONIST IN RABBIT ISOLATED HEARTS

Vanlata C. Patel, R.G. Woolfson, G.H. Neild and D.M. Yellon, Academic Department of Cardiology, Hatter Institute for Cardiovascular studies, University College Hospital, Gower Street, London W CIE 6AU.

Occlusion of a coronary artery followed by reperfusion leads to a degree of infarction within the ischaemic area. Infarction size depends on a number of factors including collateral flow, duration of ischaemia, neutrophil infiltration, and possibly the generation of free radicals. Recently the role of endogenous nitric oxide (NO) has become evident both as an important determinant of vasomotor tone as well as a potential mediator of cytotoxicity. We have previouly shown that inhibition of NO during regional ischaemia and reperfusion reduces infarct area/risk area (I/R) in the in vivo rabbit heart model (Patel et al., 1992). Recent findings by Kostic and Schrader (1991) have reported that inhibition of NO leads to a compensatory increase in adenosine in the coronary perfusate. Interestingly pretreatment with adenosine may protect the myocardium against subsequent ischaemia and reperfusion (Liu et al., 1991). In view of these findings, we have investigated whether this protection from NO inhibition is from a compensatory release in adenosine. L-NAME and SPT (8(p-sulphophenyl)theophylline) were used to block NO synthesis and adenosine receptors respectively.

New Zealand White rabbits (n=28) were anaesthetised with pentobarbitone (40 mg/kg) and ventilated. Following thoracotomy, a snare was placed round the left coronary artery prior to ligation. Hearts were excised and mounted on a Langendorff apparatus and perfused with Krebs solution. Four groups were studied: Control; L-NAME (30μM); SPT (75.5μM); and SPT+L-NAME. Regional ischaemia lasted 45 minutes and was followed by 180 minutes reperfusion. In the treatment groups, the perfusate contained SPT for the first 15 minutes prior to ischaemia only and/or L-Name which was present for 10 minutes prior to ischaemia continuing into the early reperfusion period. At the end of each experiment the artery was re-occluded and the coronary circulation perfused with fluorescent microspheres to delineate the risk area (R). Tetrazolium was used to demarcate the infarcted area (I). I/R ratios were then calculated.

	CONTROL	L-NAME	SPT	SPT+L-NAME
(n)	7	8	7	6
Area at risk cm^2	3.4±0.5	3.0±0.5	3.6±0.6	3.4±0.5
Infarct/risk %	65±4%	42±2%	58±8%	63±6%
	P<0.01		P<0.01	

NO inhibition significantly reduced infarct size in the isolated rabbit heart model, but this protection was lost in hearts preteated with SPT. These results suggest that the L-NAME may confer protection as the result of a compensatory release of adenosine.

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Patel V C, Woolfson R G, Neild G H and Yellon D M (1992) J. Mol. Cell. Cardiol. **24**, S.152

125P THE EXTENT OF LEUKOTRIENE B_4 SYNTHESIS AND NEUTROPHIL ACCUMULATION IN THE ISCHAEMIC/REPERFUSED RABBIT ISOLATED MYOCARDIUM

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The activation and infiltration of neutrophils during myocardial ischaemia and reperfusion appears to be a major determinant of tissue injury (Engler & Lucchesi, 1987). Several studies have implicated arachidonic acid metabolites as possible neutrophil chemotactic factors (Mullane et al., 1987). Leukotriene B_4 (LTB_4), a potent neutrophil chemoattractant, stimulates intravascular neutrophil-endothelial cell adhesion and diapedesis out of the vascular space via an expression of the neutrophil adhesion complex, CD11b/CD18 (Lewis et al., 1990). In the present study, we have correlated the extent of LTB_4 synthesis with the accumulation of neutrophils in isolated Krebs-perfused rabbit hearts. Additionally, we have assessed the effect of the 5-lipoxygenase inhibitor, PF-5901 (α-pentyl-3-(2 quinolinylmethoxy) benzenemethanol, Mullane et al., 1987) on LTB_4 synthesis in the same preparation.

The Langendorff-perfused (40 ml min^{-1}, 37°C) rabbit heart preparation was used to study LTB_4 synthesis following circumflex coronary artery occlusion (CAO) and reperfusion with a rabbit neutrophil suspension (2 X 10^7 cells in total over 30 min) (Lad et al., 1992). A short duration of CAO (30 min) followed by reperfusion (30 min) with the rabbit neutrophil suspension (2 X 10^7 cells in total) significantly increased LTB_4 levels (measured by Amersham International radioimmunoassay kit) in the area at risk (63.4 ± 9.0 pg g^{-1} tissue, n=6, $P<0.05$) compared to the normal zone (17.3 ± 2.4 pg g^{-1} tissue, n=6). Associated with this increase was a significant accumulation of neutrophils (as assessed by myeloperoxidase activity) in the area at risk (1.5 ± 0.3 U g^{-1} tissue as compared to 0.15 ± 0.03 U g^{-1} tissue in the normal zone, n=6, $P<0.05$). Pre-treatment with PF-5901 (3 and 10 μM) throughout the period of CAO and reperfusion significantly inhibited the cardiac LTB_4 concentrations in the area at risk (33.0 ± 2.8 pg g^{-1} tissue and 20.2 ± 8.0 pg g^{-1} tissue respectively, n=6, $P<0.05$) compared to the vehicle-treated hearts (63.4 ± 9.0 pg g^{-1} tissue, n=6). Pre-treatment of the myocardium with only the higher concentration of PF-5901 (10 μM) significantly reduced the neutrophil accumulation in the area at risk (0.54 ± 0.17 U g^{-1} tissue compared to 1.5 ± 0.3 U g^{-1} tissue in the vehicle-treated hearts, $P<0.05$, n=6).

CAO followed by reperfusion in the absence of neutrophil suspension increased LTB_4 concentrations two-fold in the ischaemic/reperfused rabbit myocardium (22.6 ± 3.9 pg g^{-1} tissue compared to 12.9 ± 0.9 pg g^{-1} tissue in the normal zone, $P<0.05$, n=6). Additionally, in both studies LTB_4 levels in the coronary perfusate were below the detection levels of the radioimmunoassay used (< 3 pg g^{-1} tissue).

It appears that synthesis of LTB_4 in the isolated Krebs-perfused myocardium following ischaemia/reperfusion associates with the accumulation of neutrophils. Moreover, a greater than 50% inhibition of LTB_4 generation was required to inhibit significantly the neutrophil accumulation in the ischaemic/reperfused rabbit isolated myocardium.

Engler, R. & Lucchesi, B.R. (1987). Fed. Proc., 46, 2395-2433.
Mullane, K., Hatala, M.A., Kraemer, R., Sessa, W. & Westlin, W. (1987). J. Cardiovasc. Pharmacol., 10, 398-406.
Lad, N., Williams, T.J. & Booth, R.F.G. (1992). Eur. J. Pharmacol., 223, 163-171.
Lewis, R.A., Austin, K.F. & Soberman, R.J. (1990). N. Eng. J. Med., 323, 645-655.

126P INHIBITION OF THE HYPERPOLARISATION ACTIVATED CATIONIC CURRENT (I_f) BY ICI D7288 IN GUINEA-PIG ISOLATED SINOATRIAL NODE CELLS

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ICI D7288 (4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride) is a sinoatrial node (SAN) modulating agent which produces a selective slowing of the heart rate (Marshall et al., 1992). We have previously reported that ICI D7288 inhibited the hyperpolarization activated cationic current (I_f) in single, freshly dissociated guinea pig SAN cells, in a concentration dependent manner (BoSmith et al., 1992).

We have now further investigated the inhibitory actions of ICI D7288 on I_f. Whole-cell I_f currents were evoked by hyperpolarizing voltage clamp steps from a holding potential of -40 mV, and were recorded using standard patch clamp procedures in a HEPES buffered Tyrode solution. The activation of I_f was investigated by measuring tail current amplitudes at +20 mV after hyperpolarizing steps to different potentials to activate the current. The reduction in I_f resulted from both a shift in the I_f current activation curve in the negative direction on the voltage axis, and also a reduction in the activation curve amplitude. ICI D7288 (0.1 μM) shifted the I_f half-maximal activation by -6.1 ± 1.1 mV (n = 8) and reduced the activation curve maximum current by 35 ± 3% (n = 8). A concentration of 0.3 μM produced a greater shift of -16.2 ± 1.8 mV (n = 5) in the half-maximal activation and a reduction of the maximum current by 52 ± 6% (n = 5). ICI D7288 did not affect the ion selectivity of the I_f channel, since the tail current reversal potential was unchanged (-35 to -25 mV) in the presence of 0.1 or 0.3 μM of the drug (n = 3). With ICI D7288 (0.3 - 1 μM) the inhibition of I_f was not use-dependent (n = 3), whereas UL-FS 49 (zatebradine) at a concentration of 1 μM (n = 3), displayed use-dependence in the block of the I_f current. These data show that ICI D7288 reduces I_f by affecting the activation characteristics of the I_f current; this inhibition may account for this agent's selective bradycardic properties.

BoSmith R.E., Briggs I., Danks P. & Sturgess N.C. (1992) Br. J. Pharmacol., 107: 381P.
Marshall P.W., Bramley J. & Briggs I. (1992) Br. J. Pharmacol., 107: 134P

127P MESSENGER RNA LEVELS OF THE α SUBUNITS OF Gi PROTEIN AND Gs PROTEIN IN β_1-BLOCKED AND NON-β-BLOCKED HUMAN ATRIUM

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Chronic β_1-blocker therapy induces hyperresponsiveness in human atrium of the β_2-adrenoceptors (β_2AR) (Hall et al.,1990). To investigate whether the β_2AR sensitisation by β_1-blockade might be due to altered gene expression of G proteins, we compared mRNA levels of Gi2α and Gsα in human right atrial appendage (RAA) from β_1-blocker treated (n=6) and non-β-blocker treated (n=6) patients undergoing cardiac surgery.

Total RNA was extracted from the RAA by the method of Chomczynski & Sacchi (1987). To correct for differences in the recovery of intact mRNA from each sample, the sense Gi2α complementary RNA transcribed from full-length Gi2 cDNA cloning vectors containing bacteriophage RNA polymerase promoters was added into each sample as an internal control before the RNA extraction. Quantitative ECL Northern Blot Analysis was established by using specific antisense cRNA probes labeled with digoxigenin and the chemiluminescent substrate luminol. The resulting bands were quantified by laser densitometry, measuring the integrated pixel volume . Initial studies demonstrated a linear relation between amount of RNA loaded and densitometric signal.

In ECL Northern Blot, the digoxigenin-labeled cRNA probe specific for Gi2 hybridized to a predominant band at 2.3Kb. The Gi3 cRNA probe detected a message of 3.4Kb in total RNA extracted from human RAA. The Gs cRNA probe revealed one predominant band at 1.9Kb, and two faint bands at 1.7Kb and at 4Kb . The expression levels of Gs mRNA in β_1-blocked RAA were not significantly different from those of non-β-blocked RAA (411.12± 97.36 vs 304.44 ± 109.93, Mean ± SD, $p>0.05$). Nor was there any significant difference in Gi2α mRNA between β_1-blocked versus non-β-blocked RAA (1029.66 ± 289.63 vs 783.46 ± 297.21, $p> 0.05$). The ratio of signal from Gi2 mRNA / Gs mRNA in β_1-blocked RAA (2.57 ± 0.80) was not significantly different from non-β-blocked RAA (2.61 ± 0.94, $p >0.05$).

These results showed there was no significant alteration of gene expression of Gi2α and Gsα proteins in the patients taking β_1-blocker treatment. This accords with our measurement of Gsα and Gi2α proteins by immunoblotting, where again no quantitative changes at the Gα protein levels were found (Brown et al.,1992). We conclude that the previously observed cross-regulation of G protein coupled receptors in human RAA is unlikely to be due to altered gene expression of G proteins.

Hall, J.A., Kaumann, A.J. & Brown, M.J. (1990) Cir. Res. 66, 1610-1623.
Chomczynski, P. & Sacchi, N. (1987) Anal. Biochem. 162,156-159.
Brown, M.J., Ferro, A., Jia, H. & Monteith, S. (1992) Br. J. Pharmacol. 105 (Suppl.), 287P.

128P EFFECTS OF AMINOGUANIDINE ON PROSTACYCLIN RELEASE AND INTERACTIONS WITH L-NAME

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We have previously shown that an infusion of angiotensin II (AII) into rat lungs isolated and perfused at constant flow results in a transient increase in perfusion pressure and release of the vasodilator prostacyclin (Eaton *et al*, 1992). In addition, by perfusion of lungs with the nitric oxide (NO) synthesis inhibitor, nitro-L-arginine methyl ester (L-NAME), we demonstrated that the vasoconstrictor response to AII was substantially attenuated by endogenous NO (Eaton *et al*, 1992). In this current study we examined the influence of aminoguanidine, a nucleophilic hydrazine compound recently reported as a putative inhibitor of NO formation (Corbett *et al* 1992), on the increases in prostacyclin release and perfusion pressure in response to AII. The interaction between these two inhibitors of NO synthesis was also investigated. Lungs were perfused (Krebs buffer, pH 7.4, 37°C, 95%O_2 5%CO_2) for a 30 min equilibration period followed by a 3 min basal period. An increase in perfusion pressure and prostacyclin release was then provoked by a 5 min infusion of AII (0.125 ml/min, final concentration 1.2×10^{-6}M), after which perfusion was continued for a 15 min recovery period. Perfusate was collected and stored at -20°C until assay of 6-keto prostaglandin $F_{1\alpha}$, the stable metabolite of prostacyclin. Perfusion pressure was monitored throughout. Lungs were either perfused with 10^{-3}M aminoguanidine, 10^{-5}M L-NAME, 10^{-3}M aminoguanidine and 10^{-5}M L-NAME or with buffer alone (control). During the basal period neither perfusion pressures or prostacyclin releases were different in any group (n=6). Prostacyclin release in response to AII was measured as the total amount (mean ± SD in ng) above basal release during the stimulation and recovery periods. In comparison to perfusion with buffer alone, perfusion with L-NAME did not influence the AII induced increase in prostacyclin release (183.8 ± 101.0 in controls vs. 147.7 ± 81.2 in L-NAME perfusions) but markedly exaggerated the peak perfusion pressure response to AII (mmHg; 15.73 ± 4.09 vs. 23.79 ± 3.77 respectively, $p<0.01$). Perfusion with aminoguanidine also significantly increased the AII induced peak perfusion pressure (25.85 ± 5.56, $p<0.01$) and, in addition, caused a threefold increase in prostacyclin release (596.7 ± 232.7, $p<0.01$) compared to controls. Perfusion pressures (27.76 ± 1.96) and prostacyclin levels (993.7 ± 526.3) of lungs perfused with both L-NAME and aminoguanidine did not differ from those with aminoguanidine alone. These results suggest that, in the lung, aminoguanidine acts as an inhibitor of NO synthesis with similar efficacy to L-NAME. However, perfusion with this compound markedly enhanced the prostacyclin release associated with AII infusion. This may be due to direct receptor stimulation or may be a consequence of the rise in perfusion pressure. If it is the latter, such an effect is not seen with L-NAME induced exaggeration of the AII pressor response.

This work was funded by Ono Pharmaceuticals, Osaka, Japan.

Eaton, J.D., Stevens, E.J., Willars, G.B. & Tomlinson, D.R. Br.J.Pharmacol. *In press* (Abstract).
Corbett, J.A., Tilton R.G., Chang, K., Hasan, K.S., Ido, Y., Wang, J.L. *et al.*, (1992) Diabetes **41**, 552-556.

129P CEREBROVASCULAR AUTOREGULATION TO HYPERTENSION INDUCED BY INHIBITION OF NITRIC OXIDE SYNTHESIS

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Inhibition of nitric oxide (NO) synthesis has been shown to attenuate carbon dioxide-induced cerebrovascular dilatation *in vivo* (Iadecola, 1992), but in contrast, cerebrovascular autoregulatory dilatation in response to hypotension appears not to be NO-dependent in anaesthetized, artificially ventilated rats (Wang et al., 1992). In this study, we have examined the effects of the NO synthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), at a dose which raises blood pressure above the upper limit of autoregulation, upon local cerebral blood flow (lCBF) and glucose use (lCGU) in the neocortex of conscious rats, and have compared the effects with angiotensin-induced hypertension.

Conscious, lightly restrained rats were injected i.v. with 30mg/kg L-NAME in 1ml saline (n=8). In a second group of rats, hypertension was induced by infusing angiotensin-II i.v. (5μg/ml) at a rate of 0.5-2.5ml/min (n=16). These rats were subsequently divided into two groups according to the final sustained mean arterial blood pressure (MABP) (146-150mmHg and >150mmHg). Control animals were infused with saline at a similar rate (n=8). Equal numbers from each treatment group were used for the measurements of lCBF or lCGU in 6 neocortical areas using the [^{14}C]-iodoantipyrine and [^{14}C]-2-deoxyglucose autoradiographic techniques respectively (McCulloch et al., 1982). The measurement of lCBF was initiated after hypertension had persisted for approximately 20 min and lCGU was initiated after 10 min.

Angiotensin-induced hypertension (MABP 146-150mmHg) did not significantly alter lCBF in any of the areas examined. At higher pressures (157±1mmHg), the lCBF was focally increased in parietal and occipital cortex (from 168±8 to 690±54 and 163±7 to 593±93 ml/100g/min respectively: mean±s.e.mean). In contrast, L-NAME-treated animals (MABP 164±1mmHg) showed no focal areas of hyperaemia; indeed, lCBF was significantly decreased in every region except parietal cortex ($P < 0.05$; Scheffe's test). No significant differences in lCGU were observed between any groups, suggesting that the effects upon lCBF represented a direct vascular effect, and were not secondary to changes in metabolic demand. With the exception of MABP, there were no significant differences in physiological parameters (rectal temperature, arterial pCO_2, or pO_2) between experimental groups.

These results suggest that by influencing cerebrovascular tone, NO may play a role in determining the upper limit of autoregulation.

C.L.T. was a Wellcome Vacation Scholar.

Iadecola, C. (1992) *Proc. Natl. Acad. Sci. (USA) 89,* 3913-3916.
McCulloch, J., Kelly, P.A.T., & Ford, I. (1982) *J. Cereb. Blood Flow Metab. 2,* 487-499.
Wang, Q., Paulson, O.B. & Lassen, N.A. (1992) *Acta Physiol. Scand. 145,* 297-298

130P EFFECTS OF L-CANAVANINE ON *E. COLI* ENDOTOXIN-INDUCED SHOCK IN THE ANAESTHETISED RAT

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Endotoxin-induced shock is associated with a loss of vascular responsiveness to endogenous and exogenous vasoconstrictors. This has been attributed to induction of nitric oxide synthase with a consequent increased formation of nitric oxide (Fleming *et al.* 1991; Wright *et al.* 1992). The present work was carried out to examine the effects on endotoxin-induced shock of L-canavanine, an inhibitor of NO-synthase which may be selective for the inducible enzyme (Umans & Samsel 1992). Male rats (260-360g) were anaesthetised with pentobarbitone sodium (60 mg kg-1 i.p. followed by continuous infusion 27 mg kg^{-1} h^{-1} i.v) and ventilated with room air (48 strokes /min; 1.5ml 100 g^{-1}). Cannulae were placed in both jugular veins, the left femoral vein for infusions/bolus injections and the right common carotid artery for blood pressure recording and blood sampling. Body temperature was maintained at 37°C.

Mean blood pressure in control rats receiving an infusion of saline (0.44 ml kg^{-1} h^{-1}) was stable over at least 5h. Infusion of endotoxin (*E coli* O55:B5, 2.5 mg kg^{-1} h^{-1}) produced a progressive fall in blood pressure, which was reduced by L-canavanine treatment (10 mg kg^{-1} h^{-1}, beginning at 1h) (Mean blood pressure at 4h, mmHg; saline 120 ± 4, endotoxin 73 ± 5, saline + L-canavanine 127 ± 4, endotoxin + L-canavanine 103 ± 5; two way ANOVA - effect of endotoxin $P< 0.01$, L-canavanine $P<0.01$, interaction $P<0.05$). However, by 6h the fall in blood pressure in endotoxin-treated rats receiving L-canavanine was parallel to that seen in rats receiving endotoxin alone. Endotoxin-induced hypoglycaemia was slightly attenuated by L-canavanine (plasma glucose at 7h, mmol l^{-1}; control 6.1 ± 0.2;endotoxin 1.3 ± 0.3; endotoxin + L-canavanine 2.2 ± 0.2, $P<0.05$). All endotoxin-treated rats but none in the group receiving L-canavanine plus endotoxin had died by 9h. Pressor responses to noradrenaline (NA, 0.1 - 0.3 ug kg^{-1}), obtained each hour, were stable in control rats from 2h of starting the infusion. In endotoxin treated rats depression of these responses was detectable by 1h and maximal by 2h and this impairment was reduced by L-canavanine (e.g. response to 0.1ug kg^{-1} NA at 4h, mmHg; saline 29 ± 5, endotoxin 15 ± 2, L-canavanine 34 ± 3, endotoxin + L-canavanine 24 ± 3; two way ANOVA, interaction $P<0.01$). This effect of L-canavanine was transient, responses to NA being depressed again by 6h.

Thus L-canavanine delays, but does not prevent, the onset of shock in rats receiving a lethal dose of *E coli* endotoxin. Further experiments are required to determine if the dose of L-canavanine used in these experiments shows selectivity for the inducible form of nitric oxide synthase, since inhibition of the constitutive enzyme will have deleterious effects in endotoxin-treated rats (Wright *et al.* 1992).

Fleming, I., Julou-Schaeffer, G., Gray, G.A., Parratt, J.R. & Stoclet, J-C. (1991). Br. J. Pharmac. **103**, 1047-1052
Wright, C.E., Rees, D.D. & Moncada, S. (1992). Cardiovascular Res. **26**, 48-57.
Umans, J.G. & Samsel, R.W.(1992). Eur. J. Pharmac. **210** 343-346.

131P REGIONAL HAEMODYNAMIC CHANGES IN CONSCIOUS BRATTLEBORO RATS DURING AND AFTER TWO WEEKS' ORAL INGESTION OF N^G-NITRO-L-ARGININE METHYL ESTER (L-NAME)

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We have described the regional haemodynamic changes during and after 1 week's oral ingestion of N^G-monomethyl-L-arginine (L-NMMA) in Brattleboro rats (Gardiner *et al.*, 1992). In the present work we investigated the possibility that longer exposure to a lower dose of the more potent nitric oxide synthase inhibitor, L-NAME, might cause sustained and irreversible haemodynamic changes. Male, homozygous, Brattleboro (i.e. vasopressin-deficient) rats were chronically instrumented with renal, mesenteric and hindquarters pulsed Doppler probes and intravascular catheters (all surgery under sodium methohexitone anaesthesia, 40-60 mg kg^{-1}, i.p.), and recordings were made before, during and after a period of 14 days in which L-NAME (0.01 mg ml^{-1}) was added to the drinking water. Table 1 summarises the results.

Table 1: Cardiovascular changes during and after oral ingestion of L-NAME in conscious, Brattleboro rats (n = 7). Values are mean ± s. e. mean.

	On L-NAME			Off L-NAME	
	1 Day	7 Days	14 Days	1 Day	2 Days
Heart rate (beats min^{-1})	-2 ± 11	-36 ± 8*	-19 ± 13	-2 ± 14	11 ± 14
Mean blood pressure (mmHg)	24 ± 4*	22 ± 5*	33 ± 6*	14 ± 6	4 ± 4
Renal flow (%)	16 ± 8	8 ± 5	4 ± 9	0 ± 7	-3 ± 5
Mesenteric flow (%)	-6 ± 6	3 ± 8	-7 ± 7	3 ± 8	-4 ± 6
Hindquarters flow (%)	-14 ± 7	-15 ± 5*	-9 ± 3	1 ± 3	-4 ± 2
Renal conductance (%)	-5 ± 7	-10 ± 3	-19 ± 8	-10 ± 9	-5 ± 7
Mesenteric conductance (%)	-22 ± 6*	-14 ± 6*	-28 ± 6*	-8 ± 8	-6 ± 6
Hindquarters conductance (%)	-28 ± 7*	-28 ± 6*	-29 ± 5*	-9 ± 6	1 ± 5

* $P<0.05$ versus baseline (Friedman's test).

Oral ingestion of L-NAME was accompanied by sustained hypertension but, as with L-NMMA (Gardiner *et al.*, 1992) this was not associated with sustained reductions in regional blood flows, although there were persistent reductions in mesenteric and hindquarters vascular conductances. Within 24 h of withdrawal of L-NAME all variables were not different from baseline values. Hence, even with inhibition of NO synthase over a period of 2 weeks there are no signs of irreversible regional haemodynamic changes.

Gardiner, S. M., Kemp, P. A., Bennett, T., Palmer, R. M. J. & Moncada, S. (1992). Eur. J. Pharmacol., **213**, 449-451.

132P HYPERCHOLESTEROLAEMIA SEVERELY IMPAIRS EDRF-INDEPENDENT COLLATERAL PERFUSION AFTER ACUTE ARTERIAL OCCLUSION

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In a newly developed model of acute arterial occlusion in the isolated rabbit ear we have recently shown that the diversion of flow through pre-existing collateral vessels is strictly dependent on EDRF (Randall & Griffith, 1992). Using this model we have now investigated the effects of dietary-induced hypercholesterolaemia on collateral perfusion.

Rabbits were fed on either a normal diet or a diet supplemented with 1% cholesterol for 4 (n=8) or 8 (n=9) weeks. The rabbits were killed, the central ear artery cannulated and the ear perfused *in vitro* with oxygenated physiological salt solution containing 10μM indomethacin. Following arterial occlusion the level of collateral perfusion was assessed by X-ray microangiography as filling of a segment of the central ear artery, ligated at its midpoint and apex (Randall & Griffith, 1992).

In control preparations the level of filling 2 mins after occlusion was 19.5±4.9% of the preocclusion value and rose to a plateau of approximately 35% after 60 mins. In the hypercholesterolaemic rabbits (4 weeks) the initial level of filling was similar to that of the controls peaking at 27.2±11.5% after 15 mins, after which time the level of collateral perfusion fell to approximately 5% and remained at this level for the remainder of the experiment. In the rabbits receiving the diet for 8 weeks the level of segmental filling 2 mins after occlusion was significantly less than in the controls (7.8±3.2%, $P<0.05$) and did not change for the entirety of the experiment. The impairment of collateral perfusion after 8 weeks, but not 4 weeks, was associated with attenuated ($P<0.01$) endothelium-dependent relaxations of intact ears to acetylcholine in the hypercholesterolaemic group where the maximum relaxation of tone was 32.6±11.6% compared to 69.5±4.6% found in the controls.

The results indicate that collateral perfusion, which is EDRF-dependent, is severely impaired in hypercholesterolaemia.

Randall, M.D. & Griffith, T.M. (1992). *Am. J. Physiol*, **263:** H752-H760.

133P PERTUSSIS TOXIN DOES NOT INHIBIT ENDOTHELIUM-DEPENDENT RELAXATIONS OF RAT AORTA TO ACETYLCHOLINE

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Some authors have investigated whether endothelium-dependent relaxations (EDR) are attenuated by the bacterial toxin pertussis toxin. EDR of guinea-pig pulmonary arteries to histamine, but not the calcium ionophore A23187 (Weinheimer & Osswald, 1989), and EDR to ACh in dog femoral vein (Millar et al.,1991) are blocked by pertussis toxin. We have previously examined EDR to ACh in KCl contracted rat aortic rings, and found that pertussis toxin pretreatment failed to attenuate these relaxations (Docherty, 1990). However, since the rat aorta may release more than one relaxant factor, it is possible that effects of pertussis toxin on one agent are masked by the continuing effectiveness of the other relaxant factor. We have re-examined the effects of pertussis toxin on EDR of rat aorta to ACh by investigating the interaction with the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) (see Rees et al., 1990) to reduce or eliminate effects of nitric oxide.

Pertussis toxin pretreatment (20 μg/kg, i.v., 3-4 days) failed to affect the contraction to noradrenaline (NA) (1 μM), but significantly reduced the contraction to KCl (40 mM) (vehicle: 0.79±0.05g; pertussis toxin treated: 0.63±0.03g; $P<0.05$).

Relaxations to ACh in aortic rings contracted with NA were unaffected by pertussis toxin, but relaxations in tissues contracted by KCl were significantly increased by pertussis toxin pretreatment (maximum relaxation to ACh; vehicle: 21.4±3.1%; pertussis toxin treated: 40.1±6.5%; $P<0.05$). However, relaxations to ACh were also significantly increased by reducing KCl from 40 to 20 mM, thus reducing the size of the contraction. L-NMMA reduced relaxations to ACh in both vehicle and pertussis toxin treated tissues contracted with NA or KCl.

It is concluded that pertussis toxin pretreatment does not directly influence endothelium-dependent relaxations to ACh in rat aorta, but reduces contractions to KCl. Changes in the contractile response to KCl influenced the degree of relaxation to ACh.

Supported by the Irish Heart Foundation, the Health Research Board (Ireland) and RCSI.

Docherty, J.R. (1990). Br. J. Pharmacol. **100**, 348-352
Millar, V.M., Flavahan, N.A. & Vanhoutte, PM. (1991). J. Pharmacol. exp. Ther. **257**, 290-293.
Rees, D.D., Palmer, R.M.J., Schulz, R., Hodson, H.F. & Moncada, S. (1990). Br. J. Pharmacol. **101**, 746-752.
Weinheimer, G. & Osswald, H. (1989). Naunyn-Schmiedeberg's Arch. Pharmacol. 339,14-18.

134P INFLUENCE OF SPONTANEOUS RELEASE OF EDRF ON THE INHIBITION OF VASCULAR CONTRACTION BY CALCIUM ANTAGONISTS

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Frombach et al. (1990) have reported that the vascular relaxation evoked by nisoldipine is not endothelium-dependent, whereas using other arteries Vilaine et al. (1991) have reported that S 11568, another calcium antagonist, evokes endothelium-dependent relaxations. Since the spontaneous release of EDRF may be variable among vessels, differences in action could be related to the vessel studied. Therefore we compared rat and rabbit aortas in wich the spontaneous release of EDRF is different (Martin et al., 1986).
Rings of aortas were incubated in the presence or in the absence of N^{ω}-nitro-L-arginine (LNNA) 0.1 mM, in physiologic solution. Contractions were evoked by an isotonic 100 mM K^+-depolarizing solution and the preparations were incubated for 90 min in the absence (controls) or in the presence of various concentrations of isradipine or verapamil before eliciting a second K^+-contraction, still in the presence of the drug.
The K^+-contractions of rat but not of rabbit aorta were significantly more sustained in the presence of LNNA ($P < 0.001$). The inhibition by calcium antagonists observed in rat preparations was significantly higher in the absence than in the presence of LNNA, whereas in the rabbit preparations it did not differ significantly (Table 1).

Table 1. Residual contraction at 120 min (expressed as % of controls).

		Rat aorta			Rabbit aorta		
		without LNNA		with LNNA	without LNNA		with LNNA
Isradipine	30 pM	31.0 ± 10.5	P<0.05	61.3 ± 4.9	81.9 ± 0.6	n.s.	83.0 ± 2.1
	100 pM	20.9 ± 6.2	P<0.01	51.1 ± 3.6	53.1 ± 2.0	n.s.	56.7 ± 3.6
Verapamil	30 nM	52.9 ± 5.8	P<0.01	84.7 ± 6.4	-		-

These results show that in rat aorta, but not in rabbit aorta, the inhibition exerted by calcium antagonists on K^+-contraction was reduced when NO production was blocked by LNNA. This indicates that the potency of calcium antagonists could be different among vessels according to the level of spontaneous release of EDRF.

Frombach, R., Reil, G.H., Schütte, C., Jost, S., Gulba, P.R. & Lichtlen, P.R. (1990) in Nisoldipine eds. Lichtlen, P.R. & Krayenbühl, H.P. pp 51-60. Stuttgart: Schattauer.
Martin, W., Furchgott, R.F., Villani, G.M. & Jothianandan, D. (1986) J. Pharmacol. Exp. Ther. **237**, 539-547.
Vilaine, J.P., Biondi, M.L., Villeneuve, N., Feletou, M., Peglion J.L. & Vanhoutte, P. (1991) Eur. J. Pharmacol. **197**, 41-48.

135P CHRONIC INHIBITION OF NITRIC OXIDE SYNTHASE: EFFECT ON ENDOTHELIUM-DEPENDENT RELAXATION AND SENSITIVITY TO SODIUM NITROPRUSSIDE IN RAT PULMONARY ARTERIES

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Endothelial function is impaired in pulmonary arteries (PA)s from patients with pulmonary hypertension [PHT] (Rabinovitch *et al.*, 1986). Here we investigated endothelium-dependent and independent relaxations in the rat PA following chronic inhibition of nitric oxide (NO) synthesis, NO being an endothelial-derived vasodilator.

Rats were treated with 1mgml^{-1} L-NAME (inhibitor of NO synthase) in their drinking water for one week. They (and their controls) were anaesthetised with 120mgkg^{-1} sodium thiopentone for recording of blood pressure. In L-NAME treated rats, mean systemic blood pressure was 113±27 mmHg (n=6) and in controls it was 63±11mmHg. PA rings were set up in organ baths, under 2g tension in Krebs with 45mmHg O_2 and 40mmHg CO_2. Rings were preconstricted with phenylephrine (10^{-5}M) and carbachol was added cumulatively to produced endothelial-dependent relaxations. These were significantly less in the rings from L-NAME-treated rats [$P<0.01$; Student's unpaired t-test] (figure 1). Conversely, the NO donor sodium nitroprusside (SNP) induced significantly greater relaxation [$P<0.01$; Student's unpaired t-test] in the arteries removed from the L-NAME-treated rats (figure 1).

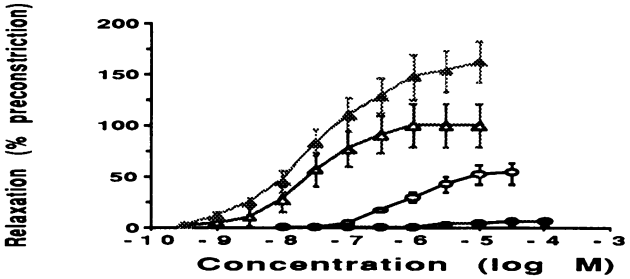


Figure 1. Effect of chronic L-NAME on endothelial-(carbachol) and non-endothelial- (SNP) dependent relaxations in rat PA rings. (●). L-NAME + carbachol; (○) carbachol controls; (▲) L-NAME + SNP; (△) SNP controls. (8 rings, 4 rats).

In man, tolerance develops to long term organic nitrate administration (Needleman & Johnson, 1973). Here we have demonstrated the opposite effect, that, in rats chronically treated with L-NAME, a reduction in NO synthesis is compensated for by a supersensitivity of the vascular smooth muscle to NO. A similar phenomenon may occur in patients with PHT where there is endothelial damage of the PAs.

This work was funded by The Wellcome Trust.

Needleman, P. & Johnson, E.M. (1973). *J. Pharmacol. Exp. Ther.*, **184**, 709-715.
Rabinovitch, M., Bothwell, T., Hayakawa, B.N. *et al.* (1986). *Lab. Invest.*, **55**, 623-653.

136P THE EFFECTS OF CAFFEINE ON IONIC CURRENTS IN RAT VENTRICULAR MYOCYCTES

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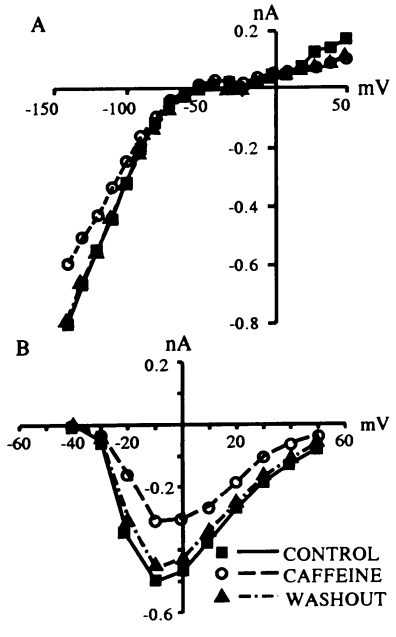


Fig.1. Steady-state (A) and peak inward (B) current-voltage relation in the absence and presence of 20 mM caffeine. Holding potential -40 mV, pulse duration 400 ms.

Caffeine is widely used as a pharmacological tool to study excitation-contraction coupling in muscle. It decreases the fast inward sodium current in cardiac muscle (Habuchi *et al.*, 1991). However little information is available about the effect of caffeine on other ionic currents in the heart. In this study we have used the whole cell configuration of the patch clamp technique to study the effects of high (20 mM) concentrations of caffeine on the action potential and ionic currents in rat ventricular myocytes.

Rapid application of caffeine released Ca^{2+} from the sarcoplasmic reticulum and induced a Ni^{2+}-sensitive transient inward current which was attributed to Na^+-Ca^{2+} exchange (Clusin, 1983). This current produced a transient depolarization of the resting potential from -72.6±0.4 to -68±0.6 mV (mean ± s.e.mean; n=4). Following these transient effects, maintained application of caffeine lengthened the action potential duration (at 90% repolarization) from 59.0±21.5 to 115.3±39.5 ms (n=3) and inhibited the inward rectifier potassium curent (Fig. 1A). The amplitude of the inward calcium current was also decreased (Fig. 1B) by caffeine and its inactivation was significantly slowed (τ_{inact} increased from 29.3±5.2 ms to 42.7±5.2 ms). All these effects were reversed upon washout.

These effects of caffeine should be recognised when it is used as a tool to study e-c coupling.

Supported by the Wellcome Trust

Clusin, W.T. (1983) Nature **301**, 248-250.
Habuchi, Y., Tanaka, H., Furukawa, T. & Tsujimura, Y. (1991) Am. J. Physiol. **261**, H1855-H1863.

137P THE VASOCONSTRICTOR EFFECT OF ENDOTHELIN AND 5-HT ON PLACENTAL VESSELS IS MEDIATED IN PART BY THROMBOXANE A_2

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Placental blood flow can be restricted in pre-eclampsia and may cause fetal distress. The activity of several vasoactive agents are known to rise in pre-eclampsia (Lindheimer & Katz 1989) and we have investigated their mechanism of action on placental vessels.

Placentae collected after normal deliveries were perfused under constant flow (5ml min^{-1}) and pressure changes were recorded after administration of graded doses of endothelin-1 (20fmols-200pmols), 5-HT (2pmols-200nmols) and the thromboxane-mimetic, 9, 11-dideoxy-11α, 9α epoxymethano-prostaglandin $F_{2\alpha}$ (U-46619; 200fmols-2nmols) in the presence and absence of GR32191B (a TX A_2 antagonist; Humphrey et al 1990) applied at 100nM-100μM. 25 ml perfusates were collected at 5 minute intervals before and after administration of an ED_{80} of the agonists given alone and TX A_2 was measured in the perfusates by ELISA assay (Amersham Ltd.,Bucks).

All concentrations of U46619 were totally blocked by all the doses of GR32191B, which supports the findings of Humphrey et al (1990) on other isolated vascular smooth muscle. The TX A_2 antagonist had a dose related inhibitory effect on the vasoconstrictor activity of endothelin-1, shifting the dose response curves significantly ($P<0.01$) to the right. A dose-response curve of 5-HT was also shifted to the right ($P<0.05$) by 1μM GR32191B. TX A_2 concentrations rose in the perfusates after administration of endothelin-1 at 15 minutes, confirming the results of Wilkes et al (1990), but not at 5 minutes as previously reported by Myatt et al (1991) (TX A_2 after endothelin-1 at 0 mins 68±13; 5 mins 71±15; 15 mins 126±10pg/l; $P<0.001$). Furthermore, we found that the levels of TX A_2 rose significantly 15 minutes after administration of 5-HT (TX A_2 at 0 mins 46±10; 5 mins 50±12; 15 mins 82±9 pg/l; $P<0.05$).

These results indicate that the vasoconstrictor effects of endothelin-1 and 5-HT are due, at least in part, to the stimulation of endogenous TX A_2 production.

Humphrey, P.P.A., Hallet, P., Hornby, E.J. et al (1990). Circ. 81 (Supp I): I-42-I-52.
Lindheimer, M.D. & Katz, A.I. (1989). Ann. Rev. Med. 40: 233-250.
Myatt, L., Langdon, G., Brewer, A.S. & Brockman, D.E. (1991). Am. J. Obstet. Gynecol. 165, 1717-1722.
Wilkes, B.M., Mento, P.F., Hollander, A.M. et al (1990). Am. J. Physiol. 258 (Endocrinol. Metab. 21): E864-E870.

138P HAEMODYNAMIC COMPARISON OF ICI D7288 AND ALINIDINE, PROPRANOLOL, VERAPAMIL AND ZATEBRADINE

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A novel heart rate modulating compound, ICI D7288, has recently been described (Marshall et al., 1992; Bosmith et al., 1992; Rouse & Johnson, 1992). Here we present haemodynamic data from experiments in anaesthetised dogs comparing ICI D7288 with compounds used to treat ischaemic heart disease, using methods as described in Rouse & Johnson, 1992. Measurements of variables were made at intrinsic and paced heart rates (180 beats/min, all animals), before and 10 min after cumulative doses of compounds shown in the title. Results (means ± SEM) at intrinsic heart rates for doses which were approximately equally effective at reducing heart rate were as follows:

DRUG & DOSE ($mg.kg^{-1}$ i.v.)		HR min^{-1}	dP_{LV}/dt $mmHg.s^{-1}$	StV ml	CO $l.min^{-1}$	A-V INTERVAL ms	TPR $dyne.s.cm^{-5}$
ICI D7288	Control	152±11	2572±178	13.0±1.7	1.90±0.13	105±6	4889±329
0.05 (n=6)	(Change)	(-33±5)**	(-2±116)	(2.5±0.4)**	(-0.09±0.04)	(-5±3)	(329±162)
Alinidine	Control	165±3	3099±183	10.1±1.2	1.67±0.17	98±3	5234±1041
0.2 (n=3)	(Change)	(-38±4)**	(-545±26)**	(0.0±0.4)	(-0.38±0.07)*	(-4±3)	(1200±179)*
Propranolol	Control	144±5	2233±163	13.9±1.0	1.99±0.16	107±6	4227±179
1.0 (n=3)	(Change)	(-21±4)*	(-296±69)*	(0.2±0.6)	(-0.25±0.03)**	(7±2)*	(317±115)
Verapamil	Control	143±6	2362±422	14.8±1.6	2.12±0.28	125±5	3965±423
1.0 (n=4)	(Change)	(-27±3)**	(-248±141)	(2.3±1.8)	(0.10±0.12)	(15±2)*	(-204±669)
Zatebradine	Control	149±11	3221±84	18.9±1.9	2.77±0.12	116±7	3340±304
0.1 (n=4)	(Change)	(-15±5)*	(95±53)	(1.5±0.4)*	(-0.06±0.07)	(0±2)	(86±148)

* $P<0.05$, ** $P<0.001$ by Student's paired t-test

Propranolol reduced myocardial contractile function and atrio-ventricular conduction velocity; stroke volume did not increase and cardiac output was not maintained. Verapamil prolonged A-V interval, preventing pacing at doses above 0.2 $mg.kg^{-1}$ i.v.. Neither propranolol nor verapamil reduced heart rate as much as alinidine, zatebradine or ICI D7288. Alinidine caused cardiac contractile depression, and increased peripheral resistance (TPR). ICI D7288 was comparable with zatebradine, selectively reducing heart rate without causing cardiac depression.

Bosmith, R.E., Briggs, I., Danks, P. & Sturgess, N.C. (1992) Br. J. Pharmacol., 107, 381P
Marshall, P.W., Bramley, J. & Briggs, I. (1992) Br. J. Pharmacol., 107, 134P.
Rouse, W. & Johnson, I.R. (1992) Br. J. Pharmacol., 107, 383P

139P PHARMACOLOGICAL ANALYSIS OF α_1 ADRENOCEPTOR BINDING SITES IN RAT LEFT VENTRICULAR TISSUE WITH SUBTYPE SELECTIVE ANTAGONISTS

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Although the existence of α_1-adrenoceptor subtypes can no longer be denied, the alignment in terms of classification of the ones identified by molecular biological techniques with their pharmacologically defined counterparts is poorly established. Since Northern blot analysis has clearly shown the coexpression of two α_1-adrenoceptor subtypes (designated α_{1A} and α_{1B}) in rat myocardium (Lomasney et al. 1991), we used rat left ventricular homogenates as a source of α_1-adrenoceptors to validate the reported α_{1A}-selectivity of the competitive antagonist 5-methyl-urapidil (Gross et al. 1988) and the α_{1B}-selectivity of the competitive antagonist spiperone (Michel et al. 1989). The effects of pretreatment of the homogenates with the alkylating agents chloroethylclonidine (CEC) and phenoxybenzamine (PBZ) on the displacement curves of the former antagonists were also investigated.

		n	n_H	pK_{iHigh} (-logM)	pK_{iLow} (-logM)	R_H(%)	R_L(%)
5-methyl-urapidil	control	6	0.85±0.06	8.31±0.27	6.65±0.06	17±3	83±3
	after CEC	12	0.59±0.02	8.80±0.15	6.95±0.16	44±4	56±4
	after PBZ	6	0.75±0.03	8.49±0.23	6.71±0.07	19±7	81±7
spiperone	control	6	0.91±0.05	8.08±0.11[a]	-	-	-
	after CEC	12	0.96±0.07	7.74±0.03[a]	-	-	-
	after PBZ	6	0.82±0.03	8.25±0.03[a]	-	-	-

[a] A two-site analysis did not result in a better fit than a one-site analysis as determined by a F-test ($p<0.05$).

Table: Displacement of specific [^{3}H]-prazosin binding (pK_d 9.57±0.07 -logM; B_{max} 193±17 fmol/mg protein) by 5-methyl-urapidil and spiperone in control preparations and after 10 min pretreatment (37°C) with the irreversible antagonists CEC (10 μM) and PBZ (0.1 nM) which resulted in the loss of 81% and 27% of the number of α_1-adrenoceptor binding sites with no significant change in the dissociation constant of [^{3}H]-prazosin.
These results confirm the presence of two α_1-adrenoceptor subtypes in rat left ventricular tissue, the proportional ratio of which can be altered by pretreatment with CEC but not with PBZ. In contrast to spiperone, 5-methyl-urapidil displays satisfactory subtype selectivity .

Gross, G., Hanft, H. & Rugevics, C. (1988). *Eur. J. Pharmacol.*, **151**, 333-335
Lomasney, J.W., Cotecchia, S., Lorenz, W., Leung, W-Y., Schwinn, D.A., Yang-Feng, T., Brownstein, M., Lefkowitz, R.J. & Caron, M.G. (1991)*J. Biol. Chem.*,**10**, 6365-6369
Michel, A.D., Loury, D.N. & Whiting, R.L. (1989). *Br. J. Pharmacol.*, **98**, 883-889

140P LOW DENSITY LIPOPROTEIN OXIDATION MAY MODIFY ENDOTHELIAL CELL FUNCTION

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Low density lipoprotein (LDL) may perturb cell signalling. Bovine aorta endothelial cells (BAEC) grown in the presence of LDL or oxidised LDL (OXLDL) show reduced uptake of [^{3}H] inositol (I) into the phosphatidylinositol cycle (Hamilton et al, 1992). We have examined the role of LDL oxidation in perturbing [^{3}H] I uptake into BAEC.
Cells, prepared by enzymatic digestion, were plated in 1 cm diameter multiwells at a density of 10^5 cells/well and grown to confluence (24hr). Growth medium was replaced by M199 for 42hr. [^{3}H] I was added for the final 18hr. LDL/OXLDL (20μg/ml) was present throughout or was added for the last 18hr only. Control cells, studied in parallel, had no LDL/OXLDL added. In some experiments N-acetylcysteine (NAC, 10μM), EDTA (1.4 mM), mannitol (M, 150mM), superoxide dismutase (SOD, 180 U/ml) or catalase (C, 200 U/ml) was added along with LDL/OXLDL. [^{3}H] phosphatidylinositol phosphates were extracted into chloroform/methanol and quantified by liquid scintillation counting.

NAC attenuated the effects of LDL but not OXLDL on [^{3}H] I uptake both in cells grown and in cells maintained in LDL/OXLDL. EDTA also modified the effects of LDL on [^{3}H] I uptake. Neither M nor SOD had any effect on [^{3}H] I uptake in the presence of LDL. However, C significantly improved [^{3}H] I uptake under these conditions (Table 1). None of these interventions modified [^{3}H] I uptake in control cells.

Table 1. [^{3}H] I uptake into phospholipids in the presence of LDL/OXLDL (% control).

OXLDL 66hr	OXLDL+NAC 66hr	OXLDL 18hr	OXLDL+NAC 18hr	LDL+M 18hr	LDL+SOD 18hr
36 ± 20	41 ± 25	25 ± 12	27 ± 7	47 ± 13	49 ± 16
LDL 66hr	**LDL+NAC 66hr**	**LDL 18hr**	**LDL+NAC 18hr**	**LDL+EDTA 18 hr**	**LDL+C 18hr**
54 ± 12	74 ± 14*	52 ± 13	87 ± 17*	73 ± 11*	83 ± 11*

n = 5-7 mean ± SD * significantly different to LDL alone (paired 't' test)

NAC and EDTA will inhibit LDL oxidation. Oxidation of LDL per se may damage cell membranes and perturb uptake of [^{3}H] I into the phosphatidylinositol cycle. The effects of C suggest a role for reactive oxygen species, in particular H_2O_2, in LDL oxidation and the related changes in intracellular processes.
Hamilton CA, McCulloch J, Godfrey EG et al. Br. J. Pharmac., 1992, 107, 176p.

141P A COMPARISON OF THE RESPONSIVENESS OF ISOLATED PULMONARY ARTERY AND TAIL ARTERY RINGS FROM HYPERTENSIVE RATS TO VASOCONSTRICTORS AND VASORELAXANTS

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There is conflicting evidence concerning the nature of the changes that occur in vascular smooth muscle from hypertensive animals. Some reports indicate that vascular muscle is supersensitive to vasoconstrictors but others suggest the opposite (Bohr & Webb, 1988). Such contradictory evidence may reflect differences in the models of hypertension or in the blood vessels used. This study examined the responsiveness of pulmonary artery and tail artery rings from spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) controls to vasoconstrictors and vasorelaxants.

Pulmonary artery and tail artery rings (3-4 mm) from SHR and WKY rats were suspended in organ baths containing Krebs buffer (37° C), gassed with a mixture of 95% O_2 and 5% CO_2. The initial tension on each ring was set at 1 g. Cumulative concentration response curves were obtained in tail artery rings to phenylephrine (PE) and in the presence of the EC_{75} for PE, to the vasorelaxant, sodium nitroprusside (SNP). Similar curves were obtained in isolated pulmonary artery rings to PE and to the vasorelaxant, carbachol (CARB). Blood pressure, measured by the tail cuff method in conscious rats, was elevated in SHR. Tail artery rings from SHR were more responsive to PE than those from WKY rats. The maximum contractile response to PE was 2.67 ± 0.42 g mg^{-1} (mean ± s.e. mean, n=8) in rings from SHR but only 1.48 ± 0.34 (n=8, 0.05>P>0.01, Student's t-test) in rings from WKY controls. Tail artery rings from SHR were less responsive to SNP than were those from WKY controls. The maximum inhibition of PE-induced tone produced by SNP (10^{-7} M) in rings from WKY controls was 97.3 ± 2.8%, n=8) but only 82.0 ± 4.1% (n=8, 0.01<P<0.001) in rings from SHR. In contrast, pulmonary artery rings were less responsive to PE and more responsive to CARB. The maximum contractile response to PE was 268.3 ± 31.2 (mg mg^{-1}, n=6) in rings from WKY rats but 136.2 ± 16.6 (n=6, 0.01>P>0.001) in rings from SHR. In contrast, the maximum relaxation to CARB was 60.8 ± 9.7% (n=8) in rings from WKY but 102.4 ± 2.4% (n=8, 0.01>P>0.001) in rings from SHR.

These results were consistent with previous observations (Sharifi et al, 1990; Dowell et al, 1991). The enhanced responsiveness of tail artery rings from SHR to PE and the reduced responsiveness to SNP may be linked and could contribute to the hypertension. The reduced responsiveness of pulmonary artery rings to PE and enhanced responsiveness to CARB may also be linked but are more likely to reflect adaptations to rather than an explanation of hypertension.

Bohr, D.F. & Webb, R.C. (1988) Ann. Rev. Pharmacol. Toxicol. **28**, 389-409.
Dowell, F.J., Aidulis, D.M., Sharifi, A.M. & Pollock, D. (1991) J. Physiol. **429**, 76P.
Sharifi, A.M., Morrison, K.J. & Pollock, D. (1990) Br. J. Pharmacol. **101**, 533P.

142P EFFECTS OF OPIOID AGONISTS IN ISOLATED RAT AORTA

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Administration of opioid agonists results in peripheral vasodilatation (Lang et al., 1982). However, the direct effects of opioid agonists and antagonists on vascular smooth muscle are poorly understood. Therefore, the present study was undertaken to examine the effects of selective opioid agonists and antagonists in rat vascular smooth muscle.

Isolated aortic helical strips (12-15 mm in length and 2-3 mm width) from male Wistar rats were incubated for isometric tension recording in Krebs solution (37ºC) under 2 g resting tension as previously described (Pérez-Vizcaino et al., 1991).

Morphine, as well as the selective δ-agonist DPDPE and selective κ-agonist bremazocine up to 10^{-5}M had no effect on resting tension. However, the selective μ-agonist DAMGO, 10^{-7}M - 10^{-6}M, induced a concentration-dependent increase in resting tension (121 ± 7 mg at 10^{-6}M which represents 14 ± 2 % of maximal noradrenaline-induced tension). In strips running in parallel pretreated with the selective μ antagonist β-funaltrexamine (10^{-6}M), this response was partially antagonized (33 ± 7 mg at 10^{-6}M DAMGO. $P < 0.01$). Noradrenaline (NA, 10^{-9}M) induced a transient contractile response (444 ± 42 mg. n = 26) which was not modified in aortic strips pretreated with 10^{-6}M morphine or 10^{-6}M bremazocine. Pretreatment with 10^{-6}M DPDPE significantly increased this contractile response (744 ± 75 mg. $P < 0.01$), this effect being completely antagonized by naltrindole (454 ± 82 mg). Morphine and DAMGO (10^{-7}M - 10^{-6}M), added when relaxation of NA-induced contractions reached about 50% of peak contraction, produced a concentration-dependent increase in tension which reached the maximum contractile response induced by 10^{-9}M NA. This increase in tension was partially inhibited by pretreatment with naloxone and β-funaltrexamine, respectively. Addition of DAMGO (10^{-7}M - 10^{-6}M) when 10^{-9}M NA-induced contractions reached a maximum, produced a concentration-dependent increase in developed tension (624 ± 52 mg at 10^{-6}M. $P < 0.01$) and this effect was blocked by β-funaltrexamine. This increase in tension was not observed with the other agonists tested.

In conclusion, μ and δ opioid agonists induced a contractile effect and modulated NA-induced tension in isolated rat vascular smooth muscle, this effect being inhibited by selective opioid antagonists.

Lang, R.E., Brückner, U.B., Kempf, et al. (1982) Clin. Exper. Hyper.-Theory Practice **A4** (1-2), 249-269.
Pérez-Vizcaíno, F., Duarte, J. & Tamargo, J. (1991) Br. J. Pharmacol. **104**, 726-730.

143P CONTRACTION OF RABBIT ISOLATED MESENTERIC ARTERY BY des-Arg^9-BRADYKININ IS MEDIATED THROUGH STIMULATION OF BRADYKININ B_1 RECEPTORS

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Des-Arg^9-bradykinin relaxes the rabbit mesenteric artery through activation of bradykinin 1 (B_1) receptors and is dependent upon the generation of endogenous prostaglandins (Churchill and Ward, 1986). We now report a contractile component to des-Arg^9-bradykinin in this preparation following the addition of indomethacin, a prostaglandin synthesis inhibitor.

The superior mesenteric artery was removed from male, New Zealand White rabbits (Froxfield) weighing 2-3kg. The endothelium was removed and ring segments, 3-4mm wide, were subsequently obtained. The preparations were suspended under a resting tension of 0.5g in Krebs' solution at 37°C, containing indomethacin (3μM) and gassed with 95% O_2/5% CO_2. Cumulative concentration-response curves to des-Arg^9-bradykinin (3nM-1μM) or bradykinin (BK, 30nM-10μM) were constructed until they were reproducible. Unlike the rabbit aorta, which requires an incubation period of approximately 2h (Regoli et al., 1981), the contractile responses to des-Arg^9-BK were immediately apparent in the rabbit mesenteric artery. Results showed that BK was approximately 14-times weaker than des-Arg^9-BK: the geometric mean concentrations (95% confidence limits) of des-Arg^9-BK or BK , required to produce 50% of each maximum response (EC_{50}), were 62 (27-144)nM and 894 (436-1834)nM, respectively (n=4).

Studies with enzyme inhibitors were performed against, des-Arg^9-BK and BK. MERGETPA (D-L-mercaptomethyl-3-guanidino-ethylpropanoic acid; 10μM, n=4), a kininase I inhibitor, captopril (1μM, n=4), a kininase II inhibitor and phosphoramidon (1μM, n=4), a neutral endopeptidase inhibitor, all had no effect on the activity of des-Arg^9-BK. However, BK-induced contraction was inhibited by MERGETPA (10μM, n=4). In the absence of these enzyme inhibitors, the specific B_1 receptor antagonist, des-Arg^9-Leu^8-BK (3, 10 and 30μM) caused parallel, concentration-related, rightward displacement of des-Arg^9-BK concentration-response curves, while D-Arg-Hyp^3-Thi^5-DTic^7-Oic^8-BK (HOE 140, 30nM, n=4), a B_2 receptor antagonist, had no effect. After a 30min incubation period with des-Arg^9-Leu^8-BK, the mean pA_2 value was 6.68 ± 0.26 (slope =1.03 ± 0.15, n=4). This value correlates extremely well with the values of 6.7 and 6.46 for antagonism of the contraction in the aorta and the relaxations in the mesenteric artery, respectively (Regoli et al., 1977; Churchill and Ward, 1986).

In conclusion, the B_1 receptor-mediated contractile response to des-Arg^9-BK, unlike the relaxant response, is not due to prostaglandin release. The rabbit mesenteric artery therefore represents a robust B_1 receptor-containing preparation, devoid of kininase I, kininase II and neutral endopeptidase enzyme activities when des-Arg^9-BK is used, with the added advantage compared to the rabbit aorta of being immediately responsive to agonists. This preparation will be useful in the investigation of B_1 receptor pharmacology.

Churchill, L. & Ward, P.E. (1986) Eur. J. Pharmacol. 130, 11-18.
Regoli, D., Barabé, J., and Park, W.K. (1977) Can. J. Physiol. Pharmacol. 55, 855.
Regoli, D., Marceau, F., and Lavigne, J. (1981) Eur. J. Pharmacol. 71, 105-115.

144P BRADYKININ-INDUCED CONTRACTIONS OF HUMAN UMBILICAL ARTERY

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Bradykinin (BK) is a potent constrictor of human umbilical artery (HUA) smooth muscle (Altura 1972). Differences in agonist and antagonist selectivity have led to the proposal that three subtypes (BK_1, BK_2, and BK_3) of BK receptor may exist in animal tissues (Farmer *et al.*, 1991). Here we have investigated possible receptor mechanisms for BK contractions of HUA.

HUA rings, 2-3 mm wide, were rubbed gently to remove endothelium and suspended for isometric recording under 2g tension between L-shaped hooks in organ baths containing Krebs buffer gassed with 95% O_2, 5% CO_2. Cumulative addition of BK (1-300nM) gave dose-dependent contractions. Responses were variable and poorly maintained, leading to desensitization at higher BK doses. Dose response curves to BK were not significantly altered by the presence of the BK_1-selective antagonist [des-Arg^9, Leu^8]-BK (1μM), or with either of the BK_2 antagonists [Thi^{5,8}, D-Phe^7]-BK and D-Arg [Hyp^3, D-Phe^7]-BK (1μM). However, 100nM HOE 140 (D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK, an antagonist of BK_2 and BK_3 responses (Field *et al.*, 1992), produced competitive antagonism with an apparent pA_2 of 7.57 ± 0.01 (mean ± s.e., n = 8). The BK_1-agonist [des-Arg^9]-BK (10nM - 10μM) was much less potent than BK, giving contractions at 10μM which were 60 ± 18% (n = 9) of those at 300nM BK. Responses to BK were unaffected by either of the cyclooxygenase inhibitors aspirin (100μM) and indomethacin (10μM), or with the 5-lipoxygenase inhibitor nordihydroguaiaretic acid (10μM). However, BK contractions were antagonized in a non-competitive slowly reversible manner by mepacrine (100μM).

These results indicate that BK-induced contractions of HUA are not clearly attributable to either BK_1 or BK_2 receptor activation, suggesting the possible involvement of another (BK_3?) receptor subtype. These responses are unlikely to be mediated by cyclooxygenase/lipoxygenase metabolites of arachidonic acid, suggesting that the inhibitory effects of mepacrine may be unrelated to its ability to inhibit arachidonic acid formation via phospholipase A_2.

Supported by Tenovus (Tayside) Scotland.

Altura, B.M. (1972) Eur. J. Pharmacol., *19*, 171-179.
Farmer, S.G., Burch, R.M., Kyle, D.J., Martin, J.A., Meeker, S.N.&Togo, J. (1991) Br. J. Pharmacol., *102*, 785-787.
Field, J.L., Hall, J.M. & Morton, K.M. (1992) Br. J. Pharmacol., *105*, 293-296.

145P BRADYKININ-INDUCED ACUTE ALGESIA IN THE GUINEA-PIG

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The nonapeptide bradykinin (BK) is one of the most potent algesic substances known, causing pain when injected into man (Whalley et al., 1987). In animal studies it has also been shown to activate and sensitise nociceptive afferent C-fibres (Dray et al., 1988). Two receptors, B_1 and B_2, have been classified which could mediate acute algesia (Regoli & Barbabé 1980). The objective of the present study was to characterise the receptors and mechanisms involved in acute algesia induced by bradykinin in the guinea-pig.

Female albino Dunkin Hartley guinea-pigs (180-200g) were injected with BK (100μl intraplantar) into the left hind paw. Increased activity (licking, biting, jumping) indicative of algesia was measured over 60 seconds using a Doppler-microwave activity monitor. Groups of 6 animals were treated with single doses of bradykinin and 5 groups used to construct a response curve. Single doses of bradykinin antagonists or vehicle and BK were co-administered 30 minutes later into the right hind paw and algesic activity in the groups of animals was measured. Comparisons were made between response curves obtained in left and right paws.

Intraplantar injection of BK (0.003-3nmol) in guinea-pigs caused significant and dose-related algesic responses: ED_{50} 0.013nmol (0.006-0.025) n=16. Time course studies demonstrated that the response peaked at 60s and had finished by 120s. Response curves to BK alone (0.03-3nmol) obtained in left and right paws were not significantly different.

The B_1-receptor agonist, des Arg^9-BK (3-100nmol), produced only a weak response, being approximately 500 times less potent than BK. A B_1-receptor antagonist, des Arg^9, Leu^8-BK (100nmol), failed to antagonise the BK-induced algesia.

The B_2-receptor antagonists, HOE 140: DArg-Hyp^3-Thi^5-$DTic^7$-Oic^8-BK (50nmol) and DArg-Hyp^3-$DPhe^7$-BK (100nmol) caused 85 and 20 fold rightward shifts respectively, of the BK dose-response curve.

Aspirin (200mgkg^{-1}; i.p. PTT^+ 30min) caused a partial inhibition of BK-induced algesia equivalent to an approximately 30 fold shift to the right of the BK-response curve. This effect was reversed by co-administration of prostaglandin E_2 (10nmol). The residual aspirin-resistant component of BK-induced algesia was further inhibited by HOE 140 (50nmol) when co-administered with the BK.

These data suggest that BK-induced algesia in the guinea-pig paw is mediated through stimulation of B_2-receptors and that prostaglandins play a significant role in this response.

$^+$ PTT - pretreatment time.

Whalley, E.T., Clegg, S., Stewart, J.M. & Vavrek, R.J. (1987) Naunyn Schmiedebergs Arch. Pharmacol., 336; 652-655.
Dray, A., Bettaney, J., Forster, P. & Perkins, M.N. (1988). Neuroscience Letters, 91; 301-307.
Regoli, D. & Barabé, J. (1980). Pharmacol. Rev., 32; 1-46.

146P THE EFFECT OF ENDOTHELIN-3 ON THE RECOVERY OF WOUNDED ENDOTHELIAL CELL MONOLAYERS *IN VITRO*

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The aim of this study was to determine whether or not endothelin-3, a member of a family of potent vasoactive peptides, influenced the repair of a wounded monolayer of human umbilical vein endothelial cells (HUVEC) *in vitro*.

HUVEC were grown to confluence on 13 mm diameter Thermanox coverslips (Miles Scientific) in 24 well plates. A series of parallel wounds was made on each coverslip using a Perspex wounding comb (Fan *et al.*, 1992) and then the coverslips were replaced in complete medium containing either peptide or vehicle. At the appropriate time, coverslips were either trypsinised and the cell number determined by trypan blue exclusion, or fixed *in situ* with 100% ethanol. The redistribution of endothelial cells into the denuded area was quantified using a VIDS III image analysis system (AMS, Cambridge) and the percentage recovery after wounding given by:

$$\% \text{ Recovery} = 100 \times [1 - (\text{Final Wound Area})/(\text{Initial Wound Area})]$$

Statistical significance was determined by Student's t-test or analysis of variance as appropriate. Statistical significance was defined as $P < 0.05$.

Endothelin-3 caused a dose dependent increase in percentage recovery between 10 nM (11.4±2.5%) and 100 nM (23.6±0.8%) relative to untreated controls at 18 h post-wounding ($n = 5$). Endothelin-3 also induced HUVEC proliferation over this time course with 10 nM, and 100 nM causing 26.7±15.2% and 34.6±5% increases in cell number respectively, compared to untreated controls (n = 4). Interestingly, endothelin-1 the parent isopeptide, had no effect in this model ($n = 4$), whilst its analogue [$Ala^{1,3,11,15}$]ET-1 was equipotent with endothelin-3 at stimulating recovery of cell denuded regions ($n = 3$). Actinomycin D (0.1 µg/ml), a selective inhibitor of mitosis, was able to abolish both the increase in cell number and the increase in rate of recovery of wounded coverslips treated with endothelin-3. Actinomycin D treated samples were indistinguishable from untreated controls ($n = 3$).

In conclusion, endothelin-3 is able to promote wound closure by inducing proliferation of HUVEC rather than by promoting migration into cell denuded regions. This effect was not mimicked by endothelin-1 implying that neither the ET_A receptor, which is primarily endothelin-1 sensitive, nor the ET_B receptor, which is equally sensitive to endothelin-1 and endothelin-3, are responsible for this effect. Instead, an endothelin-3 sensitive receptor which mediates proliferation is indicated on HUVEC, an observation which is in agreement with Yokokawa *et al.* (1991).

ADW is an MRC Research Student. We thank the Hadwen Humane Research Trust for financial support and the Marion Merrell Dow Research Institute, Strasbourg, for kind gifts of peptides.

Fan T.-P.D., Frost E.E. & Wren A.D. (1992) in *Angiogenesis 1992* ed. Steiner, R., Weisz, P.B. & Langer, R. pp 315-320. Basel: Birkhäuser.
Yokokawa K., Kohno M., Yanunari K., Murakawa K-I. & Takeda T. (1991) *Hypertension* 18, 304-315.

147P A_2 ADENOSINE RECEPTOR RELAXATION OF GUINEA-PIG AORTA

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Adenosine receptors may be divided into two major groups, A_1 and A_2, on the basis of agonist and antagonist rank order of potency. In the brain, further subdivision of the A_2 class into A_{2a} and A_{2b} receptors has been possible due to the use of a 8-benzenesulphonamide-substituted 1,3-dipropylxanthine antagonist (PD 115,199), which exhibits higher potency at the A_{2a} receptor of rat neostriatum (Jacobson *et al.*, 1992). However, A_2 adenosine receptors in the periphery have generally not been scrutinised to determine to which subclass of A_2 receptor they belong. We have therefore compared agonist potency and antagonist affinity at the A_{2b} adenosine receptor of guinea-pig cerebral cortex with the A_2 receptor mediating the relaxation of pre-contracted guinea-pig aorta (Collis *et al.*, 1987).

Guinea-pig aortal cylinders were pre-contracted with a submaximally-active concentration of phenylephrine (1 μM) and cumulative concentration-relaxation curves to 5'-N-ethylcarboxamidoadenosine (NECA), 2-chloroadenosine (2CA) or adenosine were constructed. Accumulation of [^{3}H]cyclic AMP in [^{3}H]-adenine-pre-labelled guinea-pig cerebral cortical slices was assessed as previously described (Alexander *et al.*, 1989).

NECA, 2CA and adenosine relaxed phenylephrine-pre-contracted aortal cylinders with IC_{50} values of 0.68 ± 0.06 μM (n=40), 4.3 ± 0.6 μM (n=4) and 104 ± 13 μM (n=4), with maximal relaxations of 71 ± 3 % (n=11), 98 ± 1 % (n=4) and 100 ± 1 % (n=4), respectively. In the cerebral cortex, NECA, 2CA and adenosine stimulated the accumulation of [^{3}H]cyclic AMP with EC_{50} values of 3.1 ± 0.3 μM (n=10), 11.0 ± 1.5 μM (n=8) and 87 ± 11 μM (n=4), respectively. Maximal responses of 2CA and adenosine compared to 100 μM NECA were 91 ± 2 (n=3) and 64 ± 9 % (n=4), respectively. Construction of NECA concentration-response curves in guinea-pig aorta and cerebral cortex in the absence and presence of a fixed concentration of antagonist allowed estimation of antagonist apparent dissociation constants. In the aorta, K_i's for xanthine amine congener (XAC), PD 115,199, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and 3,7-dimethyl-1-propargylxanthine (DMPX) were 17 ± 4 nM (n=4), 341 ± 64 nM (n=6), 369 ± 108 nM (n=7) and 5.5 ± 0.8 μM, respectively. Antagonist affinities at the A_{2b} adenosine receptor of cerebral cortex were 35 ± 3 nM (n=4), 407 ± 82 nM (n=5), 123 ± 21 nM (n=14) and 12.8 ± 1.8 μM (n=3), respectively.

The similar rank order of potency for agonists and antagonists at the A_2 adenosine receptor mediating relaxation of guinea-pig aorta compared to the A_{2b} adenosine receptors of cerebral cortical slices suggests that the two receptors may be equivalent.

The financial support of the Wellcome Trust is gratefully acknowledged.

Alexander, SPH, Kendall, DA & Hill, SJ (1989) *Br.J.Pharmacol.*, **98**, 1241-1247.
Collis, MG, Jacobson, KA & Tomkins, DM (1987) *Br.J.Pharmacol.*, **92**, 69-75.
Jacobson, KA, van Galen, PJM & Williams, M (1992) *J.Med.Chem.*, **35**, 407-422.

148P DIFFERENTIAL EFFECTS OF REPEATED ADMINISTRATION OF TWO SELECTIVE ADENOSINE A_1 AND A_2 AGONISTS ON BLOOD PRESSURE AND HEART RATE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHRs)

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Adenosine actions on the cardiovascular system are mediated through interaction with A1 receptors, which results in negative inotropic, chronotropic and dromotropic effects, and A2 receptors, which results in vasodilation in most vascular beds (Olsson and Pearson, 1990).
Stable adenosine analogues, which selectively stimulate either A1 or A2 receptors, have been synthesized (Olsson and Pearson, 1990). Their acute hemodynamic effects have been characterized in different studies, whereas very few data are available on effects induced by the chronic drug treatment (Webb et al., 1990; Webb et al., 1992). The present study was therefore designed to assess the effect of repeated administration of the A1 agonist CCPA (2-chloro-N6-cyclopentyladenosine) and the A2 agonist 2-hexynyl-NECA (2-hexynyl-5'-N-ethylcarboxamide adenosine) on hypertension in SHRs (Cristalli et al., 1992; Lhosen et al., 1988).
Adult SHRs were treated with CCPA (0.1 mg/kg), 2-hexynyl-NECA (0.01 mg/kg) or vehicle, intraperitoneally, twice daily over a 3-week period. Systolic blood pressure (SBP) and heart rate (HR) were measured by the tail-cuff method, at baseline (day 0) and one hour after the daily morning dose at day 1,3,7,14,21.
Baseline SBP values ranged between 197 ± 3 and 203 ± 1 mmHg. In both treated groups there was a similar decrease in SBP following single dosing (114 ± 11 and 109 ± 4 mmHg for CCPA and 2-hexynyl-NECA, respectively). This effect significantly declined over time in the CCPA group ($p<0.01$ vs control), and SBP returned to baseline value at day 21 (201 ± 2 mmHg). Conversely, 2-hexynyl-NECA maintained a significant antihypertensive effect throughout the experimental period (137 ± 12 at day 21).
Treatment with 2-hexynyl-NECA did not influence HR, whereas CCPA induced marked bradycardia (from 406 ± 7 to 210 ± 24 beats/min), which was gradually subject to tolerance, reaching, at the end of the dosing schedule, 350 ± 23 beats/min.
These results indicate that in our experimental conditions, tolerance develops to the hypotension and bradycardia produced by the adenosine A1 agonist, whereas the blood pressure lowering is maintained with the A2 agonist 2-hexynyl-NECA.

Cristalli,G., Eleuteri,A., Vittori,S. et al. (1992) J.Med.Chem. 35, 2363-2368
Lhosen,J., Klotz,K.M., Schwabe,U. et al. (1988) Naunyn-Schmiedeberg's Arch. Pharmacol. 337, 687-689
Olsson,R.A. & Pearson,J.D. (1990) Physiol. Rev. 70, 761-845
Webb,R.L., McNeal,R.B., Barclay,B.W. et al. (1990) J.Pharmacol.Exp.Ther. 254, 1090-1099.
Webb,R.L., Singer,H., Sills,M.A. et al. (1992) FASEB J. 6, A1008, 420P

149P COMPARISON OF THE PREJUNCTIONAL ADENOSINE RECEPTORS IN THE RAT ANOCOCCYGEUS AND GUINEA-PIG ILEUM

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Stone (1982) demonstrated two actions of adenosine analogues on rat isolated anococcygeus (ANO): inhibition of the contractile response to field stimulation, and contraction in preparations in which tone was already established. In this study, we have compared the pharmacological profiles of several adenosine receptor agonists in the ANO with those obtained from the guinea-pig ileum longitudinal muscle-myenteric plexus (GPI), a preparation which contains prejunctional adenosine receptors of the A_1-subtype (Paton, 1981). Preparations were field stimulated (1ms pulses, supramaximal voltage, 0.2Hz (GPI) or 10Hz for 1s every 30s (ANO)) in oxygenated Krebs' solution at 37°C. Agonist potency was assessed in GPI as described by Kennedy et al. (1992). One cumulative concentration-response curve (CRC) was obtained from each ANO, and the concentration required to reduce the response height by 50% (IC_{50}) was determined for each agonist. The following agonists were used: 5'-N-ethylcarboxamido-adenosine (NECA), the stereoisomers of N^6-phenyl isopropyladenosine (R- and S-PIA), N^6-cyclopentyladenosine (CPA) and 2[p-(2 carboxyethyl) phenylethylamino]-5-N-ethylcarboxamidoadenosine (CGS21680). The effect of the selective A_1-adenosine receptor antagonist dipropylcylopentylxanthine (DPCPX) on the inhibitory responses to NECA was assessed in paired ANO preparations, one tissue serving as control for the other which received 15 min pretreatment with antagonist. Unlike ANO, CRCs to NECA in GPI were reproducible, so the IC_{50} values obtained before and after treatment with DPCPX (15min) were compared in each tissue.
Field stimulation of ANO evoked reproducible contractile responses which were abolished by tetrodotoxin (1μM) or prazosin (10nM). The absolute potency of NECA differed on ANO and GPI, although the rank order of agonist potency was similar (Table 1). DPCPX antagonised responses to NECA to differing extents in the two preparations (pA_2 (95% confidence limits) in ANO = 9.5 (9.2-10.1) from a Schild plot with slope = 0.92 (0.71-1.13); pA_2 in GPI = 8.6 (8.4-9.0), slope = 1.10 (0.82-1.37)). The inhibitory effects of the α_2-adrenoceptor agonist xylazine were unaffected by DPCPX in both preparations. In experiments in which the ANO was not field stimulated, but was partially contracted by phenylephrine, NECA and R-PIA (10nM-10μM) did not alter basal tone.

Table 1 Comparison of the potencies of adenosine receptor agonists in ANO and GPI

	IC_{50}	Equipotent concentration ratio (NECA=1)			
	NECA	R-PIA	CPA	S-PIA	CGS21680
ANO	0.37μM	0.16 (0.04-1.24)	0.24 (0.04-2.43)	39 (19-73)*	≥196
GPI	21nM	0.95 (0.57-1.62)	0.31 (0.17-0.57)	34 (25-45)	1095 (667-1667)

Values shown as geometric mean plus 95% confidence limits. * Calculated at IC_{20} because S-PIA has intrinsic activity = 0.52 (NECA=1).

These data suggest that the prejunctional adenosine receptors on ANO are similar to those on GPI. The differing potencies of DPCPX may reflect species variation in sensitivity to this antagonist.

Kennedy, I., Gurden, M. and Strong, P. (1992). Gen. Pharmac., **23**, 303-307.
Paton, D.M. (1981). J. Auton. Pharmacol., **1**, 287-290.
Stone, T.W. (1982). J. Physiol., **335**, 591-608.

150P ACTIVATION OF THE SHEEP CARDIAC SARCOPLASMIC RETICULUM Ca^{2+}-RELEASE CHANNEL BY ANALOGUES OF SULMAZOLE

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The effects of sulmazole, a sulphur-containing benzimidazole, and several structurally-related analogues, on single Ca^{2+}-release channel conductance and gating were investigated under voltage clamp conditions (Williams & Holmberg, 1990). At an activating [Ca^{2+}] of 10μM (+)-sulmazole increased mean single-channel open probability (Po) from 0.04 to 0.35, 0.53, 0.86 and 0.99 on addition of 0.25, 0.5, 1 and 3mM of the drug to the cytosolic face of the channel, giving an approximate EC_{50} of 423 ± 35μM (±s.e.mean, n=4). The optical isomer (-)-sulmazole was as potent as the (+)-isomer with an approximate EC_{50} of 465 ± 60μM (n=4). Similarly, (+)-isomazole, an analogue of sulmazole but with a pyridine nitrogen in the 4,5c position and not the 4,5b position (as in (+) and (-)-sulmazole) was as potent as (+) and (-)-sulmazole with an EC_{50} of approximately 445 ± 49μM (n=3). The effect of (-)-isomazole has not been fully determined, but 0.25 and 0.5mM increased Po from 0.05 to 0.40 and 0.54, respectively, indicating its potency to be similar to that of the (+)-isomer. EMD 41000, an isomazole analogue with the methylsulphinyl oxygen removed, increased Po in the range 1-100μM, with an EC_{50} of 40 ± 4μM (n=5). Similarly EMD 46512, a sulmazole analogue with its methylsulphinyl oxygen removed increased Po over the same range of concentrations, with an approximate EC_{50} of 42 ± 7μM (n=3). The effects of these drugs on Ca^{2+}-release channel Po were additive with those of adenosine, suggesting discreet sites of action on the channel protein for sulmazole analogues and adenosine. Given the similarity of these drugs to caffeine, these analogues may be acting at the caffeine site on the Ca^{2+}-release channel which is distinct from the adenosine activation site (McGarry & Williams, 1991). All of the sulmazole analogues increased Po by increasing the frequency and duration of open events. The activation was synergistic with Ca^{2+}, as these drugs increased Po to a lesser degree at sub-activating concentrations of Ca^{2+}, in agreement with the earlier findings of Williams & Holmberg (1990). These compounds did not affect the slope conductance of the channel (90pS) or the $Ca^{2+}/Tris^+$ permeability ratio (12.8). These results show that analogues of sulmazole activate the sheep cardiac Ca^{2+}-release channel and that there is no stereoselectivity at the sulmazole site. They also demonstrate that neither the configuration of the methyl and oxygen groups around the sulphur atom of these compounds nor the position of the pyridine nitrogen (at the 4,5b or 4,5c position) affects the ability of these drugs to activate the cardiac Ca^{2+}-release channel. However, removal of the oxygen group attached to the sulphur atom increases the potency of the compound by approximately ten fold.

We thank E. Merck Pharmaceuticals and Boehringer Ingelheim for the supply of these drugs. Supported by the BHF.

McGarry, S.J. & Williams, A.J. (1991) J.Physiol. **446**, 597P.
Williams, A.J. & Holmberg, S.R.M. (1990) J.Membrane Biol. **115**, 167-178.

151P EFFECTS OF QUERCETIN ON CONTRACTILE RESPONSES IN ISOLATED RAT VASCULAR SMOOTH MUSCLE

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Quercetin (3,3',4',5,7 pentahydroxy-flavone) is a flavonoid that inhibits the contractions induced by phenylephrine in rat aorta (Beretz et al., 1980) as well as the contractions induced by agonists or anaphylaxis in guinea-pig ileum (Fanning et al., 1983). In addition, quercetin inhibits protein kinase C (Gschwendt et al., 1983) and cyclic AMP phosphodiesterase activity (Beretz et al., 1980). However, the mechanism of its vasodilatory effects is unclear. Therefore, the present study was undertaken to examine the inhibitory effects of quercetin in rat aorta and portal vein segments.

Experiments were performed in rat aortic strips and portal vein segments incubated in Krebs-Henseleit solution (KS) at 37°C. Contractile force was isometrically recorded as previously described (Pérez-Vizcaino et al., 1991).

Quercetin (10^{-6} M-10^{-4} M) inhibited the amplitude of the spontaneous myogenic contractions recorded in portal veins (IC_{50} = 9.1 ± 2.3 x 10^{-6} M). In rat aorta, quercetin (10^{-6} M-10^{-4} M) inhibited the contractions induced by 80 mM K (IC_{50} = 4.0 ± 0.4 x 10^{-5} M) and 10^{-5} M NA (IC_{50} = 8.6 ± 1.5 x 10^{-6} M) in a concentration-dependent manner. Quercetin also relaxed the contractions induced by both agonists. The relaxant effects on NA-induced contractions were not altered by removal of endothelium (IC_{50} = 1.6 ± 0.4 x 10^{-5} M). In addition, quercetin inhibited the contractions induced by increases in extracellular Ca (1-5 mM) in K-depolarized muscles and caused a progressive shift of Ca concentration-response curves downwards (IC_{50} = 1.8 ± 0.3 x 10^{-5} M). Quercetin also inhibited (by 89.7 ± 1.6 % at 10^{-5} M) the phasic contractions induced by NA in Ca-free KS and relaxed the contractions induced by phorbol 12-myristate 13-acetate (PMA, 10^{-7} M) in normal KS (IC_{50} = 1.1 ± 0.3 x 10^{-5} M) or Ca-free KS containing 2 mM EGTA after complete depletion of NA-sensitive Ca stores (IC_{50} = 1.1 ± 0.2 x 10^{-5} M). Isoprenaline (10^{-7} M) did not modify the relaxant effects of quercetin on NA-induced contractions (IC_{50} = 9.9 ± 2.3 x 10^{-6} M). In addition, quercetin, 3 x 10^{-6}M, did not modify the relaxant effect of forskolin on NA-induced contractions.

It is concluded that, in isolated rat aorta and portal vein, quercetin produced a vasodilator effect that seems to be related to the inhibition of protein kinase C. Inhibition of cyclic nucleotide phosphodiesterases may also contribute to the vasodilatory effect of quercetin.

This work was supported by a DGICYT (PM90-0138) Grant.

Fanning, M., Macander, P., Drzewiecki, G. & Middleton, E. Jr. (1983) Int. Arch. Allergy Appl.Immun. **71**, 371-373.
Beretz, A., Stoclet, J. & Anton, R. (1980) Planta Med. **39**, 236-237.
Gschwendt, M., Horn, F., Kittstein, W. & Marks, F. (1983) Biochem. Biophys. Res. Commun. **117**, 444-447.
Pérez-Vizcaíno, F., Duarte, J. & Tamargo, J. (1991) Br. J. Pharmacol. **104**, 726-730.

152P A STUDY OF THE PHARMACOLOGY OF MEDETOMIDINE IN THE HIND LIMB VENOUS SYSTEM OF THE HORSE

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Imidazole-derived α_2 adrenoceptor agonists are used as veterinary sedatives. Medetomidine (MT), a new member of this group of drugs (Virtanen *et al.*, 1988) has recently been licensed for veterinary use. The aim of this study was to compare the actions of MT on equine blood vessels with those previously published for the non-imidazole α_2-adrenoceptor agonist, BHT-920 (6-Allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d) azepine) (Bryant *et al.*, 1992).

Equine digital and saphenous vein (EDV & ESV) rings were prepared for isometric tension recording as before (Bryant *et al.*, 1992). Prazosin (30 nM), RX 821002 [2-[2-(2-methoxy-1,4-benzodioxanyl)] imidazoline] (10 nM), or yohimbine (0.1 μM) were added 30 min prior to the addition of MT. Cumulative dose response curves (CRCs) were constructed to MT (0.1 nM to 10 μM; n=7), in the presence of propranolol (1 μM) and cocaine (3μM). The effect of MT (0.1 to 100 nM; n=4 to 6), on electrical field stimulation (EFS; 2 ms pulse duration, 20 V, for 10 s at 8 Hz) of ESV rings was also studied in control tissues and tissues pretreated with cocaine (1 μM), corticosterone (1 μM), prazosin (10 nM), RX 821002 (3 nM) and ICI 118551 (Erythro-dl-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; 10 nM).

MT CRCs were biphasic with a plateau at 10 to 30 nM and EC_{50} values of 2.54 ± 0.75 nM and 0.48 ± 0.13 μM (n=7). Prazosin did not affect the first part of the CRC (EC_{50} 2.54 ± 0.78 nM) but increased the length of the plateau by 10 fold. RX 821002 had no inhibitory effect on the MT CRC whereas yohimbine produced a monophasic curve (EC_{50} 0.34 ± 0.06 μM). MT (0.1-100 nM) produced a significant, dose-dependent increase in response to EFS (3.0 ± 0.75 fold at 10 nM). The potentiation of EFS by MT was not inhibited by cocaine and corticosterone, ICI 118551 or RX821002, but was markedly reduced by prazosin (10 nM). MT (1 nM) did not affect responses to exogenously applied noradrenaline.

The lack of inhibition of MT by RX 821002 at a dose (10 nM) which markedly inhibited the response BHT-920 (Bryant *et al.*, 1992) and preliminary data showing vessels which are desensitised to MT remain sensitive to BHT-920, suggest these two agonists act at different prazosin-resistant postsynaptic sites. MT enhanced the response to EFS whereas BHT-920 inhibited the EFS response (Bryant et al 1992), suggesting MT acts at a different site to BHT-920 presynaptically. Imidazoline receptors and/or α_2-receptors subtypes might be proposed to explain the above findings.

CEB is a Wellcome Veterinary Fellow. We thank the Home of Rest for Horses for their financial support.

Bryant, C.E., Elliott, J., Soydan, J., Clarke, K.W. (1992) *Br. J. Pharmacol.*, **107**, 376P
Virtanen, R., Savola, J., Saano, V. & Nyman, L. (1988) *Eur. J. Pharmacol.*, **150**, 9-14.

153P REAL-TIME IMAGE ANALYSIS MEASUREMENTS OF BLOOD VESSEL RESPONSIVENESS *IN VIVO*

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Rabbit blood vessels have been previously shown *in vitro* to exhibit noradrenaline/ATP co-transmission (McGrath *et al.*, 1990; Bulloch & Starke, 1990). Examining the role of the purinergic transmitter *in vivo* using the pithed rat model is possible (Bulloch & McGrath, 1988) but is limited since pithing removes on-going vasoconstrictor tone. The objective of this study was to find and utilise a method of recording changes in arterial blood pressure and consecutive changes blood vessel tone in an intact vascular bed *in vivo* where there may be differences in the time course of responses between arteries and veins and a differential sensitivity to agents along individual vessels.

The central ear artery and marginal ear vein of the left ears of male New Zealand White Rabbits (2.5 – 3 kg) used in this study were cannulated under anaesthesia (xylocaine 0.2 mg/kg s.c.) for monitoring blood pressure and administering drugs respectively. Changes in blood vessel diameter in the intact arterial and venous vascular beds of the right ears were monitored by measuring the lumen size of the vessels in the ear network by illuminating the ear from below and recording the image of the network using a video camera (Sony DXC-151P) mounted on a dissection microscope. Sequences of digital images captured using a frame grabber (Data Translation DT2255) were displayed and analysed using a Macintosh IIfx computer (Moss, 1992). The component parts of the blood vessel network were analysed simultaneously by identifying segments of blood vessels (30 – 300 μm diameter) in the image and marking them as windows. Blood within the vessel provides the contrast in the image necessary to identify the lumen wall. Individual blood vessels were typically 5 – 50 pixels wide in the image and changes in lumen diameter as little as 1% could be measured. Sequences of up to several thousand images could be analysed and the data file visualised graphically to show vessel diameter changes with respect to time.

Real-time image analysis of vascular contractile responses (Moss, 1992) allows agonist-induced contractions to be monitored. Dose dependant vasoconstrictor responses are produced by boluses of noradrenaline (0.1 – 1 μg/kg) in both arteries and veins. Smaller arteries (less than 80 μm) are most sensitive to the contractile effects of the P_{2x}-purinoceptor desensitising agent α,β-methylene-ATP (mATP) (0.1 – 10 μg/kg). mATP (10 μg/kg) produces an increase in both systolic blood pressure of 118 ± 5.2 % from a resting value of 95.8 ± 4.66 mmHg and diastolic blood pressure of 136 ± 6.2 % from a resting value of 65.3 ± 3.0 mmHg (n=6) but this dose of mATP produces an occlusion of small arteries (30-100 μm) which lasts approximately 20s. Veins did not show this effect. The α_1-adrenoceptor antagonist prazosin (0.1 mg/kg) dilates both arteries and veins. Veins show increases in lumen size of between 80 and 140 % (n=4) whereas arteries show increases of 40 – 60 % (n=4). Prazosin (0.1 mg/kg) produces a decrease in both systolic blood pressure of 60.3 ± 9.1 % from a resting value of 95.7 ± 5.7 mmHg and diastolic blood pressure of 54.8 ± 8.3 % from a resting value of 66.2 ± 6.8 mmHg (n=6).

The results of this study using real-time image analysis of changes in vascular parameters *in situ* uncovers a difference in blood vessel responsiveness between arteries and veins and shows that it is possible to monitor the effects of blocking agents for the co-transmitters of sympathetic neurotransmission on intact vascular networks.

This work was supported by The British Heart Foundation and The Wellcome Trust.

Bulloch, J.M. & McGrath, J.C. (1988) *Br. J. Pharmacol.* **94**, 103 -108.
Bulloch, J.M. & Starke, K. (1990) *Br. J. Pharmacol.* **99**, 279 -284.
McGrath, J.C., Bulloch, J.M., Daly, C.J. & MacDonald, A. (1990) *Blood Vessels*, **27**, 44.
Moss, V. A. (1992) *J. Physiol.* **452**, 17P.

154P INTERACTIONS BETWEEN THIAZIDE DIURETICS AND CYCLIC NUCLEOTIDES IN ISOLATED SMALL ARTERIES

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The cyclic nucleotides cAMP and cGMP are important regulators of many cellular functions. Exogenously applied cAMP and cGMP have both been shown to cause smooth muscle relaxation (Kramer & Hardman, 1980). This study examines the effect of pretreatment with the thiazide diuretic hydrochlorothiazide (HTZ) and the related compounds indapamide (IND) and cicletanine (CIC) on the relaxation induced by dibutyryl cyclic nucleotides and agonists which increase intracellular cyclic nucleotide concentration.

Guinea pig mesenteric arteries (n=72, mean diameter 445±21μm) were dissected free and mounted on a microvascular myograph (Mulvany & Halpern, 1977). Dose-response curves were constructed for dibutyryl cAMP (dcAMP), forskolin (FOR: an adenylate cyclase activator), dibutyryl cGMP (dcGMP) and sodium nitroprusside (SNP: a guanylate cyclase activator) following noradrenaline preconstriction in the presence of thiazide diuretic or vehicle control. A subthreshhold dose of 100pM was selected as the pretreatment concentration of thiazide, as at this concentration HTZ, IND and CIC were found to have no direct relaxant activity.

The dibutyryl cyclic nucleotides and agonists caused dose-related relaxation of guinea pig mesenteric vessels. The log EC_{50} values of cyclic nucleotide-induced relaxation in the presence and absence of thiazide are shown in table 1.

Table 1: Effect of thiazide pretreatment on cyclic nucleotide-induced relaxation

	HTZ		IND		CIC	
	A	B	A	B	A	B
dcAMP	-7.6	-7.6	-7.1	-9.7*	-7.5	-8.4*
FOR	-7.9	-8.0	-7.6	-8.7*	-7.9	-9.6*
dcGMP	-7.4	-7.1	-7.3	-7.4	-7.4	-9.1*
SNP	-7.8	-7.3	-7.6	-7.3	-7.0	-9.4*

A and B are relaxation in the presence of vehicle control and thiazide drug respectively. * Log EC_{50} values in the presence of thiazide diuretic significantly different from control ($P<0.01$).

HTZ pretreatment had no effect on cyclic nucleotide-induced relaxation. IND potentiated the dcAMP and FOR-induced relaxations and CIC potentiated all the relaxations. It is not possible from this study to assess at which level IND and CIC affect the cyclic nucleotide pathway: it could be at the cyclase, kinase or phosphodiesterase level. However, Silver *et al* (1990) have shown that cicletanine inhibits cGMP-dependent phosphodiesterase. Further studies are in progress to assess where the thiazides affect the cyclic nucleotide pathway.

Kramer, G.L. & Hardman, J.G. (1980) in *Handbook of Physiology vol. II* ed. Geiger, S.R. pp179-200. Baltimore: Waverly
Mulvany, M.J. & Halpern, W. (1977) Circ. Res. **41**., 19-26
Silver, P.J., Buckholz, R.A., Dundore, R.L., Harris, A.L. & Pagani, E.D. (1990) J. Cardiovasc. Pharmacol. **16**, 501-505

155P EFFECTS OF HYPOXIC PERFUSION ON RELAXATION TO ENDOTHELIN PEPTIDES IN THE ISOLATED PERFUSED SUPERIOR MESENTERIC ARTERIAL BED OF THE RAT

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We have shown that the endothelium modulates the contractile activity of endothelin (ET) peptides in the isolated superior mesenteric arterial bed of the rat even when O_2 tension (PO_2) is very low (Douglas *et al.*, 1991); when indomethacin is present, this modulation is due to the action of nitric oxide. The ETs also relax preparations in which the basal tone has been increased by the α_1-adrenoceptor agonist methoxamine (Douglas & Hiley, 1991). The relaxant, but not the contractile, effects of the full 21 amino acid ETs, are shared by the C-terminal hexapeptide, ET_{16-21}, which is common to all the ETs. In this study we have investigated whether or not the relaxant effects of ET-3 and ET_{16-21} occur in the presence of only very low PO_2 in the perfusate.

The superior mesenteric arterial bed from male Wistar rats (350-450 g) was perfused at 4 ml min^{-1} with Krebs-Henseleit solution containing 10 μM indomethacin and 0.5% bovine serum albumin (Randall *et al.*, 1989). For studying vasorelaxation, tone was induced by addition of 100 μM methoxamine to the perfusate. In normoxia, the perfusing solution was bubbled with 95% O_2/5% CO_2 and for hypoxic perfusion the gas mixture used was 95% N_2/5% CO_2. The PO_2 of the perfusate was measured by a flow-through MC100 microcell and Type 1302 O_2 electrode (Strathkelvin Instruments, Glasgow) placed in the perfusion circuit just before the mesentery. During hypoxic perfusion the preparation was kept in an atmosphere of 100% N_2. ET-3 was from The Peptide Institute (Osaka, Japan) and ET_{16-21} was from Peninsula Laboratories (St Helens, Merseyside). Acetylcholine and sodium nitroprusside were from Sigma.

When bubbled with O_2/CO_2, the perfusate PO_2 was 552±3 mmHg (n = 28) as compared to 2.0±1.0 mmHg (n = 38) during bubbling with N_2/CO_2. As in normoxia (Douglas & Hiley, 1991), a bell-shaped log dose-response curve for the depressor actions of ET-3 was obtained but the peptide appeared to be less potent since the maximum response (R_{max}) was a 21.6±3.7% reduction in induced tone (n = 5) which occurred at a higher dose (30 pmol) than that in normoxia (24.3±3.8% reduction in tone at 0.3 pmol; n = 6). The log dose-response curves for ET_{16-21} were S-shaped with a significantly greater R_{max} value of 58.9±4.8% (n = 3) in normoxia than in hypoxia (21.5±2.4%; n = 4); the ED_{50} values were not different being 41.3±6.9 (normoxia) and 58.9±18.8 pmol (hypoxia). The depressor potencies of acetylcholine and sodium nitroprusside were greater in hypoxia by 3.3 and 5 fold, respectively, than in normoxia but for acetylcholine the R_{max} in hypoxia was significantly lower (63.1±1.0%; n = 9) compared to normoxia (89.8±1.7%; n = 8) whereas that for sodium nitroprusside it was significantly enhanced (79.4±1.8%, n = 5, hypoxia; 50.1±4.3, n = 6, normoxia).

Thus the relaxation responses to ET-3 and ET_{16-21} can still occur even when the PO_2 of the perfusate is very low. In normoxia, these relaxant responses are endothelium-dependent (Douglas & Hiley, 1991) and so it would seem that the nitric oxide system can still function to give endothelium-dependent relaxation when PO_2 is very low but, since the maximum responses obtained to acetylcholine and ET_{16-21} were both reduced, it would seem that the degree of dilatation that can be obtained is dependent on an adequate O_2 supply.

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Douglas, S.A., James, S. & Hiley, C.R. (1991). Br. J. Pharmacol., 103, 1441-1448.
Douglas, S.A. & Hiley, C.R. (1991). Br. J. Pharmacol., 104, 311-320.
Randall, M.D., Douglas, S.A. & Hiley, C.R. (1989). Br. J. Pharmacol., 98, 685-699.

156P CHANGES IN RAT COMMON CAROTID ARTERY (CCA) REACTIVITY TO ENDOTHELIN-1 (ET-1) AFTER BALLOON ANGIOPLASTY

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Percutaneous transluminal coronary angioplasty (PTCA) is frequently associated with life threatening coronary artery vasospasm requiring emergency bypass surgery. Since PTCA is associated with elevated levels of the vasoconstrictor ET-1 (Tahara *et al.*, 1992), this study examined changes in vascular reactivity to ET-1 after balloon angioplasty in the rat CCA model (Clowes *et al.*, 1983).

Left CCA angioplasty was performed on male Sprague-Dawley rats (350g) using a 2-F Fogarty arterial embolectomy catheter (Clowes *et al.*, 1983). A separate group of rats underwent sham operations. CCA rings, isolated either immediately or 1-14 days after surgery, were suspended in 10ml organ baths in oxygenated Krebs solution and concentration-response curves were constructed to ET-1.

Day after surgery	Sham			Angioplasty		
	EC_{50} (nM)	R_{max} (g)	n	EC_{50} (nM)	R_{max} (g)	n
0	0.76 ± 0.12	0.48 ± 0.05	12	0.36 ± 0.08†	0.41 ± 0.06	6
1	1.36 ± 0.27*	0.49 ± 0.07	9	1.06 ± 0.18*	0.33 ± 0.03	8
3	0.61 ± 0.14	0.58 ± 0.05	10	2.52 ± 0.89*†	0.45 ± 0.05	6
7	0.51 ± 0.10	0.47 ± 0.07	10	2.74 ± 0.58**††	0.42 ± 0.06	13
14	0.52 ± 0.16	0.48 ± 0.09	8	1.33 ± 0.38	0.45 ± 0.08	7

*P<0.05, **P<0.01 Student's t-test with appropriate Day 0 value; †P<0.05, ††P<0.01 ANOVA with time matched Sham value.

Neither surgical procedure altered the efficacy of ET-1, however, angioplasty caused an immediate (Day 0) increase in potency, possibly due to the loss of endothelial nitric oxide (NO•). Similar increases were not observed with noradrenaline (NA) or KCl. However, as was seen with NA and KCl, over the ensuing week (Days 3-7) ET-1 potency diminished, possibly resulting from cytokine-mediated NO• synthase induction (Joly *et al.*, 1992). Histological examination confirmed that the endothelium regenerated, however, unlike the smooth muscle cells, these cells were dysfunctional *i.e.* vessels relaxed to nitroprusside, but not to acetylcholine. Therefore, ET-1, coupled with a dysfunctional endothelium, might mediate the acute vasospasm associated with PTCA.

Clowes, A.W., Reidy, M.A. & Clowes, M.M. (1983). *Lab. Invest.*, **49**, 208-215.
Joly, G.A., Schini, V.B. & Vanhoutte, P.M. (1992). *Circ. Res.*, **71**, 331-338.
Tahara, A., Kohno, M., Yanagi, S., Itagane, H., Toda, I., Akioka, K., Teragaki, M., Yasuda, M., Takeuchi K. & Takeda, T. (1992). *Metabolism*, **40**, 1235-1237.

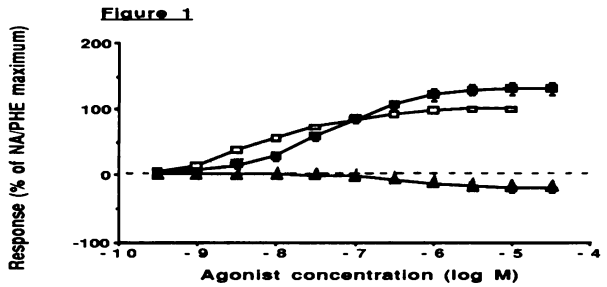
157P EFFECT OF α_2-ADRENOCEPTOR STIMULATION ON RESPONSES TO NORADRENALINE IN THE RAT PULMONARY ARTERY

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Here we have investigated the influence of α_1- and α_2-adrenoceptors on noradrenaline (NA)-induced vasoconstriction in rat pulmonary arteries (PA)s.

Rat PA rings were set up in organ baths, under 2g tension and in Krebs with 45mmHg O_2 and 40mmHg CO_2. Cumulative concentration-response curves (CRCs) to NA (α_1- and α_2-adrenoceptor agonist), phenylephrine (PHE, α_1-adrenoceptor agonist) and UK 14304 (UK, α_2-adrenoceptor agonist) were established. In seperate experiments, the CRCs were repeated with NA in the presence of the selective α_2-adrenoceptor antagonist, rauwolscine [RAU (10^{-7}M and 10^{-6}M)] and UK in the presence of 10^{-4}M L-NAME, an inhibitor of nitric oxide (NO) synthase.

In the absence of RAU, the EC_{50} for NA was -8.2 ± 0.1 [log concentration (M)]. The EC_{50} for phenylephrine was -7.35 ± 0.2 (Student's paired *t*-test $P<0.01$ compared with NA). Fig. 1 shows that UK induced vasorelaxation. L-NAME totally inhibited this relaxation. The maximum responses achieved by PHE were greater than those achieved by NA ($P<0.01$, Fig. 1). Fig. 2 shows that, in the presence of RAU, the maximum responses to PHE were now less than those to NA ($P<0.01$). In the presence of 10^{-7}M RAU, the EC_{50} for NA was -8.4 ± 0.1. In the presence of 10^{-6}M RAU, the EC_{50} for NA was -7.7 ± 0.1 ($P<0.01$ (Student's unpaired *t*-test) compared with NA in the absence of RAU). The EC_{50} for PHE in these experiments was -7.0 ± 0.2.



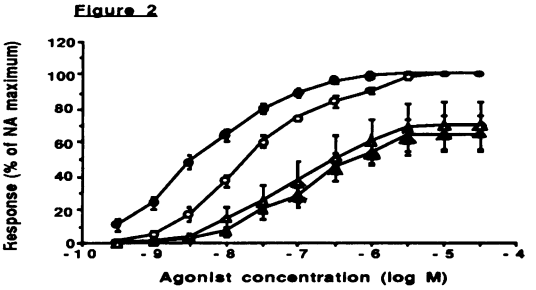


***Figure 1**. Control CRCs to NA (□,% NA max), PHE (■) and UK (▲,% PHE max) in rat pulmonary artery rings [13 rings, 9 rats].*
***Figure 2.** CRCs to NA [+ 10^{-7}M RAU (●); + 10^{-6}M RAU (○)] vs CRCs to PHE (▲ and △), [6 rings, 6 rats].*

The results show that α_2-adrenoceptor stimulation can cause vasorelaxation in rat PA rings. The maximum response to NA in the isolated rat PA was less than that acheived by PHE. This situation was reversed by the presence of RAU. This suggests that the depressor effect of NA-induced α_2-adrenoceptor stimulation opposes the pressor effect of simultaneous NA-induced α_1-adrenoceptor stimulation. 10^{-7}M RAU had no significant effect on the EC_{50} for NA. As UK-induced vasorelaxation was evidently due to endothelial NO release, 10^{-7}M RAU may, therefore, be selective for endothelial α_2-adrenoceptors in this preparation.

This research was funded by the Wellcome Trust. K.M. McCulloch was a Carnegie Trust vacational scholar.

158P PHARMACOLOGICAL EVIDENCE THAT RAT BLOOD VESSEL SMOOTH MUSCLE SEMICARBIDE-SENSITIVE AMINE OXIDASE IS AN ECTOENZYME

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Semicarbazide-sensitive amine oxidase (SSAO) enzymes exist in tissue-bound and soluble forms in many species of plants and animals. Vascular smooth muscle cells (VSMCs) exhibit particularly high SSAO activity when compared with that in other mammalian tissues (Lyles & Singh 1985). However, since little or no evidence exists to identify the physiological substrate or substrates, it has not been possible to ascribe any convincing physiological function to this enzyme activity.

In an attempt to determine some of the factors influencing the activity of SSAO in intact VSMCs, the subcellular location of its active centre, which may contain an SH group (Suva & Abeles, 1978), has been investigated in the isolated, perfused, mesenteric arterial bed of the rat and in dissociated SMCs from the aorta. The effects were thus examined of diazotised sulphanilic acid (DSA), a slowly permeating, covalent inhibitory reagent, and of *p*-chloromercuribenzene sulphonate (PCMBS), a non-permeating SH group inhibitor, on the metabolism of the permeant substrate for SSAO, benzylamine, in these two systems.

Mesenteric beds were removed from male Wistar rats and perfused with modified Krebs-Henseleit solution (Elliott *et al.*, 1989). Pressor responses to noradrenaline were obtained before and after perfusion of tissues with Krebs (controls), DSA or PCMBS for 15 min and tissues were then perfused with [^{14}C] benzylamine. The effect of removal of the vascular endothelium with the detergent, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate (CHAPS) before perfusion with PCMBS was also studied. Perfusate was collected and radioactive metabolites of benzylamine were isolated and measured as described by Elliott *et al.*, (1989). Intracellular lactate dehydrogenase (LDH) activity was measured by spectrophotometry in some vessel homogenates. SMCs dissociated from whole aortas from male Wistar rats were incubated with Krebs, DSA or PCMBS for 10 min before several washings by centrifugation and resuspension and incubation with [^{14}C] benzylamine.

Perfusion with DSA or PCMBS abolished the pressor responses to noradrenaline, at which time there was no significant reduction in LDH activity in homogenates of the vessels. Control experiments showed that complete inhibition of LDH could be produced by addition of DSA to homogenates of control vessels. However, benzylamine metabolism in control beds (2.12 ± 0.2 nmol min^{-1}; n=5) was reduced by 44% by DSA (n=5) and, although PCMBS reduced benzylamine metabolism by only 24% (n=3), prior removal of the endothelium with CHAPS allowed PCMBS to reduce metabolism to 42% of that in a CHAPS-perfused control. However, DSA and PCMBS reduced SSAO activity in dissociated aortic SMCs by 94.3 and 94.5%, respectively. Results from dissociated cell experiments therefore suggest that vascular SSAO is an ectoenzyme and that any endogenous substrate might be blood-borne. Perfused DSA and PCMBS completely inactivated extracellular α_1-adrenoceptors and penetration of DSA into VSMCs was insignificant. However, almost half of extracellular SSAO was inaccessible to these lipophobic compounds, even after removal of the endothelium, suggesting that high lipid solubility might be a further prerequisite for any putative blood-borne substrate to ensure total access to the enzyme on all VSMCs.

AH was an MRC Research Student.

Elliott J., Callingham, B.A. & Sharman, D.F. (1989) *Br. J. Pharmacol.*, 98, 507-514.
Lyles, G.A. & Singh, I. (1985) *J. Pharm. Pharmacol.*, 37, 637-643.
Suva, R.H. & Abeles, R.H. (1978) *Biochemistry* , 17, 3538-3545.

159P THE KINETICS OF METHYLAMINE METABOLISM BY SHEEP PLASMA SEMICARBAZIDE-SENSITIVE AMINE OXIDASE

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Methylamine can be produced endogenously as a result of the breakdown of either adrenaline (Schayer *et al.*, 1952) or the metabolic waste products, creatine and sarcosine (Davis & De Ropp, 1961). It can also be derived from bacterial metabolism of choline and lecithin in the intestine. Unlike most other aliphatic amines, methylamine is not a substrate for monoamine oxidase (EC 1.4.3.4) but can be metabolised by semicarbazide-sensitive amine oxidases (SSAO; EC 1.4.3.6) (see Precious *et al.*, 1988). In sheep plasma, with benzylamine as substrate, two kinetically separable enzyme activities appear to be responsible for its metabolism, with K_m values of 2.8 μM and 743 μM (Elliott *et al.*, 1992). Since methylamine, unlike benzylamine, is has been identified as an endogenous substrate of SSAO, this study has been concerned with an attempt to discover whether or not both activities were involved in its deamination.

Blood was obtained from mixed breed sheep by jugular venepuncture and centrifuged to yield platelet-poor plasma. Plasma amine oxidase activity against methylamine was estimated either by fluorimetric measurement of hydrogen peroxide production or by a radiochemical assay, where samples were incubated with [^{14}C]-methylamine and radiolabelled metabolites were separated from the substrate using Amberlite ion exchange resin (Lyles *et al.*, 1990). Kinetic analysis was by the method of Wilkinson (1961).

Only one activity responsible for methylamine deamination could be detected in sheep plasma. Hanes-Woolf kinetic plots appeared to be single regressions which could not be resolved into two components by iterative curve stripping. Fluorimetric measurement of enzyme activity allowed only substrate concentrations above 25 μM to be used. However, the radiochemical assay enabled the use of methylamine concentrations ranging from 2.5-1000 μM and a calculated K_m value of approximately 600 μM was obtained. Control experiments showed that spontaneous decomposition of the amine to its aldehyde product during incubation at 37°C was negligible.

Appreciable methylamine metabolism takes place at concentrations as low as 2.5 μM but the K_m value for the overall metabolism is approaching the millimolar range. Therefore the possibility exists that two enzyme activities are present. However, they cannot be separated kinetically. It is plausible that any activity which metabolises low concentrations of methylamine is sufficiently masked that it will not be recorded on the kinetic plot. As methylamine is possibly an endogenous substrate for SSAO, resolution of these two putative enzyme activities using selective inhibitors is of prime importance.

A.E.C. is an MRC Research Student.

Davis, E.J. & De Ropp, R.S. (1961) *Nature*, 190, 636-637.
Elliott, J., Callingham, B.A. & Sharman, D.F. (1992) *Comp. Biochem. Physiol.*, 102C, 83-89.
Lyles, G.A., Holt, A. & Marshall, C.M.S. (1990) *J. Pharm. Pharmacol;.*, 42, 322-338.
Precious, E., Gunn, C.E. & Lyles G.A. (1988) *Biochem. Pharmacol.*, 37, 707-713.
Schayer, R.W., Smiley L.R. & Kaplan H.E. (1952) *J. Biol. Chem.*, 198, 545-551.
Wilkinson, G.N. (1961) *Biochem. J.*, 125, 1149-1151.

160P EFFECTS OF ELCATONIN ON ADENOSINE TRIPHOSPHATE- AND ACETYLCHOLINE-INDUCED VASORELAXATION OF FEMORAL ARTERIAL PREPARATIONS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Elcatonin (an analogue of calcitonin) is used for treatment of osteoporosis. In addition, elcatonin reduced blood pressure in spontaneously hypertensive rats (SHR). We, therefore, determined whether or not elcatonin altered reactivity of SHR arteries to vasorelaxants. The experiments were performed on helically cut strips of femoral arteries taken from 10-week-old male SHR. The Femoral arteries were cleaned of connective tissues with special care being taken not to touch the luminal surface, and were bathed in physiological salt solution under a resting tension of 500mg. Isometric tension changes were recorded by a force-displacement transducer coupled to a pen recorder. To observe relaxation, the arterial strips were contracted with 0.5 μM noradrenaline. Elcatonin was injected subcutaneously at doses of 0.5 and 5 units/kg, six times for two weeks. Adenosine triphosphate (ATP; 1-300 μM) relaxed the arterial strips in a dose-dependent manner. The relaxation response of the strips to 100 and 300 μM ATP was increased by elcatonin (0.5 and 5 units/kg) (Table 1), and EC_{50} values for ATP remained unchanged. Acetylcholine (ACh; 1-100 nM) relaxed the arterial strips in a dose-dependent manner. Relaxation response to 10 nM ACh was increased by 5 units/kg elcatonin. Relaxation responses of the strips to histamine and adenosine diphosphate were not changed by elcatonin.

Table 1. Effects of elcatonin on ATP-induced vasorelaxation in femoral arteries of SHR.

	Concentrations of ATP (μM)				
	1	10	30	100	300
Control	23.4±8.9*	57.0±7.9	65.1±6.9	62.8±2.3	43.5±2.0
Elcatonin (0.5 units/kg)	23.6±5.9	66.9±4.0	77.0±2.2	76.9±1.3**	63.3±2.7**
Elcatonin (5.0 units/kg)	20.2±5.0	61.3±6.2	74.7±2.7	78.0±0.9**	61.6±2.5**

* The ratios (%) of relaxation responses to ATP to the relaxation induced by 0.1 mM papaverine (Means±s.e.mean of three observations). **P<0.05 compared to the control (Student's t test).

We previously reported that relaxation responses of SHR femoral arteries to ATP and ACh were mediated by P_{2y} purinoceptors and M_3 cholinoceptors, respectively (Fujimoto & Matsuda, 1991). The present results show that elcatonin can alter the relaxation responses of the SHR femoral artery to ATP and ACh.

Fujimoto, S. & Matsuda, T. (1991) *Eur. J. Pharmacol.* 202, 9-15.

161P α_1-ADRENOCEPTORS MEDIATING NOREPINEPHRINE CONTRACTION OF THE RABBIT PROSTATE *IN VIVO*

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Operational and molecular biological studies have revealed multiple subtypes of alpha$_1$-adrenoceptors (for references see Marshall et al., 1992). The present study attempted to characterise the α_1-adrenoceptor subtype(s) mediating contraction of the rabbit prostate *in vitro*. Transverse strips approximately 5mm long and 1-1.5mm in width from the dorsal prostatic capsule of male albino rabbits were mounted under 0.5g tension in organ baths containing Tyrode's solution (NaCl 139.6mM, KCl 2.7mM, $MgCl_2$ 1.0mM, NaH_2PO_4 0.4mM, glucose 5.6mM, $NaHCO_3$ 11.9mM, $CaCl_2$ 1.8mM, ascorbate 100μM, cocaine 30μM, corticosterone 30μM, indomethacin 10μm, propranolol 1μM and idazoxan 300nM) at 37°C, gassed with 95% O_2, 5% CO_2. A non-cumulative concentration response curve (CRC) to norepinephrine (NE) was constructed. Antagonists were added to the Tyrode's solution 45min prior to construction of a second CRC to NE. In studies using chloroethylclonidine (CEC; Han et al., 1987) the drug was added to the bath for 60min (10μM) or 20min (100μM) followed by washout (5x5 min).

NE caused a concentration-dependent contraction of the prostatic capsule with E_{max} and pEC_{50} values of 922±64mg and 6.18±0.05 respectively (mean±sem, 60 determinations from 30 separate experiments). The α_1-adrenoceptor antagonist prazosin and the selective adrenoceptor antagonists 5-methyl-urapidil and WB4101 (all at 30 nM), antagonised competitively NE-induced contractions yielding pA_2 estimates of 8.53±0.06, 8.60±0.08 and 8.82±0.07 (mean±sem, n=3-4). CEC at 10μM or 100μM reduced the E_{max} to NE to 81±5% (n=6) and 65±4% (n=6) of control respectively, accompanied by a small decrease in the potency of NE. Pretreatment with CEC (100μM) did not significantly alter the pA_2 estimate for WB4101 (8.77±0.07, n=3). (+)-Niguldipine (30nM) reduced the maximum response to NE to 32±8% of control with no appreciable shift in the pEC_{50} value; at 300nM, (+)-niguldipine virtually abolished the response to NE. (-)-Niguldipine was approximately 10-fold less potent at antagonising NE.

The rank order of antagonist affinity, together with the limited effect of CEC, appears similar to that seen in the prostate of man (putative α_{1C} receptor; Marshall et al., 1992). However, the low affinity of prazosin for rabbit alpha$_1$-adrenoceptors (Flavahan and Vanhoutte, 1986) prevents definitive classification of the receptor.

Flavahan, N.A. & Vanhoutte, P.M. (1986) TIPS, Sept, 347-349.
Han, C., Abel, P.W. & Minneman, K.P. (1987) Nature **329**, 333-335.
Marshall, I., Burt, R.P., Andersson, P.O., Greengrass, P.M., Wyllie, M.G. & Chapple, C.R. BPS Meeting, Dublin, Sept 1992.

162P FURTHER INVESTIGATION OF α_2-ADRENOCEPTOR SUBTYPE SELECTIVE ANTAGONISTS IN RAT KIDNEY, SUBMANDIBULAR GLAND AND VAS DEFERENS

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We have previously demonstrated that at least two different subtypes of α_2-adrenoceptor are present on adrenergic nerve terminals: an α_{2A}/α_{2D} in rat vas deferens and submandibular gland, and an α_{2B} or α_{2C} in rat atrium (see Smith & Docherty, 1992). For further classification of functional subtypes of prejunctional α_2-adrenoceptor, it is important to identify further subtype selective antagonists. In this study, we have investigated the selectivity of a series of agents between radioligand binding sites in rat kidney (α_{2B}) and rat submandibular gland ($\alpha_{2A/D}$).

Preparation of rat kidney and submandibular gland membranes was carried out as described previously (Smith et al., 1992), and antagonist K_i values are obtained from the displacement of ^{3}H-yohimbine binding. In prostatic portions of rat vas deferens, antagonist potency was expressed as a K_B from the ability of the antagonist to antagonise the inhibition by xylazine of the isometric contraction to single pulse electrical stimulation.

A series of 27 ligands were investigated for selectivity between the α_2-adrenoceptor ligand binding sites of rat kidney (α_{2B}) and rat submandibular gland ($\alpha_{2A/D}$). We have previously found that BRL 44408 shows selectivity for the submandibular binding site, and HV 723, chlorpromazine, prazosin, ARC 239 and 8-OH-DPAT show selectivity for the kidney ligand binding site (see Smith et al., 1992). In this study, two further antagonists were found to have selectivity for the kidney ligand binding site, abanoquil (UK 52,046) and spiroxatrine, with K_i values (and 95% confidence limits) of 7.18±0.32 and 7.86±0.50 in rat kidney, and 5.80±0.33 and 6.23±0.32 in rat submandibular gland, respectively.

In keeping with their low affinity for the $\alpha_{2A/2D}$ ligand binding site of rat submandibular gland, abanoquil and spiroxatrine had low potency at the prejunctional $\alpha_{2A/2D}$-adrenoceptor in rat vas deferens with K_B values of <6.0 and 6.83±0.17, respectively.

Supported by the Health Research Board, the Irish Heart Foundation, and RCSI.

Smith, K. & Docherty, J.R. (1992). Eur. J. Pharmacol. 219, 203-210.

163P ALPHA$_1$ ADRENOCEPTOR SUBTYPE MEDIATING CONTRACTIONS OF THE RAT PROSTATE GLAND

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Radioligand binding studies indicate that the bovine prostate possesses α_1-adrenoceptors predominantly (90%) with a low affinity for WB4101 (Maruyama et al.,1992). This suggests a popoulation of α_{1B}-adrenoceptors. In contrast, the human prostate appears to possess a population of predominantly α_{1C}-adrenoceptors (Marshall et al.,1992). The present study examines the α_1-adrenoceptor-mediated responses of the rat prostate gland, vas deferens (α_{1A}) and spleen (α_{1B}) (Han et al., 1987).

Rat prostate glands, vas deferens and spleen were set up in aerated Krebs-bicarbonate solution at 37°C. Cumulative concentration-response curves were obtained to phenylephrine in the absence or presence of WB4101 (1-300nM). Responses were also obtained following the incubation of tissues with chloroethylclonidine (CEC, 25μM) for 45 min. Experiments were performed in the presence of cocaine (10μM), corticosterone (10μM) and propranolol (1μM).

Table 1: pK_B and pA_2 values for WB4101 on rat prostate, vas deferens and spleen.

Tissue	pK_B	n	pA_2	Schild slope
Prostate	9.20 ± 0.15	20	9.71	0.95 ± 0.33
Vas deferens	9.44 ± 0.13	18	9.41	1.12 ± 0.33
Spleen	8.40 ± 0.08*	16	8.51	0.91 ± 0.14

*P < 0.01 compared with prostate or vas deferens.

WB4101 produced rightward shifts of concentration-response curves in all three tissues producing Schild plots with slopes similar to unity (Table 1). The pK_B values for WB4101 were similar in prostate and vas deferens, but these were significantly greater than the values obtained on the spleen. Responses of the prostate were unaffected by incubation with CEC, EC_{50} values and maximum developed tensions being similar in control [3.6(1.3-10.1)μM, 0.19 ± 0.01g] and CEC-treated tissues [8.3(3.3-20.7)μM, 0.21 ± 0.1g]. Incubation with CEC depressed responses to phenylephrine in the spleen but not the vas deferens.

These results indicate that the contractile responses of the rat prostate to phenylephrine are mediated primarily via α_{1A}-adrenoceptors which have a high affinity for WB4101 and are insensitive to inactivation by chloroethylclonidine.

HOB and AHJ are support by the MRC and BHF respectively.

Han et al. (1987) Nature 329, 333-335.
Marshall, I. et al. (1992) Br.J.Pharmacol 107 (Proc. Suppl.); 327P
Maruyama, K., et al. (1992) J. Pharm. Pharmacol., 44, 727-730.

164P SODIUM IONS DIFFERENTIALLY REGULATE AGONIST AND ANTAGONIST BINDING TO α_{2B}-ADRENOCEPTORS

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The allosteric effect of sodium ions (Na^+) on α_2-adrenoceptors is to reduce agonist affinity while increasing the affinity of antagonists for the receptor (Limbird *et al*, 1982). The amino acid residue involved in Na^+ regulation of the porcine α_{2A}-adrenoceptor has been identified (Horstman *et al.*, 1990). In this study the effects of Na^+ on the ability of subtype selective ligands to inhibit the agonist [^{3}H]-adrenaline ([^{3}H]-adr) and the antagonist [^{3}H]-RS-15385-197 ([^{3}H]-RS) binding rat neonatal lung (α_{2B}) membranes was determined. [^{3}H]-RS-15385-197 binding was carried out as previously described (MacKinnon *et al.*, 1991). [^{3}H]-adrenaline binding was carried out in a 50 mM Tris HCl buffer containing, 0.3 mM ascorbate, 5 mM $MgCl_2$, 0.8 mM dithiothreitol, 0.1 mM catechol and 10 nM propranolol to prevent binding to β-adrenoceptors.
100 mM NaCl increased the density α_{2B}-adrenoceptors labelled by [^{3}H]-RS (control B_{max} = 174±20, Na^+ = 265±14 fmoles/mg), whereas the affinity was unchanged (control K_d = 81±10, Na^+ = 92±8 pM). Conversely the affinity of [^{3}H]-adr was decreased by Na^+ (control K_d = 3.4±0.4, Na^+ = 11.1±1.7 nM), while there was an increase in the density of agonist sites labelled (control B_{max} = 77±11, Na^+ = 209±38 fmoles/mg).

	[^{3}H]-adrenaline		[^{3}H]-RS-15385-197	
	control	NaCl	control	NaCl
adrenaline	8.41±0.16	7.76±0.28*	7.05±0.04	6.25±0.05*
noradrenaline	8.42±0.14	7.49±0.21*	7.25±0.03	6.19±0.06*
oxymetazoline	8.22±0.09	8.43±0.30	6.91±0.04	6.30±0.03*
UK-14,304	7.83±0.17	7.82±0.10	7.04±0.10	6.81±0.07
prazosin	5.68±0.16	6.70±0.23*	7.46±0.18	7.66±0.02
imiloxan	5.61±0.12	6.69±0.27*	6.30±0.04	7.40±0.22*

Table 1. The effect of 100 mM NaCl on agonist and antagonist binding to α_{2B}-adrenoceptors on rat neonatal lung. The results represent the mean ± s.e. mean pIC_{50} for 3-4 determinations performed in duplicate. * $p < 0.05$ compared to control.

In competition experiments (Table 1) Na^+ decreased the affinity of the catecholamines for [^{3}H]-adr and [^{3}H]-RS binding, whilst the affinity of oxymetazoline was decreased only for [^{3}H]-RS binding. Na^+ increased the affinity of imiloxan for [^{3}H]-adr and [^{3}H]-RS binding, although prazosin affinity was only significantly increased with [^{3}H]-adr. The results demonstrate that monovalent cation regulation of agonist and antagonist binding to the α_{2B}-adrenoceptor is dependent on the agonists and antagonists used, and provides further evidence for a difference in the interaction of catecholamine and imidazoline agonists with the receptor.

Horstman, D.A., Brandon, S., Wilson, A.L., Guyer, C.A., Cragoe, E.J.Jnr. and Limbird, L.E. (1990). J. Biol. Chem. 265, 21590-21595.
Limbird, L.E., Speck, J.L. & Smith, S.K. (1982). Mol. Pharmacol. 21, 609-617.
MacKinnon, A.C., Kilpatrick, D.A., Kenny, B.A., Spedding, M. & Brown, C.M. (1992). Br. J. Pharmacol. (in press).

165P DIFFERENT EFFECTS OF ENDOTHELINS AND SARAFOTOXINS ON NEUROTRANSMISSION IN THE GUINEA-PIG ILEUM AND RAT VAS DEFERENS ARE MEDIATED BY DIFFERENT RECEPTORS

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Two receptors for endothelins have been identified. The ETA receptor is more selective for endothelin-1 (ET-1) or sarafotoxin 6b (SX6b), whereas the ETB receptor is non-isopeptide selective. The ET/SX peptides can potentiate or inhibit smooth muscle contractions induced by autonomic nerve stimulation. To distinguish which receptors are involved we have used two preparations in which the ET/SX peptides influence neurotransmission (Maggi et al., 1989) and two ET-receptor antagonists, BQ123 (ETA-selective) and PD142893 (ETA/ETB non-selective).

Guinea-pig ileum (GPI) or rat vas deferens (RVD) were mounted in organ baths with isometric transducers, under tensions of 0.5-1 g. The bathing Krebs' solution contained bacitracin 3 $\mu g\ ml^{-1}$, bovine serum albumin (0.005% w/v), indomethacin ($5x10^{-6}$ M), thiorphan (10^{-6} M), captopril (10^{-6} M) and bestatin (10^{-6} M) and was gassed with 95% O_2:5% CO_2 at a temperature of 37 °C. After an equilibration period of 60-90 min the tissues were electrically stimulated (ES) via platinum electrodes positioned for transmural stimulation of the GPI (0.1 Hz, 0.5 msec duration, 40 V) or field stimulation of the RVD (0.1 Hz, 1 msec, 100 V). When stable reproducible contractions of the tissues were obtained (approx. 10 min) BQ123 (10^{-5} M), PD142893 (10^{-5} M) or vehicle was added to the bath for 20 min before the addition of cumulative concentrations of ET-1, ET-3, SX6b or SX6c (10^{-11} to $3x10^{-7}$ M). Changes in twitch tension were calculated as a percentage of the initial stable levels. ET-1 and ET-3 were equipotent (approx. $IC_{50}=1.5x10^{-10}$) at depressing the twitch response of the GPI to ES, indicative of an ETB receptor. Neither PD142893 nor BQ123 affected the depression induced by ET-1. In addition, ET-1 (but not ET-3 up to 10^{-8} M) caused a concentration-dependent increase in the basal tone of the GPI (approx. $EC_{50}=10^{-9}$ M) which was antagonised 30-fold by either PD142893 or BQ123, suggesting that the contraction is mediated by an ETA receptor. In the RVD ET-1 or SX6b caused potentiations of the responses to ES with thresholds of 10^{-10} M whereas the threshold for ET-3 was 10^{-9} M, and SX6c ($3x10^{-8}$ M) had no effect, indicative of an ETA receptor. BQ123 and PD142893 both increased the threshold for ET-1 by 100-fold but did not depress the maximum potentiation by ET-1. SX6b ($3x10^{-8}$ M) potentiated twitch responses by 9-fold but in the presence of BQ123 there was antagonism such that there was only a 1.5-fold potentiation. BQ123 abolished the potentiation induced by ET-3 (10^{-11} to 10^{-7} M).

Thus, in the GPI, the contraction of the smooth muscle is probably mediated by ETA receptors whereas the inhibition of the responses to ES is mediated via non-ETA, possibly ETB receptors. However, the effects of ET/SX peptides on neurotransmission in the RVD are mediated by ETA receptors.

(This work was supported by the Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co. We thank Drs. A. Doherty and W. Cody of the Chemistry Department for providing the ET antagonists BQ-123 and PD142893 ((Ac-(D-Dip-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp)(D-Dip=3,3-diphenylalanine)) for these studies.

Maggi, C.A. et al. (1989). *Eur. J. Pharmacol.*, **174**, *23-31.*

166P CHARACTERISATION OF OPIOID RECEPTORS MODULATING THE FUNCTION OF A POSITIVE CHRONOTROPIC NON-ADRENERGIC, NON-CHOLINERGIC TRANSMITTER IN GUINEA-PIG ATRIA

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It has long been established that opioids are able to modulate the release of autonomic transmitters. An early report (Wilson, 1981) raised the possibility that opioids may inhibit the release of a non-adrenergic, non-cholinergic (NANC) transmitter causing a delayed positive chronotropic response(DPCR) in guinea pig atria. Mantelli *et al*, (1990) recently showed opioid inhibition of a delayed positive ionotropic response in guinea-pig left atria. The NANC transmitter responsible for this response was postulated to be calcitonin gene related peptide (CGRP) and the opioid receptor responsible was designated as belonging to the μ_1 subtype. We have now begun to characterise the receptor responsible for inhibition of the DPCR.

Spontaneously beating atrial preparations were set up in a 10ml organ bath perfused at a rate of 2ml min^{-1} with Krebs -bicarbonate solution at 37°C, gassed with 5% CO_2 in O_2. Tension and rate responses were recorded on a Grass polygraph. After an equilibration period field stimulation (Grass S88 3Hz, 3ms, 65v) was applied to the tissue via two parallel platinum electrodes (0.3mm dia, 12 mm apart) for a period of 18s every 3.7 min. All experiments were performed in the presence of atropine (1μM) and propranolol (1μM).

Following the application of field stimulation a DPCR was observed.The effect of a number of receptor selective opioid agonists on this response was then tested. Cumulative concentration-response curves were constructed, each concentration being perfused over the tissue for a period of 12 min. Of the agonists tested only the μ selective opioids fentanyl and alfentanil (n=5) inhibited the DPCR with EC_{50} values of 1.19 (1.04 - 1.34)μM and 20 (16 - 23)nM respectively. The corresponding maximum inhibitions of the DPCR were 46.8 ± 4.6 % and 68.7 ± 5.5 %.The selective κ agonist CI-977, at concentrations up to 10μM, and the selective δ agonist [D-Ser^2, Leu^5] - enkephalin - Thr^6 (DSLET), at concentrations up to 3μM, both failed to inhibit the DPCR. The effect of the μ agonists was completely reversed in the presence of 1μM naloxone and additionally tetrodotoxin (0.1μM) totally abolished the DPCR to field stimulation.

In conclusion this study shows that μ and not δ or κ selective opioids are able to inhibit specifically the effect or release of a NANC transmitter causing DPCR in guinea-pig atria. The nature of the transmitter remains unknown but it seems likely that it may be CGRP. Furthermore the fact that fentanyl is 60 times less effective than alfentanil might suggest that the μ site is atypical since in μ-binding studies fentanyl is 10 times more potent than alfentanil (Yeadon and Kitchen 1988).

Mantelli, L., Amerini, S., Rubino, A. & Ledda, F. (1990) *Eur. J. Pharmacol.* **180**, 325-330.
Wilson, A.N.A. (1981) *Br. J. Pharmacol.* **73**, 294P.
Yeadon, M. & Kitchen, I. (1988) *Neuropharmacology* **27**, 345-348.

167P VASODILATOR RESPONSES TO CGRP IN THE RAT ISOLATED PERFUSED KIDNEY ARE MEDIATED VIA $CGRP_1$ RECEPTORS

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Calcitonin gene-related peptide (CGRP), a 37 amino acid peptide, is a potent dilator of peripheral vasculature (Brain *et al.*, 1985). With the advent of newly synthesised analogues of CGRP, recent studies have proposed the existence of two CGRP receptor subtypes: $CGRP_1$ and $CGRP_2$ (e.g. Dennis *et al.*, 1989). The aim of this study was to characterise the CGRP receptors mediating vasodilatation in the rat isolated perfused kidney.

Male Wistar rats (150-200 g) were killed by cervical dislocation and exsanguination, and the right renal artery was cannulated at its origin at the aorta, followed by flushing of the vascular bed with heparinised (50 U ml^{-1}) Krebs solution. The right kidney was rapidly removed and placed on an organ-bath and perfused at 4 ml min^{-1} with gassed Krebs solution at 37°C, and changes in perfusion pressure were monitored by a precalibrated pressure transducer and Grass polygraph. After an equilibration period of 15 min, addition of agonists was made by bolus injections (< 10 µl). Antagonists were introduced via the perfusate. Drug-induced effects were measured as changes in perfusion pressure with the preparation precontracted throughout with phenylephrine (0.1 mM). Kidneys were discarded after 4 h because of oedema and changes in sensitivity.

Table 1 Activities of forms of CGRP and amylin

Compound	Relative Activity	-Log EC_{50} (s.e.mean; n)	
hCGRPα	1.00	10.51	(0.18; 9)
hCGRPβ	0.89	10.46	(0.22; 4)
rCGRPα	1.91	10.79	(0.16; 5)
Amylin	0.07	9.38	(0.26; 4)

Various forms of CGRP, and amylin which has been shown to act on CGRP receptors (Chantry *et al.*, 1991), produced dose-related vasodilatation in the perfused renal vascular bed, and the rank order of potency was rCGRP > hCGRPα ≥ hCGRPβ ≫ amylin (**Table 1**). In contrast, the $CGRP_2$ receptor-selective agonist ([Cys(ACM)^{2,7}]hCGRPα) (Dennis *et al.*, 1989) was inactive (0.1 µmol). The $CGRP_1$ receptor antagonist, hCGRPα(8-37) produced a concentration-related antagonism of the vasodilator response induced by hCGRPα with an apparent pK_B of 8.03 ± 0.21 (n=6). The inhibition was reversible after a 60 min perfusion period.

In conclusion, all forms of CGRP tested were approximately equiactive and *c.* ten-fold more potent than amylin. The $CGRP_2$-selective agonist [Cys(ACM)^{2,7}]hCGRPα was inactive, whereas the $CGRP_1$-selective antagonist hCGRPα(8-37) antagonised responses to hCGRPα. These data suggest that the predominant CGRP receptor in the rat renal vascular bed is of the $CGRP_1$ subtype.

Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R. & MacIntyre, I. (1985) *Nature*, **313**;54-56.
Chantry, A., Leighton, B. & Day, A.J. (1991) *Biochem. J*, **277**,139-143.
Dennis, T., Fournier, A., St-Pierre, S. & Quirion, R. (1989) *J. Pharmacol. Exp. Ther.*, **251**,718-725.

Supported by the Wellcome Trust. We thank Dr Ursula Ney of Celltech Limited for gifts of peptides.

168P CHARACTERIZATION OF ANGIOTENSIN RECEPTORS IN THE GUINEA-PIG COLON

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We have previously shown that the contractile responses to angiotensin II (AII) or the non-desensitising analogue, [Lys^2]-AII (Miasiro et al, 1983) in longitudinal muscle strips of the guinea-pig ileum are mediated both by presynaptic AT_1 receptors and by a direct action on AT_1 receptors located on smooth muscle (Hawcock and Barnes, 1992). In the present study, we have characterized the contractile responses to AII and [Lys^2]-AII in the guinea-pig colon. In addition, we have examined the pharmacology of the receptors using radioligand binding studies with [^{125}I]-Sar^{1}Ile^8-AII ([^{125}I]-Sarile).

In functional studies, longitudinal muscle strips of colon from male Dunkin-Hartley guinea-pigs (200-250g) were prepared and mounted in 5ml organ baths under 0.5g tension. Experiments were carried out in modified Krebs buffer, containing 10mM KCl, aerated with 5% CO_2 in oxygen at 37°C. High K^+ Krebs was used in order to reduce desensitisation to AII (Hawcock and Barnes, 1992). Responses were recorded isotonically. Cumulative concentration response curves (CRC) to AII and [Lys^2]-AII were constructed every 60min. Antagonists were applied for 45min, prior to reconstructing the curves. AII and [Lys^2]-AII caused concentration-related contractions (pD_2=8.59±0.16 and 6.66±0.13 respectively (n=5)). The maximum response (Emax) of AII relative to [Lys^2]-AII was 84±3.7% (n=4). Tetrodotoxin (TTX) (100nM) had no significant effect on the response to either agonist (Emax values in the presence of TTX relative to control responses were 99.7±5.2% (n=3) and 100±4.0% (n=3) for AII and [Lys^2]-AII respectively). The AT_1 receptor antagonist losartan (Chiu et al, 1989) (30nM) caused a parallel shift in the concentration response curves to AII (pK_B=8.44±0.02; n=4) and [Lys^2]-AII (pK_B=8.42±0.02; n=4). The sulphydryl reducing agent, dithiothreitol (DTT) (1mM), completely blocked the response to AII (n=3).

Competition binding studies were conducted in membranes prepared from guinea-pig colon using [^{125}I]-Sarile as previously described (Marshall et al, 1991). Specific [^{125}I]-Sarile binding was completely displaced by AII and [Lys^2]-AII. Displacement curves were best fitted using a two site model and indicated the presence of a low and high affinity site for both agonists (AII: Site 1 (64%) pIC_{50}=8.82±0.11, Site 2 (36%) pIC_{50}=6.25±0.1; [Lys^2]-AII: Site 1 (49%) pIC_{50}=8.04±0.1, Site 2 (51%) pIC_{50}=6.17±0.12 (n=3)). Losartan also caused a complete displacement of [^{125}I]-Sarile binding (pIC_{50}=7.3±0.05; Hill coefficient=1.01, n=3). In contrast the AT_2 receptor antagonist PD123177 (Chiu et al, 1989) failed to displace [^{125}I]-Sarile binding (pIC_{50}<5.0; n=3). DTT (10mM) reduced the specific binding of [^{125}]-Sarile by 95%.

These studies show that, in contrast to the ileum, the contractile responses to angiotensin agonists in the guinea-pig colon are mediated solely by a direct action on angiotensin receptors located on the smooth muscle. The effects of the antagonists and DTT in the functional and radioligand binding studies suggest that these are of the AT_1 type.

Chiu, A.T. et al. (1989). Biochem.Biophys.Res.Commun.**165**, 196-203.
Hawcock, A.B. and Barnes J.C. (1992). Br.J.Pharmacol.**105**, 260P
Miasiro, N. et al. (1983). Eur.J.Pharmacol.**87**, 397-406.
Marshall, F.H. et al. (1991). Br.J.Pharmacol. **104**, 425P.

169P ICI D7114 IS A PARTIAL AGONIST IN RAT WHITE ADIPOCYTES

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We have demonstrated previously that ICI D7114 is a potent and selective agonist of the atypical (β_3) adrenoceptor *in vivo*, stimulating oxygen consumption in rats at doses which have minimal effects on cardiovascular parameters (Holloway et al 1991). The maximum response elicited was indistinguishable from that of other β_3 adrenoceptor agonists tested. The current study examined the effect of the acid metabolite of D7114, ICI 215001, which is the primary active species *in vivo*, on isolated white adipocytes.

Adipocytes, isolated by collagenase digestion from rat epididymal adipose tissue, were incubated in the presence of various agonists and antagonists and the rate of lipolysis estimated by the assay of free fatty acids (FFA) released. Concentration-response curves were constructed to isoprenaline, the β_3-adrenoceptor agonist BRL 37344, and ICI 215001 essentially according to published procedures (Wilson et al, 1984). The antagonist effect of ICI 215001 was assessed against BRL 37344 and expressed as a pA_2 value. This was compared to the antagonist effects of the known β_1 and β_2 antagonists CGP 20712A and ICI 118551.
BRL 37344 was a potent agonist (EC_{50} 0.91±0.21nM) with full efficacy relative to the isoprenaline maximum response, whereas ICI 215001 only produced 34.4±1.2% of the maximum isoprenaline response. ICI 215001 competitively antagonised the lipolysis concentration-response curve to BRL 37344 (pA_2 7.26±0.14, slope not significantly different from unity, 1.18±0.13) whereas the β_1 and β_2 adrenoceptor antagonists had little effect at concentrations up to 10 and 3μM, respectively.

The current study has demonstrated that in white adipocytes, ICI 215001 is a partial agonist at the β_3-adrenoceptor. However, in adipocytes isolated from brown adipose tissue, ICI 201651, the racemic form of ICI 215001, stimulates oxygen consumption (EC_{50} 0.01μM) with efficacy equivalent to that of isoprenaline (Stock & Sudera,1988). It is possible that this difference may be a consequence of a difference in receptor reserve between the two tissue types as suggested by the work of Growcott et al, 1992 using rat and guinea pig isolated ileum. Alternatively, a partial lipolytic response may be adequate to elicit maximum thermogenic activity.

Growcott,J.W., Green,M., & Holloway,B.R.(1992) Proc.Br.Pharm.Soc. 9th-11th.Sept., P41
Holloway,B.R., Howe,R., Rao,B.S., Stribling,D., Mayers,R.M., Briscoe,M.G., & Jackson,J.M. (1991) Br.J. Pharmacol 104, 97-104.
Stock,M.J. & Sudera,D (1988) Br.J. Pharmacol 95, 741P
Wilson,C., Wilson,S., Piercy,V., Sennitt,M.V.,& Arch, J.R.S. (1984) Eur. J. Pharmacol 100, 309-319.

170P METHOXAMINE INHIBITS NORADRENALINE RELEASE THROUGH PRE- AND POST-JUNCTIONAL α-ADRENOCEPTOR MEDIATED MECHANISMS IN RAT KIDNEY

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Methoxamine is believed to be a selective α_1-adrenoceptor agonist with no effect on noradrenaline release in the rabbit pulmonary artery (Starke et al., 1975). However, methoxamine may also activate α_2-adrenoceptors in the canine saphenous vein (Guimaraes et al., 1987). The aim of the present study was to investigate the inhibitory effect of methoxamine on noradrenaline release in rat isolated kidney. After incubation with [^{3}H]-noradrenaline, there were 6 stimulations (S1-S6; each at 100 Hz for 0.06 s). Methoxamine and the α_2-adrenoceptor agonist UK 14304 induced a maximal inhibition of the stimulation induced outflow of radioactivity of 83 % (EC_{50}=1.7 μM) and 99 % (EC_{50}=0.005 μM), respectively. Since the stimulation protocol used avoids autoinhibition by endogenous noradrenaline (Bohmann et al., 1992), α-adrenoceptor antagonist affinities (pK_B values) against methoxamine and UK 14304 could be determined. The concentration response curve (CRC) of UK 14304 was shifted to the right by the α_1/α_{2B}-adrenoceptor antagonist prazosin (0.3 μM; pK_B=6.2) and by the α_2-adrenoceptor antagonist rauwolscine (0.1 μM; pK_B=8.3). In contrast, the CRC of methoxamine was shifted to the right by prazosin (0.1 μM) with a pK_B of 7.4. Rauwolscine (0.1 μM) but not rauwolscine (0.01 μM) shifted the CRC of methoxamine. However, pK_B's could not be determined, since rauwolscine (0.1 and 0.01 μM) supressed the maximum response to methoxamine to 46 % and 59 %, respectively. The CRC of methoxamine obtained in the presence of rauwolscine (0.01 μM) was shifted to the right by further addition of prazosin (0.1 μM; pK_B=8.8) as well as by the purinoceptor antagonist 8-(p-sulphophenyl)theophylline (100 μM) and the prostaglandin synthesis inhibitor indomethacin (20 μM). The results suggest that methoxamine inhibits noradrenaline release by activation of postjunctional α_1-adrenoceptors through prostaglandin and purine mediated transjunctional mechanisms. Furthermore methoxamine seems to be a poor agonist at prejunctional inhibitory α_2-adrenoceptors, which can be activated by UK 14304.

Bohmann, C., Schollmeyer, P. & Rump, L.C. (1993) Br. J. Pharmac. (in press).
Guimaraes, S., Paiva, M.Q. & Moura, D. (1987) Naunyn-Schmiedeberg's Arch. Pharmacol. **335**, 397-402.
Starke, K., Endo, T. & Taube, H.D. (1975) Naunyn-Schmiedeberg's Arch. Pharmacol. **291**, 55-78.

171P INVESTIGATION OF THE EFFECT OF PLATELET ACTIVATING FACTOR ON THE RESPONSIVENESS OF GUINEA-PIG DISTAL COLON AND BOVINE TRACHEALIS TO CARBACHOL

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Platelet activating factor (PAF) has been implicated in the pathophysiology of ulcerative colitis and mucosal levels of PAF have been shown to be increased in colitic patients (Eliakim *et al.*, 1988). Its role in the colonic motility dysfunction seen in colitis is unclear. PAF has also been implicated in asthma and a recent study in ferret tracheal smooth muscle has shown PAF-induced cholinoceptor hyperresponsiveness *in vitro* (Webber *et al.*, 1992). The aim of this study was to determine if exposure to PAF also affects the responsiveness of guinea-pig colonic smooth muscle to carbachol (CCh). We have compared this with the effects of PAF on the responsiveness of bovine trachealis to CCh.

Strips of distal colon from Tricolour guinea-pigs (250-350g) were set up in tissue baths for recording of isometric tension from either the longitudinal or circular muscle axis. Cumulative concentration-effect relationships to CCh were constructed both before and after 30 min incubation with and in the continued presence of PAF (1 & 10 μM) or equivalent vehicle. Preincubation of strips, orientated for measurement of longitudinal muscle responses, with PAF (1 & 10 μM) did not displace the CCh concentration-effect curve or affect the maximum response to CCh ($P > 0.05$, n=6). Similiarly, in strips orientated for measurement of circular muscle responses, preincubation with PAF (1 & 10 μM) did not displace the CCh concentration-effect curve or affect the maximum response to CCh ($P > 0.05$, n=5-6). PAF (1 & 10 μM) itself was without effect on the resting tension of longitudinal- and circular-orientated muscle strips.

Strips of bovine trachealis were set up for isometric tension recording. Trachealis were preincubated for 30 min with either PAF (10 μM) or equivalent vehicle before construction of a single cumulative concentration-effect relationship to CCh. The potency of CCh on strips preincubated with 10 μM PAF was not significantly different from those preincubated with the vehicle for PAF ($P > 0.05$, n=6). PAF (10 μM) itself was without effect on the resting tension of trachealis strips.

These results indicate that exposure of the guinea-pig distal colon to PAF does not result in a change in smooth muscle responsiveness to CCh and therefore may not play a role in the altered responsiveness towards spasmogens seen in colonic smooth muscle taken from both colitic patients and from animal models of colitis (Snape & Kao, 1988). The phenomenom of cholinergic hyperresponsiveness seen in ferret trachea upon exposure to PAF (Webber *et al.*, 1992) is absent in bovine trachealis indicating possible species variation in this response.

Supported by the National Association of Colitis and Crohn's Disease

Eliakim, R., Karmeli, F., Razin, E. & Rachmilewitz, D. (1989) *Gastroenterol.* **95,** 1167-1172
Snape, W.J. Jr & Kao, H.W. (1988) *Dig. Dis. Sci.* **33(Suppl 3),** 65S-70S
Webber, S.E., Morikawa, T. & Widdecombe, J.G. (1992) *Br. J. Pharmacol.* **105,** 230-237.

172P A COMPARISON OF THE INHIBITORY ACTIVITY OF MEBEVERINE (MUSCULOTROPIC) WITH CHLORDIAZEPOXIDE (ANXIOLYTIC) AND DESIPRAMINE (ANTIDEPRESSANT) IN GUINEA-PIG INTESTINE

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A wide variety of therapies are used in the management of Irritable Bowel Syndrome (IBS) (Klein 1988), a condition characterised by abdominal pain and altered bowel habit (Kellow & Langeluddecke, 1989). There is much debate as to whether the condition is primarily a central or peripheral disorder. Antidepressants and anxiolytics have been used in clinical trials for IBS and here we compare their pharmacological effects on gastrointestinal muscle with that of the most commonly prescribed spasmolytic IBS therapy, mebeverine.

Male guinea-pigs (300-400g) were either sacrificed and sections of colon or ileum removed for *in vitro* studies or anaesthetised with urethane (1.8g/kg i.p.) for *in vivo* studies. Preparations of either colon or ileum were mounted longitudinally in organ baths containing Krebs solution (NaCl, 133; KCl, 4.7; NaH_2PO_4, 1.3; $NaHCO_3$, 16.3; $MgSO_4$, 0.6; $CaCl_2$,2.5 & glucose 7.7, all mM) gassed with $5\%CO_2/95\%O_2$ and maintained at 37°C. The ability of the drugs to reduce spontaneous colonic motility *in vitro* is measured by calculating the mean frequency and amplitude of contractions before and after addition of drug. Ileal peristalsis is evoked by raising the intraluminal pressure by 2cm H_2O and measuring the height of the resultant peristaltic contraction. Recording of blood pressure and colonic motility in anaesthetised guinea-pigs was carried out by cannulation of the right carotid artery for measuring blood pressure, the left jugular vein for administering drugs and inserting a cannula into a 3cm colonic loop (inflated to 20mmHg with saline) to measure colonic motility. The pressure cannulae were connected to pressure transducers and recordings displayed on an Ormed pen recorder.

Compound	Colonic Motility *in vitro* IC_{50} μM	Ileal Peristalsis *in vitro* IC_{50}μM for ↓ height	Colonic Motility *in vivo* ↓ tone mmHg measured at 4 mg/kg iv	Blood Pressure *in vivo* % ↓ BP measured at 4 mg/kg iv
mebeverine	0.3±0.1	1±0.3	3.4±0.2	48±10 *
chlordiazepoxide	16±7	80±7	1.9±0.5	28±3
desipramine	2±0.5	3±0.7	4.4±1.2	32±4

Table 1 shows the effect of the compounds tested on inhibition of spontaneous colonic motility and ileal peristalsis *in vitro* and the inhibition of blood pressure and colonic motility *in vivo*. Values± sem are from n≥4 animals.
* followed by ↑BP 18±4% (n=3 of 4).

The data presented in Table 1 clearly shows that the anxiolytic and antidepressant compounds have peripheral activity on gastrointestinal tissues which in the case of desipramine is comparable to that of mebeverine. It is suggested that the direct effects of these compounds on gastrointestinal smooth muscle, rather than their central actions, may account for some if not all of their efficacy in IBS treatment.

Kellow, J.E. & Langeluddecke, P.M. (1989) *Med J Aust.* 151, 92-99
Klein, K.B. (1988) *Gastroenterology,* 95, 232-241

173P AN EXAMINATION OF THE ABILITY OF ISOENZYME SELECTIVE PHOSPHODIESTERASE INHIBITORS TO RELAX THE GUINEA-PIG GALL BLADDER *IN VITRO*

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Cyclic AMP and cyclic GMP are established intracellular messengers mediating relaxation through activation of cyclic nucleotide protein kinase, in a variety of smooth muscle systems including that of the gastrointestinal tract. There are at least five phosphodiesterase (PDE) isoenzymes which break down either or both cAMP or cGMP depending on their substrate preference and kinetics. In this study we have investigated the relaxant activity of isoenzyme specific PDE inhibitors as well as direct cyclase or kinase activators in the guinea-pig gall bladder.

Gall bladders removed from male guinea-pigs (300-400g) were bisected longitudinally, mounted in Krebs solution (NaCl, 133; KCl, 4.7; NaH_2PO_4, 1.3, $NaHCO_3$; $MgSO_4$; $CaCl_2$, 2.5 & glucose 7.7, all in mM) in 20ml organ baths at 37°C and gassed with 95%O_2/5%CO_2. Gall bladder tone was raised by addition of carbachol (10μM) added 20 min prior to the construction of a cumulative concentration response curve. Dose intervals were 9min except for sodium nitroprusside (SNP) where they were 1 min and tension recordings were made via Bioscience transducers connected to Ormed pen recorders. Results are expressed as a % of the maximum attainable relaxation ie: baseline, tone. The compounds tested were IBMX (a non-selective PDE inhibitor), forskolin, rolipram (low *Km* cAMP specific PDE inhibitor), SK&F 95654 (a PDE III inhibitor), di-butyryl-cAMP, SNP, zaprinast (a PDE I&V inhibitor) and 8-bromo-cGMP.

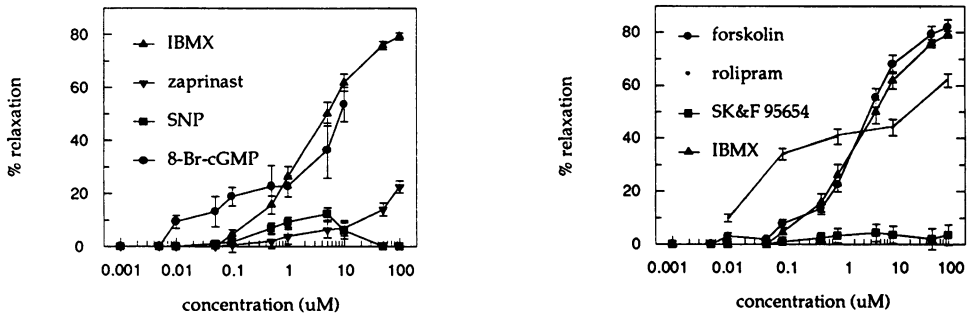


Figure 1 a&b The effect of PDE inhibitors and cyclase and kinase activators on guinea-pig gall bladder tone (n>8 preparations from 4 animals).

IBMX, forskolin, 8-bromo-cGMP and rolipram were potent relaxants. Rolipram and 8-Br-cGMP gave shallow and possibly biphasic concentration response curves. This has previously been reported for rolipram (Harris et al. 1989). Zaprinast, SK&F 95654, SNP, and dibutyryl-cAMP (data not shown) were virtually ineffective up to 100μM. The data suggests that although cAMP- and cGMP- dependent mechanisms do mediate relaxation in the guinea-pig gall bladder, the low *Km* cAMP specific PDE shows the greatest pharmacological sensitivity in this tissue.

Harris, A.L., Connell, M.J., Ferguson, E.W., Wallace, A.M., Gordon, R.J., Pagani, E.D. & Silver, P.J. (1989) *J. Pharmac. Exptl. Ther.* 251, 199-206

174P THE EFFECTS OF SPASMOLYTIC AND SPASMOGENIC PROSTANOIDS ON CYCLIC AMP LEVELS IN HUMAN MYOMETRIUM FROM PREGNANT DONORS

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Prostaglandin (PG)E_2 and the selective EP_2-receptor agonists, AH13205 and butaprost, have been shown to inhibit spontaneous contractions of human myometrium from pregnant donors (Yeardley et al., 1992), whereas, $PGF_{2\alpha}$ and the selective EP_1/EP_3-receptor agonist, sulprostone, cause only excitatory responses (Clayton et al., 1991). The aim of the present study was to investigate whether prostanoids have any effect on levels of cyclic AMP in this tissue.

Tissue samples were obtained from consenting patients undergoing elective Caesarean section at term. Strips of myometrium (5.6±1.8mg wet weight) were allowed to equilibrate for 30 min in oxygenated (95% 0_2/5% $C0_2$) Krebs solution containing indomethacin (2.8μM) and 3-isobutyl-1-methylxanthine (IBMX, 100μM). The uterine strips were then incubated for 4 min with test agonists in Krebs solution, after which, the cyclic AMP levels were determined by RIA, as described by Sumner et al. (1989).

PGE_2 (0.001-10μM), butaprost and AH13205 (both 0.001-100μM) caused concentration-related increases in cyclic AMP levels. A statistically significant elevation ($p<0.05$) over control levels (1.0-1.4pmol cyclic AMP mg protein^{-1} min^{-1}) was observed with PGE_2 and butaprost (1μM) and AH13205 (100μM) of 426±101% (n=7), 270±43% (n=6) and 614±82% (n=6) respectively. At the highest concentrations investigated, PGE_2 (10μM) butaprost and AH13205 (100μM) elevated cyclic AMP levels by 1740±615%, 3194±718% and 614±82% of control respectively. Neither $PGF_{2\alpha}$ (n=6) nor sulprostone (n=5) caused any significant change in cyclic AMP levels at concentrations of up to 10μM.

Therefore, PGE_2, butaprost and AH13205, which are all believed to exert their inhibitory effects on the human myometrium via EP_2-receptors (Yeardley et al., 1992), elevate cyclic AMP levels at concentrations similar to those which cause myometrial inhibition. In contrast, $PGF_{2\alpha}$ and sulprostone, which exert their excitatory effects via FP- and EP_1/EP_3 receptors respectively, do not increase cyclic AMP levels in this tissue. Hence it appears that cyclic AMP may serve as a second messenger to inhibitory EP_2-receptors, but not to excitatory FP-, EP_1- or EP_3-receptors. These findings are consistent with cyclic AMP being a second messenger involved in mediating inhibitory but not excitatory effects in smooth muscle.

Senior, J., Marshall, K., Sangha, R. and Clayton, J.K. (1993). Br. J. Pharmacol. (in press).
Sumner, M.J., Feniuk, W. and Humphrey, P.P.A. (1989). Br. J. Pharmacol., 97, 292-300.
Yeardley, H.L., Coleman, R.A., Clayton, J.K., Marshall, K. and Senior, J. (1992). Br. Pharmacol. Proc. Suppl., 107, 90P.

175P PRELIMINARY STUDIES ON PURINE P_2 RECEPTORS IN RAT SPLEEN

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Recently Bo & Burnstock (1990) have described the use of [^{3}H] α,β methyleneATP as a ligand for the purine P_{2X} receptor. During the course of work with this compound we have found that rat spleen contains high affinity binding sites for [^{3}H]α,β methyleneATP (Michel *et al.*this meeting). This finding prompted us to examine the effects of purine P_2 receptor agonists on rat splenic strips.

Longitudinal strips of rat spleen were prepared as described by Kenakin & Novak (1988) and suspended in modified Krebs solution at 37°C and gassed with 5%CO_2 in oxygen. Four strips were obtained from each spleen. Phenylephrine (300nM-1mM) caused concentration-related contractions of the splenic strips, maximal contraction (320±22mg, n=17) being obtained at 1mM. In the concentration range 3-300μM, α,β methyleneATP also caused concentration-related contractions. A clearly defined maximal response was not obtained, but at the highest concentration tested a contraction 135±21%(n=7) of the maximal response to phenylephrine was obtained. Responses to α,β methyleneATP were unaffected by indomethacin (5.8μM, n=2) or phentolamine (300nM, n=2), suggesting that they were not mediated by release of prostanoids or catecholamines. Splenic strips contracted in response to electrical stimulation (8Hz, 1m sec). These responses were blocked by tetrodotoxin (100 nM n=2) and phentolamine (10nM-10μM, n=2), suggesting that they are mediated by activation of noradrenergic nerves. At a concentration of 300μM, α,β methyleneATP was without effect on responses to nerve stimulation, suggesting that there are no P_2 receptors on the noradrenergic nerves. In addition to α,β methyleneATP, the effects of β,γ methyleneATP, 2-methyl thioATP and ATP were also studied. These compounds also contracted the spleen. All of these compounds were less potent than α,β methyleneATP,and because complete concentration-effect curves could not be obtained full quantitative comparisons of potency could not be made. However, the rank order of potency was α,β,methyleneATP>2-methyl thioATP=ATP>β,γ methyleneATP. Apart from the low potency of β,γ methyleneATP, these results are consistent with the presence of P_{2X} receptors (Burnstock & Kennedy,1985). These compounds also displace [^{3}H] α,β methleneATP binding from high affinity sites in the spleen (Michel *et al.*,this meeting), pIC_{50} values being 8.5±0.2, n=4 (α,β methyleneATP) 8.6±0.07, n=4 (β,γ methyleneATP), 7.2±0.29, n=4 (ATP) and 6.8±0.13, n=9 (2-methyl thioATP). There are similarities between these findings from the binding studies and those obtained in the functional studies. However, there are also two interesting discrepancies. Firstly, the pIC_{50}s are at least two orders of magnitude lower than the threshold concentrations for inducing contraction. Secondly, whilst β,γ methyleneATP has a similar affinity to that of α,β methyleneATP in the binding studies, it is the least potent of the four compounds in causing contraction. Further work will be necessary to explain these discrepancies.

Bo, X., & Burnstock, G. (1990). Br. J. Pharmacol., 101, 291-296.
Burnstock, G. & Kennedy, C. (1985). Gen. Pharmacol. 5, 433-440.
Michel, A.D., Brown, H.C., Sewter, C., Humphrey, P.P.A. & Kennedy, I., this meeting.
Kenakin, T.P. & Novak, P.J. (1988). J. Pharmacol. exp. Ther., 244, 206-212.

176P THE INFLUENCE OF PHOSPHORAMIDON AND AMASTATIN ON THE POTENCY OF SOMATOSTATIN $(SS)_{14}$, SS_{28} AND SOME SYNTHETIC CYCLIC PEPTIDES IN GUINEA-PIG ILEUM AND VAS DEFERENS

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Somatostatin $(SS)_{14}$, SS_{28} and some synthetic cyclic peptides inhibit neurogenically mediated contractions in guinea-pig ileum and vas deferens (Feniuk et al., this meeting) with different rank orders of potencies. In this study we have investigated the effects of the peptidase inhibitors phosphoramidon (1μM) and amastatin (10μM) on the potencies of SS_{14}, SS_{28}, octreotide and (cyclo-D-Trp-Lys-Thr-Phe-Pro-Phe) (peptide 1).

Vas deferens and ileum were removed from male Dunkin Hartley guinea-pigs (about 250g) and mounted in organ baths containing a modified Krebs solution (mM: Na^+ 143.4, K^+ 5.9, Mg^{2+} 0.6, Ca^{2+} 1.3, Cl^- 124.5, $H_2PO_4^-$ 1.2, SO_4^{2-} 0.6, HCO_3^- 25.0, glucose 11.1) for recording isometric tension changes. Transmural stimulation was applied to guinea pig ileum (0.1Hz 0.1ms) continuously and vas deferens (5Hz, 1.5s 0.5ms) every 30s at supramaximal current. Concentration response curves to test compounds were obtained by non cumulative addition before and after addition of phosphoramidon (1μM) and amastatin (10μM). One preparation acted as a control to monitor spontaneous changes in agonist sensitivity.

Phosphoramidon and amastatin had no effect on the potencies of SS_{14}, SS_{28}, octreotide or peptide 1 in guinea-pig ileum. The rank order of potency both in the presence and absence of peptidase inhibitors being SS_{14} = SS_{28} = octreotide = peptide 1. In contrast phosphoramidon and amastatin markedly enhanced responses to SS_{14} in vas deferens. However the rank order of potency, SS_{28} > octreotide ≥ peptide 1 > SS14 remained unchanged.

Effect of phosphoramidon (1μM) and amastatin (10μM) on the inhibitory effect of SS_{14}, SS_{28} and some synthetic cyclic peptides in guinea-pig ileum and vas deferens. Values are mean ± sem or geometric mean (95% confidence limits). CR - concentration ratio.

	Guinea-pig ileum				Guinea-pig vas deferens			
	Control		Phosphoramidon/Amastatin		Control		Phosphoramidon/Amastatin	
	EC50 (nM)	CR	EC50 (nM)	CR	EC50 (nM)	CR	EC50 (nM)	CR
SS_{14}	3.7 (1.9-7.2)	1.33±0.45	3.5 (1.8-6.7)	0.88±0.24	324 (210-498)	1.03±0.28	44 (28-70)	0.18±0.10
SS_{28}	2.8 (0.8-9.7)	1.38±0.58	2.4 (0.6-10.8)	0.75±0.09	8.9 (2.1-38.9)	1.01±0.15	4.8 (4.1-5.7)	0.65±0.16
Peptide 1	4.8 (3.3-6.9)	2.14±0.46	3.6 (1.3-9.8)	2.54±1.13	27.4 (14.9-50.4)	2.40±0.73	27.7 (15.5-49.3)	2.01±0.57
Octreotide	0.7 (0.3-1.4)	1.45±0.20	1.0 (0.3-3.4)	2.48±0.82	17.5 (3.7-84)	1.16±0.50	11.6 (2.4-57)	1.63±0.8

The data suggest that somatostatin receptors mediating the inhibitory effect of SS_{14} in guinea pig vas deferens and ileum may be different and that the activity of endogenous amino peptidases can influence the potency of SS_{14} in vas deferens but not ileum.

Feniuk, W., Humphrey, P.P.A., Humphrey, P.T., Jarvie, E., Roberts, A. and Wu, F. This meeting.

177P TACHYKININ NK_1 RECEPTOR SUBTYPES IN THE RAT URINARY BLADDER

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Recently, the existence of a novel type of septide-sensitive receptor (Septide: [Glp^6Pro^9]Substance P(6-11)) has been described in the guinea-pig ileum (Petitet et al., 1992), which may represent a tachykinin NK_1 receptor subtype. In this study we have examined the rank order of potency of non-peptide [(±)CP 96,345, RP67,580] and peptide [GR82,334] antagonists against selective NK_1 receptor agonists [Sar^9]SP sulfone (SarSP) and Septide in the rat urinary bladder (methods as described in Patacchini et al., 1992). All the NK_1 receptor antagonists competitively (slopes of Schild plot not significantly different from unity) antagonized the response to Septide with the following rank order of potency (in the table pA_2 value and 95 % confidence limits in brackets): RP67,580 > GR82,334 > (±)CP96,345.
The same rank order of potency was found when using SarSP as agonist, but the affinities of antagonists were significantly lower as compared to those measured against septide, as follows: RP67,580 > GR82,334 > (±)CP96,345 (see table).

Antagonist		[Sar^9]SP sulfone	Septide
(±)CP 96,345	pA_2	< 6	6.80 (6.63-6.97)
	slope	---	- 1.05 (0.48 - 1.62)
GR 82,334	pA_2	5.93 (5.82-6.04)	7.01 (6.89-7.13)
	slope	- 0.83 (0.52 - 1.14)	- 0.79 (0.47 - 1.12)
RP 67,580	pA_2	6.99 (6.77-7.20)	7.57 (7.38-7.76)
	slope	-1.24 (0.69 -1.79)	- 0.86 (0.51 - 1.22)

Slopes of Schild plots are shown (95 % confidence limits in brackets).
The activity of SarSP and Septide was unchanged by L 659,877 (3 μM), a selective NK_2 receptor antagonist. With both NK_1 receptor agonists tested, the rank order of antagonist potency (RP67,580>GR82,334>(±)CP96,345) is consistent with the known pharmacological profile of the rat NK_1 receptor (Garret et al., 1991). The greater affinity of all the antagonists against the Septide- than SarSP-activated response strongly suggests the existence of NK_1 receptor subtypes in the rat urinary bladder.

Garret, C., Carruette, A., Fardin, V., Moussaoui, S., Peyronel, J-F., Blanchard, J-C.& Laduron, P.M. (1991). *Proc. Natl. Acad. Sci.USA* **88**, 10208-10212.
Patacchini, R., Santicioli, P., Astolfi, M., Rovero, P., Viti, G. & Maggi, C.A. (1992). *Eur. J. Pharmacol.* **215**, 93-98.
Petitet, F., Saffroy, M., Torrens, Y., Lavielle, S., Chassaing, G., Loeuillet, D., Glowinski, J. & Beaujouan, J.C. (1992). *Peptides* **13**, 383-388.

178P PROSTAGLANDIN TP AND EP RECEPTORS IN THE GUINEA-PIG GALLBLADDER

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Prostaglandins (PGs) have been isolated from gallbladder smooth muscle and indomethacin is known to affect gallbladder motility *in vivo*, prevent gallstones and is effective in relieving biliary pain and preventing its progression to cholecystitis. However, the effects of the naturally occurring PGs on gallbladder contractility are poorly defined. We therefore examined the effects of the five main prostanoid agonists (PGD_2, PGE_2, PGI_2, $PGF_{2\alpha}$ and the thromboxane A_2 mimetic, U46619) on guinea pig gallbladder contractility *in vitro* in order to define their rank order of potency. In addition we investigated how the specific TP and EP_1 receptor antagonists, BAY u3405 (Norel et al, 1990) and AH6809 (Coleman et al, 1985) respectively, modulated prostaglandin induced contraction.
Male guinea pig smooth muscle strips were set vertically in 10ml organ baths, containing oxygenated Krebs' solution at 37°C in the presence of $3x10^{-6}$M indomethacin to prevent endogenous PG synthesis. An initial tension of 1g was applied and changes in isometric tension were measured using a force-displacement transducer. All the PG agonists produced slow sustained contractions (onset 3-5 min, plateau 5-9 min) apart from PGI_2 with produced contractions which steadly declined. The rank order of potency was U46619 >PGE_2 > $PGF_{2\alpha}$ with mean ± s.e. mean pD_2's of 7.97 ± 0.19, 7.26 ± 0.18 and 5.76 ± 0.23 respectively (3 groups significantly different from one another , $p<0.01$, n=6-8). Despite differences in potency between these 3 agonists they produced similar maximum responses. PGD_2 and PGI_2 were far weaker agonists such that their pD_2's could not be estimated. Use of the specific thromboxane/TP receptor antagonist, BAY u3405 ($5 x10^{-8}$mmol/l), produced a significant rightward shift of the U46619 concentration response curve (pD_2 with antagonist = 5.40 ± 0.17, $p<0.01$, n=6) but maximum reponse was not significantly altered. The EP_1 receptor antagonist AH 6809 ($2x10^{-5}$ mol/l) similarly produced a rightward shift of the PGE_2 dose response curve (pD_2 with antagonist = 5.24 ± 0.16, $p<0.01$) but the maximum response was significantly reduced by 39% ($p<0.025$, n=4).
These results indicate that the prostaglandin TP and EP_1 receptors are the predominant subclasses in guinea pig gallbladder smooth muscle. PG's are potent stimulants of gallbladder contraction and PGE_2 and thromboxane A_2 are the most likely agonists under physiological conditions. The thromboxane A_2 mimetic, U46619 exerts its effect via specific TP receptors and EP_1 receptors are involved in the action of PGE_2.

Norel, X., Labat, C., Gardiner, P. & Brink, C. (1990) Adv. Prostaglandin Thromboxane Leukotriene Res. 21, 473-476.
Coleman, R.A., Kennedy, I. & Sheldrick, R.L.G. (1985) Br. J. Pharmacol. 85, 273P.

179P A CHLOROFORM EXTRACT OF THE HERB FEVERFEW BLOCKS VOLTAGE-DEPENDENT POTASSIUM CHANNELS IN SINGLE SMOOTH MUSCLE CELLS DISSOCIATED FROM THE RAT ANOCOCCYGEUS

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Feverfew is a common or garden herb with a history of medicinal use as a febrifuge. Currently it is widely used as an alternative remedy for the treatment of migraine. Previous studies describe a variety of pharmacological actions and in particular, chloroform extracts of leaf have been shown to have complex effects on smooth muscle contractility (Barsby *et al.*, 1992), which may relate to the herb's anti-migraine activity. Here we report the results of an electrophysiological study of the action of a chloroform extract of fresh feverfew leaf (FF) on single smooth muscle cells dissociated from the rat anococcygeus.

Cells were prepared as detailed by McFadzean & England, (1992). Membrane currents were recorded using both the whole-cell and outside-out configurations of the patch-clamp technique.

As described previously, depolarisation of anococcygeus cells from holding potentials negative to -40 mV evoked a time and voltage-dependent outward current (McFadzean & England, 1992). In the presence of FF the current was reduced in a concentration-related manner with an IC_{50}, the concentration required to reduce the peak current by 50%, of 56$\mu g.ml^{-1}$. Under control conditions inactivation of the current on maintained depolarisation was best described by a single exponential with a time constant of 3056 ± 52ms (mean ± s.e.mean, n=4). In the presence of FF (56 μgml^{-1}) a fast component of current decay became apparent such that two exponentials were required to make the best fit, with time constants of 159 ±30ms and 3904 ±872ms (n=4). These data suggested that FF inhibited voltage-dependent potassium current by "open channel" blockade. This was supported by the results of experiments on single channels recorded in outside-out patches which showed that FF induced a flickery block of voltage-dependent potassium channels, characteristic of this mechanism of action (Hille, 1991).

Inhibition of the potassium current by FF may underlie the ability of leaf extracts to contract vascular smooth muscle, which in turn may be relevent to the anti-migraine activity of the herb.

Barsby, R.W.J., Salan, U., Knight, D.W. & Hoult, J.R.S. (1992) *Planta Medica* In the press
Hille, B (1991) *Ionic Channels of Excitable Membranes.* 2nd Edn. Sinauer Associates Inc. Sunderland Mass.
McFadzean, I. & England, S. (1992) *Pflügers Arch.* **421**, 117-124.

180P IS THE BIOCHEMICAL MACHINERY REGULATING NEUROTRANSMITTER RELEASE NEURONE-SPECIFIC?

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Activators of protein kinase C (PKC) markedly increase action potential evoked transmitter release from postganglionic sympathetic neurones in the guinea-pig vas deferens (Fig. 1B) (Wardell & Cunnane, 1993). The aim of the present investigation was to determine whether PKC is involved in the transmitter release mechanism in preganglionic sympathetic neurones.

Intracellular recordings were made from nerve cells in the guinea-pig hypogastric ganglion. Subthreshold excitatory postsynaptic potentials (EPSPs) were evoked by stimulation of the hypogastric nerve trunk about 1.5 cm from the ganglion. A ring electrode positioned midway around the vas deferens was used to activate, antidromically, axons of fibres projecting from the hypogastric ganglion. Excitatory junction potentials (EJPs) were recorded from indididual smooth muscle cells in the vas deferens. In all experiments the protein kinase C activator, phorbol 12,13 - dibutyrate (PDBu) (0.01 - 0.3 μM) had no detectable effect on either the amplitude or the time course of EPSPs (Fig. 1A).



Fig. 1. Very nice traces showing the effect of PDBu on EPSPs and EJPs evoked by single stimuli in the guinea-pig hypogastric ganglion and vas deferens

Thus, the release of acetylcholine from these sympathetic preganglionic nerves appears to be unaffected by PDBu. It would therefore appear that the depolarisation-secretion coupling mechanisms in pre - and postganglionic sympathetic nerve terminals are different. We have recently developed a novel preparation to study the release of acetylcholine at a muscarinic neuroeffector junction on an impulse to impulse basis (Searl & Cunnane, 1992). Here, the early indications are that acetylcholine release can be readily modified by protein kinase C activators and inhibitors (Searl & Cunnane, unpublished). Thus, the biochemical machinery regulating neurotransmitter release appears to be neurone rather than neurotransmitter specific.

Cunnane, T.C. & Searl, T. (1993) *J. Physiol.***459**, 200P
Wardell, C.F. & Cunnane, T.C. (1993) This meeting.

T.S. is a Bristol-Myers Squibb research fellow. A. S. is an MRC scholar

181P IS THERE A β_3-ADRENOCEPTOR IN DOG HINDLIMB VASCULATURE?

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ICI D7114 and BRL 37344 (BRL), the acid metabolite of BRL 35135A, are novel β_3-adrenoceptor agonists (Holloway et al, 1991; Arch et al, 1984). The aim of this study was to characterise the effects of the acid metabolite of D7114 (ICI215001), also a selective β_3-adrenoceptor agonist, and BRL on hindlimb perfusion pressure (HLPP) and heart rate in the anaesthetised, catecholamine-depleted dog.

Male or female Beagle dogs (12-17kg) were treated with syrosingopine to deplete catecholamines (5mg/kg/day for 2 days), anaesthetised with pentobarbitone (30mg/kg iv bolus followed by constant infusion at 1mg/min) and ventilated with room air to maintain normal blood gases. The dogs were vagotomised, heparinised (5000IU/animal) and the right hindlimb was denervated and blood perfused (blood removed from lower aorta and returning to the femoral artery) using a peristaltic pump set to match left carotid arterial pressure. HLPP was measured via a catheter in the right saphenous artery which was extended into the femoral artery.

The effects of ICI215001, BRL and isoprenaline (ISOPREN) on HLPP and heart rate in the presence or absence of β_1 and β_2-adrenoceptor blockade (Betaxolol + ICI 118551, both at 0.2mg/kg iv) are given in table below:

Table 1	ISOPREN (n=5)		ICI215001 (n=5)		BRL (n=4)	
HLPP	Control	+βBlock	Control	+βBlock	Control	+βBlock
ED50(μg/kg iv)	0.077±0.01	4.9±3.0	16.0±4.4	43.0±10.0	9.2±8.0	2.0±1.5
Potency ratio	63.6 (**)		2.7 (NS)		0.2 (NS)	
Max Effect (% ISOPREN)	100	100	26±2.0	31±3.0	46±6.0	56.0±3.0
Heart Rate						
ED50 (μg/kg iv)	0.328±0.02	9.9±1.4	9.8±0.4	824±175	35.0±5.0	1444±331
Potency ratio	30.2 (***)		84.1 (***)		41.2 (***)	
Max Effect (% ISOPREN)	100	100	48.0±2.0	34.0±2.0	69.0±3.0	51.0±3.0

Students t test (unpaired): **, 0.05<p<0.01; ***, p<0.001; NS = not significant

The results demonstrate that all three agonists decreased HLPP and increase heart rate in a dose-dependent manner with ICI215001 and BRL having lower maxima than ISOPREN. The positive chronotropic effects of the agonists were antagonised by β-blockade indicating a direct effect on cardiac β_1 and/or β_2-adrenoceptors. The HLPP effects of ICI215001 and BRL were resistant to β-blockade which suggests that the effect may occur via β_3-adrenoceptors.

Arch, J.R.S. et al (1984). Nature, 309, 163-165.
Holloway B.R. et al (1991). Br. J. Pharmacol., 104, 97-104.

182P EFFECTS OF THE NOVEL POTASSIUM CHANNEL OPENER UR-8225 ON ISOLATED RAT VASCULAR SMOOTH MUSCLE

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Potassium channel openers (PCOs) belong to a novel class of antihypertensive agents. UR-8225 is a newly synthesized naphtalenone derivative (1,2-dihydro-4-(1,2-dihydro-2-oxo-1-pyridyl)-2,2-dimethyl-1-oxonaphthalen-6-carbonitrile) which exhibits potent relaxant properties possibly related to the potassium channel opening activity of the drug (García Rafanell et al., 1992). In this study the effects of UR-8225 have been compared with those of levcromakalim in rat isolated aortae and portal veins.

Isolated aortic rings (3-4 mm in length) and portal vein segments (15 mm in length) were mounted for isometric tension recording in Krebs-Henseleit solution (KS) under 1 g resting tension (34 ºC, 95% O_2 - 5% CO_2). Membrane potential was measured with conventional glass microelectrodes (25-50 MΩ).

UR-8225 and levcromakalim at concentrations < 5 x 10^{-5}M did not modify the resting tension in aortic rings but produced a concentration-dependent inhibition of the spontaneous activity in portal veins segments, the IC_{50}s being 5.1 ± 1.4 x 10^{-8}M and 1.5 ± 0.7 x 10^{-8}M, respectively. Pretreatment with 10^{-6}M glibenclamide competitively inhibited the effects of both drugs. UR-8225 also inhibited the contractions induced by noradrenaline (NA, 10^{-5}M) in portal veins (IC_{50} = 6.9 ± 3.3 x 10^{-6}M). In aortic rings both UR-8225 and levcromakalim relaxed the contractions induced by 30 mM KCl (IC_{50}s = 1.4 ± 0.1 x 10^{-7}M and 9.4 ± 1.3 x 10^{-8}M, respectively) and this effect was competitively antagonized by glibenclamide. However, as previously described for other PCOs, at 10^{-5}M, UR-8225 and levcromakalim inhibited the contractions induced by 80 mM KCl only by 11.7 ± 4.5% and 23.5 ± 4.3%, respectively. Both drugs also inhibited the contractile responses induced by 10^{-5}M NA (IC_{50}s = 2.7 ± 0.5 x 10^{-6}M and 6.6 ± 1.3 x 10^{-7}M, respectively). However, UR-8225 and levcromakalim (10^{-5}M) inhibited only by 8.7 ± 1.2% and 9.6 ± 2.0%, respectively, the contractions induced by 80 mM KCl plus 10^{-5}M NA. Addition of 10^{-5}M UR-8225 hyperpolarized the aortic smooth muscle cell from -50.7 ± 0.7 mV to -66.0 ± 2.0 mV. UR-8225 and levcromakalim (10^{-7}M-10^{-5}M) also inhibited the contractions induced by calcium (2 mM) in the presence of NA in aortic strips previously incubated in calcium-free KS containing 0.03 mM EDTA and 10^{-5}M D600.

In conclusion the present results demonstrated that in rat vascular smooth muscle, UR-8225 exhibits vasodilator responses qualitatively similar to those of levcromakalim. This vasodilator action seems to be mediated via hyperpolarization of the membrane by activation of ATP-sensitive potassium channels.

García Rafanell, J., Rodríguez, R., Cavalcanti, F., Gómez, L.A. & Forn, J. (1992). Rev. Farm. Clin. Exp. Num. esp., 289.

183P 5,6 EET, A RENAL VASODILATOR, IS CONVERTED BY PLATELETS TO VASOCONSTRICTOR PRODUCTS

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In vivo, 5,6 epoxyeicosatrienoic acid (5,6 EET), a cytochrome P450-derived metabolite of arachidonic acid, elicits cyclooxygenase-dependent renal vasoconstriction in the rat (Takahashi et al. 1990). However, as 5,6 EET can be metabolized by platelet cyclooxygenase (Balazy 1991), it is possible that the effects following intra-arterial administration result from conversion by blood platelets to vasoconstrictor products that mask the activity of unchanged 5,6 EET. Thus, we tested the vascular effects of 5,6 EET in the rat, isolated, kidney, perfused at constant flow with Krebs' buffer and in which vascular tone was elevated with phenylephrine (7.5×10^{-7}M) to raise perfusion pressure (PP) from 70-90mmHg to approximately 200mmHg. Under these conditions, 5,6 EET produced dose-dependent vasodilatation with 3, 10 and 30ug reducing PP by 19±4, 49±4 and 70±3mmHg, respectively (n=5). Inhibition of cyclooxygenase with indomethacin (2.8uM; n=5) did not significantly affect the vasodilator response.

Subsequently, we incubated ^{14}C- 5,6 EET (50ug; specific activity 1uCi/mg) with washed, rat platelets (2.5×10^{7}) for 5 min. at 37^{0}C. Eicosanoids were extracted with methanol and separated by reverse-phase HPLC. Three peaks, eluting between 14 and 17 min., were detected by UV absorbance at 235nm which corresponded to the radioactivity in the HPLC fractions. The structure of these products is not known but they have retention times similar to HETEs. The fractions corresponding to these peaks were collected, dried and tested for vasoactivity in the isolated kidney perfused at a pressure of 70-90mmHg. Fractions eluting at 13-14, 14-15, 15-16, and 16-17 min. elevated PP by 5±2, 28±13, 81±11 and 55±16mmHg, respectively, (n=5). In constrast, these same fractions obtained from indomethacin-treated (10uM) platelets elevated PP by only 3±3, 11±9, 19±10 and 15±8mmHg, respectively, (n=5), associated with reduced radioactivity and reduced peak height on UV detection. We also incubated ^{14}C-arachidonic acid with washed, rat platelets. The major peaks of radioactivity following HPLC corresponded to thromboxane, HHT and 12-HETE. In the presence of 10uM indomethacin, platelets converted arachidonic acid to 12-HETE which eluted between 18-19 min., showing that only the cyclooxygenase pathway was affected by indomethacin.

In conclusion, 5,6 EET is a renal vasodilator that can be converted to vasoconstrictor products by platelet cyclooxygenase, results that could account for the renal vasoconstrictor effects of this eicosanoid in vivo.

Takahashi, K., Capdevila, J., Karara, A., Jacobson, H.R., Badr, K.F. (1990) Am. J. Physiol. 258: F781-F789.
Balazy, M. (1991) J. Biol. Chem. 266:23561-23567.

184P THE ADENOSINE A_1 ANTAGONIST DPCPX IS A POTENT PROLIPOLYTIC AGENT IN THE RAT ISOLATED ADIPOCYTE

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DPCPX (1,3-dipropyl-8-cyclopentylxanthine) is an adenosine antagonist with a high affinity (K_i=0.46nM) and selectivity (740-fold) for A_1-receptors (Bruns et al., 1987). We report here that in an in vitro preparation of rat adipocytes, in which adenosine A_1 receptor agonists inhibit lipolysis, DPCPX was prolipolytic in its own right. DPCPX was also tested in vivo in the pithed rat for effects on blood nonesterified fatty acid (NEFA) levels, heart rate and blood pressure.

Adipocytes were isolated from rat epididymal fat pads by collagenase digestion, using the methods described by Stratton et al.(1985). After addition of adenosine deaminase (1 unit/ml), the cells were incubated with noradrenaline (NA), DPCPX or the less potent adenosine receptor antagonist 8-phenyltheophylline (8-PT) for 60min at 37 °C under an atmosphere of 95% O_2/5% CO_2. Glycerol release into the medium was used as an index of lipolytic activity (Stratton et al., 1985). For experiments in vivo, male albino rats (250-300g) were anaesthetised with a mixture of isoflurane (5% in oxygen) and nitrous oxide (ratio 3.1), pithed and respired on room air. A carotid artery was cannulated for the measurement of blood pressure (BP) and heart rate (HR). Drugs were administered via the jugular vein. Cumulative dose-response curves to DPCPX or the A_1-agonist cyclopentyladenosine (CPA)(0.1nmol-3μmolkg^{-1}) were constructed and their effects on HR/BP observed. The effects of DPCPX (0.1 - 1.0μmolkg^{-1}) on the cardiovascular actions of CPA were investigated. For all experiments, n≥4.

In vitro, DPCPX was almost equipotent to NA in stimulating lipolysis and produced a similar maximum response (NA, EC_{50}=2.9[1.5-5.7]x10^{-8}M; DPCPX, EC_{50}=7.0[2.1-14]x10^{-8}M). 8-PT was 180-fold less potent than NA (EC_{50}=5.31x10^{-6}M) and at the highest concentration tested (1x10^{-5}M), evoked 64% of the maximal response to NA. In the pithed rat, CPA produced dose-dependent falls in HR and BP (ED_{50} values=6.33nmolkg^{-1} and 6.68nmolkg^{-1}). DPCPX dose-dependently antagonised these effects, but the highest dose did not affect acetylcholine-induced bradycardia. DPCPX (1.0μmolkg^{-1} i.v. bolus) elevated blood NEFA concentrations from 3.2±0.02x10^{-4}M to 6.1±0.07x10^{-4}M but had no effect on HR or BP.

Thus we have shown that DPCPX, in addition to its properties as an A_1-receptor antagonist, is a potent stimulant of lipolysis. The mechanism of this prolipolytic action was not investigated, but it has been suggested that some methylxanthines may stimulate adenylate cyclase through functional blockade of G_i (Ramkumar and Stiles, 1988). In light of our observations, it is clear that adequate controls must always be performed to establish whether DPCPX has any activity in its own right in a particular tissue, before it can be used a tool for the classification of adenosine receptors.

Bruns, R.F., Fergus, J.H., Badger, E.W., Bristol, J.A., Santany, L.A., Hartman, J.D., Hays, S.J. and Huang, C.C. (1987) Naunyn Schmiedebergs Arch. Pharmacol., 335, 59-63.
Ramkumar, V and Stiles, G. (1988). Biochem. Biophys. Res. Comm., 153, 939-944.
Stratton, G.D., Myles, D.D., Strong, P. and Skidmore, I.F. (1985). Biochem. Pharmacol., 34, 275-279.

185P BRL 49074, A VASCULATURE SELECTIVE POTASSIUM CHANNEL ACTIVATOR (KCA) WHICH BLOCKS THE EFFECTS OF CROMAKALIM IN AIRWAYS

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KCA's represent a novel class of smooth muscle relaxants, the majority of which have demonstrated little selectivity for relaxation of a particular smooth muscle type. BRL 49074 [(±)-N-(4-cyanophenyl)-N'-(1,2,2-trimethylpropyl) thiourea] is a compound structurally related to pinacidil. We have investigated its effects on tone in guinea-pig portal veins (GPPV) and tracheal spiral strips (GPTS) and its ability to modulate cromakalim-induced relaxations in GPTS. These studies have been supported by $^{42/43}K^+$ efflux experiments.

All experiments were performed in Krebs solution bubbled with 95% O_2/5% CO_2. GPPV and GPTS were set up under isometric conditions, 2g tension, in 10ml tissue baths. Spontaneous tone was allowed to develop (GPTS) or induced with KCl (30 or 60mM-GPPV) and BRL 49074 added in a cumulative manner. For the $^{42/43}K^+$ efflux experiments, pieces of trachealis smooth muscle or GPPV were incubated for 60 minutes in Krebs solution containing $^{42/43}K^+$ (37- 74 $MBql^{-1}$). Efflux was then performed by transferring the tissues at 3 min intervals through a sequence of tubes each containing 2ml of Krebs solution. When present, drugs were added between 27 and 39 minutes into the efflux period.

In GPPV, BRL 49074 induced concentration-dependent relaxations of K^+(30mM)- induced tone [IC_{50} with 95% confidence limits: 1.72 (0.73- 4.06)μM, (n= 7)], with an intrinsic activity relative to papaverine of 0.92 ± 0.02. Against high K^+ (60mM)-induced tone, BRL 49074 had little effect. In GPTS, BRL 49074 (20μM) had an intrinsic activity of 0.11 ± 0.04 (n= 4)relative to an isoprenaline maximum and subsequent addition of cromakalim induced only a 24.8 ± 4.4% relaxation.The (+)- and (-)-enantiomers both showed similar effects. In the $^{42/43}K^+$ efflux experiments, BRL 49074 (20μM) induced a 39 ± 8% ($p< 0.01$, n=6) increase in K^+ efflux from GPPV but had no effect in GPTS (4 ± 6%, $p >0.05$, n= 6). Cromakalim (10μM) and BRL 49074 (20 μM) in combination induced a significant (69 ± 17%, $p <0.05$, n= 6) increase in K^+ efflux in GPPV, which was not significantly different from the efflux in this tissue induced by cromakalim alone (148 ± 48%, n= 5). In GPTS, however, BRL 49074 (20 μM) significantly inhibited the cromakalim (10 μM)-induced increase in K^+ efflux (29 ± 16%, n= 6, $p.>0.05$ from controls) compared to the response to cromakalim alone (208 ± 34%, n= 6).

In conclusion, BRL 49074 has the ability to open K-channels in GPPV and induce relaxations of vascular tissue, whereas in GPTS, BRL 49074 inhibits both the relaxations and K^+ efflux induced by the KCA, cromakalim.

Thanks are due to G. Burrell, J.M. Evans, G. Nadler, G. Stemp & A. Vong for the synthesis of these compounds.

186P INDUCTION OF AIRWAY HYPERRESPONSIVENESS INCREASES THE POTENCY OF BW B70C, A 5-LO INHIBITOR, TO PREVENT ALLERGIC BRONCHOCONSTRICTION IN SENSITISED GUINEA-PIGS

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Exposure to ozone (O_3) induces bronchial hyperresponsiveness (BHR) to allergen in guinea-pigs (Yeadon & Payne, 1989). Since BHR is a characteristic feature of asthma we have explored the effect of exposure to O_3 on responsiveness to allergen in sensitised guinea-pigs, and have subsequently determined the ability of the 5-lipoxygenase (5-LO) inhibitor BW B70C (Payne, et al, 1991) to prevent allergen-induced leukotriene (LT)-dependent bronchoconstriction (BC) in normo- and hyper-reactive animals.

Male Hartley guinea-pigs (600-800g at time of challenge) were actively sensitised to ovalbumin (OV; 1mg s.c. and i.p.) and used 3wk later. Animals were exposed to O_3 (3ppm, 2h) during quiet tidal breathing, and under subsequent pentobarbitone anaesthesia, changes in pulmonary inflation pressure (PIP) resulting from inhalation of nebulised OV (0.01-10mg/ml, 5s, one response per animal, n=4-7) were recorded. O_3 exposure produced a significant (17-fold) increase in sensitivity to inhaled OV. For example, BC (cmH_2O increase in PIP, mean±sem) in response to 0.3mg/ml OV after O_3 (28±6) was of similar magnitude to that after 10mg/ml OV in controls (43±6). This is of the same order as the BHR to histamine after O_3 exposure (Yeadon, et al, 1992). In the presence of mepyramine (MEP) and indomethacin (INDO) (2mg/kg and 1mg/kg i.v., respectively, 10min pretreatment), a similar degree of reduction in the magnitude of the BC to inhaled OV was observed in both control and O_3-exposed animals, but the extent of BHR was unchanged (BC responses to 10mg/ml OV in controls=24±4; to 0.3mg/ml after O_3=17±6). The LTD_4 receptor antagonist ONO RS-411 (Nakai, et al, 1988), (1mg/kg i.v., 2min pretreatment) abolished the remaining BC after OV challenge in both groups (BC responses reduced to 1.7±1.3 and 0.6±0.3, respectively), confirming the mediation by LTs of the residual response. In further groups of control and O_3-exposed animals, BW B70C was administered at 0, 0.4, 2.0 and 10mg/kg i.v. 30min prior to OV challenge in the presence of MEP and INDO. BC responses to OV were reduced by BW B70C at 0.4mg/kg (inhibition=15±7%) and near-maximally inhibited at 2.0mg/kg (70±10%; $P<0.01$) in O_3-exposed animals. In contrast, there was no significant effect of 0.4 or 2mg/kg BW B70C in control animals (7±10% and 20±8%, respectively), with significant inhibition only observed at 10mg/kg (72±6%; $P<0.01$), a potency difference of approximately 5-fold. The difference in potency of BW B70C in control and BHR animals was highly significant ($P<0.01$).

Induction of BHR by O_3 exposure in sensitised guinea-pigs thus increases the potency of an inhibitor of 5-LO to prevent LT-dependent allergic BC. The magnitude of the increase in potency makes it unlikely that it can be explained solely by improved penetration of BW B70C into lung tissue after O_3 exposure. We suggest that greater efficacy with 5-LO inhibitors against allergen challenge in volunteer studies might be demonstrated if they are tested in atopic asthmatics with BHR rather than in atopic normals.

Nakai, H., Konno, M., Kosuge, S., et al. (1988). *J. Med. Chem.*, 31, 84-91.
Payne, A. N., Jackson, W. P., Salmon, J. A., Nicholl, A., Yeadon, M. & Garland, L. G. (1991). *Agents & Actions*, 34, 189-99.
Yeadon, M. & Payne, A. N. (1989). *Eur. Resp. J.*, 2, 2995.
Yeadon, M., Wilkinson, D., Darley-Usmar, V., O'Leary, V. J. & Payne, A. N. (1992). *Pulm. Pharmacol.*, 5, 101-112.

187P ROLE OF THE LEUKOCYTE INTEGRIN CD11b/CD18 IN MEDIATING EXPERIMENTAL LUNG INJURY

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It has been shown that LPS induces neutrophil sequestration in the pulmonary circulation *in vivo* (Haslett *et al*, 1987), but this event alone appears insufficient to precipitate lung injury. However, a combination of LPS with the neutrophil activators, C5a and FMLP, produces acute lung injury in rabbits (Worthen *et al*, 1987). Moreover, the injury is neutrophil-dependent (Worthen *et al*, 1987). The leukocyte β_2 integrin CD11/CD18 has been shown to be important in mediating stimulated neutrophil adhesion to endothelium *in vitro* (Smith *et al*, 1989), and seems to be involved in neutrophil-dependent oedema formation in several models *in vivo* (Arfors *et al*, 1987; Tuomanen *et al*, 1989). In the present study, we have established a model of acute lung injury in the mouse and have investigated whether leukocyte adhesion via the CD11b/CD18 adhesion molecule is involved in mediating the injury process.

BALB/c mice (20g) received i.v. LPS (3mg/kg) 2 hours prior to i.v. injection of zymosan (10mg/kg, i.v.) and ^{125}I-human serum albumin (HSA). ^{125}I-HSA was used as a marker of pulmonary oedema formation and its accumulation in lung tissue assessed 30 minutes after administration. The animals were sacrificed and the pulmonary circulation flushed with 15mls of heparinized saline. The activity of ^{125}I-HSA in lung tissue was measured in a gamma counter and compared with that in plasma. Oedema formation was expressed as μl of plasma retained in whole lung. The monoclonal antibody 5C6 directed against murine CD11b (Rosen *et al*, 1987) was injected (1mg, i.v.) 15 minutes before LPS.

In control mice, retention of plasma in lung tissue after 30 minutes was $3.4\pm0.9\mu$l (mean$\pm$SEM, n=6). Injection of LPS alone gave a value of $6.5\pm1.4\mu$l (n=6, n.s.) while the response to zymosan alone was increased to $6.6\pm0.6\mu$l (n=6, $p<0.05$). However, after treatment with both LPS and zymosan, ^{125}I-HSA retention was increased to $12\pm1\mu$l (n=8, $p<0.001$). The effect of 5C6 was investigated in separate groups of animals and the values determined were as follows: saline, $10.2\pm2.9\mu$l (n=5); LPS and zymosan, $21.1\pm3.1\mu$l (n=8, $p<0.05$); LPS and zymosan and 5C6, $12.9\pm2.2\mu$l (n=6, n.s. compared with saline). In the same animals, local oedema formation induced by intradermal injection of murine zymosan-activated plasma (ZAP) was inhibited by 51% by i.v. 5C6.

Our results indicate that blockade of CD11b/CD18 leukocyte adhesion molecule inhibits plasma protein extravasation into lung interstitium and so is a necessary component in this model of neutrophil-dependent lung injury.

We are grateful to the Ministry of Defence and National Asthma Campaign for their support. We would also like to thank Celltech, U.K. for their generous gift of 5C6.

Arfors,K.E.,Lundberg,C.,Lindbom,L.,Lundberg,K.,Beatty,P.G.,Harlan,J.M., (1987) Blood 69,338.
Haslett,C.,Worthen,G.S.,Gilclas,P.,Morrison,D.,Henson,J.E.,Henson,P.M., (1987) Am.Rev.Resp.Dis., 136,9-18.
Rosen,H.,Gordon,S., (1987) J.Exp.Med., 166,1685-1701.
Smith,C.W.,Marlan,S.D.,Rothlein,R.,Toman,C.,Anderson,D.C., (1989) J.Clin.Invest. 83,2008.
Tuomanen,E.I.,Saukkonen,K.,Sande,S.,Cioffe,C.,Wright,S.D., (1989) J.Exp.Med. 170,959-968.
Worthen,G.S.,Haslett,C.,Rees,A.J.,Gumbay,R.S.,Henson,J.E.,Henson,P.M., (1987) Am.Rev.Resp.Dis. 136,19-28.

188P CHARACTERISATION OF THE RECEPTOR MEDIATING THE STEROIDOGENIC AND MITOGENIC EFFECTS OF ANGIOTENSIN II IN BOVINE ADRENOCORTICAL ZONE CELLS IN PRIMARY CULTURE

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Angiotensin II (AII) stimulates cortisol secretion from bovine adrenocortical zona fasciculata/reticularis (zfr) cells in primary culture through the activation of phosphoinositidase C (PIC). In addition, AII stimulates [^{3}H]thymidine incorporation into the DNA of bovine adrenocortical cells. We have characterised the AII receptor subtype mediating these responses using selective non-peptide antagonists.

Both saralasin and DuP753, a selective AT1 receptor antagonist, dose-dependently inhibited AII (10^{-8}M)-stimulated cortisol secretion from primary cultures of bovine zfr cells, with ID_{50} values of 1.2×10^{-7}M and 3.1×10^{-6}M respectively. In [^{3}H]inositol-labelled cells, activation of PIC (as measured by the accumulation of total [^{3}H]-labelled phosphoinositols) by AII (10^{-8}M) was also inhibited by saralasin and DuP753 (ID_{50} values = 5.1×10^{-8}M and 4.9×10^{-7}M respectively). Furthermore, saralasin and DuP753 inhibited AII-stimulated [^{3}H]thymidine incorporation into zfr cells with ID_{50} values of 3.2×10^{-7}M and 5.0×10^{-8}M respectively. In contrast, PD123177, an AT2-selective antagonist, was without effect on AII (10^{-8}M)-stimulated cortisol secretion, PIC activation, or [^{3}H]thymidine incorporation at concentrations up to 10^{-5}M PD123177, suggesting that the AT1 receptor mediates both the steroidogenic and mitogenic effects of AII in zfr cells.

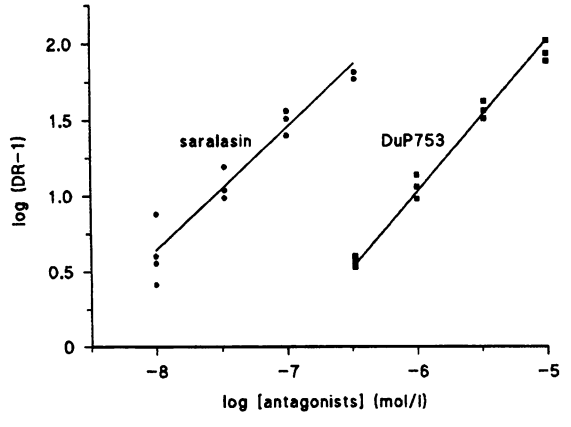


Figure 1. Schild analysis of the antagonism of AII-stimulated cortisol secretion by saralasin and DuP753

Schild analysis of the antagonism of AII-stimulated cortisol secretion by saralasin and DuP753 (Figure 1) gave pA_2 values of 8.79 ± 0.38 (95% CL) and 7.02 ± 0.13 (95% CL) respectively. While the pA_2 for saralasin agreed well with previous observations in other systems, that for DuP753 was 30-fold lower than that previously reported for inhibition of AII-stimulated contraction of rabbit aorta (Chui *et al* 1990, Liu *et al* 1992), suggesting that the DuP753-sensitive AT1 receptors present in these tissues may be different. These findings corroborate recent reports of heterogeneity of AT1 receptors between vascular smooth muscle and adrenal cortex, based both on functional data (Capponi *et al* 1991) and cloning studies (Kaker *et al* 1992).

Capponi *et al* (1991). *J. Recept. Res.* **11**, 641-651
Chui *et al* (1990). *J. Pharm. Exp. Ther.* **252**, 711-718.
Kakar *et al* (1992) *Biochem. Biophys. Res. Comm.* **183**, 1090-1096.
Liu *et al* (1992). *Br. J. Pharmacol.* **106**, 233-241.

189P THE EFFECT OF CALCIUM CHANNEL BLOCKADE ON THE AUTOREGULATION OF RENAL CORTICAL AND PAPILLARY PERFUSION OF WISTAR AND 2K1C GOLDBLATT HYPERTENSIVE RATS

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Autoregulation of blood flow through the kidney is dependent on the myogenic properties of the renal resistance vessels, the afferent and efferent arterioles. The calcium channel antagonists act at vascular smooth muscle to cause a vasodilation and there is evidence that they act preferentially at the afferent arteiole. The aim of the present study was to determine the influence of nitrendipine on autoregulation of blood perfusion through the cortex and papilla of normotensive and hypertensive rats.

2K1C Goldblatt hypertensive rats were generated from male Wistar rats (150- 180 g) by placing a silver clip on the right renal artery 4 weeks before use. Papillary windows were created in the left kidney of fluothane anaesthetised Wistar (n=15) and the 2K1C rats (n=23). Seven days later anaesthesia was induced with pentobarbitone and cannulae placed in jugular and femoral veins for drug infusion and carotid and femoral arteries for blood pressure measurement. The left kidney was placed in a cup with its dorsal surface uppermost and papilla exposed. Renal perfusion pressure (RPP) was manipulated by tightening or loosening ligatures placed around the aorta above and below the left renal artery. Saline (33µl/100g/min) was infused during preparation and was then changed to one containing adrenal hormones and vasopressin (Roman & Cowley, 1985). Laser-Doppler flowmetry was used to measure cortical and papillary perfusion (Huang et al, 1991). Measurements were taken as RPP was reduced in 10 mmHg steps from the maximum to 50 mmHg in the absence or presence of nitrendipine at either 0.125 or 0.25 µg/kg/min, iv. An autoregulatory index (AI) was determined as: AI = [(perfusion1-perfusion2)/perfusion1]/[(RPP1-RPP2)/RPP1]. The values 0 and 1 represent perfect or no autoregulation respectively. Data (mean±S.E.M.) were compared using ANOVA and Student's "t" test.

Over the pressure range 160-100 mmHg in Wistar rats, the cortical AI was 0.28±0.04 which was not significantly different from zero but during infusion of both doses of nitrendipine the values were similar to one, indicating autoregulation had been inhibited. The papilla did not autoregulate perfusion and was not affected by nitrendipine. The cortical AI in the 2K1C Goldblatt hypertensive rats from 190 - 130mmHg was 0.20±0.13 in the absence and 0.35± 0.12 in the presence of the lower dose, of nitrendipine but it was significantly ($P<0.05$) higher, at 1.13±0.04, following the higher dose of the drug. The papillary AI's of 2K1C rats were 1.34±0.11 in the absence and 1.18±0.11 and 1.35±0.15, respectively in the presence of the lower and higher dose of drug. The cortical and papillary AI's of Wistar, from 100-50 mmHg, and of 2K1C rats, from 130-50 mmHg, were not significantly different from 1 irrespective of whether nitrendipine was present.

The results showed that there was good autoregulation in the cortex over the normal physiological range in Wistar rats and over a slightly higher range in the 2K1C Goldblatt hypertensive rats. Cortical autoregulation was markedly attenuated by the lower dose of nitrendipine in the Wistar but not the 2K1C rats, while the higher dose of nitrendipine blocked autoregulation in both normotensive and hypertensive rats. Thus, the renal vasculature of the 2K1C rats is relatively insensitive to the nitrendipine.

References
Roman, R. J. & Cowley, A. W. (1985). *Am. J. Physiol.* **248**, F190-F198.
Huang, C., Davis, G. & Johns E. J. (1991). *Exp. Physiol.* **76**, 399-408.

190P OUABAIN IMMUNIZATION DOES NOT AFFECT NATRIURESIS OR PREVENT THE DEVELOPMENT OF HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Immunoreactive endogenous cardiac glycoside-like substances may have a role in natriuresis and the development of hypertension, by acting via inhibition of Na^+/K^+-ATPase (Graves & Williams, 1987). It has been reported that one of these substances, termed ouabain-like compound (OLC), may be indistinguishable from ouabain (Hamlyn *et al*, 1991). To examine this hypothesis we actively immunized spontaneously hypertensive rats (SHRs) against ouabain and subsequently monitored blood pressure and natriuresis.

One group of weanling SHRs (immunized) were injected (0.1 ml; i.m.) with a 1:1 emulsion of complete Freund's Adjuvent and ouabain-chicken albumin conjugate (2 mg/ml). With the other group (control) the conjugate was replaced by chicken albumin alone. Subsequent booster injections (0.2 ml) were given with incomplete Freund's Adjuvent. Ouabain-specific antibodies in serum were measured using an enzyme-linked immunosorbent assay. Titre was calculated as the reciprocal of the serum dilution giving an absorbance of 0.5 at 405 nm. Blood pressure was measured by the tail-cuff method. Urinary Na^+ and K^+ was assayed using a flame photometer. Means ± s.e. mean are given.

Immunization produced peak titres of 33,000 ± 11,000; the peak, mean titre in control rats was < 350. Systolic blood pressure increased with age, levelling out at 18 weeks at about 200 mmHg, but there was no significant difference between immunized and control groups. Although Na^+ and K^+ excretion decreased between 8 and 12 weeks of age, subsequent elimination stabilised and there was little change during the 12 to 16 week period. Values in immunized and control groups were similar.

Possible reasons for a lack of difference between the groups may include: insufficient cross-reactivity of the antibodies with an ouabain-*like* substance; increased production of OLC via a feedback mechanism caused by neutralization of existing OLC, so that effective OLC depletion does not occur; or lack of any role for OLC in natriuresis or the aetiology of hypertension in SHRs.

Graves, S.W. & Williams, G.M. (1987) Ann. Rev. Med. *38*, 433-444.
Hamlyn, J.M., Blaustein, M.P., Bova, S., DuCharme, D.W., Harris, D.W., Mandel, F., Mathews, W.R. & Ludens, J.H. (1991) Proc. Natl. Acad. Sci. USA, *88*, 6259-6263.

191P ANTIHYPERTENSIVE EFFECTS OF THE ANGIOTENSIN RECEPTOR ANTAGONIST, BIBR 277, IN CONSCIOUS RENAL HYPERTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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BIBR 277 is a potent, specific antagonist of angiotensin II *in vitro* (Entzeroth et al.,1993). We have examined its antihypertensive effects in conscious chronically-instrumented renovascular (2 kidney, 1 clip) (RH) and spontaneously hypertensive (SH) rats after oral administration.

Male RH rats (Chbb:THOM, about 250 g) and male SH rats (Mollegaard,Danmark, about 250 g) were implanted with a chronic-use pressure transmitter (TA11PA-C40) in the abdominal aorta under pentobarbitone sodium anaesthesia. After instrumentation with the chronic-use device, the animals were housed individually with free access to food and water. Blood pressure and heart rate were transmitted by telemetry and the signals were received by an RA 1010 General Purpose Receiver (Data Science Int., St.Paul, MN). Data acquisition was performed with DataQuest IV 1.11. BIBR 277 was dissolved in an aqueous solution consisting of diluted NaOH plus ß-cyclodextrin (1.8 w/v%) (pH=10) and applied orally by gavage. Data presented are mean values ± s.e.mean.

A single oral administration of 3 mg/kg of BIBR 277 to conscious RH rats produced a reduction in mean arterial blood pressure (MAP) by maximally -23 ± 7 mm Hg (n=4). The effects lasted for at least 24 hours. Repeated oral administration of 0.3 mg/kg BIBR 277 b.i.d. over 4 days caused a maximal reduction in MAP of -37 ± 12 mm Hg (n=4). Administration of the vehicle (n=4) had no effects on MAP. Heart rate was not influenced by the vehicle and BIBR 277. Repeated oral administration of 1 mg/kg BIBR 277 b.i.d. over the same period caused a maximal reduction in MAP of -68 ± 12 mm Hg (n=5) with no effects on heart rate. The vehicle (n=3) did not affect MAP and heart rate. Oral administration of 1 mg/kg BIBR 277 once daily over 4 days to conscious SH rats reduced MAP by maximally -22 ± 1 mm Hg (n=4) without an effect on heart rate. A higher dose of 3 mg/kg BIBR 277 once daily over 4 days reduced MAP of SH rats by maximally -38 ± 4 mm Hg (n=3). No effects on heart rate were observed. The vehicle (n=5) did not influence MAP and heart rate of the SH rats.

These results demonstrate antihypertensive activity of BIBR 277 after oral administration in two models of hypertension, (high renin) renovascular hypertensive rats and (normal renin) spontaneously hypertensive rats.

Entzeroth,M., Hauel,N., van Meel,J.C.A., Haigh,R. & Wienen,W. **This Meeting.**

192P SUBTYPES OF α_1-ADRENOCEPTORS MEDIATING CONTRACTION OF FEMORAL ARTERIES AND AORTAE IN SPONTANEOUSLY HYPERTENSIVE RATS

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α_1-Adrenoceptors (AR) have been divided into α_{1A} and α_{1B} subtypes (Minneman, 1988). In addition, molecular cloning studies have found a third α_1-AR subtype (α_{1C}-AR) in bovine brain (Schwinn et al., 1990). There is controversial evidence that the sympathetic nervous system is involved in the development of hypertension in spontaneously hypertensive rats (SHR). This study was undertaken to determine which α_1-AR subtypes were involved in the activation of rat aortic and femoral arterial preparations by α_1-AR agonists and ascertain whether α_1-AR subtypes mediating contraction in the vascular preparations of SHR were different from those in Wistar Kyoto rats (WKY). Helically cut strips of the femoral artery and aorta were suspended under optimal resting tension (femoral, 500 mg and aortic, 1 g) in physiological salt solution containing 0.2μM yohimbine, 2 μM propranolol and 0.2 μM desipramine. Contractile responses were recorded isometrically (Fujimoto & Matsuda, 1988). Noradrenaline, phenylephrine and methoxamine contracted the femoral and aortic strips in a dose-dependent manner with relative potencies of 100:27:2 in the WKY femoral artery and 100:10:0.5 in the WKY aorta. Relative potencies of these agonists in the SHR preparations were similar to those in the WKY tissues. WB 4101 at 10^{-9} M inhibited the agonist activity of phenylephrine and methoxamine and thus decreased their negative log EC_{50} values by 0.3-0.6 (log unit) in WKY and SHR blood vessels, and there was no marked difference in the antagonistic activity of 10^{-9} M WB 4101 between WKY and SHR. In WKY, Schild plot data for bunazosin, WB 4101 and 5-methylurapidil using noradrenaline as an agonist indicated that subtypes of α_1-AR mediating contraction of the femoral and aortic strips had high affinities of bunazosin (pA_2; 9.42 and 9.32) and WB 4101 (pA_2; 9.15 and 9.31) and a low affinity for 5-methylurapidil (pA_2; 7.62 and 7.74). The slopes of the Schild plot for WB 4101 and 5-methylurapidil were unity in the aorta and less than unity in the femoral artery of WKY (0.88 ± 0.11 for WB 4101 and 0.80 ± 0.11 for 5-methylurapidil). Schild plot data in the SHR preparations were similar to those in the WKY tissues. In conclusion, the predominant α_1-AR subtypes mediating contraction in the femoral artery and aorta were distinct from α_{1A} and α_{1B} subtypes. α_1-AR were heterogeneous and homogeneous in the femoral artery and aorta, respectively. α_1-AR subtypes in the SHR and WKY blood vessels were identical. Thus, it was suggested that hypertension did not alter subtypes of α_1-AR mediating contraction in the SHR blood vessels.

Fujimoto, S. & Matsuda, T. (1988) J. Pharmacol. Exp. Ther. 244, 716-723.
Minneman, K.P. (1988) Pharmacol. Rev. 40, 87-199.
Schwinn, D.A., Lomasney, J.W., Lorenz, W., Szklut, P.J. et al. (1990) J. Biol. Chem. 265, 8183-8189.

193P THE EFFECT OF AGEING AND HYPERTENSION ON CONTRACTION AND ENDOTHELIUM-DEPENDENT RELAXATION IN THE AORTA OF SPONTANEOUSLY HYPERTENSIVE RATS

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Increasing age and hypertension are both major risk factors for the development of cardiovascular disease. The effect of these risk factors on vascular reactivity as such are well known. However, the influence of simultaneous hypertension and ageing in vascular reactivity have not been studied in detail. The present study was undertaken to evaluate the effect of hypertension and increasing age on the α_1-adrenoceptor mediated contraction and on the endothelium-dependent relaxation provoked by methacholine (MCh). We studied the effect of drugs on isolated aorta preparations taken from: normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) of 18 and 28 weeks of age, and 18 weeks old spontaneously hypertensive stroke-prone rats (SHRSP). The thoracic aorta was carefully excised, cut in segments of approximately 3 mm long and placed in a 20 ml organ bath of 37°C containing a physiological salt solution, gassed with 95% O_2 and 5% CO_2. The aortic rings were connected to an isometric force transducer and equilibrated for 60 min at a resting tension of 1.0 g. Cumulative concentration-response curves for phenylephrine (PhE) and methacholine (MCh) were constructed.

Table 1 pD_2-values of the concentration-response curves of isolated aortic rings to PhE and MCh.

	WKY 18 weeks	WKY 28 weeks	SHR 18 weeks	SHR 28 weeks	SHRSP 18 weeks
pD_2 (PhE)	6.61 ± 0.04	6.35 ± 0.08	6.62 ± 0.07	6.54 ± 0.09	6.55 ± 0.08
pD_2 (MCh)	7.28 ± 0.04	7.34 ± 0.10	7.17 ± 0.01	7.35 ± 0.05	6.94 ± 0.13

Ageing (18 to 28 weeks) caused a decrease in the sensitivity to PhE contraction in the rat aorta. In preparations of 28 weeks old WKY the pD_2 significantly decreased compared to those obtained from the 18 weeks old WKY. This was not found for the 18 and 28 weeks old SHR: there were no significant alterations in pD_2 and maximal response between 18 weeks old WKY, SHR and SHRSP. Aortic ring preparations from SHRSP were less sensitive to MCh stimulation compared to those obtained from age-matched WKY and SHR. Similarly a significant decrease of the pD_2 was found for the SHR compared to the age-matched WKY. No significant age-related changes in responsiveness to MCh were observed between preparations from 18 and 28 weeks old WKY.

In conclusion, aortic ring preparations of 28 weeks old WKY are less sensitive to α_1-adrenoceptor mediated contraction compared to 18 weeks old WKY. Hypertension *per se* does not affect the contraction caused by PhE. Endothelium-dependent relaxation appears not to be affected by ageing. However, hypertension *per se* decreases the sensitivity to MCh.

194P THE INFLUENCE OF 8-SULPHOPHENYLTHEOPHYLLINE ON THE CARDIOVASCULAR AND RESPIRATORY RESPONSE TO SYSTEMIC HYPOXIA IN THE RAT

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In the spontaneously breathing rat, systemic hypoxia produces an initial hyperventilation and tachycardia which wane to secondary hypoventilation and bradycardia, a fall in arterial pressure, vasodilatation in muscle and cerebral circulation, but with a secondary fall in cerebral blood flow when ventilation, heart rate and arterial pressure reach their lowest levels. The adenosine receptor antagonist 8-phenyltheophylline greatly attenuated the secondary hypoventilation, bradycardia and the muscle and cerebral vasodilatation (Marshall & Thomas,1993) suggesting that they may be due to the central depressant effect of adenosine upon respiration and the local effects of adenosine on the sino-atrial node and vasculature. However, these changes may be interdependent so that blockade of one component may automatically attenuate the others. To study this further, we have used 8-sulphophenyltheophylline (8-SPT), an adenosine receptor antagonist that does not cross the blood-brain barrier (Daly,1982).

In 12 rats anaesthetised with Saffan (7-12 mg/kg/hr i.v.), cerebral and femoral blood flow were measured with electromagnetic probes, from the carotid artery with all but the internal carotid ligated and from the femoral artery. Breathing 8% O_2 for 10 min evoked a response comparable to that described above. 8-SPT (20 mg/kg i.v.) reduced the hypoxia-induced fall in arterial pressure at the 5th min (-33±5 vs -42±4%, $P< 0.05$ paired t-test) and 10th min (-35±4 vs -38±4%) and the increase in femoral vascular conductance at the 10th min (160±38 vs 229±48%) but 8-SPT had no significant effect on either the secondary hypoventilation or secondary bradycardia. These results support the view that adenosine mediates the muscle vasodilatation by a local action and the secondary hypoventilation by an action at a central site, but suggest that the secondary bradycardia may be due to the secondary hypoventilation rather than due to a local action of adenosine (Marshall & Thomas,1993).

T.T. holds a B.H.F. studentship

Marshall & Thomas (1993) J. Physiol (In Press) Proceedings Cambridge Meeting
Daly (1982) J. Med. Chem. 25 :197-207

195P EFFECT OF BQ123 ON REGIONAL HAEMODYNAMIC RESPONSES TO BIG ENDOTHELIN-1 IN ANAESTHETISED RATS

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The endothelins (ETs) have multiple specific binding sites through which they may exert their effects. One site has a high affinity for ET-1 but a low affinity for ET-3 and has been designated ET_A (Arai et al., 1990). Another site has equal affinity for both types of endothelin and has been designated ET_B (Sakurai et al., 1990). The location and function of these subtypes is controversial. In this study the selective ET_A receptor antagonist BQ123 (Ihara et al., 1992) has been used to examine the possible mechanism by which big ET-1, the precursor of ET-1, exerts its action on different regional vascular beds.

Alderley Park rats (300-360g) were anaesthetised with Intraval (100mg kg^{-1} iv) and the right jugular vein and carotid artery were cannulated for administration of drugs and measurement of mean arterial pressure (MAP) respectively. Heart rate (HR) was derived from the pressure signal. Regional blood flow was assessed using pulsed Doppler flow probes. BQ123 was administered 1 min prior to administration of big ET-1 and changes in MAP, HR and iliac (IC), renal (RC) and mesenteric (MC) conductance calculated.

Table 1. Effect of BQ123 on cardiovascular responses to big ET-1 (15 min post-administration, n=5-6).

	ΔMAP (mmHg)	ΔHR (min^{-1})	% ΔIC	% ΔRC	% ΔMC	
Big ET-1 (0.3 nmol kg^{-1})	12 ± 1	-17 ± 6	-21 ± 4	20 ± 4	-15 ± 5	
+ BQ123 (0.3 mg kg^{-1})	2 ± 4*	-12 ± 3	-12 ± 6	24 ± 14	-19 ± 5	* p<0.05
Big ET-1 (3.0 nmol kg^{-1})	54 ± 2	-39 ± 3	-72 ± 3	-29 ± 5	-56 ± 3	unpaired
+ BQ123 (0.3 mg kg^{-1})	19 ± 3*	-38 ± 8	-51 ± 2*	-3 ± 4*	-48 ± 7	Students
+ BQ123 (1.0 mg kg^{-1})	10 ± 9*	-10 ± 18	-38 ± 9*	-11 ± 11	-54 ± 5	t-test

Big ET-1 induced dose-related increases in MAP and decreases in HR, IC and MC. BQ123 attenuated the change in MAP and IC but not MC. The low dose of big ET-1 induced an increase in RC which was unaffected by BQ123, whereas the high dose induced a decrease in RC which was attenuated by BQ123.
The ability of BQ123 to inhibit big ET-1 induced increases in MAP and decreases in IC and RC suggest the involvement of ET_A receptors in these responses. The failure of BQ123 to inhibit the decrease in MC and increase in RC suggests the involvement of receptors other than ET_A.

Arai et al., (1990) Nature, 348, 730-732.
Sakurai et al., (1990) Nature, 348, 732-735.
Ihara et al., (1992) Life Sci., 50, 247-255.

196P CHANGES IN β-ADRENOCEPTOR-MEDIATED EFFECTS ON CYCLIC AMP LEVELS AND ON NA-INDUCED TONE AND PHOSPHATIDYLINOSITOL HYDROLYSIS IN AORTIC RINGS FROM HYPERTENSIVE RATS

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There is evidence that isolated aortic rings from spontaneously hypertensive rats (SHR) are less sensitive to vasoconstrictors and more sensitive to vasorelaxants that increase the synthesis of cyclic GMP (Sharifi et al, 1990; Young et al, 1992). This study compared the sensitivities of isolated aortic rings from SHR and Wistar Kyoto (WKY) normotensive controls to isoprenaline (ISO), whose effects on NA-induced tone, on phosphatidylinositol (PI) hydrolysis and on cyclic AMP levels were examined.

Blood pressure (BP) measured by the tail cuff method in conscious rats was higher (P<0.001, Student's t-test) in SHR (190 ± 2 mm Hg [mean ± s.e. mean] n=12), than in WKY rats (109 ± 3, n=12). Aortic rings (3-4 mm) were suspended under an initial tension of 2 g in 25 ml organ baths containing Krebs buffer (37°C), which was gassed with a mixture of 95% O_2 and 5% CO_2. Concentration curves to NA (10^{-10}-10^{-3} M) and in the presence of NA (5.6 x 10^{-9} M), to ISO (10^{-9}-10^{-6}) were obtained in rings from SHR and WKY rats. NA-induced PI hydrolysis was measured in aortic rings labelled with (myo-^{3}H)-inositol (8 μCi ml^{-1}) and then incubated in lithium chloride (10 mM) and NA (6.2 x 10^{-6}) for 1 hour. Inositol phosphates were extracted, separated chromatographically on Dowex formate resin and measured by liquid scintillation counting. In separate studies, cyclic AMP was extracted from homogenised rings in trichloroacetic acid (5%). These extracts were washed with water-saturated ether and their cyclic AMP content was measured by radioimmune assay.

Low concentrations of ISO (5 x 10^{-10}-10^{-8} M) induced smaller inhibitions of NA-induced tone in rings from SHR than in those from WKY rats but at higher concentrations there was no difference in the response to ISO. In rings from WKY rats, NA increased basal PI hydrolysis from 184.3 ± 19.7 (n=8, d.p.m. mg^{-1} tissue) to 572.2 ± 47.7 (n=8, P<0.001) and this response was inhibited by ISO to 370.5 ± 15.8 (n=8, 0.01>P>0.001). In rings from SHR, NA increased basal PI hydrolysis from 210.5 ± 41.7 (n=8) to 828.1 ± 29.7 (n=8) and this response was inhibited by ISO to 670.2 ± 16.6 (n=8). In rings from WKY rats, ISO increased cyclic AMP levels from 1100 ± 150 (n=8, p mol g^{-1}) to 3500 ± 30 (n=8, P<0.001) but in rings from SHR, ISO only increased cyclic AMP levels from 1000 ± 135 (n=8) to 2000 ± 220 (n=8, P<0.001). These results indicate that smooth muscle from SHR is less responsive to ISO than is that from WKY rats.

Sharifi, A.M., Morrison, K.J. & Pollock, D. (1990) Br. J. Pharmacol. 101, 533P.
Young, D.A., Aidulis, D.M. & Pollock, D. (1992) Br. J. Pharmacol. 107, 189P.

197P THE FUNCTIONAL ROLE OF α_1-ADRENOCEPTOR SUBTYPES IN THE REGULATION OF RENAL HAEMODYNAMICS IN DOCA-SALT HYPERTENSIVE RATS

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An increase in the sympathetic nerve activity may be involved in the development and maintenance of elevated blood pressure in several models of hypertension such as SHR and DOCA- salt hypertension.The adrenergic component of the sympathetic nervous system exerts an important control on the kidney function which may influence the level of blood pressure. Evidence has been provided to show that the renal vasoconstrictor responses in the stroke-prone spontaneously hypertensive rats and the 2K1C Goldblatt hypertensive rats are predominantly mediated by the α_{1a}-adrenoceptors (Munavvar and Johns, 1992(a),1992(b)). The aim of the present study is to examine which subtypes of the α_1-adrenoceptors are involved in the adrenergic regulation of renal heamodynamics in the low renin, volume expanded hypertensive rats. This study was undertaken in anaesthetised (sodium pentobarbital, 60 $mgkg^{-1}$ i.p.) male, DOCA- salt hypertensive rats (unilaterally nephrectomised, DOCA 15$mgkg^{-1}$ s.c. twice weekly and 0.9% saline as drinking water for 4 weeks),280-325 g. After tracheostomy, a carotid artery and jugular vein were cannulated for the measurement of blood pressure and infusion of saline. A midline abdominal incision was performed to expose the left kidney and the renal nerves isolated and dissected for stimulation. An electromagnetic flowmeter probe was fitted to the renal artery for determination of renal blood flow. The iliac artery was cannulated for close arterial administration of all drugs. The renal vasoconstrictor responses to direct renal nerve stimulation (RNS), (1, 2, 4, 6, 8, 10 Hz at 15 V and 0.2 ms), bolus doses of phenylephrine (10, 20, 30 and 40 µg) and methoxamine (20, 40, 60 and 80 µg) were determined before and after the administration of bolus doses of amlodipine (200 and 400 μgkg^{-1}), chloroethylclonidine (CEC) and 5-methylurapidil (5-MeU) at 5 μgkg^{-1} and 10 μgkg^{-1} in three different groups of rats. Throughout the experiment the animals were infused with saline, 6ml h^{-1}, containing 12.5 $mgkg^{-1}$ anaesthetic and 25 % of the respective bolus doses of the amlodipine, CEC and 5-MeU.

Table 1 Mean change in renal blood flow (ml/min) ± s.e.m.

GROUP	TREATMENT	RNS	PHENYLEPHRINE	METHOXAMINE	
A	Control	2.16±0.15	2.76±0.18	2.27±0.17	
(n= 8)	200 μgkg^{-1} amlodipine	1.48±0.11*	1.66±0.12*	1.16±0.11*	
	400 μgkg^{-1} amlodipine	1.32±0.10*	1.35±0.10*	0.86±0.10*	
B	Control	2.07±0.16	3.51±0.18	1.97±0.13	
(n=7)	5 μgkg^{-1} 5-MeU	1.50±0.10*	2.28±0.15*	1.26±0.10*	
	10 μgkg^{-1} 5-MeU	1.37±0.10*	1.86±0.16*	0.82±0.01*	
C	Control	3.15±0.20	3.73±0.25	2.16±0.17	
(n=7)	5 μgkg^{-1} CEC	2.54±0.18*	3.32±0.21	2.01±0.14	
	10 μgkg^{-1} CEC	2.42±0.16*	3.20±0.30	2.24±0.22	* P<0.05 (ANOVA)

This study showed that the adrenergic regulation of renal haemodynamics in the DOCA-salt hypertensive rats is modulated mainly by the α_{1a}-adrenoceptors as seen by their sensitivity to 5-MeU and the extracellular calcium dependency.

Munavvar, A.S. & Johns, E.J. (1992a) Br. J. Pharmacol., 105, 58P.
Munavvar, A.S. & Johns, E.J. (1992b) Br. J. Pharmacol., in press.

198P CALCIUM MOBILISATION BY α_2-ADRENOCEPTORS IN HUMAN ISOLATED SUBCUTANEOUS RESISTANCE ARTERIES

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Contractions produced by postjunctional α_2-adrenoceptor activation in human subcutaneous resistance arteries appear to be predominantly dependant on extracellular calcium (Parkinson *et al*, 1991). The direct measurement of calcium mobilisation, however, has not yet been observed and so *in vitro* studies were undertaken in human subcutaneous resistance arteries (i.d. 300-374µm, n=4) loaded with the fluorescent dye Fura-2AM.

Arteries were mounted in a single channel myograph in physiological saline solution (PSS) at 37°C as described by Mulvany & Halpern (1977). Following assesment of vessel viability, the vessels were then incubated with 6µM Fura-2AM for 2 h in PSS containing 0.1mg/ml pluronic, 0.1% cremaphor EL and 0.5% dimethyl sulphoxide. After washing in PSS, the myograph was mounted on an Axiovert 35 inverted microscope connected to a Deltascan 1 illumination unit and the tissues excited at 340nm and 380nm and emmision measured at 510nm. The vessels were exposed to high potassium depolarising solution (KDS, 118mM), 10µM NA, 10µM BHT (an α_2-adrenoceptor agonist) and 20mM caffeine in PSS containing 2.5mM calcium or calcium-free PSS containing 1mM BAPTA.

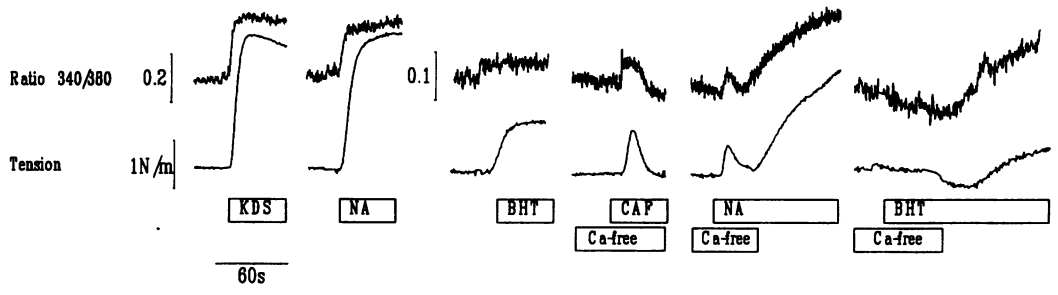


Figure 1 The effect of KDS, NA, BHT and caffeine on Ca_i and tone of a human isolated resistance artery
KDS, NA, and BHT contracted resistance arteries and increased intracellular free calcium (Ca_i) in PSS containing 2.5mM Ca^{2+}. In Ca^{2+}-free PSS, NA and caffeine caused a small contraction and a transient increase in Ca_i. In contrast, BHT failed to contract or increase Ca_i.When Ca^{2+} was reintroduced, Ca_i and contractile responses returned to control levels. We, therefore, conclude that the response to the α_2-adrenoceptor agonist BHT is totally dependent on the influx of extracellular calcium in these arteries.

Mulvany, M.J. & Halpern, W. (1977) *Circ. Res.* **41**, 19 - 26.
Parkinson, N.A., Hughes, A.D. & Sever, P.S. (1991) *Br.J.Pharmacol.* **104**, 49P.

199P EFFECTS OF VARIOUS 1,4-DIHYDROPYRIDINES ON SMALL RENAL ARTERIES

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We investigated the effect of three 1,4-dihydropyridine calcium antagonists, nifedipine, nisoldipine and manidipine, on 5-hydroxytryptamine (5-HT) and α_1-adrenoceptor agonist methoxamine-induced contractions of small isolated rat renal arteries.
Circular preparations of renal arcuate arteries with an average diameter of 305 ± 7.11 μm (n=83) were taken from 9 week old wistar rats and mounted in an isometric wire myograph. A normalization procedure (Nyborg & Mikkelsen, 1987) was used in order to standardize the degree of passive wall tension. The preparations were exposed repetitively to an iso-osmotic 120 mM potassium containing solution and once to a maximally effective concentration of the particular agonist (5-HT 3μM; methoxamine 3 μM) before the calcium antagonist was added. Because of the lipophilicity of nisoldipine and manidipine an equilibration time of 3 h was chosen before the cumulative concentration-response curve for either 5-HT or methoxamine was constructed. In control experiments it was confirmed that the magnitude of the potassium-induced contractions remained constant during this 3 h period. The experiments with 5-HT were repeated with preparations exposed 30 min to a nominally calcium-free physiological salt solution prior to the construction of the concentration-response curve. The maximal inhibition of potassium-, 5-HT- and methoxamine-induced contractions is given in Table 1.

Table 1	K^+ (120 mM) Ca^{++} 1.8 mM	5-HT Ca^{++} 1.8 mM	5-HT Ca^{++}-free	Methoxamine Ca^{++} 1.8 mM
	E_{max} (%)	E_{max} (%)	E_{max} (%)	E_{max} (%)
Nifedipine	94.1 ± 1.85	42.9 ± 4.12	43.6 ± 3.71	21.0 ± 1.59
Nisoldipine	98.1 ± 1.11	26.2 ± 0.37	26.1 ± 14.6	no effect
Manidipine	95.4 ± 2.58	93.4 ± 3.08	100.0 ± 0.00	31.7 ± 1.23

Although all three calcium antagonists were able to completely inhibit the potassium-induced spasms of rat isolated renal arteries, marked differences were found in their efficacy to suppress the agonist-induced contractions. From these results we conclude that the 1,4-dihydropyridines might influence not only the calcium-entry into the cells but also, with a compound-specific efficacy, the agonist induced calcium release. In this study manidipine was shown to be most effective in suppressing 5-HT- and methoxamine-induced spasms in rat isolated renal arteries when compared to nifedipine and nisoldipine.

Nyborg, N.C.B. & Mikkelsen, E.O. (1987) *J.Cardiovasc.Pharmacol.* **9**, 519-524

200P COMPARISON OF THE HAEMODYNAMIC PROFILES OF ELGODIPINE AND NICARDIPINE IN ANAESTHETISED DOGS

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Elgodipine is a second generation dihydropyridine calcium antagonist (Tamargo et al., 1991) currently in clinical development for the treatment of ischaemic heart disease (Suryapranata et al., 1992). In vivo, the cardiovascular effects of calcium antagonists depend upon their direct vascular and cardiac inhibitory actions (on myocardial and nodal tissues) and possible reflex cardiac stimulation mediated via the autonomic nervous system. This study compares the haemodynamic profiles of elgodipine (E) and nicardipine (N) using a model in which reflex cardiac autonomic tone is either functional or eliminated (Humphries and O'Connor, 1988).

Chloralose-anaesthetised, artificially-ventilated dogs were set up to measure heart rate (HR), mean arterial pressure (MAP), left ventricular dP/dt max, aortic and left circumflex coronary blood flows and PR interval (ECG). Total peripheral and coronary vascular resistances (TPR, CVR) and myocardial oxygen consumption index (MVO_2) were calculated. Each dog received (i.v. bolus) increasing doses (1 to 30 μg/kg) of E and N. Six dogs were pretreated with i.v. propranolol (1 mg/kg) + atropine (0.3 mg/kg), and 6 dogs were not. Results expressed as % changes at peak effects of E and N are summarized for the two highest doses.

	NON PRETREATED DOGS (n=6)				PRETREATED DOGS (n=6)			
Dose (i.v.)	10 μg/kg		30 μg/kg		10 μg/kg		30 μg/kg	
Treatment	E	N	E	N	E	N	E	N
TPR	-39 ± 4*	-40 ± 6*	-43 ± 4*	-44 ± 4*	-25 ± 5*	-27 ± 5*	-26 ± 7*	-29 ± 6*
HR	8 ± 4§	16 ± 5*	1 ± 2§	16 ± 5*	-12 ± 3*§	- 5 ± 2*	-21 ± 3*§	- 8 ± 2*
dP/dt max	14 ± 9*§	23 ± 10*	18 ± 5*	27 ± 7*	-14 ± 2*	-11 ± 3*	-25 ± 4*§	-18 ± 2*
CVR	-47 ± 6*	-44 ± 3*	-51 ± 6*	-50 ± 5*	-38 ± 4*	-32 ± 4*	-30 ± 5*	-32 ± 4*
MVO_2	6 ± 4§	19 ± 7*	9 ± 4§	23 ± 9*	-15 ± 3*	-13 ± 3*	-25 ± 3*§	-17 ± 2*
PR	- 7 ± 3*	- 7 ± 2*	5 ± 4§	- 3 ± 3	9 ± 3*§	4 ± 1	28 ± 5*§	12 ± 3

*: P<0.05 vs. predrug values, §: P<0.05 vs. nicardipine

E and N caused similar, dose-related falls in MAP, TPR and CVR i.e. the two compounds were equipotent as systemic and coronary vasodilators. N produced dose-related reflex tachycardia, maximal at 10 μg/kg i.v., whereas E caused much smaller increases in HR with no change at all at the highest doses. Experiments conducted in pretreated dogs show this can be largely explained by a greater intrinsic inhibitory effect of E on sinus rate. A further consequence of the direct cardiac effects of E was reduced reflex increases in MVO_2. Coronary vasodilatation associated with less reflex tachycardia and a reduced tendency to increase MVO_2 suggests that E will have a favourable effect on myocardial oxygen supply/demand balance, an appropriate profile for the treatment of angina.

Humphries R.G. and O'Connor S.E. (1988) J. Cardiovasc. Pharmacol.: **11**, 332-338
Suryapranata H., Maas A., MacLeod D.C., de Feyter P.J., Verdouw P.D. and Serruys, P.W. (1992) Am. J. Cardiol. **69**, 1171-1177.
Tamargo J., Lopez-Sendon J., Delpon E., Gonzalez-Morales M. and de Miguel E. (1991) Arzneim. Forsch. **41**, 895-900.

201P THE ROLE OF LYMPHOCYTES IN ARTERIAL REACTIVITY TO NORADRENALINE *IN VITRO*: COMPARISON BETWEEN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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Several lymphocyte-associated immune responses are depressed in spontaneously hypertensive rats (SHR) compared to normotensive rats (WKY). Peripheral blood lymphocytes may be altered in hypertension; depressed responsiveness to lectin stimuli and changes in lymphocyte subpopulations have both been reported. These changes may cause a different cytokine production by lymphocytes. Several cytokines are reported to cause a hyporeactivity to noradrenaline in rat aorta *in vitro*. We investigated the effect of supernatants produced by lymphocytes taken from normotensive and hypertensive rats on noradrenaline reactivity in aorta also taken from age-matched normotensive and hypertensive rats.

In this study we used 8 and 16 weeks old SHR and WKY rats. Lymphocytes were isolated from rat blood using a Percoll density gradient. Lymphocytes were cultured unstimulated or stimulated with phytohaemagglutinin (100 µg/ml) in RPMI medium. The aortas were cut into rings of 2 mm length. The rings were incubated for 20 hr in 250 µl lymphocyte supernatant and 500 µl fresh medium. All incubations were performed at 37 °C in an humidified atmosphere containing 5 % CO_2 under sterile conditions. The incubated rings were suspended in an organ bath and the tension to a dose response curve of L-noradrenaline (10^{-9} - 10^{-5} M) was measured isometrically.

In 8 week old rats a clear hyporeactivity was found when aorta rings were incubated with all supernatants. Although a decrease in maximal contraction after incubation with supernatant of unstimulated lymphocytes from WKY rats was observed, this decrease was not significant. In 16 week old rats a significant hyporeactivity to noradrenaline was only induced in aorta rings of WKY rats by the superatant of lymphocytes from SHR rats. However, the lymphocyte supernatant of WKY rats produced a decrease in the pD_2-value (-Log EC_{50} = 7.55 ± 0.17 for control vs. 7.03 ± 0.15 and 6.96 ± 0.18 respectively for unstimulated and stimulated lymphocytes).

Table 1. Maximal contraction (mean ± s.e.mean; g) to noradrenaline in rat aorta after incubation with supernatant of rat lymphocytes

Rat aorta	Control	Lymphocyte supernatants			
		WKY unstimulated	WKY stimulated	SHR unstimulated	SHR stimulated
8 weeks old					
WKY	1451 ± 109	1179 ± 207	632 ± 153 **	944 ± 161 *	775 ± 150 **
SHR	1136 ± 198	791 ± 297	531 ± 152 *	704 ± 152	496 ± 137 *
16 weeks old					
WKY	2555 ± 80	2168 ± 235	2120 ± 280	1866 ± 258 *	1520 ± 303 **
SHR	1857 ± 228	1799 ± 307	1724 ± 227	1642 ± 182	1473 ± 156

Significant differences from control were calculated using the students t-test (* $p<0.05$, ** $p<0.005$). n=7-10.

We conclude, that in aortas taken from 8 week old rats stimulated lymphocyte supernatants from both normotensive and hypertensive rats induce hyporeactivity. Interestingly, at 16 weeks only the lymphocyte supernatants of hypertensive rats, stimulated and unstimulated, induced significant decreases in reactivity to noradrenaline.

SJDK is supported by the Netherlands Heart Foundation (NHS 88.248).

202P SPONTANEOUSLY HYPERTENSIVE RATS WITH DIABETES: CONSEQUENCES FOR MEAN ARTERIAL PRESSURE AND THE CONTRACTILE RESPONSE OF ISOLATED SMALL ARTERIES

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It is well established that diabetes mellitus is associated with pathological changes in the macro- and microvasculature. Accordingly, the reactivity of vessels to pharmacological substances may be altered. In the present study, we investigated the effects of various vasoconstrictors on small arteries of spontaneously hypertensive rats (SHR) with simultaneous diabetes. SHR and normotensive Wistar-Kyoto rats (WKY) of 12 weeks of age with streptozotocin-induced diabetes (60 $mg \cdot kg^{-1}$ streptozotocin) were used. Mean arterial pressures (MAP) of both non-diabetic (mean values ± SEM, $n \geq 30$, 171.4 ± 4.0 mmHg) and diabetic (160.0 ± 4.7 mmHg) SHR were significantly ($p<0.05$) higher compared to both non-diabetic (101.6 ± 2.5 mmHg) and diabetic (107.6 ± 4.4 mmHg) WKY. However, diabetic SHR (160.0 ± 4.7 mmHg) had significantly lower MAP's compared to non-diabetic SHR (171.4 ± 4.0 mmHg). Mesenteric arteries were isolated and the preparations were normalized to their individual optimal lumen diameter in a wire myograph. Active wall pressures were calculated by using Laplace's equation (Ålkjær *et al*, 1979). Individual optimal lumen diameters (approximately 240 µm) showed no significant differences between the various groups. Passive wall pressures ($mN \cdot mm^{-2}$) of preparations from diabetic SHR ($n \geq 40$, 2.7 ± 0.2) and non-diabetic WKY (2.5 ± 0.2) were significantly lower compared to non-diabetic SHR (3.8 ± 0.3). Cumulative concentration-response curves were constructed for noradrenaline (in the presence of propranolol 1 µM, desipramine 1 µM and cortisol 0.1 µM), potassium chloride and serotonin. Subsequently, the viability of the endothelium was established by determining the ability of precontracted arteries to relax in reponse to 0.3 µM methacholine. Concentration-response curves of noradrenaline showed no significant differences for the four groups of preparations, except for the maximal active pressures ($mN \cdot mm^{-2}$) from diabetic SHR ($n \geq 6$, 32.8 ± 1.2) compared to diabetic WKY (25.1 ± 2.0). For potassium chloride, concentration-response curves were the same, except for the maximal active pressures of diabetic SHR (24.1 ± 2.5) and diabetic WKY (22.2 ± 1.4) compared to non-diabetic SHR (30.8 ± 1.8). For serotonin, -log EC_{50}-values of preparations from diabetic SHR (6.43 ± 0.05 M) were significantly different compared to preparations from non-diabetic SHR (6.65 ± 0.05 M). The maximal active pressures of preparations from diabetic SHR (30.4 ± 1.8) were significantly different from diabetic WKY (21.1 ± 1.4), and the latter differed significantly from preparations from non-diabetic SHR (28.4 ± 1.8). From these experiments we may conclude that mean arterial pressures of diabetic and non-diabetic hypertensive rats are significantly higher compared to appropriate normotensive control rats. Preparations from diabetic SHR are less sensitive to serotonin compared to non-diabetic SHR. In general, small arteries from normotensive and hypertensive diabetic rats tend to have equal or lower maximal active pressures, except for serotonin.

Ålkjær, C. & Mulvany, M.J. (1979) Acta Physiol. Scand. 107, 309-312.

203P EFFECT OF POLYMYXIN B ON α-ADRENERGIC RESPONSES OF CANINE BLOOD VESSELS

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Alpha$_1$-adrenoceptor stimulation results in increased turnover of inositol trisphosphate and diacylglycerol. The latter then activates protein kinase C (PKC) which mediates numerous cellular events and transmembrane ionic movements. To what extent α_1-adrenoceptor stimulation involves PKC activation is unknown, although it is thought that PKC is responsible for the maintenance of the sustained component of the α_1-adrenoceptor induced contraction. Polymyxin B is a known inhibitor of PKC and tumour promoting phorbol esters (e.g. phorbol dibutyrate; PDBu). This study examines the effect of polymyxin B on α_1-adrenoceptor induced contractions of canine saphenous vein and dorsal pedal artery. Furthermore the effect of polymyxin B on phorbol dibutyrate-induced contractions is also evaluated.

Concentration-effect curves were constructed (in the presence of yohimbine [10^{-7} M] to inhibit α_2-adrenoceptor-mediated responses) to noradrenaline (NA), phenylephrine (PE), BHT 920 (B2) and PDBu on vascular rings of from 5 normal dogs. Once completed, rings were washed frequently and were then treated with polymyxin B (80 units) before repeating the concentration-effect curves to the agonists. The whole procedure was repeated one more time using 200 units of polymyxin B.

The results are shown in table 1, for maximum response (max; mean + s.e.mean) and EC_{50} (uM; geometric mean with 95% confidence limits) and * $P < 0.05$ versus control.

		Max - NA - EC_{50}		Max - PE - EC_{50}		Max - B2 - EC_{50}		Max - PDBu - EC_{50}	
Vein:	control	19.3±13	3.2(2.2-4.5)	21.8±1.8	1.4(0.5-3.5)	12.3±1.0	1.1(0.4-2.8)	27.5±6.3	.03(.02-.06)
	poly 80	16.9±1.0	1.6(1.1-2.3)	25.4±2.1	4.0(2.0-8.1)	7.7±0.9*	4.4(1.6-12.3)	11.3±3.9*	.04(.02-.09)
	poly 200	11.8±0.5*	2.4(1.1-5.0)	19.8±1.1	2.2(1.2-4.2)	5.6±0.9*	29.6(17.8-49.2)*	7.1±3.8*	.17(.12-.25)
Artery:	control	26.8±1.0	2.0(1.1-3.2)	29.4±2.3	4.0(2.9-5.4)	9.8±1.4	5.9(1.9-18.5)	24±5.4	.23(.09-.61)
	poly 80	23.3±1.6	1.4(1.1-1.7)	27.0±1.7	4.6(2.3-9.2)	14.3±2.0	8.7(4.9-14.9)	12±4.0*	.89(.35-.92)
	poly 200	22.6±1.0	10.6(5.4-20.7)*	24.3±1.3	5.7(3.8-8.7)	3.5±1.2*	44.6(32.3-61.6)*	7.0±2.6*	1.50(.57-2.51)

These data show that polymyxin B had no effect on PE-induced contractions. Polymyxin antagonised responses to PDBu in a dose-dependent manner in both the vein and the artery. In the saphenous vein and dorsal pedal artery, polymyxin B inhibited responses to BHT 920 by decreasing the maximum response and increasing the EC_{50}. Against NA, there was a decreased maximum in the saphenous vein and an increased EC_{50} in the dorsal pedal artery with only the highest concentration of polymyxin B.

It can be concluded that in the normal saphenous vein and dorsal pedal artery, NA and BHT 920-induced contractions involve PKC which is unlikely to be α_2-adrenoceptor mediated since this activation process does not involve the PKC pathway. Therefore the PKC limb may be a useful approach for studying the differentiation and characterization of α_1-adrenoceptor subtypes in various tissues.

Supported by the Heart and Stroke Foundation of Ontario.

Forder, J., Scriabne, A. & Rasmussen, H. 1985. J. Pharmacol. Exp. Ther. 235;267-273.

204P ELECTROPHYSIOLOGICAL EFFECTS OF LEVCROMAKALIM IN THE RAT AND RABBIT MESENTERIC ARTERY AND RAT PORTAL VEIN

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Single cell studies have recently shown that levcromakalim (formerly BRL 38227) opens a small conductance, glibenclamide-sensitive K-channel in the spontaneously active rat portal vein (Noack *et al.*, 1992). The present study has compared the effects of levcromakalim on resting membrane potential in rat portal vein and the electrically quiescent rabbit mesenteric artery using standard microelectrode recording techniques, and further examined the electrophysiological profile of this agent in single cells from rat small mesenteric vessels.

Rat portal veins and rabbit mesenteric arteries were mounted for intracellular recording using conventional techniques. Microelectrodes of resistance 50-80MΩ, filled with 3M KCl, were employed for recording of membrane potential from tissues superfused with a standard physiological salt solution maintained at 37°C. In single cell experiments, cells from rat small mesenteric arteries were freshly dispersed (Clapp & Gurney, 1991) and currents were recorded in the whole cell mode of the patch clamp technique. The composition (mM) of the solutions was; pipette (internal) Na^+ 5, K^+ 122.4, Mg^{2+} 1.2, Cl^- 129.8, $H_2PO_4^-$ 1.2, glucose 11, HEPES 10, EGTA 1.2, oxalacetic acid 5, sodium pyruvate 2, sodium succinate 5, pH 7.3: bath (external) Na^+ 125, K^+ 6, Mg^{2+} 3.7, $H_2PO_4^-$ 1.2, Cl^- 137.2, glucose 11, HEPES 10, EGTA 1, pH 7.3 gassed with O_2.

The resting membrane potential in cells from the rat portal vein was -59 ± 4mV. Addition of levcromakalim (0.1μM) produced a rapid and complete inhibition of spontaneous activity of the tissue within 1-2 min without affecting resting membrane potential. In the concentration range 0.5-5μM levcromakalim also produced a marked concentration-dependent hyperpolarisation reaching a maximal plateau value of -84 ± 3mV . Recordings obtained from the rabbit mesenteric artery (resting membrane potential -61 ± 4mV) showed that levcromakalim (0.1-5μM) elicited a concentration-dependent hyperpolarisation. At the highest concentration of levcromakalim (5μM) the membrane potential was -76 ± 4mV. All values are mean ± s.e. mean (n=6-8).

Experiments carried out in single cells from rat small mesenteric arteries showed that levcromakalim (10μM) induced a non-inactivating outward current (I_{KCO}) (75.8 ± 4.2pA at a holding potential of -10mV, n=5) which was slowly developing (3-4 min to maximal level) and was associated with increased current noise. A more detailed analysis of the effects of levcromakalim in mesenteric arterial cells was derived from construction of current-voltage relationships under control conditions and in the presence of levcromakalim with holding potentials of -90mV and -10mV. Levcromakalim (10μM) increased non-inactivating currents (derived from a holding potential of -10mV) crossing the control curve at approximately -80mV. In addition, when cells were held at -90mV, levcromakalim was also found to inhibit a transient outward delayed rectifier current (I_{TO}), which became apparent at potentials positive to -30mV. All effects of levcromakalim were fully reversible by glibenclamide (10μM).

The electrophysiological profile of action of levcromakalim thus appears to be similar in both portal vein and small, quiescent blood vessels. It is concluded therefore that the generation of I_{KCO} and consequent hyperpolarisation play an important role in the mechanoinhibitory effects of levcromakalim in the studied vessels.

DNC is supported by SmithKline Beecham.

1. Clapp, L.H. & Gurney, A.M. (1991) *Exp. Physiol.* **76**, 677-693
2. Noack, T., Deitmer, P., Edwards, G. & Weston, A.H. (1992) *Br. J. Pharmacol.* **106**, 717-726.

205P THE EFFECTS OF HYPERTENSION AND DIABETES ON THE VASCULAR REACTIVITY OF RESISTANCE ARTERIES

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Hypertension and diabetes mellitus frequently coincide. Therefore we have studied the effects of both hypertension and streptozotocin (STZ)-induced diabetes mellitus on vascular reactivity in perfused mesenteric resistance arteries taken from spontaneously hypertensive rats (SHR) and from STZ (60mg/kg, i.v.)-treated SHR. We compared these preparations with those obtained from normotensive Wistar-Kyoto rats (WKY) and from STZ (60mg/kg, i.v.) treated WKY.
Concentration-response curves for the α_1-receptor agonist methoxamine and the endothelium-dependent vasodilators metacholine, histamine, adenosine diphosphate (ADP) and the endothelium-independent vasodilator sodium-nitroprusside (SNP) were constructed. Concentration-response curve parameters for the different preparations are listed in the table.

	WKY		SHR		WKY_{STZ}		SHR_{STZ}	
	pD_2 (M)	E_{max} (mmHg)	pD_2 (M)	E_{max} (mmHg)	pD_2 (M)	E_{max} (mmHg)	pD_2 (M)	E_{max} (mmHg)
methoxamine	5.07±0.06	170±5.0	5.20±0.01	234±13	4.88±0.02	137±3.3	4.99±0.04	199±6.7
metacholine	7.30±0.04	11.2±2.0	7.46±0.06	10.4±1.6	6.50±0.04	11.3±2.2	6.67±0.03	11.8±2.2
histamine	6.15±0.06	11.6±1.7	5.92±0.02	13.0±1.6	5.46±0.06	10.8±2.1	5.23±0.06	10.8±1.4
ADP	8.36±0.04	9.2±2.0	8.39±0.05	14.3±3.9	7.70±0.03	11.3±0.8	7.31±0.08	13.7±5.4
SNP	6.57±0.18	12.2±1.6	6.89±0.08	14.4±5.5	6.33±0.09	15.4±5.6	6.57±0.03	10.2±4.7

For methoxamine, the mesenteric preparations taken from SHR yielded stronger maximal responses than those taken from WKY. STZ treatment resulted in a decreased reactivity to methoxamine in both SHR and WKY compared to their respective control preparations. Endothelium-dependent vasodilation was not affected by hypertension. However, STZ-induced diabetes mellitus resulted in a decreased sensitivity to the three endothelium-dependent vasodilators. Neither hypertension nor STZ treatment affected the nitroprusside-induced endothelium-independent relaxation.
In conclusion, STZ-induced diabetes mellitus results in changes to both methoxamine-induced vasoconstriction and endothelium-dependent vasodilation in perfused mesenteric arteries. Diabetes mellitus decreases the responsiveness to methoxamine and the endothelium-dependent vasodilators to the same extent in mesenteric resistance arteries obtained either from hypertensive- or from normotensive rats.

206P HIGH POTENCY OF TRANDOLAPRIL ON HYPERTENSION, CARDIAC HYPERTROPHY AND TISSUE ACE ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS

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Many studies indicate that during treatment of hypertensive subjects with angiotensin converting enzyme (ACE) inhibitors, there is a poor correlation between plasma ACE inhibition and blood pressure (BP) fall but a rather good correlation between tissue ACE inhibition and BP lowering (Kaplan *et al.* 1984 ; Ohmura *et al.* 1985 ; Aono *et al.* 1988). However, few attempts have been made to establish complete *in vivo* dose-response curves for ACE inhibitors and to compare their relative effects on BP, serum and tissue ACE activity and cardiac hypertrophy.
The aim of this study was therefore to determine the minimum active dose of trandolapril on BP, circulating and tissue ACE activity and cardiac hypertrophy and to compare the dose-response curves for these 3 parameters after short term treatment in spontaneously hypertensive rats (SHR). Enalapril was chosen as a reference drug.
Male SHR (aged 22-26 weeks) were treated during 14 days with trandolapril at 10 different dosages (from 0.1 µg/kg/day to 3 mg/kg/day p.o. in drinking water, 7 to 20 rats/group). At the end of treatment, arterial BP was measured in anaesthetized rats, then the heart was excised and quickly weighed ; the atria were isolated and ventricles dissected into several parts to measure ACE activity. ACE activity was also measured in aorta and serum. Enalapril (10 µg/kg/day to 10 mg/kg/day) was used as a reference compound under similar conditions.
Trandolapril produced a dose dependent decrease in BP and cardiac hypertrophy with a threshold dose at 30 µg/kg for both parameters. At 3 mg/kg mean BP was decreased by 39% and cardiac hypertrophy by 17%. A dose dependent inhibition of ACE activity was also observed after trandolapril treatment in all the tissues, the ID_{50} (µg/kg) (at 90% confidence limits) being : serum 63.7 ± 39.2 ; aorta 0.66 ± 0.18 ; right atrium 1.32 ± 1.77 ; left atrium 1.07 ± 2.55 ; right ventricular wall 15.1 ± 6.59 ; apex 7.9 ± 7.98 ; septum 7.4 ± 7.2 ; left ventricle, epicardium part 3.4 ± 0.3 ; left ventricle, endocardium part 2.9 ± 1.9. Enalapril was much less active than trandolapril since at 10 mg/kg mean BP was decreased by only 24% (which is similar to the effect of trandolapril at 0.3 mg/kg), cardiac hypertrophy by 9% and serum ACE activity by 34% ; the ID_{50} for tissue ACE inhibition after enalapril being (mg/kg) : aorta 0.26 ± 0.0 ; right atrium 0.32 ± 0.61; left atrium 1.05 ± 0.10 and > 10 mg/kg for all parts of ventricle.

In conclusion, trandolapril is a very potent ACE inhibitor, which is about 30 times more active than enalapril in decreasing BP and 250 to 1000 times more active than enalapril in inhibiting cardiac and vascular tissue ACE, thus demonstrating its very marked tissue penetration.

Kaplan, H.R., Cohen, D.M., Essenburgh, A.D. (1984) Fed. Proc., 43 : 1326 - 1329
Ohmura, I., Maki, E., Naruse, T. (1985) Folia Pharmacol., 86 : 303 - 313
Aono, J., Koga, T., Yamazaki, T., Shiraki, Y., Sakai, K. (1988) Arch. Int. Pharmacodyn. Ther., 292 : 203 - 222

207P SYMPATHOINHIBITORY ACTION OF RILMENIDINE IN CONSCIOUS SINOAORTIC DENERVATED RATS

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It is well documented that hypotensive action of rilmenidine relates more to the interaction with imidazoline receptors (IRs) than α2-adrenoceptors in anesthetized normotensive rats (see review by Bousquet *et al.*, 1992). To evaluate the contribution of each receptor subtype to the hypotensive and sympathoinhibitory effects of rilmenidine in conscious sinoaortic denervated rats, we measured the effects of rilmenidine, with or without idazoxan or yohimbine pretreatment, on mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA).

Intracisternal (i.c.) injection of rilmenidine (300 μg/kg) significantly decreased MAP, HR and RSNA (Table 1). I.c. pretreatment with idazoxan (1 μg/kg, i.e. 5 nmol/kg), an α2-antagonist with imidazoline structure, significantly attenuated the hypotensive and sympathoinhibitory effects of rilmenidine. In contrast, i.c. pretreatment with equimolar dose of yohimbine, an α2-antagonist with low affinity to IRs, did not significantly alter the effects of rilmenidine on MAP and RSNA. Furthermore, i.c. injection of yohimbine at a 10 - fold higher dose again failed to modify the cardiovascular effects of rilmenidine. However, both idazoxan and yohimbine did not affect rilmenidine-induced bradycardia.

Table 1. Effects of rilmenidine on MAP, HR and RSNA with or without idazoxan or yohimbine pretreatment (5 nmol/kg).

Treatment	MAP (mm Hg)			HR (beats/min)			RSNA (%)		
	Before Antagonist	After Antagonist	After Rilmenidine	Before Antagonist	After Antagonist	After Rilmenidine	Before Antagonist	After Antagonist	After Rilmenidine
Vehicle	107±5	108±8	81±4*	449±11	448±16	390±10*	100	96±2	65±5*
Idazoxan	108±6	103±6	96±3*#	453±15	443±14	394±14*	100	94±3	86±3*#
Yohimbine	109±3	108±3	85±4*	423±13	420±13	361±12*	100	100±3	61±5*

Values are means ± s.e. mean, n = 6 - 10; * $P < 0.05$ compared with pre-rilmenidine values, # $P < 0.05$ compared with vehicle and yohimbine groups (Kruskal-Wallis test).

We concluded that, in conscious sinoaortic denervated rats, 1) rilmenidine acts on two different sites controlling separately the sympathetic tone and HR, 2) the sympathoinhibitory and hypotensive action of rilmenidine may relate more to the interaction with IRs than α2-adrenoceptors.

Bousquet, P., Bricca, G., Dontenwill, M., Feldman, J., Greney, H., Belcourt, A., Stutzmann, J. & Tibiriça, E. (1992) Fundam. Clin. Pharmacol. **6**, Suppl 1, 15S-21S.

208P MUSCARINIC RECEPTOR-MEDIATED INHIBITION OF ADENYLYL CYCLASE AND ITS ROLE IN THE FUNCTIONAL ANTAGONISM OF ISOPRENALINE IN AIRWAY SMOOTH MUSCLE

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Using both animal and human airway smooth muscle preparations, we have recently found a significant relationship between the potency of muscarinic agonists to induce inositol phosphates production and their capacity to reduce pD_2 and E_{max} values of (ISO)-induced relaxation, indicating that cross-talk between (M_3-receptor-mediated) phosphoinositide (PI) metabolism and (β-adrenoceptor-mediated) activation of adenylyl cyclase (AC) may underlie the functional antagonism of cholinergic contraction by β-agonists (Van Amsterdam et al, 1989; 1990). Another mechanism that could be involved in or contribute to the reduction of β-adrenergic responsiveness by muscarinic agonists is G_i-mediated inhibition of AC through stimulation of M_2 muscarinic receptors (Yang et al, 1991), which are abundant in airway smooth muscle (Roffel et al, 1988). To study this hypothesis, we investigated methacholine (MeCh)-induced inhibition of AC in membranes of isolated, enzymatically dispersed bovine tracheal smooth muscle (BTSM) cells and its relation to changes in pD_2 and E_{max} of ISO-induced BTSM relaxation in the presence of increasing concentrations (10 nM - 1 mM) of this agonist. In addition, we investigated the effects of the selective M_2-antagonist gallamine on the ISO dose-relaxation curves in the presence of the same MeCh concentrations.

MeCh caused a concentration-dependent inhibition of 10μM forskolin-stimulated AC activity with a maximum response of 27.7 ± 1.8% (from 490 ± 78 pmol/mg/min to 354 ± 58 pmol/mg/min) and a pD_2 of 6.16 ± 0.19 (n = 6). Significant correlations were found between the inhibition of AC and the decrease of both pD_2 and E_{max} values of ISO-induced relaxation at increasing MeCh concentrations ($r = 0.992$; $p < 0.001$ and $r = 0.977$; $p < 0.01$, respectively), suggesting a possible role for MeCh-induced AC inhibition in the decrease of β-adrenergic relaxability. However, quantitatively similar relationships of the pD_2 and E_{max} values with much lower levels of inositol phosphates production were observed, indicating a more prominent role of PI metabolism than of G_i-mediated AC inhibition in the functional antagonism of muscarinic BTSM contraction by β-agonists. This was supported by the observation that gallamine (30 μM) had no significant effect on the ISO dose-response curves (pD_2 and E_{max}) at all MeCh concentrations used.

In conclusion, the results indicate that M_2 muscarinic receptor-mediated AC inhibition plays only a minor, if any, role in the functional antagonism of muscarinic BTSM contraction by β-adrenoceptor agonists.

Van Amsterdam, R.G.M., Meurs, H., Brouwer, F., Postma, J.B., Timmermans, A. & Zaagsma, J. (1989) *Eur. J. Pharmacol.* 172, 175-183.
Van Amsterdam, R.G.M. Meurs, H., Ten Berge, R.E.J., Veninga, C.C.M., Brouwer, F. & Zaagsma, J. (1990) *Am. Rev. Respir. Dis.* 142, 1124-28.
Roffel, A.F., Elzinga, C.R.S., Van Amsterdam, R.G.M., De Zeeuw, R.A. & Zaagsma, J. (1988) *Eur. J. Pharmacol.* 153, 73-82.
Yang, C.M., Chou, S.-P. & Sung, T.-C. (1991) *Br. J. Pharmacol.* 104, 613-618.

209P CYCLOSPORIN A AND THE ACUTE ALLERGIC REACTION IN THE GUINEA-PIG AND RAT

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Low concentrations (nM range) of cyclosporin A (CYA) inhibit activation of human lung mast cells in vitro (Triggiani et al., 1989). This action of CYA has been observed in other species (Ezeamuzie & Assem, 1990) so that it is possible to determine whether such activity in-vitro is predictive of anti-allergic efficacy in vivo.

Concentrations of CYA within the therapeutic range (50-500 nM) were without effect upon Schultz-Dale reactions in guinea-pig trachea. In other experiments, guinea-pigs (400-600g) were passively sensitised by intravenous injection of guinea-pig antiserum (1.0 ml) to OA, anaesthetised with phenobarbitone (100 mg kg^{-1}) and pentobarbitone (30 mg kg^{-1}), paralysed by gallamine (10 mg kg^{-1}) and ventilated with an air/oxygen mixture (50:50) to allow recording of resistance (R_L) and compliance (C_{dyn}). Following intraduodenal injection of CYA (100 mg kg^{-1}), intratracheal installation of ovalbumin (1,10 & 100 μg $animal^{-1}$) induced acute airway obstruction (ΔR_L ± sem, 193±13, 221±43, 829±176; ΔC_{dyn} ± sem, 0.72±0.09, 0.68±0.09, 0.35±0.12, n=10), which was not significantly different from changes in vehicle-treated animals (ΔR_L ± sem, 188±20, 250±52, 786±153;ΔC_{dyn} ± sem, 0.64±0.08, 0.6±0.08, 0.24±0.09, n=10) respectively. Similarly, passive cutaneous anaphylactic reactions (diameter mm ± sd, n=5) in rats (15±2) were unaltered by prior (1 and 24 hrs) treatment with CYA (100 mg kg^{-1}) (16±2,16±1 respectively, n=10). In animals in which delayed-type hypersensitivity (DTH) had been induced by intraperitaneal injection of bovine serum albumin conjugated with arsenate (BSA-AS) in Freund's complete adjuvant, airway obstruction (ΔR_L ± sem, 125±7; ΔC_{dyn} ± sem, 0.5±0.04, n=10) was evident 24 hours after inhalation of BSA-AS (1 mg ml^{-1} for 30 min). Such airway obstruction was significantly ($p=<0.05$) suppressed (ΔR_L ± sem, 141±12, 103±5, 95±5; ΔC_{dyn} ± sem, 0.45±0.07, 0.7±0.05, 0.97±0.06, n=10) following prior (1hr) oral administration of CYA (10, 32 and 100 mg kg^{-1} p.o.) respectively. Similarly, delayed type hypersensitivity responses (diameter mm ± sd) to BSA-AS (10μg kg^{-1}) in skin (vehicle, 15±1, n=10; CYA, 13±2, 11±2, 1±2, n=6) were also reduced in a dose-dependent fashion.

The present experiments indicate that the capacity of CYA to inhibit secretion of allergic mediators from isolated mast cells is not predictive of anti-allergic activity in other in-vitro or vivo tests.

Triggiani, M., Cirillo, R., Lichtenstein, L.M. & Marone, G. (1989). Int. Arch. Allergy Appl. Immunol. 88, 253-255.
Ezeamuzie,I.C. & Assem, E.S. (1990). Agents Actions. 30, 110-113.

210P CHARACTERISTICS OF AIRWAY HYPERREACTIVITY FOLLOWING INTRAVENOUS INFUSION OF ENDOTOXIN IN THE GUINEA-PIG

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It has previously been reported that inhalation of endotoxin (LPS) in rats (Pauwels et al., 1990) or guinea-pigs (Nagai et al, 1991) evokes both increased airway reactivity as well as intrapulmonary accumulation of inflammatory cells. The present study has characterised LPS-induced airway hyperreactivity and related such changes to leukocyte accumulation in the guinea-pig lung.

Guinea-pigs were anaesthetised with phenobarbitone (100 mg kg^{-1} i.v.) and pentobarbitone (30 mg kg^{-1} i.v.), paralysed with gallamine (10 mg kg^{-1} i.m.) and ventilated with air and oxygen (50:50). R_L and C_{dyn} were measured electronically and airway responsivity to intravenous spasmogens defined as the ratio (post/pre) of the dose-effect slopes (SR) for R_L. For histamine (hist), SRs (1.0, 1.1, 3.9, 4.0 & 4.2) increased in a dose-dependent fashion following infusion of LPS (0, 0.1, 1, 10 & 100 μg/kg/hr,i.v., n=10 per group) consequently a dose of 10 μg kg^{-1} was selected for study of other spasmogens. By comparison with infusion of vehicle, infusion of LPS (10 μg kg^{-1} i.v.) increased SR for substance P (8.9 v 0.6), hist (4.0 v 1.0), prostaglandin $F_{2\alpha}$ (3.4 v 1.8), leukotriene E_4 (2.4 v 1.0), acetylcholine (1.8 v 1.2) and leukotriene C_4 (0.5 v 0.2). Expression of airway hyperreactivity to hist following LPS (10 μg kg^{-1} i.v.) was not associated with intrathoracic accumulation of labelled (^{111}Indium) platelets or neutrophils, was reduced by prior (24 hrs) injection of anti-neutrophil antiserum and was abolished by prior (24 hrs) injection of anti-platelet antiserum (0.1 ml i.v.) and by infusion of a PAF-receptor antagonist SRI 63-675 (0.1 mg kg^{-1} hr).

The present results indicate that airway hyperreactivity following infusion of LPS in the guinea-pig is spasmogen selective, the greatest increase in sensitivity being observed for substance P. Moreover, the increased airway reactivity observed in this species appears to be a consequence of PAF formation and subsequent platelet activation, and is independent of intra-thoracic accumulation of either platelets or neutrophils.

Nagai, H., Tsuji, F., Goto, S. & Koda, A. (1991). Arch. Int. Pharmacodyn. 313, 161-175.

Pauwels, R.A., Kips, J.C., Peleman, R.A. & Van Der Straeten, M.E.(1990). Am.Rev.Resp.Dis. 141, 540-545.

211P INHIBITORY EFFECTS OF PHOSPHODIESTERASE III AND IV INHIBITION ON HISTAMINE-INDUCED TONIC AND PHASIC TONE IN BOVINE TRACHEAL SMOOTH MUSCLE

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We have previously shown that the histamine (HIST)-stimulated inositol phosphate response of bovine tracheal smooth muscle (BTSM) is inhibited by rolipram, a selective inhibitor of low K_m, cyclic AMP-specific phosphodiesterase (PDE IV), and that this inhibitory effect could be potentiated by co-addition of the PDE III inhibitor ORG 9935 (Adams et al., 1992). Here, we have attempted to establish whether the enhanced inhibitory effect of PDE III/IV combinations are also evident at a functional level by investigating the effects of PDE IV/III inhibition on histamine (HIST)-induced tonic and phasic tone in BTSM strips.

BTSM strips (10 x 2 x 2 mm) were suspended in oxygenated Krebs-Henseleit buffer (KHB) and maintained at 37°C under a resting tension of 0.5 g for 90 min. Following initial challenge with spasmogen, strips were exposed to different concentrations or rolipram and/or ORG 9935 for 20 min prior to re-challenge. To assess the effects of PDE inhibition on phasic contraction, BTSM strips were pre-equilibrated as above and then exposed to Ca^{2+}-free KHB containing 2 mM EGTA for 10 min prior to addition of 30 μM HIST. Following maximal phasic contraction, strips were rapidly washed with normal KHB (Tagliente et al., 1992).

HIST caused a dose-dependent increase in tension (T_{max} 28 ± 1 g, EC_{50} 5.5 ± 0.4 μM (n=6). Increasing concentrations of rolipram progressively inhibited the response to HIST, with the effectiveness of rolipram being reduced at higher HIST concentrations. Thus, rolipram (10 μM) inhibited the contraction caused by 10, 30 and 100 μM HIST by 61 ± 5, 42 ± 4 and 31 ± 3% respectively. ORG 9935 (1 μM) had little effect on HIST-stimulated contraction (9.7 ± 5.2% inhibition of response to 10 μM HIST). In contrast, combinations of rolipram and ORG 9935 had greater than additive inhibitory effects against this spasmogen. Rolipram (0.1 μM) and ORG 9935 (1 μM) completely suppressed the contractile response to 10 μM HIST, and inhibited the response to 100 μM HIST by 47 ± 4%. Under Ca^{2+}-free conditions, 30 μM HIST caused a phasic contraction (5.9 ± 0.8 g), which slowly returned to baseline. Pre-incubation with rolipram (10 μM) or ORG 9935 (1 μM) inhibited the phasic response by 39 ± 16 and 34 ± 11% respectively; however, combined addition of 0.1 μM rolipram with 1 μM ORG 9935 completely abolished the HIST-induced contractile response.

These results suggest that although selective PDE IV inhibition can decrease the phasic and tonic responses of BTSM to HIST, combined additions of ORG 9935 and rolipram, to inhibit both PDE III and IV, result in greater than additive anti-spasmogenic effects against HIST-induced contractions. The results suggest that PDE III/IV inhibitor combinations may offer some potential as a therapeutic strategy in asthma.

We gratefully acknowledge a Science & Engineering Research Council CASE award to D. Adams.

Adams, D., Nicholson, C.D. and Challiss, R.A.J. (1992) Br. J. Pharmacol. **107**, 165P
Tagliente, T.M., Dalton, B.A. and Ben-Harari, R.R. (1992) J. Pharmacol. Exp. Ther. **261**, 755-763.

212P NEGATIVE HOMOTROPIC ALLOSTERIC INTERACTIONS BETWEEN TWO VINBLASTINE BINDING SITES OF P-GLYCOPROTEIN

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P-glycoprotein (P-gp) is a 170 kDa ATPase which pumps a diverse range of cytotoxics out of tumour cells rendering them multidrug resistant (MDR). Drugs which reverse MDR by preventing cytotoxic drug efflux, such as verapamil, nicardipine and cyclosporin A can reverse MDR (Endicott and Ling, 1989). It has been demonstrated that P-gp possesses a vinca-alkaloid selective domain (receptor site1) and a 1,4-dihydropyridine selective domain (receptor site 2) which are allosterically coupled (Ferry et al, 1992).

We have used the human lung cancer cell line H69/LX4 which hyperexpresses P-gp and has a classical MDR phenotype (Reeve et al, 1990) as a source of surface membranes. [^{3}H]-Vinblastine (8.3 Ci/mmol) in 50 mM Tris HCl, pH 7.4 was mixed at 12 or 23° C in a volume of 0.25 ml with 10-20 µg of membrane protein before bound and free radioligand were separated by filtration through Whatman GF/C filters. As previously reported [^{3}H]-vinblastine bound to 31 pmol/mg of membrane protein with a K_D of 9 nM under these conditions (Ferry et al 1992).

The concentration-dependence of vinblastine to induce self dissociation from P-gp at 12° C was investigated. 300 fold dilution induced a monophasic dissociation with a rate constant of 0.008 min^{-1}. 300 nM unlabelled vinblastine induced a monophasic dissociation of [^{3}H]-vinblastine from P-gp with a rate constant of 0.0124 ± 0.0029 min^{-1} (n=3). At 3 µM vinblastine induced a monophasic dissociation with a k_{-1} of 0.0226 ± 0.0053 min^{-1} (n=3), $p < 0.05$. At higher vinblastine concentrations self induced dissociation appeared to be biphasic. At 30 µM fitting a large pool of data from n=7 experiments the k_{-1} for a monophasic decay was 0.0559 ± 0.009 min^{-1}, with R = 0.59. The same data fitted better to an equation describing a double exponential decay, with a minor fast component of 28 ± 13% with a k_{-1} of 3.3 ± 8 min^{-1}, and a dominant slow component of 72 ± 6% with a k_{-1} of 0.028 ± 0.006 min^{-1}, with R = 0.87. Plotting k_{-1} for vinblastine concentrations up to 3 µM and the k_{-1} of the slow component at higher concentrations the EC_{50} for vinblastine to accelerate its own dissociation from P-gp was 597 ± 350 nM. The plot of vinblastine concentration against % fast component did not saturate by 30 µM vinblastine.

We propose that P-gp possesses two binding sites for vinblastine which are allosterically coupled, but distinct from the 1,4-DHP-selective domain, these have apparent K_D values of 9 nM and 500 nM, the low affinity site accounting for vinblastine self induced dissociation acceleration. The biphasic self induced dissociation by vinblastine at > 3 µM could represent vinblastine binding to the 1,4-DHP site. The possession of two vinblastine binding sites within P-gp has interesting implications for this fascinating transporter, perhaps if the high affinity site is facing the intracellular milieu, then transfer to the low affinity, outward facing domain would be thermodynamically favourable and help facilitate transport.

Endicott, J.A. & Ling, V. (1989) Annu Rev Biochem. **58**, 137-171.
Ferry, D.R., Russell, M.A. and Cullen, M.H. (1992) Biochem. Biophys. Res Commun. **188**, 440-445.
Reeve, J.G., Rabbitts, P.H. and Twentyman, P.R. (1989). Br J Cancer. **60**, 339-342.

213P SUPERNATANTS FROM HUMAN LUNG MAST CELLS PRIME HUMAN BASOPHILS *IN VITRO*

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Priming with cytokines such as granulocyte macrophage colony stimulating factor and interleukin 3 (IL-3) will augment the responsiveness of human basophils to IgE-dependent and independent degranulation stimuli (Hirai *et al* 1988) and may be relevant to the pathogenesis of asthma. Histamine releasing factors have been isolated from lung alveolar macrophages (Schulman *et al* 1985) and there is some evidence that cytokines are released from stimulated human mast cells. Thus, we decided to investigate whether supernatants from challenged human lung mast cells were capable of priming human basophils, enhancing the degranulation response to anti-IgE and IL-3. Human lung mast cells (n=5) were incubated with sheep anti-human IgE (anti-IgE) for 45 minutes and the resulting supernatant was used to prime human basophils (n=9) for 15 minutes prior to stimulation with 2 ng/ml IL-3 or 1μg/ml anti-IgE. Unchallenged control supernatants were tested simultaneously. The supernatants from challenged lung mast cells caused some histamine release when used alone. The supernatants also enhanced histamine release in basophils challenged with anti-IgE or IL-3. The most marked increase was observed when basophils were challenged with IL-3. Challenged mast cell supernatants increased histamine release by 90± 12% whereas unchallenged control supernatants only increased histamine release by 9±7%. The priming effect was observed with mast cells from all 5 lungs tested and with basophils from different donors (n=9), however the extent of enhancement varied from 90-950% in different donors.

In summary, it was found that anti-IgE challenged mast cells produce a factor(s) capable of enhancing histamine release in basophils. It is likely that the factor(s) released is a cytokine or histamine releasing factor however because of the limited number of human cells available further characterisation will be difficult.

Hirai, K., Morita,Y., Misaki,Y., Ohta, K., Takaishi, T., Suzuki, S. *et al* (1988) *J. Immunol.* **141**, 3958-3964.
Schulman, E., Liu, M., Proud, D., MacGlashan, D.W. Jr. *et al* (1985) *Am. Rev. Respir. Dis.* **131**, 230-235.

214P POSSIBLE ROLE OF PHOSPHOLIPASE D IN IgE-MEDIATED SIGNAL TRANSDUCTION IN HUMAN LUNG MAST CELLS AND BASOPHILS

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The signal transduction pathways which lead to the release of histamine from human mast cells and basophils remain unclear. We have used two drugs, wortmannin and propranolol, to assess the role of phospholipase D (PLD) in histamine release from both lung mast cells and basophils. Wortmannin, which is thought to prevent activation of PLD (Reinhold *et al*, 1990), inhibited histamine release (HR) from basophils challenged with 1μg/ml sheep anti-human IgE (anti-IgE) (Warner *et al*, 1988) with an IC_{50} of 0.6±0.07nM (mean±sem., n=13, control HR=45±6% of total cellular histamine). The inhibition of histamine release was >95% at concentrations of 3nM and above. Wortmannin also inhibited the release of histamine from basophils challenged with 1μM f-met peptide though the concentrations required were 100 fold greater (n=6, IC_{50} =58 ±11nM, control HR =58±4%) However, wortmannin was both less potent and less effective when 10ng/ml phorbol myristate acetate (PMA)(n=6, maximum inhibition =64±10%, control HR =37±9%) or 1μg/ml A23187 (n=6, maximum inhibition =44±12%, control HR =81±3%) were used to initiate release. Wortmannin was much less potent against anti-IgE induced histamine release in human lung mast cells (n=6, IC_{50} =56±13nM, control HR =18±4%) but failed to inhibit A23187 induced release in these cells (n=5, maximum inhibition =2±3%, control HR =59±12%). Propranolol, which at high concentrations inhibits the conversion of phosphatidic acid to diacylglycerol (Pappu & Hauser, 1983) also affected the release of histamine from human basophils. The IC_{50} was 46±10μM when anti-IgE was used to initiate histamine release (n=13, control HR= 28±4%) The drug was also effective against PMA induced release (IC_{50} =119±24μM, n=7, control HR =52±9%) but failed to affect histamine release in basophils challenged with 1μM f-met peptide (n=4, control HR =45±15%) or 1μg/ml A23187 (n=4, control HR =43±11%). Propranolol was much less effective in human lung mast cells challenged with anti-IgE (n=8, IC_{50} =256 ±66μM, control HR = 25±6%). Studies with the isomers of propranolol indicate that the inhibition was not due to effects on β adrenergic receptors, though we are unable to exclude the membrane stabilising actions of propranolol.

In summary, the crosslinking of IgE may lead to the activation of PLD in both human lung mast cells and basophils. Both wortmannin and propranolol appear to be more potent against anti-IgE induced histamine release than other receptor or non-physiological stimuli.

Warner, J.A., Lichtenstein, L.M. & MacGlashan, D.W., Jr. *J. Pharm. Exp. Ther.* **247**: 218-22, 1988.
Pappu, A.S. & Hauser, G. *Neurochem. Res.* **8**:1565-1575. 1983.
Reinhold, S.L., Prescott, S.M., Zimmerman, G.A. & McIntyre, T.M. *FASEB J.* **4**:208-214, 1990.

215P REGULATION OF HISTAMINE RELEASE FROM HUMAN MAST CELLS AND BASOPHILS BY ESTRADIOL

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The relationship between the sex hormones and the incidence of allergic disease is complex. We have examined the effects of estradiol on mast cells from human lung and uterus and basophils from the peripheral blood. Mast cells were isolated by collagenase digestion and basophils by dextran sedimentation. They were exposed to estradiol (1μM-1pM) for 15 minutes at 37°C and then challenged with 10μg/ml sheep anti-human IgE (anti-IgE) for a further 45 minutes. Estradiol (1pM) enhanced anti-IgE induced histamine release from uterine mast cells by 68±14% (mean±sem, n=7, control histamine release =10±2% of total cellular histamine, $P<0.05$) but failed to affect lung mast cells (n=6, control histamine release =12±3%). Both 10 and 100pM estradiol had similar effects on mediator release from uterine mast cells, though higher concentrations (1μM-1nM) had no significant effect on histamine release. As all the uterine tissue came from pre-menopausal women (average age 40±2 years) while the lung tissue came from both older men and women we decided to see if there were similar differences between the blood basophils of men and women. Basophils from 6 women (average age 23±2 years) who were not using the contraceptive pill showed a similar increase in histamine release in the presence of 1pM estradiol (average enhancement =42±17%, control histamine release =12±5%, $P<0.05$). Intriguingly, basophils from a group of women taking the contraceptive pill failed to show any enhancement (n=6, average age = 23±1 years, average enhancement 8±2%, control histamine release =28±8%, P=NS). Basophils from 6 male donors also failed to show any increase in histamine release when incubated with estradiol (average enhancement =6±15%, control histamine release = 43±7%, P=NS). Enhancement of histamine release was most marked during the first seven days of the menstrual cycle when oestrogen levels are lowest. Testosterone failed to affect anti-IgE induced histamine release from either uterine or lung mast cells or the basophils of any of our groups of donors suggesting that this effect was specifically associated with estradiol and not a general effect of sex steroids.

In summary, physiologically relevant concentrations of estradiol may increase histamine release from mast cells and basophils of pre-menopausal women. These effects may be related to changes in circulating levels of estrogens in female donors which may affect baseline histamine release.

216P HERBIMYCIN A MAY AFFECT PROTEIN KINASE C AS WELL AS TYROSINE KINASES IN HUMAN MAST CELLS AND BASOPHILS

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Recent investigations have implicated tyrosine kinases as second messengers in the allergic response. We have shown that tyrosine kinases in human lung mast cells and basophils have different pharmacologic profiles (Lavens et al 1992). We have therefore examined the effect of herbimycin A, a broad spectrum tyrosine kinase inhibitor, on both cell types.

Basophils or human lung mast cells were incubated with herbimycin A (50, 100 and 500 ng/ml) at 37°C for various time points up to a maximum of 24 hrs. Basophils were washed twice prior to stimulation with either 1μg/ml anti-IgE, 1μM F-met peptide, 10ng/ml phorbol myristate acetate (PMA) or 1μg/ml calcium ionophore, A23187. Mast cells were washed and challenged with 10μg/ml anti-IgE or 1μg/ml calcium ionophore, A23187.

A 16 hour incubation with 500ng/ml herbimycin A inhibited both anti-IgE (54±6%, mean ± sem, n=6, $P<0.0001$ using a paired t test) and PMA (37±5%, n=6, $P<0.0001$) induced histamine release in the basophil. Neither f-met peptide or calcium ionophore, A23187 induced histamine release was affected. There was significant inhibition of anti-IgE induced histamine release (45±7%, n=4, $P<0.05$) in the lung mast cell, but again calcium ionophore was unaffected. Lower concentrations of 50 and 100ng/ml herbimycin A had no significant effect in either cell type.

In summary, the tyrosine kinase inhibitor, herbimycin A, inhibits anti-IgE induced histamine release in both the human basophil and the lung mast cell. However, our experiments with PMA suggest that the drug may modulate protein kinase C (PKC) in addition to tyrosine kinase.

Lavens, S.E., Peachell, P.T. & Warner, J.A.(1992) *Am. J. Res. Cell. Biol.* 7(6): 647-652

Supported by the Wellcome Trust and the National Asthma Campaign.

217P OVALBUMIN CHALLENGE POTENTIATES BRONCHOCONSTRICTION BY U-46619 BUT INHIBITS 5-HT AND METHACHOLINE IN CONSCIOUS GUINEA-PIGS

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Bronchconstrictors including 5HT and acetylcholine are commonly used to demonstrate airway hyperreactivity in anaesthetized guinea-pigs, following bronchial anaphylaxis (Daffonchio, Lees, Payne & Whittle, 1987). The aim of this study was to use conscious guinea-pigs to determine whether direct inhalational challenge with aerosolized antigen leads to hyperreactivity to inhaled 5HT, methacholine (MCh) or the thromboxane analogue U-46619.

Bronchoconstrictor responses were measured in unanaesthetized, spontaneously breathing guinea-pigs previously sensitized with 1ml i.p. of a suspension containing 10µg ovalbumin and 100mg aluminium hydroxide in normal saline. 14-21 days after sensitization, guinea-pigs were exposed to a macroshock (20mg/ml) of ovalbumin in 0.9% NaCl, by inhalation in a sealed box for 2 min. Animals were protected against fatal anaphylaxis with mepyramine (30mg/kg i.p.) 30 min before exposure.

To determine any hyperreactivity, bronchoconstrictor responses were measured as changes in specific airway conductance (sGaw) determined by whole body plethysmography (Griffiths-Johnson, Nicholls and McDermott, 1988). Guinea-pigs were exposed for 1 min to an aerosol of either 5HT (500µg/ml), MCh (100µg/ml) or U-46619 (0.03µg/ml) and sGaw measured before and at 2, 5, 10 and 20 min after exposure. The sGaw values measured at 18-24 h after macroshock were compared with those obtained in the same animal the day prior to macroshock.

Bronchoconstriction due to 5HT and MCh peaked at 2 min (-65.6±10.1 and -57.5±12.1% of control respectively, n=4). At 18-24 h after macroshock, the bronchoconstriction was significantly reduced ($P<0.05$), the peak values at 5 min being -13.5±11.0 and -19.3±5.2% of controls. In contrast, the bronchoconstriction to U-46619, with a peak reduction of sGaw at 5 min after exposure (-22.1±5.3%, n=6), was significantly potentiated ($P<0.05$) after the macroshock (-38.1±3.9%). These results were compared with a control group of sensitized animals receiving an NaCl challenge instead of ovalbumin. In those, the bronchoconstrictor responses to 5HT, MCh and U-46619 before and after NaCl challenge were virtually superimposed.

Thus, in contrast to anaesthetized guinea-pigs, ovalbumin challenge of conscious animals inhibits 5HT- and MCh-induced bronchoconstriction. This apparently masks the airway hyperreactivity observed with U-46619. Ovalbumin challenge may interfere with an indirect component of responses to 5HT and MCh but not with U-46619 acting directly on airway smooth muscle.

Daffonchio, L., Lees, I., Payne, A N. & Whittle, B J R. (1987) Br J Pharmacol 90 141P
Griffiths-Johnson, D A., Nicholls, P J. & McDermott, M. (1988) J Pharmacol Meth 19 233-242

218P THE EFFECTS OF HYPOXIA ON VASODILATATION TO BRL 38227 IN THE ISOLATED PERFUSED RABBIT EAR

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We have recently shown that BRL 38227, the active enantiomer of cromakalim, selectively increases collateral perfusion after acute arterial occlusion and we proposed that this specificity may be associated with hypoxia experienced by collateral vessels (Randall & Griffith, 1992). We have now investigated whether hypoxia influences vasorelaxation to this agent which acts via opening of potassium channels sensitive to ATP.

Rabbit ears were cannulated and perfused *in vitro* with Holman's solution containing 10µM indomethacin and gassed with either 95% O_2/5% CO_2 (normoxia) or 95% N_2/5%CO_2 (hypoxia). The preparations were preconstricted by addition of 5-HT and histamine (both 1µM) and the vasorelaxant properties of BRL 38227 were assessed. Under normoxic conditions the EC_{50} = 343±41nM and the maximum relaxation (R_{max}) = 80.3±4.6% (n=8), while under hypoxic conditions there was a significant ($P<0.01$) leftward shift in the concentration-response curve (EC_{50}= 118±16nM and R_{max}= 89.9±2.7%, n=12). Neither vasorelaxation to sodium nitroprusside nor verapamil was affected by hypoxia. In another set of preparations perfused under normoxic conditions impairment of oxidative phosphorylation with the metabolic inhibitor carbonyl cyanide m-chlorophenylhydrazone (400nM) caused a similar significant ($P<0.01$) leftward shift in the concentration-response curve to BRL 38227, which had an EC_{50} value of 120±20nM and R_{max}=91.4±2.9% (n=7).

The results show that vasorelaxation to BRL 38227 is augmented by hypoxia and this may be related to a reduction in intracellular ATP. Therefore it appears that BRL 38227 may selectively dilate hypoxic blood vessels.

Randall, M.D. & Griffith, T.M. (1992). *Br. J. Pharmacol.*, **106:** 315-323.

This work was funded by a grant from the British Heart Foundation.

219P ASSESSMENT OF THE SMOOTH MUSCLE RELAXANT ACTIVITY OF THE HUMAN MAJOR URINARY METABOLITES OF LEVCROMAKALIM (BRL 38227)

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The disposition and pharmacokinetics of an oral dose of levcromakalim have been determined in man (Kenworthy et al., 1992). The main metabolites appearing in the urine were phase I and II oxidative products, a glucuronide conjugate and unchanged levcromakalim (Table). The aim of the present work was to assess the relaxant activity of these metabolites in comparison with levcromakalim in rabbit isolated mesenteric artery (RIMA), guinea-pig isolated tracheal spirals (GPITS) and on blood pressure in the rat.

RIMA were set up as previously described (Clapham and Wilson, 1987). After equilibration (2h), a tonic contraction to noradrenaline (100 μM) was established and cumulative concentration response curves to levcromakalim or its metabolites constructed. GPITS were set up according to Foster et al (1983). After equilibration, GPITS were incubated with aminophylline (1mM) for 10 min to determine point of zero tone, washed twice and spontaneous tone allowed to develop (30 min). Once tension had developed drugs were administered as described for RIMA. Values are expressed as IC_{50} (conc. producing 50% inhibition) or the % inhibiton at the highest concentration used. Female spontaneously hypertensive rats (205-295g) were anaesthetised with pentobarbitone sodium (60 $mg.kg^{-1}$ i.p.). A carotid artery and jugular vein were cannulated for blood pressure measurement and drug administration respectively. Data are expressed as % fall in diastolic blood pressure (DBP, mmHg) at 5-10 min post dose. The effects of levcromakalim and its metabolites are shown in the Table .

COMPOUND	% * in urine	RIMA IC_{50} (μM ; n= 3 to 6)	GPITS IC_{50} (μM ; n= 3 to 6)	I.V. DOSE $mg.kg^{-1}$	% CHANGE DBP ±SEM
Levcromakalim	20.6 ± 5.8	0.40 (0.20 - 0.81)	0.78 (0.19 - 3.18)	0.05	-52.7 ± 6.0 (n=6)
Glucuronide	30.0 ± 5.5	26%[a]	25%[c]	1.6	-1.3 ± 1.8 (n=6)
Amidocarboxylic acid	20.0 ± 2.6	26%[a]	9%[c]	1.0	-1.6 ± 0.9 (n=5)
3'R hydroxy	4.6 ± 0.8	4.27 (0.87 - 20.89)	6.76 (2.99 - 15.31)	NT	
Amidohydroxy	1.3 ± 0.2	31%[b]	36%[d]	NT	

* Data from Kenworthy et al, 1992. a = 300 μM, b = 100 μM, c = 30 μM, d = 10 μM. 95% confidence limits in parenthesis and NT = not tested.

The data show that the major urinary metabolites of levcromakalim, the glucuronide and the amidocarboxylic acid, have negligible biological activity at 40-700 times the concentration of levcromakalim *in vitro* and at 20-30 times the levcromakalim dose halving DBP. The 3'R hydroxy metabolite relaxed both RIMA and GPITS but was 10 times less potent than levcromakalim. This metabolite accounted for only 5% of the total radiometabolite pattern. These data suggest therefore that the biological activity of levcromakalim resides solely in the parent compound with no contribution from metabolic products.

Clapham, J.C. and Wilson, C. (1987) J. Auton. Pharmacol., 7, 233-242.
Foster, R.W.,et al., (1983) Br. J. Pharmcol., 79, 255-263.
Kenworthy, D., et al., (1992)Br. J. Clin. Pharmacol., 34, 449P

220P OZONE EXPOSURE IN GUINEA-PIGS INDUCES BRONCHIAL HYPERREACTIVITY TO HISTAMINE AND SALBUTAMOL, BUT NOT TO LTD_4

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Non-specific bronchial hyperreactivity (BHR) to bronchoconstrictor agents is generally held to be a characteristic feature of asthma. However, it has been variously reported that BHR to leukotriene (LT) D_4 is either present (Smith, et al, 1985) or absent (Griffin, et al, 1983) in asthmatics. In principle, BHR to bronchodilators may also occur although this has not been reported. Ozone (O_3) exposure induces BHR in guinea-pigs to a range of bronchoconstrictor agents (Yeadon, et al, 1992). In this study we have compared the airway reactivity to inhaled LTD_4, histamine (H) and salbutamol (S) in anaesthetised guinea-pigs after exposure to either air or O_3.

Guinea-pigs were exposed to either air (controls) or O_3 (3ppm, 2h) then anaesthetised with sodium pentobarbitone. During artificial ventilation, changes in pulmonary inflation pressure (PIP) resulting from inhalation of LTD_4 (10-320ng/ml) or H (1.25-320μg/ml), 10s nebulisation (DeVilbiss Pulmosonic) were recorded. In studies with LTD_4 and H, the concentration of each required to provoke a $20cmH_2O$ rise in PIP (PC_{20}) was determined by interpolation, and used as a measure of reactivity. In separate experiments, the ability of S (1-1000μg/ml, 10s nebulisation, 1min pretreatment, one concentration/animal) to reduce bronchoconstriction induced by nebulised H (measured as area under the curve of increased PIP for 5min) was determined in control and O_3 exposed animals.

In controls, the PC_{20} (mean±sem, n=5-6) for H was 130±15μg/ml, but in O_3 exposed animals, PC_{20} was reduced to 13±2μg/ml ($P<0.001$), confirming that BHR was induced. In contrast, reactivity to LTD_4 (control PC_{20}: 150±18ng/ml) was not increased after O_3 exposure, and bronchoconstrictor responses were characterised by a reduced maximal elevation (less than $20cmH_2O$) in PIP after O_3.

In both control and O_3 exposed animals (n=4-8), S reduced the bronchoconstrictor effect of H in a concentration-related manner. In controls, the maximum effect was obtained at 100μg/ml S, with a small effect after 10μg/ml S. In contrast, in O_3 animals the maximum effect was observed after 10ug/ml S, with no effect at 1ug/ml. The effects of S were compared at concentrations of H (1250μg/ml in controls, 250μg/ml in O_3 animals) which produced comparable, sub-maximal bronchoconstriction. In controls, 10 and 100ug/ml S produced 13±8% and 67±7% inhibition of H-induced bronchoconstriction, whilst in O_3 animals, 1 and 10ug/ml S produced -1±3% and 49±12% inhibition. The effect at 10ug/ml S was significantly greater ($P<0.01$) in O_3 exposed guinea-pigs than in controls.

We have shown that O_3 exposure produces BHR to inhaled H but not to inhaled LTD_4, demonstrating that the phenomenon of BHR is not agonist non-specific in this model, which may parallel the BHR of asthma. Further, we have shown that BHR to the bronchodilator salbutamol also occurs after O_3. It is intruiging that O_3 exposure does not potentiate responses to LTD_4, and it remains to be established whether the mechanisms underlying O_3 induced BHR to H and S are the same.

Yeadon, M., Wilkinson, D., Darley-Usmar, V., O'Leary, V. J. & Payne, A. N. (1992). Pulm. Pharmacol., **5**, 101-112.
Smith, L.J., Greenberger, P.A., Patterson, R., Krell, R.D. & Bernstein, P.R. (1985). Am. Rev. Resp. Dis., **131**, 368-372.
Griffin, M., Weiss, J., Leitch, A.G., McFadden, E.R., Corey, E.J., Austen, K.F. & Drazen J.M. (1983). N. Eng. J. Med., **308**, 436-439.

221P IL-4 GENE DELETION PROFOUNDLY ATTENUATES LUNG EOSINOPHILIA IN PARASITE-INFECTED MICE BY IMPAIRING T-CELL COMMITMENT TO AN IL-5 PRODUCING (CD4+ Th2) PHENOTYPE

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In a number of *in vivo* experimental models eosinophilia is governed by production of interleukin 5 (IL-5), a cytokine essential for the terminal differentiation of eosinophils, produced predominantly by Th2 T lymphocytes bearing the CD4+ surface marker. Th2 cells have a distinct cytokine production profile producing IL-4 and IL-5 but little IFNγ. The *in vivo* determinants of T cell commitment to a Th2 cytokine pattern are not known but *in vitro* IL-4 is itself an essential signal to induce T cell IL-4 synthesis [1]. In order to identify molecular targets for pharmacological intervention in the therapy of asthma we have studied the role of IL-4 in the induction of IL-5 producing T-cells by deleting the genomic IL-4 gene using embryonic gene targeting in mice (C57B6 x 129cv, age matched). Mice were infected s.c. with 750 stage 3 larvae of *N. brasiliensis*, a helminth that induces an intense systemic and pulmonary eosinophilia and elevated IgE. Differential counts of peripheral blood made over 13 days showed strong leukocytosis and eosinophilia in control (+/+) mice and an attenuated response in gene targeted (-/-) mice on day 13 (+/+, 6.0E3; -/-, 2.3E3 eosinophils/ml, $p<0.05$, *t*-test). Differences in bronchoalveolar eosinophils were marked (+/+,1.8±1.1E6; -/-, 4.0±1.1E5, total eosinophils recovered from 1.2 ml lavage, day 13, $p<0.05$) and less eosinophils were observed histologically in lung inflammatory cell infiltrates in (-/-) mice. Serum IgE was undetectable in (-/-) mice. Lung cytokine mRNA levels (transcripts/μg RNA) measured on day 7, before the onset of peak eosinophilia, were determined by quantitative PCR and are tabulated:

mouse type	IL-4	IL-5	IL-6	IL-2	IFNγ	TNFβ	TNFα	IL-1α
+/+	50	150	2000	1.6	8	1.6	1000	1000
-/-	0	20	400	1	8	1.6	1000	1000

Cytokine protein determined in (-/-) mice by bioassay or ELISA from supernatents of lymphoid T cells sorted for CD4 or CD8 surface markers and restimulated *in vitro* with anti-CD3 antibodies showed weak IL-5 production by CD4+ T cells and the induction of a CD8+ pool of T cells weakly producing IL-5. These data indicate the IL-4 is a dominant, but not obligatory, determinant of T cell commitment to Th2 phenotype. Neither IL-4 nor IgE are necessary for transmigration of eosinophils into the lung. The presence of IL-5 producing CD8+ cells suggests a biological "escape" from strictly Th2 mediated eosinophilia.

[1] LeGros et al., (1990). J. Expt Med., 172, 921-929.

222P INTRAVENOUS PAF CAUSES EOSINOPHIL RECRUITMENT TO THE LUNGS OF NORMAL AND ALLERGIC HORSES

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Allergen challenge of horses with allergic respiratory disease leads to an increase in neutrophil numbers in bronchoalveolar lavage fluid, together with an increase in eosinophils in some animals (Derksen *et al*, 1985). We have previously demonstrated that i.v. administration of PAF alters airway function and causes radiolabelled neutrophil accumulation in the lungs of both normal and allergic horses (Fairbairn *et al*, 1992a and b). The aim of the present study was to compare the effects of i.v. PAF on eosinophil distribution in normal and allergic horses, in clinical remission at the time of the study.

Equine peripheral blood eosinophils (10^8) were radiolabelled with 500μCi ^{111}-indium-merc and reinfused back into the donor horse. Radiolabelled cell distribution *in vivo* was monitored externally by an automated isotope monitoring system with probes placed over the lungs and a control site, the leg. Airway function was measured using an oesophageal balloon to detect maximum changes in pleural pressure (ΔPplmax) and respiratory (resp.) rate.

PAF (5ng/kg; i.v.) caused a rapid influx of radiolabelled eosinophils into the lungs of both normal (n=5) and allergic (n=5) horses which was maximal at 5min (mean ± s.e.mean % increase in radioactive counts; 9.6 ± 4.6% and 13.1 ± 2.4 %, respectively). Maximal decreases in radiolabelled eosinophils in the leg were detected at 7min (-10.6 ± 2.4%) in normal horses and at 1min (-4.7 ± 2.0%) in allergic horses. In addition, both ΔPplmax and resp. rate were transiently increased in normal (ΔPplmax: 2.9 ± 0.4 and 8.8 ± 2.7 cmH_2O; resp. rate: 17.0 ± 4.0 and *39.0 ± 4.5 breaths/min, pre and 1min after PAF, respectively) and allergic horses (ΔPplmax: 3.6 ± 0.8 and *7.6 ± 1.6 cmH_2O; resp. rate: 15.0 ± 3.1 and *26.0 ± 1.9 breaths/min, pre and 1min after PAF, respectively; * = $p < 0.05$, paired Student's t test). There were no significant differences between the responses of normal and allergic horses to PAF.

Systemic administration of PAF thus caused recruitment of equine eosinophils to the lungs. Eosinophils from asymptomatic allergic horses showed no difference in sensitivity to PAF at the dose rate used in this study.

We are grateful to the Horserace Betting Levy Board for financial support .

Derksen F.J., Scott J.S., Miller D.C., Slocombe R.F. and Robinson N.E. (1985) Am. Rev. Respir. Dis., **132**, 1066-1070
Fairbairn S.M., Lees P., Page C.P. and Cunningham F.M. (1992a) Br. J. Pharmacol., **105**, 124P
Fairbairn S.M., Lees P., Page C.P. and Cunningham F.M. (1992b) Br. J. Pharmacol., **107**, 394P

223P TRACHEAL HYPERREACTIVITY AND PULMONARY INFLAMMATION IN A DELAYED-TYPE HYPERSENSITIVITY (DTH) REACTION IN THE MOUSE LUNG

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Increased reactivity of the airway smooth muscle to various stimuli and pulmonary inflammation are prominent features in asthma. Although the mechanisms involved in the pathogenesis of asthma are still not fully understood, the lymphocyte has been proposed to play a critical role. We have recently developed delayed-type hypersensitivity (DTH) reactions in the mouse lung using picryl chloride as the sensitising hapten (Garssen et al 1991). Interestingly, these reactions were found to be T lymphocyte-dependent. In this study DTH reactions were investigated further in the mouse lung using dinitrofluorobenzene (DNFB) as the sensitising hapten.

DNFB (0.5 %, 100 µl) or vehicle control were applied epicutaneously to the shaved thorax and feet of anaesthetised (sodium pentobarbitone 30 mg/kg) mice (Balb/c) on two consequetive days. Five days thereafter the mice were challenged intranasally with dinitrobenzene sulphonic acid (DNS; 0.6 %, 50 µl). Pulmonary responses were measured 2, 24 and 48 h after challenge. Tracheal reactivity was assessed by measuring isometric contractions (mg) of the isolated trachea to the cholinergic agonist carbachol. Leukocyte accumulation and Evan's blue (EB) leakage in the bronchoalveolar lavage (BAL) fluid were assessed by perfusing the airways with saline. Total leukocyte accumulation (cell/lung) was measured using a haemocytometer. Leukocytes were also differentiated, after eosin/thiazine staining, by light microscopy. To assess leakage into the airways EB (1.25 % in sterile saline, 50 µl) was administered intravenously (i.v.) 2 h before taking the BAL fluid. A few minutes before the lavage, heparin was also injected i.v. and a blood sample was taken. The concentrations of EB (µg/ml) in the BAL fluid and the plasma were evaluated by measuring the samples spectrophotometrically (620 nm). Leakage was expressed as % EB in the lavage fluid compared to EB in 1 ml of plasma.

Trachea taken from sensitised (DNFB + DNS) mice showed marked hyperreactivity (increase in E_{max}) to carbachol 24 and 48 h (but not 2 h) after challenge when compared to trachea taken from non-sensitised (vehicle + DNS) mice. (Results E_{max}: non-sensitised; 2133 ± 187 mg, 1944 ± 156 mg, 1941 ± 114 mg: sensitised; 2021 ± 220, 2663 ± 147, 2496 ± 148 mean ± s.e.mean n=6-12 mice/group for 2, 24 and 48 h after challenge respectively.) Leakage of EB and mononuclear leukocyte accumulation were also elevated 24 h after challenge in the BAL taken from sensitised mice but both these parameters had returned to control levels 48 h after challenge.

In summary, the DNFB-induced DTH reaction in the mouse lung is characterised by airways hyperreactivity which is associated with mononuclear leukocyte accumulation and leakage in the airways 24 h after challenge. At 48 h after challenge the signs of pulmonary inflammation had diminished but hyperreactivity was still evident. We are currently using this model to investigate the complex relationship between pulmonary inflammation and airways hyperreactivity.

TLB is in receipt of a Royal Society European Exchange Fellowship. ADK is supported by a Glaxo (UK) studentship

Garssen J, Nijkamp FP, Van der Vliet H, Van Loveren, H. Am.Rev.Respir.Dis. 1991 144: 931-938.

224P THE EFFECTS OF LOW FREQUENCY, LOW AMPLITUDE MAGNETIC FIELDS ON GRAMICIDIN CHANNELS

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Channels formed by gramicidin A provide a well defined system for investigating effects of low frequency, low amplitude magnetic fields on ion transport through pores. Small changes in the summed current through many channels can be detected using a bridge circuit (Hainsworth & Hladky, 1987). Sufficient gramicidin was added to produce about 10^4 pores in glyceryl monooleate +n-hexadecane bilayers. The static magnetic field at the membrane was nulled and the conductance of the reference arm was adjusted to match that of the membrane during 10msec, 75mV pulses applied at 10Hz. A combination of static and varying magnetic fields were then applied and the frequency varied from 10 to 200Hz over 1 to 2 minutes, and any change in the conductance noted. Under the conditions of the survey, the resolution was between 0.2 and 0.5%. With LiCl, CsCl and KCl many combinations of field strengths between 50µT and 5mT were used with static and varying fields oriented parallel or perpendicular to each other and parallel or perpendicular to the membrane (Wang & Hladky, 1992). In no case did the summed conductance during the application of the magnetic fields differ from the control. Galt et al (1992) found no effect greater than approximately 2% for 0.1M KCl.

Opening rate, open time, single-channel conductance, frequency of flickers persisting for two or more data samples, and the average flicker duration for the longest lived population of flickers can each be determined in patched clamp bilayers. Currents are recorded in patches with up to five channels open using an Axopatch 200 amplifier with internal filter at 5kHz, recorded on digital audio tape, transferred via an 8 pole Bessel filter at 2kHz, sampled at 4kHz with a CED 1401 interface, and analysed using the continuous analysis module of the CED Patch software version 5.5. Transitions between results are detected as crossings of a series of equally spaced thresholds. Flickers and openings are easily distinguished. Most flickers persist for less than two data points (0.5msec), a few last as long as 5msec (compare Ring,1986 and Sigworth et al,1987) while channel opentimes are hundreds of milliseconds or seconds. Artifactual, frequent small transitions or 'flutters' occur in the results file when a (rare) non-standard conductance level coincides with a threshold. These are edited out of the results file by an auxiliary program that detects the repetition of small, brief fluctuations. By visual inspection the results file is then a faithful copy of the data except that many single-point flickers are missed because they do not cross a threshold and occasional, brief sojourns in a partly closed state on the way to full closure are merged with the closed state. Preliminary experiments with 1M KCl are in agreement with the results of Galt et al (1992) in finding no effect of applied magnetic fields

This work was supported by a grant from the National Grid Company plc.

Galt,S., Sandblom,J., Hamnerius,Y., Höjevik,P., Saalman,E. & Nordén,B. (1992) *Bioelectromagnetics*, in press.
Hainsworth,A.H. & Hladky,S.B. (1987) *Biophys.J.* 51,27-36.
Ring, A. (1986) *Biochim.Biophys.Acta* 856,646-653.
Sigworth,F.J., Urry,D.W. & Prasad,K.U. (1987) *Biophys. J.* 52,1055-1064.
Wang,K.-W. & Hladky,S.B. (1992) *Ann. Rev. Research. on Biological Effects of Electric and Magnetic Fields*. U.S. Dept. of Energy.

225P PROTEIN KINASE C ACTIVATION IS INVOLVED IN AGONIST-STIMULATED SECRETION OF t-PA, BUT NOT PAI-1, FROM CULTURED ENDOTHELIAL CELLS

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The vascular endothelium regulates fibrin clot lysis by balanced secretion of tissue-type plasminogen activator (t-PA) and of its specific inhibitor plasminogen activator inhibitor type 1 (PAI-1). Agonists and several other agents, including the protein kinase C activating phorbol ester phorbol 12-myristate 13-acetate (PMA) modulate the secretion of one or both of these proteins (Levin & Santell, 1988). We have now undertaken studies to determine if protein kinase C plays a role in agonist-stimulated secretion of t-PA and PAI-1.

Experiments were performed using passage 2 human umbilical endothelial cells (HUVEC) cultured in 16mm wells. After exposure to agonists or other agents for 24 hours at 37°C in serum-free medium the accumulation of t-PA and of PAI-1 antigens in the cell culture supernatant was measured using specific ELISAs.

Incubation with PMA (50nM) caused significant increases in the secretion of t-PA (348±32% control; mean±S.E., n=16) and of PAI-1 (151±11% control; n=7). Incubation with 4α-phorbol 12,13-didecanoate (α-PDD) (50nM), which is unable to activate protein kinase C, did not enhance secretion of t-PA above basal level, but stimulated secretion of PAI-1 to a level which was similar to that obtained with PMA (177±22%; n=3). Ro31-7549, a selective inhibitor of protein kinase C (Davies et al.,1989), added 15 minutes before PMA, dose-dependently inhibited t-PA secretion with an IC_{50} of 1μM, but over the same concentration range had no effect on PAI-1 secretion induced by PMA. Ro31-7549 (1μM) caused no significant reduction in t-PA secretion induced by 10nM thrombin , but reduced t-PA secretion induced by 10μM histamine to basal level.

These results indicate that activation of protein kinase C is required to observe t-PA secretion induced by histamine, but that this enzyme is not involved in thrombin-induced t-PA secretion. Furthermore, although an agent which activates protein kinase C stimulated the secretion of PAI-1, this effect was not due to the activation of protein kinase C and resulted from some other effect of the phorbol ester, which remains to be defined.

Davies,P.D., Hill,C.H., Keech,E., Lawton,G., Nixon,J.S., Sedgwick,A.D., Wadsworth,J., Westmacott,D. & Wilkinson,S.E. (1989). *FEBS letts.* **259**:61-63
Levin,E.G. & Santell,L (1988). *J. Biol. Chem.* **263**:9360-9365

226P THE EFFECT OF A THROMBOXANE MIMETIC AND A THROMBOXANE RECEPTOR ANTAGONIST ON OESTROGEN-DEPENDENT CELL GROWTH, USING THE MCF-7 CELL LINE

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The MCF-7 cell line was first described in 1973 as being derived from a primary pleural effusion from a breast cancer carcinoma, since then it has been characterised for oestradiol receptivity and tumourigenicity. Previous studies in this laboratory have suggested a thromboxane involvement in oestrogen dependent growth (Kerr *et al.*, 1991). To investigate further the pharmacological properties of a thromboxane mimetic and a thromboxane receptor antagonist on the MCF-7 cell line, cells were routinely grown in RPMI medium (without phenol red, which has been found to be oestrogenic, and as such elicits a marked effect on the cellular responses to oestrogenic compounds), with 5% dextran-charcoal stripped foetal bovine serum, glutamine, insulin and antibiotic/antimycotic solution. Cultures were passed every 7-10 days, at confluence, and were used between passage 570 and 574. Cells were plated at 2-3 x 10^4 cells per ml into multi-well plates two days before drug addition (i.e. day -2); media was changed to phenol red minus RPMI on day -1. Drugs were added on day 0 from stock 10^{-3}M ethanolic solutions, and ethanol concentration never exceeded 0.3% in the growth media. Media was changed every two days and cells were counted on day 4 of growth using the electronic sensing zone technique (the Coulter principle). Duplicate wells were counted, and data shown is from three separate experiments. Results are expressed as percentage growth relative to controls.

U46619, a thromboxane mimetic, caused an increase in cell growth (133% of control growth ±16%) at 10^{-9}M. EP092, a thromboxane antagonist, attenuated cell growth at 10^{-6}M, to 29% of control ±13%. In order to assess the relative effects of these compounds, oestradiol was used to demonstrate classical oestrogen receptor effects. Oestradiol caused an increase in cell growth up to 121%±11% of control at 10^{-10}M.

In combination studies it was found that EP092, at all concentrations tested, was able to overcome the stimulation induced by U46619 at 10^{-9}M, a more marked effect was seen at 10^{-6}M EP092, as before. Conversely, EP092 (10^{-6}M), when used with U46619 10^{-9} to 10^{-6}M, caused a more marked cell regression than that elicited by EP092 10^{-6}M alone, to less than 20% of control growth. However, EP092 at 10^{-6}M overcame the oestradiol induced stimulation in cell growth. The proliferative effects of U46619 have been previously reported on rat epithelial mammary cancer cell line (Nigam & Zakrzewicz, 1990). This study supports a role for thromboxane in oestrogen dependent cell growth and raises the question of an interaction between the oestrogen receptor (ER) and thromboxane receptor agonists and antagonists. Radioligand binding studies are in progress to elucidate whether there is a thromboxane receptor present on these cells and also to assess if any direct competition takes place between oestradiol and the thromboxane receptor agonists and antagonists for the ER.

Kerr, M.B., Marshall, K., Senior, J. (1991) *Br. J. Pharmacol.* **102**, 742-746
Nigam, S. & Zakrzewicz, A. (1990) *Advances in Prostaglandin, Thromboxane and Leukotriene Research* **21**, 925-928

227P PHARMACOLOGICAL PROFILE OF [^{3}H]-VINBLASTINE BINDING TO THE PARENT LUNG CANCER CELL LINE H69/P WHICH DO NOT EXPRESS P-GLYCOPROTEIN

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The human lung cancer cell line H69/LX4 was derived from the parent cell line H69/P by increasing the concentration of doxorubicin in the culture medium up to 760 nM. H69/LX4 cells hyperexpress P-glycoprotein (a 170 kDa ATPase). As a consequence these cells are resistant to anthracyclines, vinca alkaloids, epipodophyllotoxins and actinomycin D, but this is reversed by e.g. verapamil and cyclosporin A and termed multidrug resistance (MDR), (Twentyman et al, 1986).

Partially purified surface membranes were prepared from the resistant (H69/LX4) and sensitive (H69/P) cell lines by homogenising ≈2 x 10^8 cells in ice cold Tris HCl pH 7.4 supplemented with 0.1 mM PMSF with an ultraturrax. Membranes were then isolated by centrifugation as previously described (Ferry et al, 1992). Binding assays were performed in 50 mM Tris HCl in a volume of 0.25 ml at 23° C allowing 60 min for equilibrium to be reached. For experiments with H69/LX4 or H69/P membranes 10-20 µg and 90-120 µg of membrane protein were employed respectively.

At 9.4 nM [^{3}H]-vinblastine, defining specific binding with 30 µM unlabelled vinblastine H69/LX4 membranes bind ≈ 10 pmol/mg, whereas H69/P membranes bind 0.7 pmol/mg [^{3}H]-vinblastine. In H69/LX4 membranes the K_D of [^{3}H]-vinblastine was 9.8 ± 1.5 nM and B_{max} 31 ± 9 pmol/mg. The affinity of [^{3}H]-vinblastine for H69/P membranes was low and therefore the saturation isotherm constants had to be determined by titration of [^{3}H]-vinblastine specific activity by unlabelled drug, the K_D in H69/P membranes was 584 ± 210 nM (n=3) and B_{max} 65 ± 33 pmol/mg of membrane protein. The pharmacological specificity of saturable [^{3}H]-vinblastine binding was explored in both membrane preparations. In the H69/LX4 membranes the following cytotoxics (with K_i values in parentheses ± SEM for n=3) inhibited binding: vincristine (600 ± 180 nM), actinomycin D (3020 ± 1540 nM), doxorubicin (18200 ± 4500 nM) and etoposide (20 000 nM, n=2). For the MDR reversing agents: cyclosporin A (17 ± 2.2 nM), quinidine (274 ± 119), quinine (1912 ± 473 nM), verapamil (600 ± 180 nM), nicardipine (153 ± 47 nM, n=5), nifedipine (5000 nM, n=2) were active. In contrast in H69/P membranes only the vinca alkaloids vinblastine (K_i 584 nM), vincristine (K_i 1233 ± 222 nM, n=3) and vindesine (890 ± 435 nM) were active drugs, the other cytotoxics and MDR reversing agents were inactive at 100-1000 fold K_i to inhibit [^{3}H]-vinblastine binding to P-glycoprotein.

Although the structural differences between vinblastine (methyl) and vincristine (aldehyde) are small , this confers high selectivity for vinblastine to bind to P-gp. The identity of the vinblastine binding site in the H69/P membranes is unknown, but as only the three vinca alkaloids are active it may be a vinca binding cytoskeletal element, perhaps membrane associated tubulin, as the K_D of vinblastine to bind to tubulin is reported to be 6.6 µM (Bai et al, 1990).

Bai, R., Pettit,G.R. & Hamel, E. (1990), J Biol Chem, **265**, 17141-17149.
Ferry, D.R., Russell, M. & Cullen M.H. (1992) Biochem and Biophys Res Commun. **188**, 440-445.
Twentyman, P.R., Fox, N.E., Wright, K.A. & Bleehen, N.M. (1986), Br J Cancer, **53**, 529-537.

228P COMPARATIVE STUDIES OF THE ANGIOGENIC ACTIVITY OF PLATELET-DERIVED ENDOTHELIAL CELL GROWTH FACTOR AND BASIC FIBROBLAST GROWTH FACTOR IN A RAT SPONGE MODEL

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Earlier *in vitro* work showed the chemotactic and mitogenic activities of platelet-derived endothelial cell growth factor (PD-ECGF) to be specific for endothelial cells. Recent studies suggest that it may also stimulate angiogenesis by an indirect mechanism through its thymidine phosphorylase activity (Usuki, *et al.*, 1992). Indeed, PD-ECGF has been reported to stimulate thymidine uptake in endothelial cells without causing them to proliferate *in vitro* (Moghaddam & Bicknell, 1992). Here we used a rat sponge model to characterise the angiogenic effect of *E. coli* recombinant PD-ECGF and compared it with that of basic fibroblast growth factor (bFGF).

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (150-200g) and, using a ^{133}Xe clearance technique (Andrade, *et al.*, 1987), neovascularisation was assessed as a function of blood flow through the implants every two days over two weeks. The effects of test substances on angiogenesis were confirmed histologically and morphometrically. Using a computer-assisted image analysis system, the fibrovascular growth areas of the sponges were measured by the axial strip sampling technique of Mayhew & Sharma (1984). Length density of blood vessels was calculated as described by Gundersen *et al.* (1988).

Daily administration of 10 pmol recombinant human PD-ECGF into the sponges caused an intense neovascularisation. The 6 min ^{133}Xe clearance values obtained on Day 6, 8, 10, 12 for the PD-ECGF group and the control group were 25.9±2.0, 32.6±1.7, 38.5±0.8, 42.9±1.2 and 15.7±0.5, 21.6±1.1, 30.8±1.1, 36.0±1.1 %, respectively ($P < 0.01$, $n = 6$). Histological studies of sponge sections showed massive infiltration of neutrophils and monocytes and intense angiogenesis in the PD-ECGF-treated sponges as compared to controls. Morphometric analysis showed the fibrovascular growth areas on Day 8 for the control group and the PD-ECGF group were 16.3±2.3 and 60.8±6.5 % respectively ($P < 0.01$, $n = 3$) and length density of blood vessels on Day 8 for the control group and the PD-ECGF group were 10.7±1.1 and 62.5±4.6 mm mm^{-3} sponge, respectively ($P < 0.01$, $n = 3$). Daily injection of 6 pmol recombinant human bFGF into the sponges also produced an angiogenic response, but without significant inflammatory cell infiltration. Lower doses of PD-ECGF (0.1 and 1 pmol) or bFGF (0.06 and 0.6 pmol) produced no apparent effect on sponge-induced neovascularisation. Further studies showed the neovascular response induced by bFGF, but not PD-ECGF, was markedly suppressed by co-administration of 3 mg suramin or by a single i.v. injection of 200 mg kg^{-1} on Day 1 after sponge implantation.

In conclusion, our data indicate that PD-ECGF and bFGF elicit angiogenesis by distinct mechanisms. This appears to be consistent with the recent finding that bFGF, but not PD-ECGF, stimulates endothelial proliferation *in vitro* (Moghaddam & Bicknell, 1992). The mechanism by which PD-ECGF stimulates angiogenesis remains to be elucidated.

This work is supported by the Wellcome Trust.

Andrade, S.P., Fan, T.-P.D. and Lewis, G.P. (1987) *Br. J. Exp. Path.* **68**, 755.
Gundersen, H.J.G., Bendtsen, T.F., Korbo, L. *et al.* (1988) *APMIS* **96**, 379.
Mayhew, T.M. & Sharma, A.K. (1984) *J. Anat.* **139**, 45.
Moghaddam, A. & Bicknell, R. (1992) Biochemistry (in press).
Usuki, K., Saras, J., Waltenberger, J. *et al.* (1992) *Biochem. Biophys. Res. Commun.* **184**, 1311.

229P EFFECTS OF SELECTED CYTOKINE ANTIBODIES AND IL-1 RECEPTOR ANTAGONIST ON ANGIOGENESIS IN THE RAT

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We have previously shown that daily administration of recombinant human basic fibroblast growth factor (bFGF), tumour necrosis factor (TNF-α), interleukin-1 (IL-1α) or IL-8, caused intense neovascularisation in a rat sponge model (Hu *et al.*, 1992). Here we tested if the angiogenic activity of these cytokines could be manipulated by their respective antibodies or receptor antagonist.

Sterile polyether sponges were implanted subcutaneously in male Wistar rats (180-200g) and neovascularisation was assessed as a function of blood flow through the implants every two days over a period of 14 days, using a ^{133}Xe clearance technique.

Daily doses of 100 ng bFGF, 50 ng TNF-α, 25 ng IL-8, or 50ng IL-1α, caused an intense neovascularisation. These cytokine-induced neovascular responses were inhibited by co-administration of antibody to bFGF (DG2; 1000 ng), TNF-α (TNF-ab; 500 ng), IL-8 (IL-8-ab; 1:1000) or IL-1 receptor antagonist peptide (IRAP; 50μg), respectively. Histological studies of sponge sections showed that both the cellular infiltration and neovascularisation in the bFGF-, TNF-α-, IL-8- or IL-1α-treated sponges were profoundly suppressed by DG2, TNF-ab, IL-8-ab or IRAP, respectively. The 6 min ^{133}Xe clearance values mean ± s.e. (n = number of animals) in various groups are as follows:

	Day 6		Day 8		Day 10		Day 12	
	+PBS	+ AB or IRAP	+PBS	+AB or IRAP	+PBS	+AB or IRAP	+PBS	+AB or IRAP
Control	15.6±1.1 (22)		21.1±1.0		30.2±1.0		35.9±1.1	
bFGF	23.6±1.9 (8)	15.7±1.7 (6)*	30.9±0.7	21.0±1.8*	40.3±1.0	31.0±1.4*	42.0±1.0	37.0±1.0*
TNF-α	23.4±1.2 (8)	16.5±1.0 (6)*	31.1±0.8	23.8±1.0*	40.3±0.5	31.5±1.2*	43.1±0.5	36.9±1.0*
IL-8	24.3±1.0 (14)	15.6±1.5 (4)*	31.9±0.8	21.0±1.0*	38.1±1.7	31.1±0.8*	41.6±1.6	34.7±1.1*
IL-1α	24.3±1.0 (16)	16.6±2.0 (4)*	31.1±1.1	22.9±1.5*	39.8±1.6	32.7±0.8*	42.7±1.6	34.1±1.2*

* P<0.01; PBS = phosphate buffered saline; AB = antibody to the cytokine in use.

In the absence of exogenous peptides, the sponge-induced angiogenesis was not modified by DG2, TNF-ab, IL-8-ab or IRAP alone. However, this neovascular response was significantly inhibited by the combination of these four reagents. The 6 min value of control group on Day 8, 10, 12, 14 were 20.7±1.0, 29.8±1.1, 36.3±1.0, 40.2±1.0% (n = 8) while co-administration group were 16.4±1.1, 19.6±3.4, 22.7±2.5, 24.7±1.6%, respectively (n = 4, $P < 0.05$). Histological studies confirmed a profound inhibition of angiogenesis.

Thus, these results highlight the involvement of various cytokines in angiogenesis. Further studies are necessary to delineate the exact role of cytokines in vascular development and to explore the therapeutic potential of cytokine antibodies and antagonists.

We thank the Wellcome Trust for support and Drs T.M. Reilly (Du Pont Merck, USA), R.Thorpe (National Institute of Biological Standards and Control, England) and R.C.Thompson (Synergen Inc., USA) for the gifts of DG2, IL-8-ab and IRAP, respectively.

Hu, D.E., Hori, Y. & Fan, T.-P.D. (1992) *Br. J. Pharmacol.*, **106**, 103P.

230P IL-5-INDUCED EOSINOPHILIA IN THE RAT PLEURAL CAVITY: THE EFFECT OF DEXAMETHASONE AND INDOMETHACIN

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Interleukin 5 (IL-5) is a 115 amino acid protein that specifically facilitates the terminal differentiation and proliferation of eosinophils (Eos), as well as the survival, viability and antibody dependent cytotoxicity of mature Eos. Furthermore, this protein is a potent chemotactic factor for murine and human Eos *in vitro* (Yamaguchi *et al*, 1988a, Yamaguchi *et al*, 1988b). We have used this mediator to generate a model of Eos migration in the rat pleural cavity and to investigate the effects of dexamethasone and indomethacin on the Eos infiltration.

Female Wistar rats (200-220g) were anaesthetised and injected intrapleurally with 25 units recombinant murine IL-5 (rmIL-5, Genzyme) in 0.1ml of pyrogen free saline with 0.1% BSA. To determine the degree of Eos infiltration, the animals were killed by anaesthetic overdose and the contents of the pleural cavity lavaged with 1ml of citrated saline. Total and differential cell counts were then used to determine the percentage and total Eos. Dexamethasone or indomethacin, were given at 1mg/kg i.v. in 0.5 ml pyrogen free saline at the time of IL-5 challenge and again (1mg/kg) 6 hours later i.p. Groups of 6 animals were used and the results analysed for statistical significance using analysis of variance.

Time course studies demonstrated a similar cell infiltration profile to that produced by platelet activating factor (PAF) Silva *et al* 1991. There is an early neutrophil infiltration which was maximal at 6 hours and had returned to normal by 48 hours. Mononuclear cells peak at 48 hours but the increase is not statistically significant. The peak Eos infiltration is at 24 hours and remains elevated until 48 hours. Mononuclear cells predominate in control and IL-5 treated pleural cavities 24 hours post injection, $4.7\pm0.6 \times 10^6$ and $6.6\pm0.8 \times 10^6$ cells respectively. Neutrophils and Eos represent a much smaller proportion of the normal washout ($0.52\pm0.1 \times 10^6$ and $0.26\pm0.08 \times 10^6$ cells respectively) and increase to $2.7\pm0.2 \times 10^6$ and $1.54\pm0.18 \times 10^6$ cells 24 hours after IL-5 injection. Treatment with dexamethasone resulted in a significant reduction in Eos at 24 hours of 68.2±8.4 % ($p<0.05$) whereas indomethacin at 24 hours produced only a 17±17% reduction which was not significant.

These results demonstrate that intrapleural injection of rmil-5 is able to induce a significant Eos infiltration which can be inhibited by a steroid but not by indomethacin. We believe that this model may prove of value for the development of compounds which have a selective anti-inflammatory action on Eos for the treatment of diseases in which these cells are thought to play a role.

Yamaguchi *et al*, 1988a. J.Exp.Med. 167. p43
Yamaguchi *et al*, 1988b. J.Exp.Med. 167. p1737
Silva *et al*, 1991. J.Pharm.Exp.Therapeutics. 257. p1039.

231P DISTRIBUTION OF PERFLUOROCHEMICAL EMULSION DROPLETS IN RAT TISSUES

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Perfluorochemical (PFC) emulsions have applications as intravascular oxygen carriers and imaging agents, and have been approved for oxygenating the myocardium during coronary balloon angioplasty (Lowe, 1992). Following injection, PFC emulsion droplets accumulate in lymphoid tissues, causing an increase in organ weight in proportion with dose administered. The responses to PFC emulsions also depend upon the formulation injected and species studied (Lowe, 1988). To further understand the biokinetic behaviour of PFCs, we have recovered emulsion droplets from tissues, and have studied their uptake and disposition following injection into rats.

Male Wistar rats (body weight (b.w.); 283-321 g; n = 13) were injected i.p. with 10 ml/kg b.w of either a PFC emulsion (mean droplet diameter 226 nm, polydispersity 0.23) containing 30% (w/v) perfluorodecalin (FDC; Rhône-Poulenc, U.K.) and 4% (w/v) purified Pluronic F-68 (Bentley *et al.*, 1989), or saline (0.9% w/v; controls). 72 h or 7 d after injection, animals were killed by cervical dislocation and their livers, spleens, and lungs removed, rinsed with saline, and frozen (-20°C). Blood samples were also obtained by cardiac puncture and assayed immediately. Tissue or blood samples (1-5 g or 1.0 ml) were homogenized for 1 min in 30 ml of d. H_2O using a Silverson homogenizer at 2000 rpm. 2 ml of sodium hypochlorite (35%; Rhône-Poulenc, U.K.) were added and the homogenization repeated. Samples were then centrifuged at 3000 g for 10 min, the supernatant discarded, and residual tissue fragments rinsed from the pellet of PFC droplets. Droplets were then counted and their size distribution measured using a Coulter Multisizer.

At 72 h after emulsion injection, the mean (± s.e.m.; n = 3 throughout) diameter of droplets recovered from the liver and spleen were 2.24 ± 0.04 μm and 2.78 ± 0.10 μm respectively; droplets recovered from the lung after 72 h (mean: 1.73 ± 0.13 μm) were significantly smaller ($P < 0.01$). At 7 d after injection, the mean diameter of droplets recovered from the liver had risen to 3.31 ± 0.13 μm ($P < 0.01$) and those in the lung to 2.71 ± 0.14 μm ($P < 0.01$); in contrast, the mean diameter of droplets from the spleen after 7 d (2.22 ± 0.09 μm) was similar to that measured after 72 h. The mean percentage of the injected PFC dose recovered from the liver after 72 h was 2.2 ± 0.4% and this had fallen to 0.07 ± 0.05% ($P < 0.01$) after 7 d. A smaller decrease in the percentage of injected PFC in the spleen also occurred over the same period (72 h: 1.9 ± 0.3%; 7 d: 0.8 ± 0.5%; $P < 0.01$). The percentage of the PFC in the lung was considerably smaller than in the liver or spleen and did not change significantly between 72 h (0.007 ± 0.004%) and 7 d (0.005 ± 0.001%). FDC was undetectable (< 0.001%) in all blood samples. These results show that coalescence of FDC droplets occurs in the liver, spleen and, to a lesser extent, the lung between 72 h and 7 d following injection. The maximum increase in droplet diameter was *ca.* 10 times that at preparation. The small quantity of FDC retained in the lung suggests that, in contrast to liver or spleen, the excretion rate from this organ is relatively high. This accords with previous findings (Yamanouchi *et al.*, 1975) that PFCs are rapidly eliminated from the body by expiration. The absence of detectable levels of FDC in the blood supports previous findings that the vascular half life of this compound is *ca.* 26 h (Yamanouchi *et al.*, 1975). The greater rate of FDC elimination from the liver than from the spleen may be related to differences in the rates of reticuloendothelial system processing between these organs.

Bentley, P.K. *et al.* (1989) J. Pharm. Pharmacol. 41, 661-663.
Lowe, K.C. (1988) In Oxygen Transport to Tissue, Vol. X (eds. Mochizuki,, M. *et al.*), pp. 655-663. New York, Plenum.
Lowe, K.C. (1992) Clin. Hemorheol. 12, 141-156.
Yamanouchi, K. *et al.* (1975) Chem. Pharm. Bull. 33, 1221-1231.

232P THE EFFECTS OF TOPICAL LIDOCAINE ON THE DEVELOPMENT OF TRINITROBENZENE SULPHONIC ACID (TNBS)-INDUCED COLITIS IN RATS

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In man, treatment of ulcerative colitis with topical application of the local anaesthetic, lignocaine (800mg/day for up to 8 months) reduced severity, restored mucosal integrity and reduced mucosal T cell infiltration (Björck et al., 1989). We have tested the effects of lignocaine on the development and maintenance of colitis in a rat model (Morris et al., 1989). Male Wistar rats (175-200g) received vehicle (2.5% carboxymethylcellulose) alone or containing lignocaine hydrochloride (Astra, 2% gel; 5-100mg/kg) intracolonically. Thirty minutes later, TNBS (0.5ml of 60mg/ml in 50% ethanol) was administered in the same way. Animals were killed 24h after treatment, the colons were removed, samples were taken for estimation of myeloperoxidase (MPO) activity (Units/mg tissue) and scored for damage as previously described (Morris et al., 1989). Twenty-four hr after TNBS rats developed colitis and elevated MPO activity (Table 1). Lignocaine at all doses significantly improved (* $p<0.05$) clinical score. Doses of 10mg/kg and 100mg/kg also reduced MPO activity.

Group		Colitic Score	MPO Activity	n
Vehicle		13.0±0.4	40±4	18
Lignocaine	5mg/kg	7.4±1.3*	36±6	10
	10mg/kg	6.9±1.0*	23±6*	5
	25mg/kg	6.6±1.3*	28±6	5
	100mg/kg	3.7±0.9*	16±5*	5

In a separate experiment, rats received vehicle or lignocaine (10mg/kg) 30min before TNBS, then once a day thereafter for 7 days. They were then weighed, killed and clinical score, MPO activity and a histological examination was performed in which mucosal integrity and mast cell number were assessed. TNBS (vehicle treated) animals lost 9±9g whereas with lignocaine treatment rats gained 31±9g. Similarly, clinical scores were 13.3±0.8 in vehicle treated rats and 5.8±2.1 in rats given lignocaine. MPO activity was 61±9 and 26±11 U/mg in vehicle- and lignocaine-treated groups respectively. Histologically, lignocaine treatment improved mucosal integrity in 5 of 10 rats examined and mast cell hyperplasia observed in colitis was also reduced (17±6 cells/mm length of section, vehicle ; 5±2, lignocaine). When lignocaine treatment was started 3 days after TNBS, the results obtained were similar to when it was given throughout the experiment. These results suggest that treatment with lignocaine can block the development of acute colitis in a dose-dependent manner and can partially reverse the chronic colitis. This model may be valuable in determining the possible role of neural mechanisms in the development and maintenance of intestinal inflammation.

Björck, S., Dahlström, A. & Ahlman, H. (1989). Scand. J. Gastroenterol., 24, 1061-1072.
Morris, G.P., Beck, P.L., Herridge, M.S., Depew, W., Szewczuk, M.R. & Wallace, J.L. (1989). Gastroenterology 96, 795-803.

233P THE EFFECT OF VERAPAMIL AND DILTIAZEM ON THE CYTOTOXICITY OF VARIOUS ANTITUMOUR AGENTS IN THE YEAST *SACCHAROMYCES CEREVISIAE*

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Verapamil (VRP), a voltage-sensitive calcium channel (VSCC) blocker acting at the phenylalkylamine site, has been reported to enhance the cytotoxic effect of some antitumour agents (Tsuruo *et al*, 1983). This study has investigated the role of VRP and diltiazem (DIL) in the cytotoxicity of adriamycin (ADR), amsacrine (AMSA), 5-fluorouracil (5-FU) and melphalan (MEL) in the yeast, which has been used already as a model for studying antitumour drug action (White, 1982; Tiligada & Delitheos, 1990).

S. cerevisiae ATCC 2366 was incubated in yeast broth at 27 °C for 2 h at a concentration of $2x10^7$ cells/ml. Various concentrations of each antitumour agent (6.25 to 50 μg/ml) were then added either alone or in combination with 22μM VRP or 100μM DIL and further incubated at 27 °C for 22 h. The cytotoxicity of each preparation was measured in terms of cell survival following plating of a culture sample on agar after 22 h incubation. Cell survival was not significantly affected by the presence of VRP or DIL. Survival was therefore calculated as the percentage of yeast survival in the absence of drug and VRP or DIL for each set of experiments and expressed as the average of 6-20 observations.

S. cerevisiae was sensitive to ADR and 5-FU but resistant to AMSA and MEL (Table 1). DIL, a VSCC blocker acting at the benzodiazepine site, had no effect on the action of any antineoplastic agent. Incubation of yeast cells with VRP in the presence of ADR or 5-FU induced no change in the cytotoxicity of these agents. VRP in combination with AMSA, a DNA intercalating agent, significantly ($P<0.05$) decreased yeast survival whereas the resistance of yeast cells to the alkylating agent MEL was significantly ($P<0.05$) enhanced by the presence of VRP. These modifying effects of VRP were observed at all concentrations of the two agents (Table 1).

Table 1: Percentage survival ±s.e.mean of *S. cerevisiae* in the presence or absence of various antitumour drugs or verapamil after 22h incubation (*P<0.05, **P<0.01, ***P<0.001 against % survival in the presence of verapamil alone).

Antitumour drug concentration	ADRIAMYCIN	AMSACRINE	5-FU	MELPHALAN	ADRIAMYCIN	AMSACRINE	5-FU	MELPHALAN
	in the absence of VERAPAMIL				in the presence of 22μM VERAPAMIL			
0	100	100	100	100	84±3%	84±3%	84±3%	84±3%
6.25μg/ml	15±3%	87±7%	6±1%	100±5%	11±1%	61±7%**	6±2%	120±7%***
12.5μg/ml	0.5±0.3%	109±11%	1±0.5%	84±8%	0.3±0.1%	68±6%*	2±1%	115±8%***
25μg/ml	0	103±6%	0.4±0.2%	92±4%	0	76±3%*	2±1%	113±6%***
50μg/ml	0	110±8%	0	94±7%	0	73±5%*	0	128±9%***

These data suggest that blockade of the L-type VSCC by VRP is able to modify the action of the antineoplastic drugs to which *S. cerevisiae* is resistant. This modifying effect of VRP on AMSA and MEL suggests that the phenylalkylamine site of the channel may play a differential role on the mechanism of action of each group of chemotherapeutic agents in yeast.

Tiligada, E. & Delitheos, A. (1990) *Anticancer Res.* 10 (5B), Abstr 251.
Tsuruo, T., Iida, H., Tsukagoshi, S. & Sakurai, Y. (1983) *Cancer Res.* 43,2267-2272
White, R.J. (1982) *Ann. Rev. Microbiol.* 36, 415-433.

234P CHARACTERISATION OF THE MUSCARINIC CHOLINOCEPTOR SUBTYPE MEDIATING ACh-INDUCED CORTISOL SECRETION FROM BOVINE ADRENOCORTICAL INNER ZONE CELLS IN PRIMARY CULTURE

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Acetylcholine (ACh) stimulates steroid secretion from the perfused calf adrenal and from both isolated adrenocortical zona glomerulosa and fasciculata cells. Cholinergic regulation of adrenal steroidogenesis also occurs *in vivo* (Stern *et al* 1989). The muscarinic cholinoceptor subtype present in the adrenal cortex has not been characterised, and we have therefore obtained pA_2 values for a range of selective antagonists of ACh-stimulated cortisol secretion from bovine adrenocortical zona fasciculata/reticularis (zfr) cells in primary culture.

Zfr cells were isolated from the bovine adrenal cortex and maintained in primary culture for 48 h before use. Basal cortisol secretion was 10.2 ± 1.4 pmol/well/30 min (n = 24 experiments). ACh stimulated cortisol secretion with an ED_{50} of 1.1 μM, and was maximally effective at 100 μM, at which concentration cortisol secretion was 143.4 ± 12.9 pmol/well/30 min (n = 24). Hexahydro-sila-difenidol (HHSiD), p-fluoro hexahydro-sila-difenidol (p-FHHSiD), pirenzepine and methoctramine antagonised this response in a competitive manner. The pA_2 values and slope of the Schild regressions for these antagonists are shown in Table 1.

Table 1 : Affinity profiles of muscarinic antagonists in bovine adrenocortical zfr cells

Antagonist	pA_2 ± S.E.	slope ± S.E.	Published pA_2*		
			M1	M2	M3
pirenzepine	6.95 ± 0.28	1.06 ± 0.06	8.24	6.82	6.88
methoctramine	6.06 ± 0.27	1.01 ± 0.04	6.85	7.69	6.17
HHSiD	8.68 ± 0.28	1.02 ± 0.10	7.92	6.53	7.96
p-FHHSiD	7.69 ± 0.29	0.95 ± 0.05	6.68	6.01	7.84

*Lambrecht *et al* (1988,1989)

The pA_2 values determined in this study (HHSD, p-FHHSiD - high affinity; pirenzepine - moderate affinity; methoctramine - low affinity) are consistent with the antagonist profile of the M_3 cholinoceptor subtype. We conclude that ACh-stimulated cortisol secretion occurs via the M_3 receptor in primary cultures of bovine adrenocortical zfr cells.

Lambrecht, G. *et al* (1988). *Eur. J. Pharmacol.* **152**, 193-104.
Lambrecht, G. *et al* (1989). *Eur. J. Pharmacol.* **168**, 71-80.
Stern, N. *et al* (1989). *J. Clin. End. Metab.* **69**, 294-298.

235P ETHANOL ENHANCES SUBERYLDICHOLINE'S INTRINSIC EFFICACY AT THE NICOTINIC RECEPTOR OF *TORPEDO* ELECTROPLAQUES

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Ethanol increases the apparent affinity with which full agonists elicit a response at the nicotinic acetylcholine receptor (nAcChoR) by increasing either the affinity with which agonists bind to the receptor or the opening probability, p_{open}, of the occupied receptor, or both (Forman *et al.*, 1989). Partial agonists fail to elicit a full response even when they occupy all receptors. They might differ from full agonists because either fewer occupied receptors open (reduced p_{open}) or more of the open channels are blocked by partial agonists (self-inhibition) (Marshall *et al.*, 1991). Here we define ethanol's kinetic site of action by examining its perturbation of suberyldicholine's concentration-response curve.

Postsynaptic membrane vesicles from *Torpedo nobiliana* electroplaques were preincubated with α-bungarotoxin to remove spare response and loaded with $^{86}Rb^{+}$. Following treatment with 0.1 mM diisopropylfluorophosphate to inhibit acetylcholinesterase, the vesicles were simultaneously mixed with agonist and ethanol and the flux subsequently quenched using a rapid quenched-flow technique. Free radioactivity was measured after filtration of the vesicles and results corrected for time dependent leak.

Suberyldicholine-stimulated cation efflux was linear from 3 to 20 msec in the presence and absence of ethanol. Using a 10 msec flux time, we found that suberyldicholine's action on the nAcChoR followed a bell-shaped concentration-response curve. Increasing the ethanol concentration from 0 to 1.5 M decreased the concentration which elicited a half maximal response (EC_{50}) of suberyldicholine 5.5-fold from 33 ± 5.1 μM to 6 ± 0.3 μM and increased the maximum releasable flux (F_{max}) from 0.18 ± 0009 to 0.82 ± 0.012. The latter F_{max} was not different from that elicited by acetylcholine. Self-inhibition occurred at higher suberyldicholine concentrations with a half maximal inhibiting concentration (IC_{50}) of 16 ± 4.8 mM in the absence of ethanol increasing to 32 ± 6.2 mM in the presence of 1.5 M ethanol.

Within the framework of the sequential model (Marshall *et al.*, 1991), the enhancement of suberyldicholine's efficacy by ethanol could not be accounted for simply by increasing the affinity at the agonist binding site and simultaneously decreasing the affinity of the agonist for the inhibition site, although this did result in a small increase in F_{max}. Nonlinear least squares analysis showed that the major effect of ethanol was to enhance suberyldicholine's normally low p_{open} in a concentration dependent manner until it achieves a value close to that for the full agonist acetylcholine.

Supported by the U.S. National Institute on Alcohol Abuse and Alcoholism. P. H. T. is a NATO research fellow.

Forman, S.A., Righi, D.L. & Miller, K.W. (1989) Biochim. et Biophys. Acta **987**, 95-103
Marshall, C.G., Ogden, D.C., & Colquhoun, D. (1991) J. Physiol. **433**, 73-93

236P THE ANTI-INFLAMMATORY ACTIONS OF FRIEDELANOL AND GLUTIN-3-ONE TWO TRITERPENES FROM *POLYGONUM BISTORTA*

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Following observations of anti-inflammatory activity in extracts of *P. bistorta*, two pentacyclic triterpenes, friedelanol and glutin-3-one isolated from the active fraction of the extract (Duwiejua, 1992), were tested for anti-inflammatory activity. Suspensions (in 10% v/v acacia mucilage) of the compounds were administered p.o. 18 and 2 h prior to the induction of carrageenan-induced paw oedema (Winter *et al*, 1962) in male Sprague Dawley rats. Oedema was monitored as percent increase in paw diameter.

Table 1: Effect of friedelanol and glutin-3-one on carrageenan-induced rat paw oedema

Drug	Dose Moles/Kg	%Reduction of Peak Oedema	%Reduction Mean Area under Time-course Curve	Drug	Dose Moles/Kg	%Reduction of Peak Oedema	%Reduction Mean Area under Time-course Curve
Friedelanol	$1.9x10^{-4}$	18.8*	17.9*	Indomethacin	$3.5x10^{-6}$	28.0*	27.0*
Glutin-3-one	$5.9x10^{-5}$	24.0*	21.0*		$7.0x10^{-6}$	40.0*	46.8*
	$1.2x10^{-4}$	55.9*	52.3*		$1.4x10^{-5}$	58.7*	61.0*
	$2.4x10^{-4}$	61.1*	55.0*	Prednisolone	$3.5x10^{-6}$	33.0*	31.9*
					$7.0x10^{-6}$	52.0*	55.8*
					$1.4x10^{-5}$	63.0*	67.0*

Asterisks indicate differences from vehicle controls at $P< 0.05$, N= 6 (one way ANOVA plus Newman-Keuls range test).

Friedelanol (1.9×10^{-4} moles.kg^{-1}) and glutin-3-one ($1.2x10^{-4}$ moles.kg^{-1}) significantly ($p<0.05$) inhibited the maximal oedema response by 18.8 and 55.9 respectively. Glutin-3-one, the more active compound, had not previously been reported to show anti-inflammatory activity. It produced dose-dependent suppression of carrageenan-induced neutrophil infiltration (monitored as myeloperoxidase activity) and paw oedema and was 16 and 23 times less potent (maximal oedema inhibition) than indomethacin and prednisolone respectively. Glutin-3-one ($1.2x10^{-4}$ moles.kg^{-1}.day^{-1}) also reversed the onset of joint swelling and neutrophil infiltration in adjuvant arthritis in male Wistar rats (Pearson, 1956), when administered from 8 days *after* its induction ($p< 0.05$). Daily administration (p.o.) for 10 days produced reductions of 44.6% and 74.3% respectively in the adjuvant-injected and contralateral paw volumes compared with vehicle-treated controls.

MD was supported by the Association of Commonwealth Universities.

Duwiejua, M. (1992) Ph.D thesis, University of Strathclyde.
Pearson, C.M. (1956) Proc. Soc. Exp. Biol. **91**, 95-101.
Winter, C.A., Risley, E.A. & Nuss, G.W. (1962) Proc. Soc. Exp. Biol. (N.Y) **111**, 544-547.

237P THE EFFECT OF A SPECIFIC OXYTOCIN ANTAGONIST ON THE RENAL RESPONSES TO OXYTOCIN OF THE SALINE-INFUSED RAT

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Although oxytocin is known to be natriuretic in the rat (Windle & Forsling, 1991), the underlying mechanism is not clear. An oxytocin antagonist [Pen ^{1},Phe(Me)^{2},Thr ^{4},Orn ^{8}]OT has been reported to prevent development of the negative sodium balance associated with chronic oxytocin administration (Verbalis et al., 1991). Here we describe the effects of another specific oxytocin receptor antagonist [Mpa ^{1}, D-Tyr(et) ^{2}, Thr ^{4},Orn ^{8}]OT (kindly supplied by Ferring Research Institute, Malmo, Sweden) on the renal responses to oxytocin infusion in the conscious rat.

Male Sprague Dawley rats (250-300g) were implanted with jugular vein cannulae under fentanyl citrate , fluanisone and diazepam anaesthesia (0.32, 10 & 2.5mg/kg bw respectively) The rats, given a minimum of 48h to recover, were then placed in metabolism cages and infused with 0.077mol/NaCl/l at 150µl/min for an equilibration period of not less than 3.5h. Following this each spontaneous urination was timed and collected. After a control period of at least 30min the infusate was supplemented with the oxytocin antagonist at a rate of 0,1,5 or 10 ng/min for 60min. Oxytocin was then added to this infusate at a rate of 0.1ng/min (a dose known to produce plasma levels within the physiological range) for a further 60min. Finally the rats were returned to saline infusion for 30min. Urine volume and electrolyte concentrations were measured and the renal responses determined.

During control infusion the rates of urine flow and sodium excretion approximated those from saline administration and the analogue had no signficant effect on salt and water excretion. Oxytocin alone produced significant natriuretic and diuretic responses of 41.8±8.3% and 19.8±2.8% respectively (paired t-test $P<0.05$). Infusion of the antagonist at 5 & 10ng/min significantly reduced the natriuretic response which was 18.5±6.15% with the highest dose. However, no signficant effect on the oxytocin induced changes in urine flow were observed with the antagonist. Therefore the natriuretic action of oxytocin may be mediated by specific oxytocin receptors and may be dissociated from the urine flow changes.

Supported by the Special Trustees of St. Thomas' Hospital and the Central Research Fund of London University.

Verbalis, J.G., Mangione, M.P. & Stricker, E.M. (1991) Endocrinology **128**, 1317-132
Windle, R.J. and Forsling, M.L. (1991) J. Physiol. and Pharmacol. **42**, 417-426.

238P EVALUATION OF THE USE OF COMPUTER SIMULATIONS OF EXPERIMENTS IN TEACHING UNDERGRADUATE STUDENTS

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Although the use of computer simulations of traditional practical classes in physiology and pharmacology has increased considerably in recent years few evaluations of their effectiveness have been reported. Here we report the results of a study which combined several modes of evaluation. It was carried out at Sheffield Hallam and Liverpool John Moores universities involving 65 undergraduate students on a number of courses and using a number of computer simulation programs (e.g. Ileum, Nerve, Muscle, Frog Heart: Sheffield Bioscience Programs) designed to offer an alternative to the traditional approach.

The performance of students (measured by the marks awarded for their practical reports) taught either by using CAL or the traditional approach was compared retrospectively. There was no statistical significance of difference in the mean marks of both groups at the 95% level but mean marks of students using the computer simulations were lower at the 90% level (variances of the mean marks were significantly different: $P<0.05$).

Student questionnaires and interviews were used to assess the ease with which students learned to use the CAL interface and to navigate through the programs. The responses indicated that students felt comfortable with and learned to use the menu-driven interface very quickly; of 22 students who were novice users of computers 21 were able to master the interface within 5 minutes. They did not require tutor support to use the programs.

Students were also observed in the classroom working both individually and as small groups. It was clear that students working in dyads gained much from the interaction: they were seen to plan a protocol for recording the data, to discuss the results they had obtained and frequently to dispute each others interpretations. In contrast students asked to work alone had little dialogue with colleagues. The role of the lecturer was also studied and students noticeably made more use of staff (usually to resolve disputes) when they worked in small groups. It was also apparent that students working with computer simulation programs without supervision and with no guidance often follow an invalid protocol and clearly this approach is not appropriate if study design is an important learning objective.

In summary the study indicates that computer simulations may offer a realistic alternative to traditional practical classes for undergraduate students. Clearly the learning potential of this approach is maximised by defining learning objectives, encouraging students to work in small groups and by a proactive tutoring role. Such considerations should be taken into account both at the software design and the delivery stage.

239P PHARMACOLOGY OF GR138950, A NOVEL ANGIOTENSIN AT_1 RECEPTOR ANTAGONIST

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The effects of GR138950 (1-[[3-Bromo-2-[2-[[(trifluoromethyl)sulphonyl]amino]phenyl]-5-benzofuranyl]methyl]-4-cyclopropyl-2-ethyl-1H-imidazole-5-carboxamide) have been determined at angiotensin AT_1-binding sites in rat liver and at AT_1-receptors in rabbit isolated aorta and conscious rats and dogs. In addition, its effects on blood pressure and heart rate were measured in conscious normotensive rats and dogs and in spontaneously hypertensive and renal artery ligated hypertensive (RALH) rats (for methods see Hilditch et al., 1991; Hunt et al., 1991; Marshall et al., 1991; Robertson et al., 1991).

GR138950 displaced [^{3}H]-angiotensin II from binding sites in rat liver membranes (pK_i=8.4; n_H=0.98; n=3). GR138950 (1-10nM) inhibited contractile responses to angiotensin II (AII) in strips of the rabbit isolated aorta; concentration-response curves to AII being displaced rightwards and downwards. The pK_B (Kenakin, 1984) for GR138950 was ~9.0 (n=4). GR138950 (1μM), however, had no effect on phenylephrine or 5-HT-induced contractile responses. Administration of GR138950 (1mg kg^{-1} ia or po) to conscious, normotensive rats or dogs inhibited pressor responses produced by intra-arterial administration of AII; AII dose-pressor response curves being displaced maximally 76 and 37 fold (rats) or 33 and 16 fold (dogs) to the right, in a parallel manner, after systemic or oral administration of GR138950, respectively. The inhibitory effect of GR138950 was of long duration (7-24h, rats; 7->24h, dogs). In separate studies, GR138950 (1mg kg^{-1} ia) produced small reductions in diastolic blood pressure (9-15mmHg) in conscious normotensive rats and spontaneously hypertensive rats. GR138950 (0.1-3mg kg^{-1} ia or po) administered to RALH rats produced more marked, dose-related decreases in diastolic blood pressure. Peak effects (53-93mmHg ia; 39-85mmHg po) were seen 7h or more after administration and persisted for >24h. GR138950 had little effect on heart rate in conscious, normotensive rats or hypertensive rats.

These experiments show that GR138950 is a potent ligand at angiotensin AT_1 binding sites and a potent and specific antagonist at angiotensin AT_1 receptors *in vitro*. GR138950 is also effective *in vivo* after both oral and intra-arterial administration, suggesting that it has high oral bioavailability. In addition, GR138950 is a potent, long-lasting antihypertensive agent.

Hilditch, A ., Akers, J.S., Travers, A., Hunt, A.A.E., Robertson, M.J., Drew, G.M., *et al.* (1991) Br. J. Pharmacol. **104**, 423P.
Hunt, A.A.E., Hilditch, A., Robertson, M.J., Drew, G.M., Gardner, C.J., Twissell, D.J., *et al.* (1991) Br. J. Pharmacol. **104**, 424P.
Kenakin, T.P. (1984) Pharmacol. Rev. **36**, 165-222.
Marshall, F.H., Barnes, J.C., Brown, A.D., Michel, A.D. & Tyers, M.B. (1991) Br. J. Pharmacol. **104**, 425P.
Robertson, M.J., Middlemiss, D., Ross, B.C., Drew, G.M., Scopes, D.I.C. & Dowle, M.D. (1991) Br. J. Pharmacol. **104**, 300P.

240P *IN VITRO* PHARMACOLOGY OF BIBR 277, A POTENT AND HIGHLY SELECTIVE NONPEPTIDE ANGIOTENSIN ANTAGONIST

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BIBR 277, 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid, is a novel nonpeptide angiotensin AT_1 receptor antagonist. In receptor binding studies, the compound inhibited ^{125}I-angiotensin II binding to rat lung AT_1 sites (Entzeroth & Hadamovsky, 1991) with a K_i of 3.7 ± 1.7 nM (n=3). This and all other values are given as mean ± SEM. Radioligand binding to AT_2 sites in rat adrenal medulla was not affected up to 10 μM. Affinity constants for DuP 753 in these assays were 23.7 ± 2.5 nM (n=3) and > 10,000 nM, respectively. Other components of the human renin-angiotensin system such as serum angiotensin I-converting enzyme or plasma renin were not inhibited by BIBR 277 (10 μM). Furthermore, BIBR 277 did not interact with other receptors involved in cardiovascular regulation such as adenosine, alpha- and beta-adrenergic, dopamine, neurokinin, neuropeptide Y or serotonin up to 10 μM.

In isolated rabbit aorta, BIBR 277 (10, 100 and 1,000 nM) produced a parallel rightward shift of the concentration-contractile-response curve to angiotensin II. However, in the presence of the compound a significant decrease of the Hill-slope (control: 2.12±0.08 (n=18); + BIBR 277: 1.76±0.22, 1.37±0.08 and 1.66±0.09 (each n=6), respectively) and the maximum contractile force (45±3%, 52±3% and 41±3% (each n=6), respectively) was observed. This phenomenon was due to a specific interaction with the AT_1 receptor since the insurmountable antagonism could be reversed concentration-dependently in the presence of the surmountable angiotensin II antagonist DuP 753 as shown recently with other insurmountable antagonists (Wienen et al., 1992). The K_B value of BIBR 277 was calculated according to Kenakin (1987) and was 3.3±0.9 x 10^{-10} M. In this tissue preparation, BIBR 277 (10 μM) did not interfere with norepinephrine or K^+-induced contractions or with acetylcholine-induced relaxation in precontracted rings with intact endothelium.

In conclusion, BIBR 277 selectively and potently interacts with angiotensin AT_1 receptors. In functional experiments, the compound was shown to be an effective and insurmountable antagonist of angiotensin II induced contractions.

Entzeroth, M. & Hadamovsky, S. (1991) *Eur. J. Pharmacol. - Mol Pharmacol Sect.* **206**, 237-241.
Kenakin, T. (1987). In *Pharmacologic Analysis of Drug Receptor Interaction.* ed. T. Kenakin. pp. 205-244. New York: Raven Press.
Wienen, W., Mauz, A.B.M., Van Meel, J.C.A. & Entzeroth, M. (1992), *Mol. Pharmacol.* **41**, 1081-1088.

241P HIGH AFFINITY AMYLIN BINDING SITES IN RAT BRAIN

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Amylin is a peptide hormone secreted by pancreatic beta cells which is proposed to regulate fuel metabolism, at least in part through actions upon skeletal muscle glycogen metabolism (Cooper *et al.*, 1989). Amylin is composed of 37 amino acids, is amidated at the C-terminal, and is structurally related to calcitonin gene-related peptide (CGRP) and, less so, to calcitonin. While circulating concentrations of amylin in plasma are reported to be in the range of 2 to 100 pM in healthy humans and rodents (see e.g. Koda *et al.*, 1992), binding sites with affinity for amylin in this range have not previously been identified.

Rat amylin, [^{125}I] Bolton-Hunter labeled at the N-terminal lysine (^{125}I-BH-amylin), was incubated at concentrations of 13-17 pM in HEPES buffer containing bovine serum albumin and bacitracin with membranes prepared by centrifugation from rat brain regions. Incubations were carried out for 1 h at 23 °C; membranes were then collected by filtration over glass fiber filters. Nonspecific binding was measured in the presence of 100 nM rat amylin. Greatest density of binding was present in nucleus accumbens, with less in midbrain and hypothalamus, and least in cortex and cerebellum. Scatchard plots of ^{125}I-BH-amylin binding to nucleus accumbens membranes yielded a K_d of 27 pM and a Bmax of 24 fmol/mg protein. Related peptides competed for this binding with the following K_is: rat amylin (28 pM), salmon calcitonin (30 pM), rat βCGRP (80 pM), rat αCGRP (370 pM), rat calcitonin (>100 nM). Autoradiographic studies demonstrated that binding sites were present throughout nucleus accumbens, and with a distribution in brain similar to that of a subclass of CGRP binding site ("C3") with high affinity for salmon calcitonin, first described by Sexton *et al.* (1988).

These results describe high affinity amylin binding sites with a binding profile (amylin ≥ salmon calcitonin > βCGRP > αCGRP >> rat calcitonin) distinct from CGRP receptors (αCGRP > βCGRP >> amylin) that mediate cardiovascular actions of these peptides.

Cooper, G.J.S., Day, AJ., Willis, A.C., Roberts, A.N., Reid, K.B.M. et al. (1989) *Biochim Biophys. Acta* 1014:247-258.
Koda, J.E. Fineman, M., Rink, T.J., Bailey, G.E., Muchmore, D.B. & Linarelli, L.G. (1992) *The Lancet* 339:1179-1180.
Sexton. P.M., McKenzie, J.S. & Mendelsohn, F.A.O. (1988) *Neurochem. Int.* 12:323-335.

242P EXCITATORY AMINO ACIDS STIMULATE THE RELEASE OF ARGININE VASOPRESSIN BUT NOT CORTICOTROPHIN RELEASING FACTOR-41 FROM THE RAT HYPOTHALAMUS *IN VITRO*

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Recent evidence that glutamate induces the release of neurohormones from axons terminating in both the median eminence and the posterior pituitary (Van den Pol et al., 1990) has suggested that excitatory amino acids may play an important role in the control of neuroendocrine function. Thus, we have used an in vitro model to examine the effects of excitatory amino acids on the release of the two corticotrophin releasing factors arginine vasopressin (AVP) and corticotrophin releasing factor-41 (CRF-41) from the rat hypothalamus. Hypothalami, dissected post-mortem from male Sprague Dawley rats were preincubated for 1h in artificial cerebrospinal fluid (csf) with 2 medium changes. They were then subjected either to two 15 min incubations, the first in medium alone and the second in medium +/- excitatory amino acid, or stimulated repeatedly with N-methyl-D-aspartate (NMDA) with two 15 min washes between the first and second stimulations. Solutions from all incubations were kept at -20°C for determination of immunoreactive (ir)-AVP and ir-CRF-41 by radioimmunoassay.
L-glutamate (30mM) produced a significant ($p<0.05$) 5 fold increase in ir-AVP release, although lower concentrations (100μM - 10mM) were without effect. This stimulatory response was mimicked by NMDA (30 & 45mM) and kainic acid (30mM), with a submaximal concentration of NMDA (30mM) causing 2-4 fold increases in ir-AVP release. The stimulatory response to NMDA (30mM) was not affected by removal of Mg^{2+} or Ca^{2+} from the medium, although basal ir-AVP release was significantly ($p<0.05$) reduced with Ca^{2+} free medium. Hypothalami also responded to repeated stimulation with NMDA (30mM) with increases in ir-AVP release, the magnitude of the response to the second pulse of NMDA was approximately 2.5 fold higher ($p<0.005$) than the first. The response to the second pulse was reduced by approximately 60% by addition of the selective NMDA antagonist AP5 (100μM) to the medium immediately after the first NMDA stimulation.
In contrast, parallel measurements of ir-CRF-41 revealed that neither L-glutamate (30mM) nor kainic acid (30mM) influenced the release of this peptide from isolated hypothalami in vitro while in 2/5 experiments the NMDA (30mM) caused a significant ($p< 0.05$) fall in ir-CRF-41 release. Regardless of treatment all tissues responded to subsequent stimulation with K^+ (56mM) with a normal rise in ir-CRF-41 secretion.
These results support the concept that excitatory amino acids stimulate the release of AVP but not CRF-41 from the isolated rat hypothalamus in vitro.

We are grateful to the Wellcome Trust for financial support.

Van den Pol, A.N. et al. (1990). Science, **258,** 1276-1278

243P MUSTARD OIL-EVOKED ACTIVATION OF RAT DORSAL HORN NEURONS IS INHIBITED BY NK_2 BUT NOT NK_1 RECEPTOR ANTAGONISTS

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We have previously reported that the responses of dorsal horn neurons to brief noxious thermal (but not mechanical) cutaneous stimuli are inhibited by NK_2 but not NK_1 receptor antagonists (Fleetwood-Walker *et al*, 1991). In the *in vitro* spinal cord/dorsal root preparation, neuronal activity evoked by brief application of capsaicin to the dorsal root ganglia is also inhibited by a selective NK_2 antagonist (Urban *et al*, 1992). It is not clear, however, whether NK_1 and/or NK_2 receptors are important in the sustained activation of dorsal horn neurons by prolonged noxious stimulation which results in altered sensitivity and receptive field properties of those neurons (Woolf and King, 1990). Topical cutaneous application of the chemical irritant 3-isothiocyanato-prop-1-ene (mustard oil) causes prolonged activation of C- (and briefly also Aδ-) afferents (Heapy *et al*, 1987) and brings about a state of increased responsiveness in dorsal horn neurons (Woolf & King, 1990).

Extracellular recordings were made from laminae IV/V dorsal horn neurons in segments T_{12} - L_4 of α-chloralose/urethane-anaesthetised rats, as described previously (Fleetwood-Walker *et al*, 1991). Neuronal receptive fields were located using brief innocuous and noxious stimuli and then mustard oil (5 - 20% in paraffin oil) was topically applied to a restricted area (usually about 3 cm^2) within this region. Neuronal responses to this stimulus were recorded using the central barrel of a glass microelectrode, with ionophoresis of drugs from the side barrels. The NK_1 antagonist L-668169 and the NK_2 antagonist L-659874 were prepared as 0.15 mM aqueous solutions with 0.3% dimethylformamide, pH 4.5 - 5.0 (Fleetwood-Walker *et al*, 1991). Ionophoresis of this vehicle alone had no effect. The NK_1 antagonist GR 82334 (Hagan *et al*, 1991) was prepared as a 1 mM aqueous solution, pH 4.5 - 5.0.

In virtually all multireceptive neurons tested, mustard oil evoked a large and prolonged increase in activity with firing rates of 149 ± 36 fold of background (mean ± s.e. mean) being consistently maintained. In most cases, a steady elevated firing rate was maintained for at least 30 min by repeated application of mustard oil to the same site. Drugs were tested after the mustard oil-evoked activity was stable and had been continuous for at least 1 - 2 min. In 19 out of 21 neurons tested with L-659874 (10 - 80 nA), there was a clear and maintained inhibition of mustard oil-evoked activity to 52 ± 6% of prior control (mean ± s.e. mean) with an onset delay of 0 - 120 seconds. Recovery was readily observed after terminating ionophoresis of L-659874 in nearly all cases. In about half of the cases, this inhibition began to decline after 159 ± 34 s (mean ± s.e. mean) even during continued ionophoresis. In contrast, L-668169 and GR 82334 (20 - 80 nA) rarely inhibited mustard oil-evoked activity (in 1 out of 8 and 3 out of 9 cases respectively). In 14 cells tested with both NK_2 and NK_1 antagonist, there was only one example where both were effective.

These results provide further evidence for a role of NK_2 receptors in transducing nociceptive afferent inputs to dorsal horn neurons. NK_2 receptors appear to be important not only in the acute responses of dorsal horn neurons to brief noxious thermal stimuli, but also in their sustained activation by noxious chemical stimuli.

Fleetwood-Walker, S.M., Hope, P.J., Parker, R.M.C. & Mitchell, R. (1991) Br. J. Pharmacol. **104**, 472P.
Hagan, R.M., Ireland, S.J., Bailey, F. McBride, C., Jordon, C.C. & Ward, P. (1991) Br. J. Pharmacol. **102**, 168P.
Heapy, C.G., Jamieson, A. & Russell, N.J.W. (1987) Br. J. Pharmacol. **90**, 164P.
Urban, L., Maggi, C.A., Nagy, I. & Dray, A. (1992) Neuropeptides **22**, 68.
Woolf, C.J. & King, A.E. (1990) J. Neurosci. **10**, 2717-2726.

244P THE EFFECT OF i.v. ADMINISTRATION OF CCK_4 ON LEVER PRESSING RATES OF RATS ON AN OPERANT RANDOM INTERVAL SCHEDULE

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It has been established that an intravenous injection of the selective CCK_B receptor agonist, CCK-4 (the C-terminal tetrapeptide of CCK) induces emotional and physical symptoms of panic in patients with panic disorder (PD) and in normal volunteers (Bradwejn *et al*, 1990; De Montigny, 1989). In the present experiment, we examined the effects of a bolus injection of CCK-4 on operant responding in rats. Earlier behavioural studies have shown a rat will "freeze" when presented with an aversive stimulus and this behaviour can be indexed by the degree to which behaviour is suppressed on a free operant baseline (Estes and Skinner, 1941). In this experiment, we assessed the aversive properties of an i.v. infusion of CCK-4 on free operant responding.

Polyethylene tubing catheters were implanted into the jugular vein of 16 male hooded-Lister rats (213-262 g; Bantin & Kingman, Hull U.K.) under halothane anaesthesia (N_2O:O_2 carrier). The catheters were flushed daily with heparinised saline. The rats were trained to press a lever for food pellets that were delivered according to a Variable Interval (VI) 30 sec schedule. When lever-pressing rates had stabilised, rats were attached to a motorised pump via a free-moving swivel to the catheter. In the 17th minute of the 30 min session, 0.35 ml of saline (drug vehicle) was infused at a steady rate over a 10 sec period. The dependent measure, lever-pressing rate, was collected in 30 successive one-minute time bins. On test days, one of three doses of 0.3, 1.0 and 3.0 µg/ml of CCK-4 or drug vehicle was given in a latin square design (these doses are equivalent to those which induce panic-like attacks in humans). Rats in which the catheter became blocked in the course of the experiment were excluded.

The mean lever-pressing rates 5 min before (T1) and 5 min after (T2) infusions are given in table 1. The data were analysed using an analysis of variance (ANOVA) with repeated measures. Although the response rates of all the groups declined across the session, there was no statistically significant effect of treatment.

Table 1 Lever-pressing rates (per min) before and after i.v. CCK-4 infusion (means ± s.e. mean, n=13)

CCK-4 (µg/ml)	T1	T2
Vehicle	11.92 (± 2.55)	8.51 (± 1.98)
0.3	12.05 (± 2.76)	10.74 (± 2.71)
1.0	9.92 (± 2.08)	7.92 (± 1.66)
3.0	10.66 (± 2.50)	9.20 (± 2.07)

In conclusion, these results suggest that rats may not be sensitive to the effects of peripherally administered CCK-4, a discrepancy with the clinical findings in humans which requires further investigation.

Bradwejn, J., Koszycki, D., Shriqui, C. and Meterissian, G. (1990) *Can. J. Psychiatry* **35**, 83-85
Estes, W. and Skinner, B. (1941) *J. Exp. Psychol*, **29**, 390-400
De Montigny, C. (1989) *Arch. Gen. Psychiatry*, **46**, 511-517

245P 5-HYDROXYTRYPTAMINE CAUSES ARRHYTHMIAS IN ISOLATED HUMAN ATRIUM

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5-Hydroxytryptamine (5-HT) increases contractile force and hastens the onset of relaxation (lusitropic effect) of isolated human right and left atrium through activation of 5-HT_4 receptors (5-HT_4R). The mechanisms are consistent with the cascade: 5-HT → 5-HT_4R → G_s protein → adenylyl cyclase → cyclic AMP → protein kinase A (PKA) → phosphorylation of L-type Ca^{++} channels, troponin I and phospholamban → increase in L-type Ca^{++} current (I_{Ca}++) → increase in cellular Ca^{++} → positive inotropic and lusitropic effects (Kaumann et al., 1990 & 1991; Sanders & Kaumann, 1992). Stimulation of 5-HT_4R by 5-HT, of β-adrenoceptors by (-)-isoprenaline or of PKA by cyclic AMP, each increases I_{Ca}++ sevenfold in human atrial myocytes (Ouadid et al., 1992). Such marked increase in I_{Ca}++ conceivably may cause atrial cell Ca^{++} overload. Both cyclic AMP (Opie et al., 1979) and Ca^{++} overload (Thandroyen et al., 1991) facilitate arrhythmias. We have searched for the appearance of arrhythmias in isolated right atria of man. Atrial appendages were obtained from patients usually undergoing coronary bypass operations. The tissues were set up in modified Krebs solution and paced at 0.5 Hz at 37°C as described (Kaumann et al., 1990). A single cumulative concentration-effect curve to 5-HT was obtained per tissue and the incidence of arrhythmia recorded. The incidence of 5-HT-induced arrhythmia was compared with both the incidence of spontaneous arrhythmia and (-)-isoprenaline-induced (200 μM) arrhythmia. We present results of 209 preparations from 59 patients. 2.4% of the preparations showed spontaneous arrhythmias, 15.8% (-)-isoprenaline-induced arrhythmias and 10.5% 5-HT-induced (0.1 - 200 μM) arrhythmias. The arrhythmias consisted mainly in extrasystoles and on occasion ectopic tachycardia. The relevance of our findings is that 5-HT-induced arrhythmias may degenerate into atrial fibrillation, the most common cardiac arrhythmia. We propose that platelet-derived 5-HT may induce atrial fibrillation and other atrial arrhythmias. We suggest that 5-HT-evoked arrhythmias may be mediated by 5-HT_4R and 5-HT_4R antagonists may be useful to treat and prevent atrial fibrillation.

We are grateful to the surgeons of Papworth Hospital for supply of atrial appendages.

Kaumann, A.J., Sanders, L., Brown, A.M., Murray, K.J. & Brown, M.J. (1990) Br. J. Pharmacol. 100, 879-885
Kaumann, A.J., Sanders, L., Brown, A.M., Murray, K.J. & Brown, M.J. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol. 344, 150-159
Opie, L.H., Nathan, D. & Lubbe, W.F. (1979) Am. J. Cardiol. 43, 131-148
Ouadid, H., Seguin, J., Dumuis, A., Bockaert, J. & Nargeot, J. (1992) Mol. Pharmacol. 41, 346-351
Sanders, L. & Kaumann, A.J. (1992) Naunyn-Schmiedeberg's Arch. Pharmacol. 345, 382-386
Thandroyen, F.T., Morris, A.C., Hagler, H.K., Ziman, B., Pai, L., Willerson, J.T. & Buja, L.M. (1991) Circ. Res. 69, 810-819

246P CHARACTERIZATION OF 5-HYDROXYTRYPTAMINERGIC DEPOLARIZATIONS IN THE ISOLATED RAT VAGUS NERVE

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Previous studies have shown that serotonergic depolarizations of the isolated rat vagus nerve are largely mediated by 5-HT_3 receptors (Ireland & Tyers, 1987). An additional component of the depolarization has recently been attributed to activation of 5-HT_4 receptors (Rhodes *et al.* 1992). We sought to confirm the presence of the 5-HT_4 receptor in this preparation and study its coupling mechanism to ion channels. Extracellular recordings of d.c. potentials in the desheathed rat vagus nerve were made using standard "grease-gap" techniques. One section of each nerve was continuously superfused with control or drug containing Krebs solution warmed to 35°C. Agonist exposures were for 1 min with 12 min washes, and tissues were superfused with antagonists for at least 40 min prior to subsequent agonist application. All experiments were conducted in the presence of 1 μM spiperone to block 5-HT_{1A} and 5-HT_2 receptors. Only one concentration-response curve (CRC) was obtained from each nerve. All values are mean ± s.e.mean.

5-HT ($1x10^{-9}$-$1x10^{-4}$ M) produced concentration-dependent depolarizations of the vagus nerve that yielded a triphasic CRC. At lower concentrations ($1x10^{-9}$-$1x10^{-7}$ M), depolarizations developed slowly and were long-lasting. At high concentrations ($1x10^{-6}$-$1x10^{-4}$M), latencies to peak responses were considerably shorter, and peak responses were followed by a sustained plateau. At the two highest concentrations tested ($3x10^{-5}$ & $1x10^{-4}$ M), peak responses were slightly reduced, perhaps due to receptor desensitization. The 5-HT_3 agonist, 2-methyl-5-HT, yielded a monophasic CRC that did not include high potency, slow to peak responses. 2-methyl-5-HT produced a maximum response of 342±40 μV at 100 μM, in contrast to the 5-HT maximum of 442±29 μV at 10 μM. The 5-HT_3 specific antagonist ondansetron (1 μM) reduced the average response to 10 μM 5-HT by 66% to 150±14 μV. Depolarizations in the presence of ondansetron were elicited by low concentrations of 5-HT ($1x10^{-9}$-$1x10^{-7}$ M) and displayed only slow kinetics. The 5-HT_4 selective antagonist, RS-23597-190 (Eglen *et al.* 1992), at 1 μM shifted the 5-HT CRC to the right by eliminating the high potency, slow to peak response portion of the curve without affecting the large rapid responses.

5-HT_4 receptors are positively coupled to adenylyl cyclase in several systems (Bockaert *et al.* 1992), and thus we attempted to mimic the slow depolarization with stimulators of cAMP-dependent protein kinase (PKA). Superfusion of 300 μM 8-Br-cAMP for 4 min caused a slow depolarization (223±39 μV) that peaked several minutes after application. In order to obtain quicker responses, we turned to forskolin. Exposure to forskolin (1 or 10 μM; 1 min) evoked depolarizations that were kinetically similar but larger (220±18 μV and 525±42 μV, respectively) than those caused by 5-HT in the presence of ondansetron. The inactive analogue 1,9-dideoxy-forskolin (10 μM) produced negligible effects. After depolarizations elicited by 10 μM forskolin had stabilized, 10 μM 5-HT (with 1 μM spiperone and 1 μM ondansetron present) caused additional small depolarizations (147±19 μV) that strongly resembled those evoked by 5-HT alone. IBMX, at 300 μM, did not potentiate 5-HT_4 responses elicited either before or during forskolin applications.

In conclusion, our data support the finding of Rhodes et al. (1992) that both 5-HT_3 and 5-HT_4 receptors contribute to serotonergic depolarizations of the isolated rat vagus nerve. However, even though forskolin and 8-Br-cAMP produced effects qualitatively similar to 5-HT_4 receptor activation, the failure of stimulation of PKA to alter 5-HT_4-induced responses raises the possibility that 5-HT_4 receptors in the vagus nerve may be coupled to ion channels through mechanisms other than PKA.

Bockaert, J., Fozard, J., Dumuis, A. & Clarke, D.E. (1992) Trends Pharmacol. Sci. **13**, 141-145.
Eglen, R.M., Bonhaus, D.W., Clark, R., Hegde, S., Leung, E. & Whiting, R.L. (1992) Br. J. Pharmacol., in press.
Ireland, S.J. & Tyers, M.B. (1987) Br. J. Pharmacol. **90**, 229-238.
Rhodes, K.F., Coleman, J. & Lattimer, N. (1992) Naunyn-Schmiedeberg's Arch. Pharmac. **346**, 496-503.

247P THE EFFECT OF THE 5-HT$_4$ RECEPTOR ANTAGONIST DAU 6285 ON A COMPONENT OF 5-HT EVOKED DEPOLARIZATION OF THE RAT ISOLATED VAGUS NERVE

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Depolarization of the rat isolated vagus nerve evoked by 5-hydroxytryptamine (5-HT) is mediated predominantly by 5-HT3 receptors (Ireland & Tyers, 1987). However a minor component of the total response to 5-HT (approximately 20%) has been shown to be resistant to blockade by 5-HT3 receptor antagonists such as ondansetron and to be absent in tissues preincubated with 5-methoxytryptamine at a concentration known to cause tachyphylaxis of 5-HT4 receptor-mediated responses (Rhodes *et al.*, 1992). In this study the recently available 5-HT4 receptor antagonist DAU 6285 (DAU) has been used to characterize the depolarizing mechanisms. Extracellular recording of depolarization of desheathed cervical vagus nerves was carried out using a grease-gap technique (Ireland & Tyers, 1987; Rhodes *et al.*, 1992). Male Sprague-Dawley rats (180-280 g) were killed by a blow to the head and cervical dislocation. The nerve was placed in a two chamber tissue bath, one chamber being perfused with Krebs solution (2.5 mM $CaCl_2$, at 27°C, gassed with 5% CO_2 in O_2) to which drugs were added. The potential difference between the chambers was recorded. Two non-cumulative concentration-response curves (CRC's) to 5-HT were constructed in each tissue using a 3 min agonist contact time and 10-15 min wash period. The first CRC was obtained in the absence of antagonist the second in the presence of either 1 μM ondansetron or ondansetron (1μM) together with DAU (0.3-3 μM). Antagonists were equilibrated for 50 min before constructing the second CRC in each tissue.

In the first CRC 5-HT (10 nM-30 μM) evoked concentration-dependent depolarizations with a geometric mean EC_{50} of 0.95 (0.79-1.15) μM. The mean maximum depolarization was 556 (506-606) μV (n=18). In the presence of ondansetron, the CRC to 5-HT was biphasic with responses to 10 nM-3 μM 5-HT being resistant to ondansetron blockade. Responses to higher concentrations of 5-HT were antagonized by ondansetron. The geometric mean EC_{50} for the first phase was 120.2 (83.2-166.0) nM (n=8) and the maximum depolarization of the first phase was 20.9 (17.5-24.3) % of the first curve maximum response to 5-HT. The first phase of the 5-HT CRC was displaced to the right in a concentration-related manner in the presence of DAU. Schild analysis gave a pA_2 value of 6.8 (6.7-7.1) for DAU with a slope value of 1.00 (0.77-1.23, n=12). No effect of DAU on the second phase of the CRC was observed.

The pA_2 and Schild plot slope for DAU are consistent with a competitive 5-HT4 receptor antagonist mechanism (Schiavone *et al.*, 1992). Although DAU has significant 5-HT3 receptor affinity (K_i = 322 nM, Dumuis *et al.*, 1992), this is unlikely to influence the present study in the presence of the potent 5-HT3 receptor antagonist ondansetron (Butler *et al.*, 1988). Dumuis *et al.*, (1992) have shown DAU to have low affinity at 5-HT1A, 1B, 1C, 1D and 5-HT2 sites making it unlikely that DAU is acting through these receptors. The antagonist action of DAU is consistent with a component of the 5-HT evoked depolarization of the rat vagus nerve being 5-HT4 receptor-mediated.

DAU 6285 was kindly supplied by Dr C.A. Rizzi of Boehringer Ingelheim Italia.

Butler A., Hill, J.M., Ireland, S.J., Jordan, C.C. & Tyers, M.B. (1988) *Br. J. Pharmacol.* **94**, 397-412.
Dumuis, A., Gozlan, H., Sebben, M., Ansanay, H., *et al.*, (1992) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **345**, 264-269.
Ireland, S.J. & Tyers, M.B. (1987) *Br. J. Pharmacol.* **90**, 229-238.
Rhodes, K.F., Lattimer, N. & Coleman, J. (1992) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **346**, 496-503.
Schiavone, A., Giraldo, E., Giudici, L.,Turconi, M. & Sagrada, A. (1992) *Life Sci.* **51**, 583-592.

248P 5-HT RECEPTORS IN CULTURED HUMAN VASCULAR SMOOTH MUSCLE CELLS AND ENDOTHELIAL CELLS

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Serotonin (5-hydroxytryptamine, 5-HT) has been shown to mediate complex effects leading to dilatation/contraction of blood vessels through multiple/different receptor subtypes located on different cells. In a previous study we showed mRNA for the 5-HT_{1A} receptor in human epicardial coronary artery and in cultured human vascular smooth muscle cells (VSMC) and endothelial cells (HUVEC) (Sun *et al.*, 1991). The presence of serotonin receptors in cultured VSMC and HUVEC was further investigated in the present study by examining the effects of drugs on binding of [^{125}I]-iodo-lysergic acid diethylamide (LSD) to membranes and on intracellular cAMP formation.

VSMC and HUVEC were both isolated from human umbilical vein. VSMC were identified by immunofluorescent study with anti- α-Isoactin antibody; and HUVEC by anti-Factor VIII antibody and accumulation of DiI-acetyl-LDL. Cell membranes were prepared from the first passaged confluent VSMC or HUVEC. 50 μg membrane protein was used for each binding assay and 20 μg for adenylyl cyclase (AC) activity measurement.

In VSMC membranes, binding of [^{125}I]-LSD (0.5 nM) was inhibited by 5-HT, 5-carboxamidotryptamine (5-CT), methiothepin, methysergide and ketanserin. 1 μM ketanserin produced 79% of the inhibition obtained with 0.1 mM 5-HT or 5-CT. The IC_{50}s for 5-HT, 5-CT and ketanserin were 60 nM, 800 nM and 6 nM, respectively. Non-linear regression curve fitting of the inhibition data showed that both 5-HT and 5-CT inhibited the [^{125}I]-LSD binding to VSMC membrane at two sites. 5-HT showed higher affinity than 5-CT on both sites. Sumatriptan and 8-OH-DPAT inhibited the [^{125}I]-LSD binding to VSMC membranes only at concentrations > 1 μM. The ketanserin-sensitive site is consistent with the existence of 5-HT_2 receptors. In binding-inhibition experiments with HUVEC membranes, ketanserin and spiperone reduced [^{125}I]-LSD binding by approximately 35%. The nature of the [^{125}I]-LSD binding sites on HUVEC is unknown. Methiothepin and methysergide inhibited the binding only at high concentrations (> 1 μM).

Neither 5-HT (1 μM) nor 8-OH-DPAT (1 μM) inhibited or stimulated the AC activity in membrane preparations from VSMC or HUVEC. Neither of them influenced the forskolin (10 μM)- or alpha-hCGRP (1 μM)-mediated increase in AC activity. In VSMC membranes, 5-HT inhibited the adrenaline (1 μM)-induced increase in cAMP formation by 10% and 14% in two separate experiments ($p < 0.01$); 8-OH-DPAT inhibited by 8% ($p < 0.05$). 5-HT inhibited forskolin-stimulated cAMP accumulation by 10% in membranes prepared from HUVEC which had been cultured in the presence of 100 nM noradrenaline, whereas the basal AC activity in the membranes was unaltered.

This study suggests that there is more than one type of serotonin receptor in the cultured human VSMC and HUVEC. VSMC receptors appear predominantly to be of 5-HT_2 nature.

Sun, B., Monteith, S. & Brown, M.J. (1991) *Clin. Sci.* **81** (suppl. 25), 14P.

249P EFFECT OF SUMATRIPTAN AND 5-HT IN BOVINE PULMONARY AND MESENTERIC ARTERY

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Sumatriptan (GR43175) is a selective 5-HT_1 receptor agonist effective in the acute treatment of migraine. McIntyre *et al* (1992) have shown that this agent, when given subcutaneously to patients undergoing diagnostic coronary angiography, will produce a greater percentage rise in pulmonary arterial resistance when compared with overall systemic resistance. This study was conducted to determine whether there was any difference in sensitivity between a pulmonary and systemic vessel and also whether there was any involvement of the endothelium in modulating the response.

Lobes of bovine lung and proximal segments of the small intestine with attached mesenteric fat were obtained from freshly killed cattle and transferred to the laboratory in oxygenated Krebs solution. The bovine main pulmonary artery with branches attached was dissected from the lung and vessels of 3-5mm in diameter removed. Similar sized mesenteric arteries which were of the third order were also taken. Rings of artery 2-3mm in length were set-up in organ baths in Krebs solution under 1.5grammes tension (optimum for contraction to 100mM KCl in both tissues), at 37°C in 95% O_2, 5% CO_2 and allowed to equilibrate for 1 hour before experimentation. Tissues were then exposed to cumulative additions of either 5-HT or sumatriptan (3nM-10μM) and washed three times with a period of 45 minutes equilibration before the procedure was repeated. In some arteries the endothelium was removed by gentle rubbing of the intimal surface. The effect of L-NAME (100μM), a nitric oxide synthase inhibitor and selective 5-HT antagonists ketanserin(5-HT_2) and methiothepin(5-$HT_{1/2}$) were studied on the second cumulative concentration response curve to either agonist with responses expressed as % of the control curve. A second cumulative concentration curve was found to be not significantly different from the first.

Responses to 5-HT were similar in both pulmonary and mesenteric arteries with pD_2 values of 7.1 and 6.95 respectively. Removal of the endothelium and/or inclusion of L-NAME had no effect in either tissue on sensitivity or maximum response to 5-HT. Responses to sumatriptin were obtained only in arteries from the pulmonary vascular bed. In six mesenteric arteries which contracted normally to 5-HT sumatriptan up to 10μM had no effect. In the pulmonary artery sumatriptan contracted the vessel in a concentration dependent manner with 10μM still not reaching an absolute maximum response. Sensitivity was slightly reduced compared with 5-HT although an accurate measurement could not be obtained. The maximum response approximately 50% of that to 5-HT (1224±170mg.wt., compared with 2440±300mg.wt.). Inclusion of L-NAME caused an increase in sensitivity and maximum response to sumatriptan to 174 ± 24%. Removal of the endothelium also increased the sensitivity of the response to sumatriptan. Ketanserin 0.1-1μM antagonised responses to 5-HT in both pulmonary and mesenteric arteries. In addition it almost completely blocked responses to sumatriptan, maximum response reduced to 9.7±4% with 1μM. Methiothepin 1nM had no effect on responses to 5-HT in both arteries, but significantly inhibited responses to sumatriptan, maximum response reduced to 48±9%.

In conclusion sumatriptan will contract pulmonary arteries with a similar sensitivity to 5-HT but with a smaller maximum response. Since it is unable to contract the mesenteric artery this may indicate a relatively selective effect on the pulmonary vasculature in this animal model at least. In addition the response is inhibited by either the selective 5-HT_1 or 5-HT_2 antagonist indicating that both receptors are involved in the response in this artery to sumatriptan.

This work was supported by the Wellcome Foundation. Sumatriptan was kindly donated by Glaxo

McIntyre,P.D., Gemmill,J.D., Hogg,K.J., Bhargava,B. & Hillis,W.S. *Br.J.Pharmacol.*, 1992, **107**, 155P .

250P EFFECTS OF SUMATRIPTAN ON PLASMA PROTEIN EXTRAVASATION AND CAROTID VASCULAR RESISTANCE DURING TRIGEMINAL GANGLION STIMULATION IN THE ANAESTHETISED RAT

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Sumatriptan is an effective anti-migraine drug which is a potent vasoconstrictor of intracranial vessels which, it is thought, may be dilated during a migraine attack (Humphrey *et al*, 1990). Sumatriptan also blocks plasma protein extravasation in the dura mater produced by trigeminal ganglion stimulation (Buzzi & Moskowitz, 1990) an action which has also been implicated in the aetiology of migraine. This study compared the effects of sumatriptan on carotid vascular resistance (CVR) and extravasation in the dura mater during trigeminal ganglion stimulation in anaesthetised rats.

Male Sprague-Dawley rats (280-350 g) were anaesthetised with sodium pentobarbitone (70 mg/kg, i.p.) and the right femoral artery and vein cannulated for continual recording of mean arterial blood pressure (MABP) and drug administration respectively. Both common carotid arteries were excised and fitted with Transonic flow probes (1mm). Animals were then injected with sumatriptan (300 μg/kg, i.v., n=6) or saline (n=6) and placed in a stereotaxic frame with the incisor bar at -2.5 mm. Bipolar electrodes were lowered into the trigeminal ganglia (3.5 mm AP, ± 3.0 mm L and 9.5 mm V from Bregma) and the animal was injected with 50 mg/kg, i.v. fluorescein-labelled BSA (FITC-BSA). Five min later the right trigeminal ganglion was stimulated for 3 min (1.2 mA, 5 msec duration, 5 Hz). MABP and carotid flow were monitored before and during stimulation and CVR calculated as MABP/flow (expressed as mmHg/ml/min). Rats were then perfused with saline at 120 mmHg for 3min via the left cardiac ventricle, the brain removed and the dura from each side excised, rinsed and incubated in 2 ml of saline (pH11) for 18-24 h. The dura were then blotted dry and weighed and the [FITC-BSA] in the incubation solution measured using a Perkin-Elmer LS-30 luminescence spectrometer. Results are expressed as the ratio of [FITC-BSA] (in ng/mg tissue) on the stimulated side compared with the unstimulated side. Statistical comparisons were performed using a 3 factor nested ANOVAR and Student's t-test for paired data (basal vs stimulated) or unpaired data (sumatriptan vs control).

Trigeminal stimulation increased MABP from 116±2.6 to 152±3.4 mmHg ($p<0.001$) in control rats and from 110±1.8 to 140±3.7 mmHg ($p<0.001$) in sumatriptan treated rats. Neither the basal blood pressure nor the increase was significantly affected by sumatriptan. Trigeminal stimulation decreased CVR on the stimulated side (from 14.1±1.0 to 4.9±0.5, $p<0.001$) but had no effect on CVR on the unstimulated side (from 14.4±0.6 to 15.3±1.9). Sumatriptan had no effect on either the basal or stimulated CVR on either side (from 15.4±2.4 to 4.9±0.4 ($p<0.01$) on the stimulated side and from 16.4±2.6 to 14.4±2.2 ($p<0.05$) on the unstimulated side). Trigeminal stimulation produced plasma protein extravasation into the dura mater. In control animals the ratio of [FITC-BSA] between the two sides was 1.8±0.18. In sumatriptan treated animals this ratio was 1.27±0.04 ($p<0.02$).

These data confirm that sumatriptan (300 μg/kg) significantly blocks plasma protein extravasation following trigeminal ganglion stimulation in the anaesthetised rat and suggest that FITC-BSA is a useful alternative to the [^{125}I]-BSA used as a marker by Buzzi & Moskowitz (1990). The results also show that trigeminal stimulation decreases ipsilateral CVR (presumably due to vasodilatation) but has no effect on contralateral CVR. Sumatriptan had no effect on either the basal or the stimulated CVR, neither did it have any effect on the basal MABP or the stimulation induced increase in MABP. Therefore, the rat appears to differ from other species in which sumatriptan has been shown to increase CVR (Humphrey *et al*, 1990). One possible explanation for this difference is that the proportion of sumatriptan-sensitive vessels in the total carotid vasculature of the rat may be too small to have any measurable effect on overall CVR.

Buzzi, M.G. & Moskowitz, M.A. (1990) *Br. J. Pharmacol.* **99**, 202-206.
Humphrey, P.P.A., Feniuk, W., Perren, M.J., Beresford, I.J.M. & Skingle, M. (1990) *Ann. New York Acad. Sci.* **600**, 587-600.

251P EFFECTS OF ZACOPRIDE AND CISAPRIDE ON PIGLET ATRIAL 5-HT$_4$ RECEPTORS

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A putative 5-HT$_4$ receptor mediates the positive inotropic effects of 5-HT in piglet and human atria (Kaumann *et al.*, 1991; Lorrain *et al.*, 1992). Zacopride and cisapride, gastroprokinetic benzamide derivatives, are 5-HT$_3$ receptor antagonists which also show 5-HT$_4$ agonist properties in gastrointestinal and CNS models (Eglen *et al.*, 1990; Baxter *et al.*, 1991; Dumuis *et al.*, 1989). We have studied the effects of zacopride and cisapride on the piglet atrial 5-HT$_4$ receptor.

Piglet left atrial strips, bathed in Krebs solution at 37°C, 95 %O_2, 5% CO_2, containing dl-propranolol (1 µM) and cocaine (10 µM) were paced at 1 Hz with supramaximal voltage. A single concentration-response curve (CRC) to 5-HT or one of the benzamides was performed in each tissue and positive inotropic effects expressed as % of the response to supramaximal concentration of isoprenaline (200 µM).

5-HT is a potent inotropic agent in this model (pD_2 6.73 ± 0.1, E_{max} 55 ± 4 % of isoprenaline maximum) and its effects are antagonised competitively by SDZ 205-557 (pA_2 7.30, Lorrain *et al.*, 1992). Cisapride produced inotropic effects over a similar concentration range (pD_2 6.38 ± 0.07) but was a partial agonist (E_{max} 22 ± 3 %). In the presence of cisapride, the 5-HT CRC was displaced rightwards in a parallel fashion (pA_2 6.68 ± 0.34). Zacopride was a much weaker inotropic agent producing small increases in tension from 0.3 µM to 1000 µM. These responses were sensitive to SDZ 205-557 3 µM over the lower portion of the CRC but not at higher concentrations, making it impossible to calculate an appropriate E_{max}. Zacopride antagonised inotropic responses to 5-HT (pK_b estimate 6.3) thus confirming its affinity at 5-HT$_4$-receptors, but did not inhibit the isoprenaline CRC at 30 µM.

Hence, at piglet atria 5HT$_4$ receptors, cisapride and zacopride are partial agonists with cisapride the more potent of the two. Zacopride also possess an additional inotropic mechanism at high concentrations.The low intrinsic activity of cisapride and zacopride appears to be a characteristic of cardiac 5-HT$_4$ receptors mediating inotropy since it applies to both piglet atria (our data) and human atria (Kaumann *et al.*, 1991) whereas these benzamides are almost full agonists in other 5-HT$_4$ models (Baxter *et al.* 1991).

Baxter G.S., Craig D.A. & Clarke D.E. (1991) Naunyn-Schmiedeberg's Arch Pharmacol. **343**, 439-446.
Dumuis A., Sebben M. & Bockaert J. (1989) Naunyn-Schmiedeberg's Arch Pharmacol. **340**, 403-410.
Eglen R.M., Swank S.R, Walsh L.K. & Whiting R.L.(1990) Br. J. Pharmacol. **101**, 513-520.
Kaumann A.J., Sanders L., Brown A.M., Murray K.J. & Brown M.J. (1991) Naunyn-Schmiedeberg's Arch Pharmacol. **344**, 150-159.
Lorrain, J., Grosset, A. & O'Connor, S.E. (1992) Eur. J. Pharmacol. 229, 105-108

252P STEPWISE ADMINISTRATION PREVENTS THE FENFLURAMINE-INDUCED DEPLETION OF BRAIN 5-HYDROXYTRYPTAMINE IN THE RAT

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Repeated administration of high doses of d-fenfluramine (d-FEN) produces long-lasting depletion of brain 5-HT analogous to the depletion of dopamine by high doses of d-amphetamine (Kleven and Seiden, 1989; Ellison and Eison, 1983). However, Robinson and Camp (1987) reported that exposure to gradually increasing doses of d-amphetamine attenuated its dopamine-depleting effect. Therefore, we report the effect of escalating doses of d-FEN on the d-FEN-induced depletion of cerebral 5-HT levels in the rat.

Male Wistar rats (138-186g) were treated with either (1) sub-acute d-FEN (5mg/kg i.p b.i.d for 4 days) or (2) escalating doses of d-FEN (0.5, 1, 1.5, 2, 3, 4 and 5mg/kg i.p. b.i.d., each dose given for 4 days before increasing to the next dose). Control rats for each group received saline (1ml/kg i.p. b.i.d. for the period of drug administration). Food intake and body weight were measured throughout the period of drug administration. Six rats from each group were weighed and killed 1, 15 and 30 days after the last dose of d-FEN. 5-HT and 5-HIAA concentrations were determined in the hypothalamus (HYP), hippocampus (HIPP) and cortex (CORT) by HPLC.

Food intake was reduced during both regimes of d-FEN adminstration. Body weight was reduced from control values only on day 1 after withdrawal from d-FEN in both treatment groups. Following sub-acute administration of d-FEN, 5-HT and 5-HIAA levels were reduced in all tissues studied up to 30 days, the HYP being least severely affected (Figure). Following the administration of escalating doses of d-FEN, 5-HT and 5-HIAA levels were only reduced compared to control values on day 1 in the CORT, no alterations in the HYP or HIPP were observed (Figure).

In agreement with Caccia et al (1992), these data suggest that stepwise increases in d-FEN administration produce desensitization to the 5-HT-depleting effect of a high dose of the drug without apparently altering serotonergic function in rats.

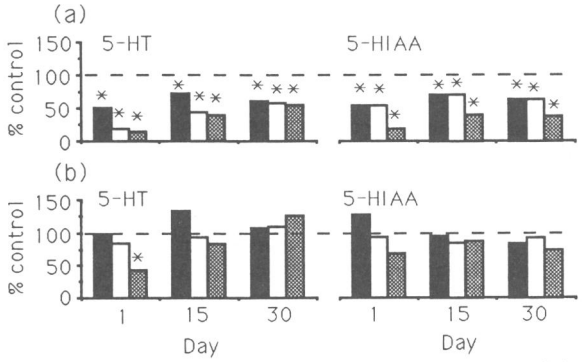


Figure Effect of (a) sub-acute administration or (b) escalating doses of d-FEN on 5-HT and 5-HIAA. * $p<0.05$ vs control. HIPP HYP CORT

Kleven, M.S. & Seiden, L.S. (1989) Brain Res. 505: 351-353.
Ellison, G.D. & Eison, M.S. (1983) Psychol. Med. 13: 751-762.
Robinson, T.E. & Camp, D.M. (1987) Pharmacol. Biochem. Behav. 26: 821-827.
Caccia, S., Sarati, S., Anelli, M. & Garattini, S. (1991) Neuropharmacol. 31: 875-879.

253P INTERACTION OF YOHIMBINE WITH DIAZEPAM AND ONDANSETRON TO MODIFY EXTRACELLULAR LEVELS OF 5-HT IN THE RAT FRONTAL CORTEX

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Previous studies have demonstrated that yohimbine possesses anxiogenic activities in man and in animal models of anxiety (Charney et al., 1984; Pellow et al., 1985). In the present study, we have investigated the effects of yohimbine and its interaction with diazepam and ondansetron on the extracellular levels of 5-HT in rat frontal cortex using the technique of microdialysis.

Microdialysis probes were implanted into chronically indwelling cannulae located in the frontal cortex of male hooded Lister rats (300-400g) 12 hr prior to the experiment. Probes were perfused with artificial CSF at a rate of 2 µl/min. 20-min samples were collected and analysed for 5-HT by HPLC-ECD according to the method of Barnes et al. (1992). After assessment of basal levels (t=0), the animals were treated with either yohimbine (Yohim., 5.0 mg/kg, i.p.) or vehicle (0.9% w/v NaCl, 1.0 ml/kg, i.p.). Diazepam (Diaz, 2.5 mg/kg, i.p.) or ondansetron (Ond, 100 µg/kg, i.p.) was administered alone or as a 5 min pretreatment prior to yohimbine.

Table 1. The effect of yohimbine and its interaction with diazepam and ondansetron on the extracellular level of 5-HT in the rat frontal cortex.

	Collection time (min)								
Treatment	0	20	40	60	80	100	120	140	320
Vehicle	100±4	100±3	100±5	96±4	97±3	91±2	92±3	90±5	94±3
Yohim	102±5	101±7	110±6	143±10*	164±15**	175±20**	231±20**	176±20**	120±10
Diaz	100±2	96±3	65±7*	66±8*	49±6**	46±5**	51±6**	52±5**	88±8
Yohim+Diaz	96±7	97±6	94±8	91±8	94±10	92±8	94±9	81±10	98±7
Yohim+Ond	93±5	86±9	88±11	89±7	92±7	91±8	100±9	99±12	98±5

Data represent mean±SEM (% of the basal levels), n=6-8, *P<0.05, **P<0.01 significant increases or decreases compared to the basal levels (one way ANOVA, followed by Dunnett's t test).

Yohimbine significantly increased the extracellular levels of 5-HT which were antagonised by diazepam and ondansetron. Diazepam caused a reduction in the extracellular 5-HT levels when administered alone whilst ondansetron has been previously demonstrated to fail to modify the 5-HT levels (Barnes et al., 1992). The data may indicate a 5-HT involvement in the actions of yohimbine to exacerbate behavioural responding to aversive situations in animals and in its interaction with diazepam and 5-HT_3 receptor antagonist.

Barnes N. M., Cheng C. H. K., Costall B., Ge J., Naylor R. J. (1992) Br. J. Pharmacol., **107**, 233-239.

Charney D. S., Heninger G. R. and Breier A (1984) Arch. Gen. Psychiatry, **41**, 751-763.

Pellow S., Chopin P., File S. E., Briley M. (1985) Methods, **14**, 149-167.

254P BRL 46470A, A SELECTIVE 5-HT_3 RECEPTOR ANTAGONIST, ATTENUATES THE INCREASED SEIZURE SUSCEPTIBILITY OBSERVED DURING DIAZEPAM-INDUCED WITHDRAWAL IN MICE

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In man, withdrawal from repeated benzodiazepine (BDZ) administration induces a number of symptoms including anxiety and enhanced seizure susceptibility (Marks, 1985). We have previously shown the selective 5-HT_3 receptor antagonist, BRL 46470A (Blackburn et al 1992), to reverse diazepam-induced withdrawal anxiety in rats (Kennett et al., 1992). The present study investigated the effect of BRL 46470A on another aspect of diazepam withdrawal; lowered seizure threshold. Groups of male CD mice (n=25) were administered diazepam (1 mg/kg) or vehicle (10 ml/kg) i.p. at 9 am & 5 pm for 7 days and on day 8 were dosed at 9 am only. On day 9 (23h after the last chronic dose) animals received BRL 46470A (0.001-1 mg/kg p.o.) or vehicle (10 ml/kg p.o.) and 60 min later were infused intravenously with pentylenetetrazol (PTZ: 8 mg/ml, 0.5 ml/min). Latencies to the induction of generalised clonic and tonic extensor seizures were recorded and the threshold dose of PTZ required to elicit each particular seizure type was calculated.

Table 1 Effect of BRL 46470A on diazepam-induced withdrawal in the PTZ infusion model

Pretreatment Days 1-8 (mg/kg i.p.)	Treatment Day 9 (mg/kg p.o.)	Mean ± SE Convulsive dose of PTZ (mg/kg i.v.) Clonic Seizure	Tonic Seizure
Vehicle	Vehicle	51.3 ± 1.6	131.9 ± 5.7
Diazepam 1	Vehicle	43.5 ± 1.6**	97.9 ± 5.5**
Diazepam 1	BRL 46470A 0.001	47.4 ± 1.4	131.9 ± 3.8^{++}
Diazepam 1	BRL 46470A 0.01	50.9 ± 1.6^{+}	122.1 ± 5.4^{++}
Diazepam 1	BRL 46470A 0.1	50.5 ± 1.9^{++}	126.3 ± 6.7^{++}
Diazepam 1	BRL 46470A 1	53.7 ± 1.9^{++}	120.9 ± 6.8^{++}

Significantly different from vehicle + vehicle group** $P < 0.01$; from diazepam + vehicle group^{+} $P < 0.05$, $^{++}$ $P < 0.01$ by Newman-Keuls test following significant 2 way ANOVA.

A significant decrease in the dose of PTZ required to induce clonic and tonic seizures was observed in the mice undergoing withdrawal from diazepam (Table 1). This proconvulsant effect of diazepam-induced withdrawal was significantly attenuated by BRL 46470A over a wide dose-range (0.001-1 mg/kg p.o.) (Table 1). The precise mechanism underlying this action of BRL 46470A is presently unclear as the 5-HT_3 receptor antagonist was not found to possess anticonvulsant activity per se in the PTZ infusion test (data not shown). However, the present findings do suggest that BRL 46470A may reduce the likelihood of seizure activity occurring during withdrawal from BDZ treatment in man.

Blackburn, T.P., Baxter, G.S., Kennett, G.A., King, F.D. et al. (1993) Psychopharmacol. in press
Kennett, G.A., Grewal, S.S., Upton, N. and Blackburn, T.P. (1992) Br. J. Pharmacol. 105 (suppl), 173P.
Marks, J. (1985) in The benzodiazepines, use, overuse, abuse ed. Marks, J. pp 33-38. Lancaster: MTP Press Ltd.

255P EFFECTS OF THE PUTATIVE 5-HT_{1A} RECEPTOR ANTAGONIST SDZ 216,525 IN TWO MODELS OF SOMATODENDRITIC 5-HT_{1A} AUTORECEPTOR FUNCTION

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It was recently suggested that SDZ 216,525 is a selective and silent 5-HT_{1A} receptor antagonist (Hoyer et al. 1991). However, a number of other putative antagonists have been shown to act as postsynaptic 5-HT_{1A} receptor antagonists and presynaptic 5-HT_{1A} receptor agonists. As yet the effects of SDZ 216,525 at the somatodendritic 5-HT_{1A} autoreceptor are unknown. Therefore, we examined the potential intrinsic agonist activity of SDZ 216,525 in two *in vivo* models of 5-HT_{1A} somatodendritic autoreceptor function: release of 5-HT in the rat hippocampus using microdialysis and increased food intake of satiated animals. The effects of SDZ 216,525 were compared to those of 8-OH-DPAT, buspirone and the silent 5-HT_{1A} receptor antagonist (±)-WAY100135 (Fletcher et al. 1991).

In neurochemical studies, 24 hours after implantation of guide cannulae into the ventral hippocampus of anaesthetised male Sprague-Dawley rats (280-350g), dialysis probes were inserted for measurement of extracellular 5-HT. Probes were perfused with artificial cerebrospinal fluid pH7 (1.0 µl/min) and following a 3 hr stabilisation period samples were collected at 20 min intervals. 3 control samples were taken prior to s.c. administration of vehicle (25% PEG, saline or 0.3% methyl cellulose), SDZ 216,525, (±)-WAY100135, buspirone or 8-OH-DPAT. Samples were collected for a further 3 hr. In antagonist studies, administration of (±)-WAY100135 or vehicle was followed 30 min later by SDZ 216,525 or vehicle and samples were collected for a further 2.5 hr. In behavioural studies, the food intake of free feeding rats following the administration (s.c.) of SDZ 216,525, (±)-WAY100135, 8-OH-DPAT and buspirone was studied 1 and 3 hr after injection at 10.00 a.m.

SDZ 216,525 (1 mg/kg), 8-OH-DPAT (0.1 mg/kg), and buspirone (5 mg/kg) significantly ($p<0.05$) decreased extracellular 5-HT, to 38.1 ± 10.3, 19.2 ± 9.9 and 39.9 ± 15.0 % of preinjection control values respectively. In contrast, (±)-WAY100135 had no significant effect on 5-HT release. Pretreatment with (±)-WAY100135 (10 mg/kg s.c.) had no effect on SDZ 216,525-induced inhibition of 5-HT release. The food intake of satiated rats over 1-3 hr was increased by 8-OH-DPAT (0.1 mg/kg, intake = 1.7 g), buspirone (3 mg/kg, intake = 2.8 g) and SDZ 216,525 (10 mg/kg, intake = 1.5 g) but unaffected by (±)-WAY100135 (10 mg/kg, intake = 0.1 g) when compared to vehicle controls (intake = 0.3 g).

The decrease in extracellular levels of 5-HT and the increase in feeding induced by SDZ 216,525 suggest that this compound may be a 5-HT_{1A} receptor partial agonist. However, the inability of the 5-HT_{1A} antagonist (±)-WAY100135 to antagonise the SDZ 216,525-induced decrease in 5-HT release is inconsistent with an agonist action at the 5-HT_{1A} receptor. It is possible that these effects are mediated via α_1-adrenoceptors as SDZ 216,525 has high affinity for this site (Khawaja, unpublished data) and the α_1-adrenoceptor antagonist prazosin has been shown to decrease 5-HT release *in vivo* (Claustre et al. 1991). Further studies are required to elucidate the mechanism of action of SDZ 216,525 on central 5-HT neurones.

Claustre, Y., Rouquier, L., Serrano, A., Benavides, J. & Scatton, P. B. (1991) Eur. J. Pharmacol. **204**, 71-77.
Fletcher, A., Bill, D.J., Bill, S.J., Brammer, N.T., Cliffe, I.A., Forster, E.A., Reilly, Y. & Lloyd, G.K. (1991) Soc. Neurosci. Abstr., **17**, 92P.
Hoyer, D., Schoeffter, P., Palacios, J.M., Kalkman, H.O., Bruinvels, A.T., Fozard, J.R., Siegl, H., Seiler, M.P. & Stoll, A. (1991) Br.J.Pharmacol., **105**, 29P.

256P MODIFIED ASSAY CONDITIONS GREATLY IMPROVE THE AFFINITIES OF AGONISTS, BUT NOT ANTAGONISTS, AT 5-HT_3 RECEPTORS

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Although most studies in vitro indicate that 5-HT has low (>100 nM) affinity for $5HT_3$ receptors, some suggest a high affinity (e.g. Paudice and Raiteri, 1991). We now report assay conditions in which the affinities of agonists (but not antagonists) for the 5-HT_3 receptor are much improved compared to those found in previous studies. Experiments were performed on cortex of male Sprague-Dawley 200-300g rats using two methods. In method 1 (based on Nelson and Thomas, 1989), cortex was homogenised in 10 volumes of 50 mM HEPES buffer (pH 7.5, 4°C), centrifuged (10 min, 48,000 g, 4°C), and the pellet washed 3 times by resuspension in 10 volumes of buffer and centrifugation. The final pellet was resuspended in 20 volumes of the buffer. Membranes (~1 mg prot) were incubated (30 min, 25°C) in 2 ml of 50 mM HEPES buffer, with ~0.4 nM [^{3}H]BRL 43694 and test drug, or 100 nM RS-zacopride for nonspecific binding, then filtered over polyethylenimine-soaked GF/B filters. In method 2, tissue was homogenised in 10 volumes of sucrose (0.32 M, 4°C), centrifuged (10 min, 1,000 g, 4°C), and the supernatant stored on ice. The pellet was resuspended in 10 volumes of cold sucrose and recentrifuged as above. Supernatants were mixed, and centrifuged (20 min, 48,000 g, 4°C). The pellet was washed 6 times by resuspension in 20 volumes of 50 mM phosphate buffer (pH 7.4, 4°C), followed by centrifugation (20 min, 48,000 g, 4°C), with a 10-minute incubation at 37°C during the fourth wash, and storage overnight at -85°C before the last wash. The final pellet was resuspended in 20 volumes of HEPES 10 mM buffer (pH 7.4). Membranes (~0.5 mg prot) were incubated (30 min, 25°C) in 2 ml HEPES 10 mM buffer with ~0.1 nM [^{3}H]BRL 43694, and test compound, or 100 nM RS-zacopride for nonspecific binding, and filtered over polyethylenimine-soaked GF/C filters. In both methods, filters were washed with 2 x 7.5 ml buffer at 25°C, and counted by liquid scintillation. Using both methods, saturation analysis of [^{3}H]BRL 43694 binding showed a single saturable binding site with characteristics in the range found previously (Nelson & Thomas, 1989). When displacement studies were performed, 4 antagonists had approximately the same affinities. In contrast, the IC_{50} values of 4 agonists were 13 to 22 times lower ($P<0.001$ in each case, Student's t-test) with the second method (table 1). Calculation of Ki values was precluded when [^{3}H]BRL 43694 was used as radioligand, as Hill coefficients (nH) for the agonists were all >1.8 with method 1, and between 1.2 and 1.6 with method 2. When [^{3}H]S-zacopride (~0.2 nM) was used as radioligand in rat cortex or NG 108-15 cells, a similar shift in affinity of 5-HT was seen (Ki = 141 ± 10 nM for method 1, and 21 ± 4 nM for method 2) without any change in nH, but the affinity of ICS 205-930 was unaltered (Ki = 0.8-1.5 nM with either method). Thus, the affinities of 5-HT_3 agonists, but not antagonists, are highly dependent on the assay conditions. This may suggest the existence of independent agonist and antagonist states of the 5-HT_3 receptor, or the possible induction by agonists in the conditions used in method 2 of allosteric desensitisation phenomena at 5-HT_3 receptors (by analogy with those already found with the nicotinic receptor). The present results also suggest a means in vitro of determining whether a ligand is an agonist or an antagonist at 5-HT_3 receptors.

	RS-Zacopride	ICS 205-930	MDL 72222	METO	5-HT	2-Me-5-HT	PBG	m-Cl-PBG
Method 1	0.24 ± 0.03	0.7 ± 0.2	16 ± 4	273 ± 44	145 ± 21	173 ± 24	123 ± 29	3.2 ± 0.6
Method 2	0.09 ± 0.02	0.6 ± 0.1	18 ± 7	143 ± 46	9.5 ± 0.8	13 ± 2	6 ± 2	0.15 ± 0.04

Table 1 : IC_{50} (nM ± s.e. mean, n ≥ 3) values of ligands in the two assay conditions. (METO = metoclopramide, PBG = phenylbiguanide)

D.R. Nelson & D.R. Thomas, Biochem. Pharmacol., (1989), 38, 1693-1695.
P. Paudice & M. Raiteri, Br. J. Pharmacol. (1991), 103, 1790-1794.

257P COMPARISON OF [^{3}H]-MESULERGINE BINDING TO THE CLONED RAT 5-HT$_{1C}$ RECEPTOR EXPRESSED IN COS AND 293 CELLS

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Many cloned neurotransmitter receptors have been studied after stable or transient expression in various cell lines. However, the effect of expression in different cell lines is not known. We have therefore compared the profile of [^{3}H]-mesulergine (Mes) binding to the cloned rat 5-HT$_{1C}$ receptor transiently expressed in COS (primate kidney) and 293 (human embryonic kidney) cells.

The binding of [^{3}H]-Mes (0.5nM final in drug competition experiments; essentially according to the method of Pazos et al,1984) was studied using washed membranes prepared from the cells. Specific binding was that displaced by mianserin (10^{-6}M). Data was analysed using LIGAND for saturation experiments and ALLFIT (4-parameter-logistic) for drug displacement studies.

In both cell lines [^{3}H]-Mes (0.01-8nM) bound to a single site [K_D 0.78nM±0.28(3) and 0.48±0.12(4);Bmax 8.85 pmol/mg Pr±2.0 and 25.9±3.4; COS and 293 cells respectively(x±SEM(n)]. Similar drug potency was seen for both agonists and antagonists (Table 1).

Table 1: Inhibition of [^{3}H]-mesulergine binding to cloned rat 5-HT$_{1C}$ receptor expressed in COS and 293 cells

Compound	COS		293	
	pK_I	slope	pK_I	slope
5-HT	7.51 ± 0.42	0.84 ± 0.14	7.44 ± 0.15	0.76 ± 0.09
Cyproheptadine	8.31 ± 0.05	1.02 ± 0.06	8.41 ± 0.12	0.94 ± 0.16
ICI 169,369	8.07 ± 0.11	0.99 ± 0.09	8.25 ± 0.09	1.17 ± 0.16
mCPP	6.92 ± 0.03	1.03 ± 0.10	7.10 ± 0.02	1.06 ± 0.06
Mesulergine	8.46 ± 0.08	1.04 ± 0.03	8.79 ± 0.08	1.01 ± 0.04
Mianserin	8.57 ± 0.04	1.24 ± 0.21	8.94 ± 0.04	1.21 ± 0.08
SCH 23390	7.58 ± 0.17	0.91 ± 0.04	7.95 ± 0.05	0.92 ± 0.07
Spiperone	6.47 ± 0.18	1.05 ± 0.15	6.53 ± 0.01	1.05 ± 0.06

All results are means from 3-5 experiments.

In conclusion COS and 293 cells are useful expression carriers for the 5-HT$_{1C}$ receptor and they probably contain the necessary G-protein compliment. Furthermore the pharmacological profile was not altered by high levels of expression.

Pazos A.,Hoyer D. & Palacios J.M. (1984) Eur.J.Pharmacol. 106,539-546

258P EXPOSURE TO THE ELEVATED X-MAZE RESULTS IN AN INCREASE IN VENTRAL-HIPPOCAMPAL 5-HT IN SOCIALLY-REARED RATS BUT NOT IN ISOLATION-REARED RATS

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Rats reared from weaning in social isolation demonstrate various behavioural and neurochemical disturbances including anxiogenic behaviour on the elevated x-maze and post-synaptic 5-HT$_{1A}$ receptor supersensitivity (Wright *et al* 1991a,b). The present study determines whether the rise in extracellular hippocampal 5-HT observed in group reared rats placed on the elevated x-maze (Wright *et al* 1992) also occurs in isolation reared rats.

Male Lister Hooded rats were reared from weaning (21 days) either in social groups (5/cage) or in isolation. After four to six weeks (200-320g) the rats were implanted with a concentric microdialysis probe. Rats were anaesthetised with O_2/N_2O(2:1)/halothane (2%) and a probe, perfused with artificial CSF at 1.0µl/min, was implanted into the ventral hippocampus (L+4.6, R-4.8 relative to bregma; V-8.5 to dura). 18 hours after surgery, 20 min perfusate samples were collected for analysis of 5-HT by HPLC with electrochemical detection. After 3 basal samples had been taken the rat was transferred to an elevated x-maze for a 20 minute period when the rat was returned to the holding cage for the collection of a further 5 samples.

In agreement with studies using both rats (Wright *et al.* 1992) and guinea-pigs (Rex *et al.* 1993), exposure to the elevated x-maze induced a significant increase in the level of dialysate 5-HT in the socially-reared rats, (18.3 ± 4.1 fmol/10µl (mean ± sem) for the 3rd basal sample to 37.4 ± 6.6 fmol/10µl for the x-maze sample; n=8; $p<0.05$, Students t-test). This represents a significant 85.5% increase over the mean basal 5-HT. In contrast, dialysate 5-HT did not increase significantly in the isolation-reared rats during a 20 minute period on the maze (14.7 ± 3.4 to 20.3 ± 4.5 fmol/10µl; n=7).

It has previously been shown that the 5-HT$_{1A}$ agonist 8-OH-DPAT and the 5-HT$_2$ agonist DOI produce significantly greater behavioural responses in isolation-reared rats, suggesting enhanced 5-HT$_{1A}$/5-HT$_2$ post-synaptic sensitivity respectively (Wright *et al.* 1991a). The present results suggest that this post-synaptic 5-HT receptor is associated with reduced pre-synaptic 5-HT release indicating that social isolation may cause an inbalance between pre- and post-synaptic 5-HT function.

MJB is a SERC CASE student in conjunction with Wyeth Research (UK) Ltd.

Rex, A., Marsden, C. A. & Fink, H. (1993). Psychopharmacology (In press).
Wright, I.K., Ismail, H., Upton, N. & Marsden, C.A. (1991a). Psychopharmacology **105**: 259-263.
Wright, I.K., Upton, N. & Marsden, C.A. (1991b). Physiology and Behaviour **50:** 1129-1132.
Wright, I.K., Upton, N. & Marsden, C.A. (1992). Psychopharmacology **109**: 338-346.

259P [^{3}H]KETANSERIN BINDING IN THE CEREBRAL CORTEX OF MATURE AND AGED SPRAGUE-DAWLEY RATS

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Previous studies have demonstrated an age-related increase in 5-HT$_2$ receptor mediated (Fone *et al.*, 1989), 1-(2, 5-dimethoxy-4-iodomethyl)-2-aminopropane (DOI)-induced wet dog shakes in aged, compared to mature, Sprague-Dawley rats (Gower and Gobert, 1992). Radioligand binding studies were performed to determine whether age-related changes in cortical 5-HT$_2$ receptor number or affinity were present in SD rats.

Mature (3-4 months, 370-590g) and aged (18-19 months, 540-890g) male SD rats were decapitated, and the brains dissected rapidly on ice and maintained at -80°C until use. Membranes from each individual animal were prepared using the method of Leysen *et al.* (1982). Tissues were incubated in glass test tubes for 15min at 37°C in the presence of [^{3}H] ketanserin (60.0 Ci/mmol) at concentrations of 0.07-0.54nM in saturation studies and 0.1nM in competition studies. 5μM methysergide was used to define non-specific binding. Competition curves with DOI (0.3nM-0.1mM) were generated in the presence and absence of 100μM GTP. Membrane-bound radioactivity was recovered by filtration under vacuum through Whatman GF/B filters, presoaked in 0.3% polyethyleneimine, using a Brandel cell harvester. Radioactivity was determined using liquid scintillation counting at an efficiency of approximately 30%. Tissues from mature and aged rats were assayed on the same day.

Saturation and competition binding studies in the cerebral cortex of mature and aged SD rats

	Saturation studies			Competition studies				
	Kd±s.e.m	Bmax±s.e.m (fmoles/mg protein)	n	Log IC50 (nM)±s.e.m -GTP	+GTP	%High affinity sites -GTP	+GTP	n
Mature	0.75±0.09	248.6±20.6	6	7.13±0.07	6.81±0.10$^+$	78.8±4.45	57.90±10.72	8
Aged	0.87±0.26	257.3±16.4	5	6.95±0.06	6.86±0.07	44.9±14.8*	68.91±7.46	7

Aged versus mature $^*p<0.05$, 2-tailed, unpaired Student's t-test. GTP versus control $^+p<0.05$, 2-tailed, paired Student's t-test.

Saturation studies showed there to be no significant age-related changes in the affinity or number of 5-HT$_2$ receptors. Competition curves with DOI were best fitted to a two-site model (Graph Pad). There was no age-related change in the affinity of the receptors for DOI. In the samples from mature rats GTP significantly reduced the IC50, increased the slope of the competition curve (0.82±0.03 to 0.91±0.06) and reduced the percentage of high affinity sites. In samples from the aged animals GTP failed to displace the competition curve or increase the slope (0.89±0.11 to 0.82±0.05). The proportion of receptors in the high affinity state was reduced in the aged compared to mature animals.

These data suggest there are no age-related changes in 5-HT$_2$ receptor number or affinity but indicate possible changes to the receptor effector coupling mechanism. The reduction in the number of high affinity binding sites on its own fails to explain the enhanced behavioural 5-HT$_2$ receptor function observed *in vivo*.

L.Robson is a MRC funded student in conjunction with UCB.

Fone, K.C.F., Robinson, J. and Marsden, C.A. (1991) *Br. J. Pharmacol.* **104**, 1547-1555.
Gower, A.J. and Gobert, J. (1992). *J. Psychopharmacol.* **6**, 128.
Leysen, J.E., Niemegeers, C.J.E. , Van Neuten, J.M. and Laduron, P.M. (1982) *Mol. Pharmacol.* **163**, 145-149.

260P PHARMACOLOGICAL ACTIVITY *IN VIVO* OF GR85548, A SELECTIVE 5-HT$_1$ RECEPTOR AGONIST

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GR85548 (N-methyl-3-(1-methyl-4-piperidinyl)-1H-indole-5-ethanesulphonamide) is a selective agonist for the 5-HT$_1$ receptor mediating vasoconstriction and inhibition of neuronally mediated contractions, being about 4-fold more potent than sumatriptan *in vitro* (Connor et al, this meeting). This study examines the pharmacological profile of action of GR85548 in anaesthetised dogs and additionally, investigates the pharmacokinetic profile of GR85548 in rats and dogs. Anaesthetised beagle dogs were prepared for measurement of arterial blood pressure and heart rate; vascular resistance was measured in the carotid vascular bed using a Statham electromagnetic flow probe as described by Feniuk et al, (1989). GR85548 was administered via the femoral vein using a cumulative dosing schedule. Bioavailability studies were conducted in conscious rats and dogs; drug was administered intravenously or orally. Terminal blood samples were taken from groups of rats (n=3) and serial blood samples were taken from dogs (n=2) at various times after dosing. The concentrations of drug in plasma were determined by a specific HPLC assay and the bioavailability determined from comparison of the plasma area under the curve (AUC) data obtained following oral and intravenous administration.

GR85548 (1-300μg/kg iv) caused a dose-dependent, long lasting decrease in carotid arterial blood flow associated with an increase in carotid vascular resistance (CVR) in anaesthetised dogs; the maximum effect, obtained at 300μg/kg iv GR85548, was a 92±15% increase in CVR (n=4), which was associated with a decrease in carotid blood flow of 44±6%. The intravenous dose of GR85548 producing 50% of its maximum carotid vasoconstrictor response (CD_{50}) was 19±3μg/kg iv. This compares to a CD_{50} for sumatriptan of 39±8μg/kg iv (Feniuk et al, 1989). GR85548 did not cause increases in arterial blood pressure: indeed at the lower doses (1-10μg/kg iv) small decreases in diastolic blood pressure were obtained (-12±2mmHg at 3μg/kg iv). This suggests that GR85548 does not have a generalised vasoconstrictor effect. GR85548 also produced a small, dose-dependent bradycardia (maximum change -26±4bpm at 100μg/kg iv). Oral bioavailability data for GR85548 in rats and dogs are summarised in Table 1. They show GR85548 to have high oral bioavailability in both species.

Table 1 Bioavailability of GR85548 in dog or rat

	Species	Dose (mg/kg po)	Bioavailability (% AUC po/iv)	Clearance ml/min/kg Plasma	Renal	$t_{1/2}$ (h)
GR85548	Rat	10	71	42	20	1.7
	Dog	0.5	95	24	3	1.7

Thus, in anaesthetised dogs GR85548 has a similar profile of action *in vivo* to sumatriptan, but is about 2 fold more potent. In addition, in rat and dog, GR85548 has high oral bioavailability.

Connor HE et al (1992) This meeting.
Feniuk W, Humphrey PPA and Perren MJ (1989) Br J Pharmacol 96: 83-90.

261P EFFECTS OF BUSPIRONE ON FOOD INTAKE IN FOOD DEPRIVED RATS

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It has previously been shown that drugs, such as 8-OH-DPAT and buspirone, that display agonist properties at central $5HT_{1A}$ receptors, increase food intake in non-deprived rats (Fletcher and Davis, 1990; Ebenezer, 1992a). In contrast however, it has been demonstrated that 8-OH-DPAT decreases both operant and non-operant feeding in food deprived rats (Ebenezer, 1992b). The present study was undertaken to investigate the effects of the clinically available anxiolytic drug buspirone on food intake in food-deprived rats.

Experiment 1. 22h food deprived male Wistar rats (n=8, b.wt. 220 - 300g) were injected s.c. with saline or buspirone (0.5 - 2 mg kg^{-1}) and 15 min later placed individually in experimental cages where they had free access to food and water. The amount of food consumed was measured 30 and 60 min after food presentation. A repeated measures design was used with each animal receiving all doses of drug. 4 - 5 day separated successive trials.

Experiment 2. Female Wistar rats (n=6, b.wt. 200 - 240g) were deprived of food for 22h a day and trained to press a lever in an operant chamber for food pellets on a fixed ratio schedule of 4 (FR-4), as described previously (Ebenezer, 1992a). During the sessions that followed, the rats were injected s.c. with either saline or buspirone (0.25 - 1 mg kg^{-1}) and placed seperately in the operant chamber for 30 min. Operant food intake was measured in 10 min bins during this period.The results obtained from Experiments 1 and 2 are shown in Tables 1 and 2 respectively.

Table 1. Effects of buspirone(B) on non-operant food intake measured 0 - 30 and 0 - 60 min after food presentation.

	Mean Food Intake(g) ± s.e.m	
	0 - 30 min	0 - 60 min
Saline	5.3 ± 0.4	9.3 ± 0.7
B (0.5 mg kg^{-1})	5.0 ± 0.4	10.0 ± 0.5
B (1.0 mg kg^{-1})	2.2 ± 0.3**	7.9 ± 0.3
B (2.0 mg kg^{-1})	0.6 ± 0.1**	1.6 ± 0.4**

Table 2. Effects of buspirone on operant food intake in food deprived rats measured 0 - 10, 0 - 20 and 0 - 30 min after administration.

	Mean Food Intake (g) ± s.e.m.		
	0 - 10 min	0 - 20 min	0 - 30 min
Saline	3.2 ± 0.5	5.9 ± 0.3	7.6 ± 0.6
B (0.25 mg kg^{-1})	2.8 ± 0.5	3.7 ± 0.2**	5.8 ± 0.5*
B (0.5 mg kg^{-1})	2.2 ± 0.5	3.0 ± 0.4**	4.1 ± 0.7**
B (1.0 mg kg^{-1})	0.93 ± 0.3**	1.3 ± 0.4**	1.5 ± 0.5**

*P<0.05 **P<0.01

The results of this study extend previous findings (Ebenezer, 1992b) and show that buspirone has acute dose-dependent depressant effects on food intake in food-deprived rats in both operant and non- operant paradigms. It is of interest to note that similar doses of the drug have previously been shown to increase food intake in non-deprived rats (Fletcher and Davis, 1990). Further work is necessary to identify the mechanisms involved.

Ebenezer, I.S. (1992a) NeuroReport, 3, 62 - 64.
Ebenezer, I.S. (1992b) NeuroReport, 3, 1019 - 1022.
Fletcher, P.J. and Davis, M. (1990) Br. J. Pharmacol., 99, 519 - 525.

262P TRH ANALOGUE MK771 INDUCES FOREPAW LICKING: EVIDENCE FOR 5-HYDROXYTRYPTAMINERGIC MODULATION

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Administration of the thyrotrophin-releasing-hormone (TRH) analogues CG3509, CG3703 and RX77368 to rats has been shown to induce fore-paw-licking behaviour (FPL) (Fone *et al.*, 1987,1989 Johnson *et al.* 1989). Administration of the 5-HT2 / 5-HT1C antagonist ritanserin has been shown to attenuate FPL behaviour in rats (Fone *et al.* 1989). We have therefore investigated the effects of a systemically active TRH analogue MK771 (Veber et al., 1976) on FPL and, since 5-HT1A agonists attenuated blinking behaviour in mice (McCreary and Handley, 1992) we have begun to investigate the role of the serotonergic system in modulating this behaviour.

Male MF1 mice (20-30g, n=6 per treatment group) were treated with MK771 (5.0 mg/kg i.p.) or saline and the number of FPL bouts counted in 5 minute bins for 30 minutes following injection. Subsequently, the 5-HT1A agonists 8-OH-DPAT (0.1 and 1.0 mg/kg i.p., n=5-11 per treatment group) and buspirone (1.0, 5.0 and 10.0 mg/kg i.p., n=5-14 per treatment group) were administered 7.5 minutes prior to MK771 (2.5 mg/kg i.p.) and behaviour was recorded 7.5 minutes later for 5 minutes. Behaviour was recorded on video tape and analysed later with the observer unaware of the treatment each mouse had received.

MK771 induced a behavioural profile consisting of: blinking (unilateral and bilateral), FPL, fore-paw tremor, ear-scratch, abdominal contractions with vertical tail movements, tail elevation and tremor and infrequent back muscle contractions. MK771 induced FPL (*eg.* 5.0 mg/kg from 3.9±1.2 (basal±s.e.m.) to 30.7±11.3, over 30min. ($p<0.01$) *cf.* saline treated mice) which peaked during the second and third bins (5-9.9 and 10-14.9 minutes). Pretreatment of MK771 treated mice (2.5 mg/kg) with 8-OH DPAT dose dependently attenuated FPL (0.1 mg/kg, 11.1±2.6 (control±s.e.m.) to 5.4±1.9 (drug±s.e.m.)($p<0.10$); 1.0 mg/kg, 12.5±3.5 to 0.7±0.5 ($p<0.01$); over 5min.) as did pretreatment with buspirone (1.0 mg/kg, 14.2±3.6 to 3.8±1.6($p<0.01$); 5.0 mg/kg, 11.1±3.0 to 0.4±0.2 ($p<0.01$); 10.0 mg/kg, 6.4±2.3 to 0.6±0.3 ($p<0.05$); over 5min.), (analysed using two and one-tailed Mann-Whitney U-test).

Thus systemic administration of the TRH analogue MK771 induced FPL in mice consistent with the action of other TRH analogues in rats, and this effect may be modulated by 5-HT1A receptors.

Acknowledgements. We thank the Tourette's Syndrome Association (U.S.A.) for financial support.

Fone K.C.F. *et al.* (1987). Neuropharmacol. **26**:8, 1147-1155.
Fone K.C.F. *et al.* (1989). Brit. J. Pharmacol. **96**: 599-608.
Johnson J.V. *et al.* (1989). Neurosci. **29**:2, 463-470.
McCreary A.C. and Handley S.L. (1992). Brit. J. Pharmacol. (**107S**: 450P).
Veber D.F. *et al.* (1976). Proc. 14th Eur. Peptide Symp. (Ed. A. Loffet), Univ. Brussels Press, Belgium.

263P THE INFLUENCE OF CONSTANT NICOTINE INFUSION ON BEHAVIOURAL AND ADRENOCORTICAL RESPONSES TO NICOTINE

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Constant nicotine infusions abolish the stimulation of nucleus accumbens dopamine secretion seen in rats repeatedly exposed to bolus injections of the alkaloid (Benwell & Balfour 1992). The present study examined the possibility that nicotine-induced behavioural and plasma corticosterone (PC) responses are also desensitised by chronic infusions of the drug.

Male Sprague-Dawley rats received saline or nicotine (4mg/kg/day) from subcutaneous Alzet minipumps (2ML2) for 14 days. On days 9-14, half of the saline and nicotine infused rats were injected subcutaneously once daily with saline (1ml/kg sc) or nicotine (0.4mg/kg sc). On day 14, the animals were placed on an elevated plus maze 3 minutes after the last injection. Open and enclosed runway entries were recorded for 30 minutes using infra-red photocells located along the arms of the maze. At the end of the trial, the rats were killed and blood removed for subsequent estimation of PC. Statistical analyses were performed using an ANOVA followed by Duncan's test where appropriate. The data are summarised in table 1. In agreement with previous studies (Balfour et al, 1986), the bolus administration of nicotine, to animals pretreated with nicotine boli, increased ($p < 0.01$) total entries (entries into all 4 arms) but had no effect on the ratio of open/total runway entries (O/T ratio). Nicotine infusions abolished the stimulation of activity evoked by the nicotine injections but did not influence the O/T ratio. The infusions also suppressed ($p < 0.01$) the PC levels in both the saline and nicotine treated groups. The data suggest that infusions of nicotine, at a dose which inhibits the locomotor response to nicotine injections, influences the PC levels in rats tested in an elevated plus maze in a manner similar to a number of anxiolytic drugs (Pellow et al, 1985) although it does not increase the O/T ratio.

TABLE 1 - Influence of constant nicotine infusions on behavioural and corticosterone responses to nicotine.

Infusion	Injection	PC (µg/100ml)	Total Entries	O/T Ratio
sal	sal	56.5 ± 9.3	20 ± 3	0.256 ± 0.046
sal	nic	46.3 ± 2.4	44 ± 2	0.256 ± 0.051
nic	sal	39.8 ± 5.7	24 ± 6	0.217 ± 0.059
nic	nic	40.8 ± 2.2	25 ± 5	0.243 ± 0.039

Results are means ± SEM of 6 observations in each group.

The study was supported by a grant from Forschungsrat Rauchen und Gesundheit.

Balfour, D.J.K., Graham, C.A & Vale, A.L. (1986). Psychopharmacology, 90: 528-532.
Benwell, M.E.M. & Balfour, D.J.K. (1992). J. Psychopharmacol., In Press.
Pellow, S., Chopin, P., File, S.E. & Briley, M. (1985). J. Neurosci. Meth., 14, 149167.

264P INFLUENCE OF NMDA ANTAGONISTS ON BEHAVIOURAL AND NEUROCHEMICAL ADAPTATION TO REPEATED NICOTINE ADMINISTRATION

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The non-competitive N-methyl-D-aspartate (NMDA) antagonist dizocilpine (MK 801) can attenuate the sensitization of locomotor activity increases seen after chronic nicotine treatment in rats (Shoaib & Stolerman, 1992). The present study has examined (a) the effects of MK801 and the competitive NMDA antagonist, 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid (D-CPPene), on the development of tolerance to the locomotor depressant effects of nicotine and (b) the influence of MK801 on the nicotine-induced sensitized dopamine release in the nucleus accumbens reported by Benwell & Balfour (1992).

In saline control tests, locomotor activity of rats in photocell cages did not differ significantly between groups (range 68 ± 4 – 87 ± 4 cage crosses in 30 min, means ± s.e.m.). In experiment 1, acute administration of (-)-nicotine (0.4 mg/kg sc) reduced activity to 49% of control ($P<0.001$ by analysis of variance); activity was 12% above control after daily injection of nicotine for 7 days (tolerance); after pretreatment with MK801 (0.1 or 0.3 mg/kg ip) given 30 min before all chronic injections of nicotine, nicotine (0.4 mg/kg) reduced activity to 77-82% of control; thus, tolerance was attenuated ($P<0.05$). In experiment 2, nicotine (0.4 mg/kg), given acutely, reduced activity to 68% of control ($P<0.001$) and activity was 25% above control after chronic nicotine. After pretreatment with D-CPPene (2 and 8 mg/kg ip), given 30 min before all chronic injections of nicotine, activity after nicotine was 88-89% of control; again, tolerance was attenuated ($P<0.01$). Microdialysis studies showed that daily injections of nicotine (0.4 mg/kg sc) for 5 days caused sensitization of the dopamine response to a subsequent nicotine injection resulting in an increase to 295 ± 89% of baseline release (0.108 ± 0.045 pmoles/20 µl dialysate). Pretreatment with MK801 (0.3 mg/kg ip 30 min before all daily injections of nicotine) attenuated ($P<0.01$) this response to nicotine to 145 ± 17% (0.068 ± 0.024 pmoles/µl dialysate), a concentration not significantly above baseline. Locomotor activity studies in the same rats showed that the enhanced locomotor activity (199 ± 20 photobeam crossings) was also attenuated ($P<0.05$) to 65% of the enhanced response by administering MK 801 (0.3 mg/kg) prior to daily nicotine treatment.

The results suggest there may be a common mechanism underlying both tolerance and sensitization after chronic administration of nicotine. This mechanism seems to involve NMDA receptors and may also be linked to changes in the mesolimbic dopamine system.

Acknowledgements: we thank MRC and Forschungsrat Rauchen und Gesundheit for financial support, and Merck Sharp & Dohme and Sandoz for donating MK801 and D-CPPene.

Benwell, M.E.M. & Balfour, D.J.K. (1992) Br. J. Pharmacol. **105,** 849-856.
Shoaib, M & Stolerman, I.P. (1992) Br. J. Pharmacol. **105,** 514-515.

265P NBQX REDUCES LESION VOLUME IN A RAT MODEL OF FOCAL CEREBRAL ISCHAEMIA

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Excitotoxic damage is important in the neural degeneration which follows cerebral ischaemia (Meldrum & Garthwaite, 1990). Study of the role of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor in post-ischaemic cell damage has been aided by development of selective non-NMDA excitatory amino acid receptor antagonists. Of these, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), has been shown to be neuroprotective in both a gerbil global ischaemia model (Sheardown et al, 1990) and a rat transient ischaemia model (Nellgard & Wieloch, 1992). As approximately 85% of strokes are caused by thrombotic arterial occlusions (Biller, 1992), the aim of the present study was to determine whether NBQX is efficacious in a rat photothrombotic cortical lesion model.

Male Lister hooded rats (250-280g) anaesthetised with halothane, were positioned in a stereotaxic frame. Rectal temperature was maintained at 37°C. The skull was exposed and a bifurcated fibre optic light guide (3.0 mm diameter) from a 300W xenon arc lamp was positioned on the skull at bregma in the anterior-posterior plane in a holder designed to centre the heads of the light guide 2.5 mm to left and right of the midline. Rose bengal (20 mg/kg) was injected into a lateral tail vein. The skull was illuminated for 5 minutes, after which the wound was sutured and anaesthesia discontinued. NBQX (30 mg/kg i.p.) was administered at 60, 70 and 85 minutes post illumination, following a dosing regime shown (Sheardown et al, 1990) to be effective in the gerbil global ischaemia model. Control animals received vehicle (saline). Seven days post-operation the rats were sacrificed and perfusion fixed. Serial coronal sections 60 μm thick were cut through the lesions and stained with cresyl fast violet. The total area of each lesion was determined by digital planimetry and volumes calculated using Simpson's rule.

The results show that administration of NBQX caused marked decreases in lesion volumes in both hemispheres (p=0.005; see Table 1). There was no statistically significant difference in lesion volumes between left and right hemispheres in either group (p=0.17); neither did NBQX have a significantly different effect between hemispheres (p=0.53). Statistical analysis was by analysis of variance.

Table 1: Lesion volumes in mm^3 (mean ± SEM) for left and right hemispheres in NBQX and vehicle-treated groups

	Left hemisphere	Right hemisphere	Mean of treatment	Comparison of drug vs vehicle
NBQX	4.4 ± 1.1 (n=10)	3.8 ± 1.0 (n=10)	4.1 ± 0.7 (n=10)	p=0.005
Vehicle	8.5 ± 0.9 (n=11)	6.7 ± 1.0 (n=11)	7.6 ± 0.7 (n=11)	
Comparison of left vs right (within rats)	p=0.17			

The present study has demonstrated that photothrombotic lesions of the parietal cortex induced by rose bengal can be significantly reduced in volume by the AMPA receptor antagonist NBQX. This would suggest that excitotoxic neurodegeneration of the cortex by AMPA receptor activation represents an important contributory factor in neuronal damage following ischaemia.

Biller, J. (1992) Neurologic Clinics 10:1; 63-85
Meldrum, B. & Garthwaite, J. (1990) TIPS 11, 379-387
Nellgard, B. & Wieloch, T. (1992) J. Cereb. Blood Flow Metab. 12, 2-11
Sheardown, M.J., Nielsen, E.Ø., Hansen, A.J., Jacobsen, P. & Honoré, T. (1990) Science 247, 571-574.

266P PHENYLGLYCINE DERIVATIVES AS COMPETITIVE ANTAGONISTS AT GLUTAMATE METABOTROPIC RECEPTORS (mGluRs)

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The possible physiological roles of G-protein-coupled metabotropic glutamate receptors (mGluRs) within the CNS are currently receiving much attention (Conn & Desai, 1991; Baskys, 1992). The diversity of actions of agonists at these receptors probably reflects receptor heterogeneity. (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (ACPD) is generally considered to be a selective agonist for mGluRs; however, selective antagonists have been lacking. We report here the competitive antagonist actions of (RS)-4-carboxyphenylglycine (4C-PG) and (RS)-α-methyl-4-carboxyphenylglycine (αM4C-PG) on ACPD-stimulated phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis.

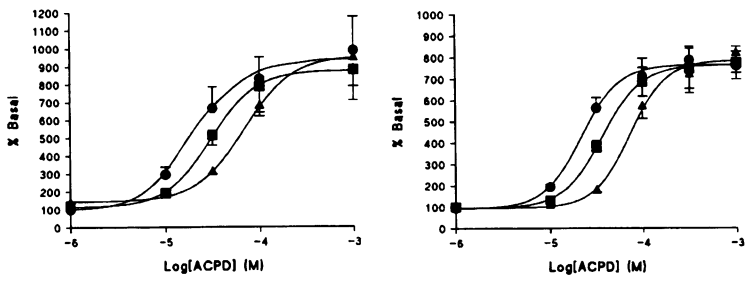


Figure 1: Stimulation of PI hydrolysis in rat pup cerebrocortical slices by (1S,3R)-ACPD in the absence (●) or presence of 1mM (■) and 3mM (▲) (RS)-4C-PG (left panel) and (RS)-αM4C-PG (right panel). Methods were as described previously (Porter *et al.*, 1992). Data points are means ± s.e. mean of 4 experiments.(RS)-4C-PG and (RS)-αM4C-PG produced parallel shifts to the right of the ACPD curves, yielding K_B values of 1.7 and 1.2 mM respectively.

Both compounds have also been shown to antagonize depolarizations of neonatal rat spinal cord motoneurones produced by (1S,3R)-ACPD (Jones *et al.*, 1992). These results identify two novel competitive antagonists of mGluR receptors coupled to PI hydrolysis in rat brain . They should prove to be valuable tools in elucidating both structure-activity relationships and the physiological roles of these receptors.

Supported by Research into Ageing, the MRC and US Public Health Service NS 26540

Baskys, A. (1992) Trends Neurosci. **15**, 92-96.
Conn, P.J. & Desai, M.A. (1991) Drug Dev. Res. **24**, 207-229.
Porter, R.H.P., Roberts, P.J., Jane, D.E. & Watkins, J.C. (1991) Br. J. Pharmacol. **106**, 509-510.
Jones, P.L. St. J., Birse, E.F., Jane, D.E., Jones, A.W., Mewitt, K.N., Pook, P.C-K., Sunter, D.C., Udvarhelyi, P.M., Wharton, B. & Watkins, J.C. (1991) Br. J. Pharmacol. (this meeting)

267P THE ACTION OF ENDOGENOUS EXCITATORY AMINO ACIDS (EAAs) AT ACPD-DEPOLARIZING RECEPTORS IN NEONATAL RAT MOTONEURONES

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The metabotropic glutamate receptor (mGluR) agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (ACPD) produces a transient ('desensitizing') depolarization in neonatal rat motoneurones (Sunter *et al*, 1991). Such depolarizations are insensitive to the mGluR antagonist L-2-amino-3-phosphonopropionate (L-AP3), the N-methyl-D-aspartate (NMDA) antagonist, D-2-amino-5-phosphonopentanoate (D-AP5) and the kainate/α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), but are suppressed by (S)-3-hydroxyphenylglycine (3H-PG), (S)-4-carboxyphenylglycine (4C-PG) and (S)-4-carboxy-3-hydroxyphenylglycine (4C3H-PG) (Roberts *et al*,1992; Jones *et al*, 1993). We report here that in the presence of a mixture of antagonists to prevent responses mediated by NMDA and AMPA/kainate receptors motoneuronal depolarizations produced by a series of endogenous amino acids are differentially depressed in the same pattern by all the phenylglycine-derived ACPD antagonists.

Recordings of amino acid-induced motoneurone depolarizations were made from ventral roots of hemisected spinal cords from neonatal rats (1-5 days old) bathed in medium containing tetrodotoxin (10^{-7} M), Mg^{2+} (2 mM), D-AP5 (50 μM) and CNQX (100 μM). Depolarizations of approximately equal magnitude (0.5-1 mV) were induced by L-glutamate (2 mM), L-aspartate (3 mM), L-cysteine sulphinate (5 mM), L-homocysteine sulphinate (5 mM) and L-homocysteate (5 mM) as well as by quisqualate (20 μM) and 1S,3R-ACPD (50 μM). To prevent desensitization of ACPD receptors, each amino acid agonist was added for a period of only 1 min and an interval of at least twenty minutes was allowed between agonist responses.

When added to the superfusion medium (S)-3H-PG (500 μM), (S)-4C3H-PG (300 μM) and (RS)-4C-PG (1 mM) all produced a similar pattern of depression of endogenous amino acids in that responses to L-glutamate were always the least affected, and those to L-homocysteate the most. The effects of (S)-3H-PG (% control $\pm$ s.e.mean, n) were: L-glutamate, 91.2$\pm$5.6(7), L-cysteate, 80.1$\pm$5.9(4), L-aspartate, 65.4$\pm$4.8(3), L-cysteine sulphinate, 58.9$\pm$2.1(3), L-homocysteine sulphinate, 58.9$\pm$4.5(3), L-homocysteate, 48.6$\pm$7.3(5), quisqualate 43.2$\pm$6.9(6), (1S,3R)-ACPD, 0(5).

These results indicate that under the conditions of these experiments, which precluded responses mediated by NMDA and AMPA/kainate receptors, depolarization-mediating receptors of the ACPD type contributed a considerably larger proportion of the residual response in the case of L-homocysteate than in the case of L-glutamate. With all the endogenous amino acids, however, and particularly with L-glutamate, a large proportion of the response mediated by non-NMDA, non-AMPA/kainate receptors could not be attributed to ACPD-like depolarization-mediating receptors.

Sunter,D.C.,Edgar,G.E.,Pook,P.C-K.,Howard,J.A.K.,Udvarhelyi,P.M. & Watkins,J.C. (1991) *Br.J.Pharmac.* **104** 377P.
Roberts,P.J.,Birse,E.F.,Eaton,S.A.,Jones,P.L.St.J.,Pook,P.C-K.,Porter,R.H.P.,Udvarhelyi,P.M.,Wharton,B.,Salt,T.E. & Watkins,J.C. (1992) *Soc.for Neurosci.Abstr.* **18** 648.
Jones,P.L.St.J.,Birse,E.F.,Jane,D.E.,Jones,A.W.,Mewett,K.N.,Pook,P.C-K.,Sunter,D.C.,Udvarhelyi,P.M.,Wharton,B. & Watkins,J.C. (1993) this meeting.

268P INHIBITION OF A_{2b} ADENOSINE RECEPTOR-ELICITED AND FORSKOLIN-STIMULATED CYCLIC AMP ACCUMULATION BY L-2-AMINO-4-PHOSPHONOBUTYRATE (L-AP4)

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L-Amino-4-phosphonobutyrate (L-AP4) potently inhibits presynaptic transmission in guinea-pig hippocampus (Lanthorn *et al.*, 1984), yet the molecular basis of this inhibitory action remains uncharacterised. Receptors mediating presynaptic inhibition of transmitter release are often negatively linked to adenylyl cyclase and we now report that L-AP4 elicits an inhibitory effect on A_{2b} adenosine receptor-mediated and forskolin-stimulated cAMP accumulation in guinea-pig cerebral cortical slices.

[^{3}H]-cAMP formation was measured in cross-chopped cerebral cortical slices from Dunkin Hartley guinea-pigs (either sex) by the pre-labelling method previously described by Donaldson *et al* (1990). Forskolin-stimulated accumulation of cAMP was inhibited by L-AP4 in a concentration-dependent manner (IC_{50} value 124 $\pm$ 80μM). The forskolin response was completely inhibited by L-AP4 at a concentration of 1mM (n=3). D-AP4 and DL-AP4 were less effective, eliciting inhibitions of approximately 25% (n=2) and 35% (n=2) respectively at concentrations of 100μM. D-AP4 failed to reverse the inhibitory action of L-AP4. The inhibition of forskolin-stimulated cAMP accumulation by L-AP4 was not reversed by the excitatory amino acid (EAA) antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX, 100μM), L-(+)-2-amino-5-phosphonopentanoate (L-AP5, 100μM) and DL-2-amino-3-phosphonopropionate (DL-AP3, 1mM). These data suggest that this inhibitory action of L-AP4 is mediated by a receptor with similar properties to that mediating the inhibition of forskolin-stimulated cAMP formation by the isomers of the EAA receptor agonist 1-aminocyclopentane -1,3-dicarboxylate (ACPD) (Cartmell *et al.*, 1992).

In contrast to their inhibitory effects on the forskolin-stimulation of cAMP, 1S,3R- and 1S,3S-ACPD isomers potentiate the A_{2b} adenosine receptor-elicited cAMP response in guinea-pig cerebral cortical slices (Cartmell *et al.*, 1992). L-AP4 inhibited the cAMP response to the adenosine receptor agonist 5' N-ethylcarboxamidoadenosine (NECA,10μM) in a concentration-dependent manner (IC_{50} value 120 $\pm$ 20μM) eliciting a maximal inhibition of 86 $\pm$ 4% (n=3) at a concentration of 1mM. D-AP4 and DL-AP4 were not as effective as L-AP4 in eliciting this inhibitory effect. The inhibitory effects of L-AP4 on NECA-stimulated cAMP accumulation were resistant to the EAA receptor antagonists DNQX (100μM), L-AP5 (100μM) and DL-AP3 (1mM). It remains to be determined whether this response to L-AP4 is mediated by one of the family of metabotropic receptors or by a distinct receptor subtype.

Jayne Cartmell is a MRC/CASE award recipient.

Cartmell,J., Curtis,AR., Kemp,JA., Kendall,DA. and Alexander,SPH.(1992). *Br J Pharmacol* **107**; 57P.
Donaldson,J., Kendall,DA. and Hill,SJ. (1990). *J Neurochem* **54**; 1484-91.
Lanthorn,TH., Gangong,AH. and Cotman,CW. (1984). *Brain Res* **290**; 174-178.

269P FURTHER EVIDENCE THAT THE EFFECTS OF KAINIC ACID, DOMOIC ACID AND AMPA ARE MEDIATED VIA TWO DISTINCT RECEPTORS

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Recently, Zeman and Lodge (1992) have shown that in baby rat spinal cord (BRSC), 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F)quinoxaline (NBQX) antagonizes only part of the depolarization due to kainic acid, but all of the α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) response. This, suggests that kainic acid could activate a receptor subtype that is insensitive to AMPA. We have now extended this investigation to rat cerebellar slices (RCS) from adult animals using kainic acid, domoic acid and AMPA as agonists and NBQX as antagonist.
Depolarizations of hemisected spinal cord of 6-11 old day rats and sagittal cerebellar slice (wedge like form) from adult rats (150-250g) were recorded in a Mg^{++} free Krebs'solution at room temperature (22°C) using the grease-gap technique as described by Zeman and Lodge (1992) and Garthwaite et al. (1987). The antagonist, NBQX, was added to the tissues 15 min before the concentration response curve (CRC) to the agonist was undertaken and it remained for the duration of the experiment. Depolarizations were measured by $Ag^{+}/AgCl$ electrodes. Kainic acid, domoic acid and AMPA, depolarized both BRSC and RCS with the same rank order of potencies, i.e. domoic acid $\geq$ AMPA > kainic acid. In BRSC kainic acid and AMPA behaved as full agonists, whereas in RCS AMPA reached only 34% of the response obtained with kainic acid. Furthermore, domoic acid reached 66% of the response obtained with kainic acid in both preparations.
In both BRSC and RCS, NBQX (0.1-1μM) only antagonized the upper part of the CRC to kainic acid and domoic acid. This antagonism appeared to be competitive against kainic acid in both BRSC and RCS with Schild plot slopes (±s.e) of 0.98±0.23 and 0.89±0.16 respectively. The estimated pK_B (± s.e.) were 7.30±0.20 (d.f.10) in BRSC and 7.14±0.20 (d.f.8) in RCS. Against domoic acid in RCS the antagonism of the upper part of the curve appeared competitive with Schild plot slope (±s.e.) of 1.28±0.36 and with an estimated pK_B (±s.e.) of 7.05±0.23 (d.f.7). In BRSC the antagonism of NBQX was complicated by a bell-shaped CRC to domoic acid. However, the data could be simulated using a pK_B of 7.3 for NBQX as antagonist of the upper ascending part of the curve. When AMPA was used in both preparations, NBQX (0.3-3μM) antagonized all of the CRC in a seemingly competitive manner (parallel right shift of the CRC). In RCS at the highest concentration of NBQX (3μM) there was a reduction in the maximal response. The pK_B(s.e.) estimated in BRSC for NBQX was 6.90±0.10 (d.f 10) and the pA_2 obtained in RCS was 6.90±0.30 (d.f 15) .
This study extends the hypothesis to RCS that kainic acid, domoic acid and AMPA cause depolarization through activation of two distinct receptors. In both BRSC and RCS there is present one receptor site activated by all three agonists and blocked by NBQX with a pK_B between 6.9 and 7.3 and one receptor site activated by kainic acid and domoic acid but insensitive to both AMPA and NBQX.

Garthwaite, G., et al. (1987). Dev. Brain Res. 36, 288-292.
Zeman, S. & Lodge, D. (1992). Br.J.Pharmacol. 106, 367-372.

270P MEASUREMENT OF EXCITATORY AMINO ACIDS AND NITRIC OXIDE RELEASE FROM ADULT RAT CEREBELLAR SLICES

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We have recently reported that N-methyl-D-aspartate (NMDA) potentiates the release of endogenous aspartate from neonatal rat cerebellar slices, this effect being mediated via production and subsequent intercellular diffusion of nitric oxide (NO) (Dickie et al., 1992). In this study we applied equimolar concentrations of NMDA, kainate, quisqualate and (S)-α-amino-3-methyl-4-isoxazolepropionic acid (S-AMPA) to slices obtained from adult rat cerebellum, and monitored release of endogenous NO and the excitatory amino acids aspartate and glutamate.

Parasaggital slices (250μm) were prepared from the cerebellar hemispheres of female Wistar rats (200-250g) and superfused (1ml/min) with magnesium-free artificial cerebrospinal fluid gassed with 95% O_2 /5% CO_2 at 37°C. Four-minute aliquots of perfusate were collected for analysis of glutamate and aspartate by high performance liquid chromatography; NO was measured by a chemiluminescent assay (Dickie et al., 1990). Drugs were applied as three, four-minute "pulses". NMDA, kainate, quisqualate and S-AMPA (all 100μM) failed to significantly induce release of NO or aspartate above baseline values. S-AMPA, however, significantly elevated levels of glutamate in the perfusate, from 15.8 ± 1.4 pmol/mg tissue to peak values of 34.2 ± 3.4 pmol/mg ($p<0.05$), whereas NMDA, kainate and quisqualate had no significant effect. Prior application of 100μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to the perfusion medium reduced the S-AMPA-evoked release of glutamate by 70%.

The results show that the NMDA-stimulated release of endogenous NO and aspartate observed, under identical conditions, in neonatal slices is not detectable in adults; this is likely to be linked to the marked reduction in NMDA responsiveness observed during postnatal cerebellar maturation (Garthwaite et al., 1987). The ability of S-AMPA to increase glutamate levels and the antagonistic action of CNQX may be due to the postsynaptic excitation of granule cells at the mossy fibre-granule cell synapse (Garthwaite & Brodbelt, 1989) and the corresponding release of glutamate from parallel fibre terminals.

Dickie, B.G.M., Lewis, M.J. & Davies, J.A. (1990). Br. J. Pharmacol. 101, 8-9.
Dickie, B.G.M., Lewis, M.J. & Davies, J.A. (1992). Neurosci. Lett. 138, 145-148.
Garthwaite, G., Yamini, B. & Garthwaite, J. (1987). Dev. Brain Res. 36, 288-292.
Garthwaite, J. & Brodbelt, A.R. (1989). Neuroscience. 29, 401-412.

271P MODULATION OF THE INTRACELLULAR FREE CALCIUM RESPONSE TO NMDA BY GLYCINE IN CULTURED RAT CEREBELLAR GRANULE CELLS

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N-Methyl-D-aspartate (NMDA) receptor activation is a major mechanism for elevating neuronal cytoplasmic free calcium concentration ($[Ca^{2+}]$) (Rothman & Olney, 1987). NMDA receptor activation can be regulated by a complex array of modulators which exert their effects through distinct recognition loci within the receptor/channel structure. In the present study we have investigated the modulatory action of glycine upon NMDA-mediated changes in $[Ca^{2+}]$ in small populations of cerebellar granule cells.

Cerebellar granule cells were prepared from neo-natal rats as described previously (Whitham et al., 1991) and cultured on glass cover-slips for up to 10 days in vitro (DIV). Cells were incubated with 2 μM Fura-2 AM for 20 min, gently washed for 30 min and epifluorescence microscopy used to observe groups of 6-8 cells for on-line recording of $[Ca^{2+}]$. Cells were constantly perfused (1.0 ml/min) with Mg^{2+}-free Krebs-Henseleit buffer (KHB) and additions of agents were made via a multi-line puffer pipette.

The $[Ca^{2+}]$ response to NMDA was concentration-dependent and varied considerably with DIV. In the vast majority of experiments NMDA evoked an initial transient increase in $[Ca^{2+}]$ followed by a sustained plateau, however the precise temporal relationship between peak and plateau varied considerably. Comparison of cells cultured for different periods demonstrated that with increasing DIV the EC_{50} for the plateau response increased by about 10 fold between 3 and 8 DIV whilst the maximal peak and plateau responses also increased in magnitude. In all cases, no $[Ca^{2+}]$ response to NMDA was observed in nominally Ca^{2+}-free KHB, and the NMDA response could be completely antagonized by 100 nM MK-801. Co-addition of glycine (10 μM) potentiated the NMDA response, without apparently affecting the EC_{50} at all DIV studied, suggesting that the NMDA-receptor glycine site is not saturated in cerebellar granule cell cultures. Co-addition of 5,7-dichloro-kynurenate (dcKYN), a selective glycine-site antagonist, concentration-dependently inhibited the NMDA-mediated $[Ca^{2+}]$ response. In the presence of 100 nM dcKYN, the increase in $[Ca^{2+}]$ response to 200 μM NMDA was suppressed by 97.6 $\pm$ 3.4%. This inhibition could be reversed by increasing concentrations of glycine. The AMPA-receptor antagonist, 6-cyano-2,3-di-hydroxy-7-nitroquinoxaline (CNQX), was also active at the glycine site (IC_{50} 4 μM for reversal of $[Ca^{2+}]$-response to 200 μM NMDA).

These results indicate that the generation of maximal $[Ca^{2+}]$ responses to NMDA in granule cells requires supplementation with glycine. Furthermore, the fact that selective blockade of the NMDA-receptor glycine binding site by dcKYN leads to complete suppression of the NMDA $[Ca^{2+}]$-response suggests that glycine acts as a co-agonist at the NMDA-receptor/channel complex.

We thank the Wellcome Trust for the award of a Toxicology studentship to P.B. Simpson.

Rothman, S.M. & Olney, J.W. (1987) Trends Neurosci. **10**, 299-302
Whitham, E.M., Challiss, R.A.J. & Nahorski, S.R. (1991) Eur. J. Pharmacol. **206**, 181-189

272P NOVEL AMINOALCOHOLS RELATED TO IFENPRODIL POSSESS SIGMA AND NEUROPROTECTIVE ACTIVITY

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In the course of a study of polyamine-sensitive sites linked to the NMDA receptor, we recently reported a series of ifenprodil-like aminoalcohols: RCH(OH)CH(CH_3)NHR' where R = thiochromane or benzothiazine, while R' was piperidinyl-$CH_2C_6H_5$ or $(CH_2)_4C_6H_5$ with minor changes in the alkyl chain (Beart *et al.*, 1992). In view of their structural relationship to the polyamine NMDA antagonist and anti-ischaemic agent ifenprodil, which is also known to possess sigma activity (Benavides *et al.*, 1992), we have evaluated these aminoalcohols for sigma and neuroprotective activities.

Binding at the sigma site was determined on rat brain membranes using the radioligand R(+)-[^{3}H]3-[3-hydroxyphenyl]-N-(1-propyl)piperidine ([^{3}H]3-PPP) (Beart *et al.*, 1989). The aminoalcohols were also evaluated against the binding of [^{3}H]-(N-1-[thienyl]cyclohexyl)-piperidine ([^{3}H]TCP) to the non-sigma related channel site. Binding data were analysed by iterative curve-fitting (Beart *et al.*, 1989). All aminoalcohols fully inhibited the specific binding of [^{3}H]3-PPP with inhibition constants being in the sub-micromolar range, but slope factors were considerably < 1. Inhibition data were significantly better described by a two site model of binding with 60-80% of total sites in the high affinity state. The two most potent members of the series (R' = piperidinyl-$CH_2C_6H_5$) were both analogues of ifenprodil and all three substances possessed very similar affinity for the sigma site; high- and low-affinity inhibition constants were 5-20 nM and > 5 µM, respectively. All of the heterocyclic aminoalcohols exhibited very low affinity for the phencyclidine-site labelled by [^{3}H]TCP (< 50% inhibition at 10 µM). Neuroprotective activity of the heterocyclic aminoalcohols was assessed in a mouse model which examines the methamphetamine-induced damage of nigrostriatal dopaminergic neurones. White Swiss mice (20-25 g) received intraperitoneal injections of methamphetamine (4 x 10 mg/kg) and aminoalcohols or ifenprodil (15 min before and 4 h after methamphetamine), and striatal tyrosine hydroxylase activity was measured at 7 days as an index of the integrity of nigrostriatal dopaminergic neurones (Sonsalla *et al.*, 1991). All aminoalcohols studied significantly (analysis of variance, $P < 0.001$) attenuated methamphetamine-induced decrements in striatal tyrosine hydroxylase activity. Protection afforded by the aminoalcohols relative to methamphetamine-treated mice was as follows: tibalosine (erythro conformation; 100 mg/kg, 17%), threo-tibalosine (50 mg/kg, 42%), ifenprodil (50 mg/kg, 45%) and the benzothiazine analogue of ifenprodil (50 mg/kg, 100%).

Our study describes the sigma activity of a novel series of heterocyclic aminoalcohols, which are neuroprotective in a model of excitotoxicity. All of these molecules, like ifenprodil, inhibit the polyamine-sensitive binding of [^{125}I]ifenprodil and the polyamine-stimulated binding of [^{3}H]TCP (Beart *et al.*, 1992). The present data, however, do not indicate whether neuroprotective activity is due to actions at sigma and/or polyamine-related NMDA sites, but do reveal the therapeutic potential of the heterocyclic aminoalcohols in ischaemic/hypoxic disorders.

Beart, P.M., O'Shea, R.D. & Manallack, D.T. (1989) *J. Neurochem.* 53, 779-788
Beart, P.M., Mercer, L.D., Ryan, M.C., Loiacono, R.E. & Jarrott, B. (1992) *Mol. Neuropharmacol.* 2, 113-118
Benavides, J., Peny, B., Allen, J. & Scatton, B. (1992) *J. Pharmacol. Exp. Ther.* 260, 896-901
Sonsalla, P.K., Riordan, D.E. & Heikkila, R.E. (1991) *J. Pharmacol. Exp. Ther.* 256, 506-512

273P BLOCKADE OF NMDA RESPONSES BY THE PROTEIN KINASE C ANTAGONIST CALPHOSTIN C

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We have studied the effects of calphostin C, an antagonist of the regulatory subunit of protein kinase C, on the mechanisms of long-term potentiation (LTP) and the function of NMDA (N-methyl-D-aspartate) receptors in rat hippocampal slices.

Hippocampal slices were prepared from 5-8 week old Sprague Dawley rats and maintained in a perfusion chamber. A stimulation electrode was placed on the Schaffer collaterals and an extracellular recording electrode in the dendritic layer of the CA1 area. Excitatory post synaptic potentials (epsp's) were recorded at a stimulus frequency of 0.02 Hz. LTP was induced by theta burst stimulation (10 trains, each composed of 4 pulses at 100 Hz, at 5 Hz). Potentiation was quantified as the change in the epsp amplitude determined before and 60 min after the induction of LTP. All data are presented as mean ± s.e.mean.

Application of calphostin C ($1x10^{-6}$ M) did not affect pre-established LTP, but pretreatment with calphostin C ($1x10^{-6}$ M) reduced LTP from a control value of 43 ± 6.6 (n = 6) to 12.2 ± 6.1 % (n = 6). Calphostin C ($1x10^{-6}$ M) did not affect the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptor component, but reduced the NMDA receptor-mediated component of epsp's evoked by normal stimulation at 0.02 Hz by 74 ± 5 % (n = 6). In addition, calphostin C ($1x10^{-6}$ M) significantly reduced the NMDA receptor mediated component of responses evoked by theta burst stimulation from 19.1 ± 1.3 to 1.3 ± 1.1 % (n = 4). Similarly, calphostin C ($1x10^{-6}$ M) completely abolished the effect of ionophoretic application of NMDA (100 mM, 20 sec).

The effects of calphostin C upon synaptic transmission and LTP are most likely the result from a direct interaction with the NMDA receptor complex.

274P CONDITIONED AVOIDANCE RESPONDING AND ENDOGENOUS DOPAMINE RELEASE FROM RAT BRAIN STRIATAL SLICES: A COMPARISON OF ELECTRICAL AND NMDA STIMULATION

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It has been suggested that learning and memory in relation to conditioned behaviour are associated with striatal/limbic interfacing via the nucleus accumbens and ventral tegmental areas (Mogenson, 1987). Previously we have shown that operantly learned behaviour, namely conditioned avoidance responding (CAR) correlated with a significant reduction in *electrically* stimulated release of endogenous dopamine (DA) from corticolimbic and corticostraital slices. However, the *electrically* stimulated release of [^{3}H] DA from prelabelled tissue showed no significant difference between naive and conditioned rat (Patel & Turnbull, 1989) thus apparently confirming the findings of Herdon *et al.* (1985) who showed that the prelabelling process appears to alter the intracellular source from which subsequent release occurs and endogenous release can be affected quite differently.

As glutamate is known to stimulate DA release, studies with *NMDA* were initiated; the stimulated release of [^{3}H] DA in striatal slices was significantly reduced in conditioned rat brain slices in comparison with release from naive rat brain slices,(Smith *et al.* 1991). We have now made direct comparisons of electrical and *NMDA* stimulated release of endogenous DA in conditioned and naive rat brain.

Male rats, aged 35 days, which had been reared under reversed light conditions to facilitate learning, were trained to avoid an aversive stimulus by responding to an auditory cue. Rat corticostriatal slices were taken immediately postmortem, the slice sections were placed in a perspex insert within the chamber and superfused at 0.4ml/min with magnesium-free modified Krebs' bicabonate solution (pH 7.4, 37°C), gassed with 95% O_2/5% CO_2. The net efflux of endogenous DA was measured by HPLC with electrochemical detection. Electrical field stimulation was performed using biphasic rectangular pulses (10Hz, 2ms, 20mA). Two 2 min stimulations (S1 & S2) were applied 50 mins apart; basal release was measured before each stimulation. S2 stimulations were achieved by current alone; NMDA 5mM; NMDA 5mM + current, and in the presence and absence of its antagonist 2 amino-5-phosphono valerate (APV) 10μM. The results (mean+/- sem) are summarised in Table 1 for n slice sections. * $p<0.05$, ** $p<0.01$ versus naive rats of same tissue preparation (Mann Whitney U test).

Table 1

Treatment		Net efflux endogenous DA pg/mg fresh tissue			
		Naive		Conditioned	
S1	S2	S1	S2	S1	S2
Current	Current	57.43 (4.86) n=30	92.8 (7.3) n=6	15.28 (3.2) n=6 **	38.96 (10.0) n=4 **
↓	NMDA		67.1 (10.5) n=4		30.37 (7.8) n=4 *
↓	NMDA + current		241.6 (20.7) n=4		54.1 (13.4) n=4 **
↓	NMDA, APV + current		225.6 (39.3) n=4		30.28 (8.5) n=4 *
↓	APV		6.25 (1.2) n=4		

These results suggests that whether the DA is radiolabelled or endogenous, NMDA releases less from the conditioned rat brain than from naive brains. This is a different picture compared with that seen with electrically stimulated release of radiolabelled DA where there is no difference. Thus the store from which the two treatments release DA appears to be different.

Herdon, H., Strupish, H., & Nahorski,S.R. (1985) *Brain Res.* **348**,309-920.
Mogenson, G. (1987). Progress in Psychobiology and Physiological Psychology. 117-170 Academic Press N.Y.
Patel, D.R, Turnbull, J.M.(1989) *J.Neurosci. Methods.* **29**, 3, 272.
Smith, M., Rakhit, D., Patel., D.R.,Turnbull, J.M.(1991) *J.Physiol.* **438**,43.

275P 8-OH-DPAT AND 5-METHOXY-N,N-DIMETHYLTRYPTAMINE HAVE OPPOSITE EFFECTS ON DOPAMINE D_2 RECEPTOR ANTAGONIST-INDUCED CATALEPSY

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Catalepsy induced by antipsychotics is reported to be modified by alterations in central 5-HT function, but the results obtained have been inconsistent (see Waldmeier & Delini-Stula, 1979; Elliott *et al*, 1990; Wadenberg & Ahlenius, 1991). We have therefore now examined the effects of 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) on the catalepsy produced by three antipsychotics: two selective D2-antagonists and one typical neuroleptic.

Male rats (130-190g; Charles River) were injected ip with raclopride (0.75-12mg/kg), BRL 34778 (exo-4-amino-5-chloro-2-methoxy-N-[9-(4 fluorophenylmethyl)-9-azabicyclo(3.3.1)non-3-yl]-benzamide; 0.125-4mg/kg) or haloperidol (0.125-4mg/kg) . Catalepsy was assessed at three timepoints, by placing each rat paw on a 45mm rubber bung, in turn. A score of 1 was given for every paw that remained on the bung for 15s. 8-OH-DPAT (1mg/kg), 5-MeODMT (5mg/kg) or vehicle were administered sc 20 min prior to the final reading (4h for BRL 34778 and haloperidol, 1.5h for raclopride) . Affinities of drugs for 5-HT2, D1 and D2 receptors were determined versus ^{3}H-ketanserin (0.5nM), ^{3}H-SCH 23390 (0.25nM) and ^{3}H-sulpiride (1nM).

Table 1. Antipsychotic drugs: D2 and 5-HT2 receptor affinities and the effects of 5-MeODMT and 8-OH-DPAT on catalepsy

	Catalepsy (ED50; mg/kg)			Ki (nM)		
	Vehicle	8-OH-DPAT	5-MeODMT	D1	D2	5-HT2
Haloperidol	0.44 (0.14-1.34)	1.25 (0.57-2.79)	0.32 (0.15-0.70)	236 ± 10.7	2.1 ± 0.23	51.2 ± 12.1
Raclopride	3.73 (1.81-7.68)	16.5 (4.10-65.8)	0.98 (0.32-3.04)*	>1000	38.4 ± 3.48	>1000
BRL 34778	0.77 (0.34-1.74)	8.1 (1.41-46.5)*	0.16 (0.07-0.41)*	>1000	4.0 ± 1.15	38.2 ± 7.42

Each ED50 ± 95% confidence limits was obtained from 5 groups of 5 rats ; statistical comparison of ED50 values used the method of Litchfield & Wilcoxon; * = $p \leq 0.05$ vs drug + vehicle. Ki values are mean ± s.e. of mean of 3 determinations using 6 concentrations.

8-OH-DPAT attenuated all cataleptic responses; the ED50 of BRL 34778 was significantly increased (>10-fold). Conversely, the actions of the D2-selective drugs, raclopride and BRL 34778, were clearly potentiated by 5-MeODMT, whereas those of the typical neuroleptic, haloperidol, were unaltered. These findings confirm and extend those of Wadenberg & Ahlenius (1991) on the effect of 8-OH-DPAT on raclopride-induced catalepsy. In addition, 5-MeODMT, like 5-HT uptake inhibitors (Waldmeier & Delini-Stula, 1979), potentiated the cataleptic response to D2-selective antipsychotics. These findings are, however, not consistent with the reduction of catalepsy seen following the administration of the more selective 5-HT2/1C agonist , DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane), reported by Elliott *et al* (1991).

Elliott, P.J., Walsh, D.M., Close, S.P., Higgins, G.A. & Hayes, A.G. (1990) *Neuropharmacology* **29,** 949-956.
Wadenberg, M.L & Ahlenius, S. (1991) *J. Neural Transm.* **83,** 43-53.
Waldmeier, P.C. & Delini-Stula, A.A. (1979) *Eur. J. Pharmacol.* **55,** 363-373.

276P EFFECT OF DIRECTLY AND INDIRECTLY ACTING DOPAMINE AGONISTS ON LOCOMOTOR ACTIVITY IN THE GUINEA-PIG

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It has recently been shown that guinea-pig locomotor activity (LMA) can be increased by administration of the dopamine (DA) D_2 receptor agonist, quinpirole (Brent, 1991). To date, the locomotor effects of compounds which increase extracellular DA concentrations such as amphetamine and cocaine, have not been investigated in the guinea-pig. In this study we have extended the work of Brent (1991) by investigating the behavioural effects of the D_1 receptor agonist, SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine; Pendleton et al, 1978), the D_2 receptor agonists PHNO ((+)-4-propyl-9-hydroxynaphthoxazine; Martin et al, 1984) and quinpirole, together with amphetamine and cocaine.

Male guinea-pigs (Interfauna, 200-250g) were habituated to test boxes for 30min prior to an injection of either drug or vehicle (n = 6-8 per group). Motor activity (photocell beam breaks = counts) was subsequently recorded for 60min. Data were analysed using a oneway ANOVA followed by a post hoc Dunnett's test to assess significance.

Table 1 summarises the LMA scores for animals treated with PHNO (0.01-1.0mgkg^{-1}, sc) and quinpirole (0.01-3.0mgkg^{-1}, ip). Both D_2 receptor agonists evoked significant (* $p < 0.05$ wrt vehicle) and dose-related increases in LMA. The stimulation induced by 0.1mgkg^{-1} PHNO was significantly (+ $p < 0.05$) antagonised by the DA receptor antagonist, haloperidol (0.5mgkg^{-1}, sc) {vehicle: 109±20; 0.1mgkg^{-1} PHNO: 1755±164; 0.5mgkg^{-1} haloperidol: 78±28; haloperidol plus PHNO: 318±52$^+$}. In contrast, the D_1 receptor agonist, SKF 38393 (1.0-10mgkg^{-1}, sc), had no effect on LMA. Surprisingly, neither amphetamine (0.25-20mgkg^{-1}, ip) nor cocaine (1.0-50mgkg^{-1}, ip) had any effect on LMA over a wide dose range.

Table 1

	Dose (mgkg^{-1}) of agonist						
	vehicle	0.01	0.03	0.1	0.3	1.0	3.0
PHNO	234 ± 34	687 ± 70	1715 ± 151*	1715 ± 225*	1862 ± 322*	3068 ± 331*	N/D
Quinpirole	157 ± 42	167 ± 39	128 ± 28	136 ± 16	253 ± 81	637 ± 231*	1442 ± 421*

Data are expressed as mean ± SEM; * $p < 0.05$ wrt vehicle; N/D: not determined; wrt: with respect to.

These results have extended the findings of Brent (1991), showing that LMA in guinea-pigs can be stimulated by directly-acting D_2 receptor agonists. The most important finding from this study was that administration of amphetamine and cocaine had no effect on guinea-pig LMA. This is in marked contrast to the stimulant effects of both compounds observed in rats and mice. Thus, it appears guinea-pig LMA can be stimulated by D_2 receptor agonists, but not by drugs which increase the concentration of DA in the synapse. As yet, we do not have an explanation for the lack of effect of amphetamine and cocaine on LMA. We are currently investigating whether these agents mediate other behavioural effects in guinea-pigs.

Brent, P.J. (1991) Psychopharmacol., 104, 201-207.
Martin, G.E., Williams, M., Pettibone, D.J., Yarbrough, G.G., Clineschmidt, B.V. & Jones J.H. (1984) J. Pharmacol. Exp. Ther., 230, 569-576.
Pendleton, R.G., Samler, L., Kaiser, C. & Ridley, P.T. (1978) Eur. J. Pharmacol., 51, 19-28.

277P FURTHER CHARACTERISATION OF HUMAN D_2 AND D_3 DOPAMINE RECEPTORS: GppNHp SHIFTS ARE EXPLAINED BY THE PRESENCE OF MORE THAN ONE BINDING SITE IN EACH CLONE

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Coldwell *et al.* (1992) identified two binding sites in human (h) D2 and D3 receptor clones, by coanalysis of inhibition curves against two radioligands. Sokoloff *et al.* (1992) described two binding sites for dopamine in human clones by analysing shifts in inhibition curves caused by 5'-guanylylimidodiphosphate (GppNHp). We show here that antagonist and agonist inhibition of [^{125}I]-iodosulpride binding to hD2 and hD3 was modified by GppNHp and that this can be explained by the presence of two binding sites. Radioligand binding was as described by Coldwell *et al* (1992) with the exception that incubations were in the absence or presence of 0.1mM GppNHp and 1μM ascorbic acid was present in incubations with dopamine.

GppNHp decreased specific [^{125}I]-iodosulpride binding to hD2 (control 4377 ± 379 dpm; +GppNHp 1846 ± 300 dpm; n=10) and hD3 (control 12577 ± 1711 dpm; +GppNHp 7966 ± 1281 dpm; n=10) clones and produced rightward shifts in the inhibition curves of dopamine (D), quinpirole (Q), iodosulpride (I) and spiperone (S). These shifts were less pronounced for the hD3 clones. We analysed the data from 10 experiments, each comprising 4 or 6 inhibition curves, according to a model that assumed two states of each receptor (Coldwell *et al.* 1992). The analysis also assumed the simplest model to explain shifts in inhibition curves, i.e. that GppNHp converted the high affinity state into the low affinity state without affecting equilibrium constants. In contrast, Sokoloff *et al.* reported different ligand affinities in the absence and presence of GppNHp. The Kd values for Q, S and I (Table 1) were close to those obtained previously with a different experimental strategy (Coldwell *et al.* 1992), supporting the applicability of the model. The proportion of hD2 receptors in the high affinity state without GppNHp was 47 ± 12% of the maximal binding. In the presence of GppNHp this fell to 2 ± 0.7% and accounted for the shifts in the inhibition curves and the fall in binding. The control proportion of the high affinity site for hD3 was similar to hD2 at 49 ± 8% but with GppNHp 13 ± 3% remained unconverted. This, together with the smaller differences between the affinities of the compounds for the two states in the hD3 receptor, led to smaller shifts than for hD2.

Table 1. Equilibrium constants (nM) from coanalysis of data obtained in the presence and absence of 100 μM GppNHp

	hD2		hD3	
	SITE1	SITE2	SITE1	SITE2
D	2600 ± 400	83000 ± 11000	65 ± 3	800 ± 50
Q	720 ± 90	24000 ± 3000	32 ± 2	65 ± 4
S	0.039 ± 0.005	0.13 ± 0.02	0.27 ± 0.02	0.87 ± 0.06
I	1.0 ± 0.2	22 ± 3	1.1 ± 0.1	5.0 ± 0.3

These results provide further evidence for the presence of two binding sites for dopaminergic ligands in both hD2 and hD3 receptor clones.

Coldwell, M.C., Bowen, W.P., Brown, F., Browne, M.J., Fears, R.B., Hadley, M.S., Hicks, F.R., Mannix, C. & Riley, G.J. (1992) Biochem. Soc. Trans. **21**, 56S
Sokoloff, P., Andrieux, M., Besançon, R., Pilon, C., Matres, M-P., Giros, B. & Schwartz, J-C. (1992) Eur. J. Pharmacol. **225**, 331-337

278P CORRELATION OF PLASMA WITH CSF DOPA AND DOPAMINE CONCENTRATIONS IN THE RAT AFTER L-DOPA ADMINISTRATION

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In man there is a good correlation between plasma dopa levels and improved motor function in Parkinson's disease (PD)(Kempster *et al.*, 1989) whilst experimentally systemic dopa is known to increase striatal dopamine (DA) (Brannan et al., 1989). Plasma levels of dopa have not been correlated, however, with those in the extracellular fluid of the striatum. We have used intracerebral microdialysis to monitor striatal levels of dopa and DA as well as their metabolites after administration of L-DOPA alone or with the extracerebral dopa decarboxylase inhibitor (ExCDDI), benserazide. These were then compared with their plasma concentrations.

Male Sprague-Dawley rats (200-300g) were anaesthetized with halothane and controlled at 37°C. Cannulae were inserted into a femoral artery and vein, the rat held in a stereotaxic frame and a laboratory-made dialysis probe (vitafiber, Amicon, UK) implanted into the striatum (A+0.7, L2.7, V7.0 mm relative to bregma). Artificial CSF (mM: Na^+147, Ca^{2+} 2.3, K^+4 and Cl^-155.6, pH 6.5) was perfused through the probe at 3μl/min and dialysate samples collected every 20 min and assayed by HPLC with electrochemical detection for dopa, DA, DOPAC and HVA. Blood (50μl) was collected 5,10,15 min after drug administration and thereafter at 20 min intervals from the femoral artery, treated with 0.1% $HClO_4$, centrifuged and analysed by HPLC. DA and benserazide were dissolved in normal saline, L-DOPA in acidic saline and all administered intravenously.

Basal levels of dopa, DA and their metabolites were not clearly detectable in plasma but in the striatal dialysates (CSF) DA(0.08±0.04, mean±s.e.), DOPAC(6.73±2.11), HVA(2.65±0.49) (pmole/20min), but not dopa were detected. After L-DOPA (15mg/Kg) was injected (n=4) its plasma level peaked after 5 min (125±39)(pmole/50μl) and remained above 50pmole for more than 3h but little dopa (0.10±0.00) was detected in CSF. Dopamine also rose in plasma within 10 min to 523±143 before falling after 1h and this was paralleled by a significant rise in its striatal level (peak:1.88±0.80, $p<0.05$) over the same time course. Plasma DOPAC (139±85) and HVA (72±19) also peaked within 15 min falling to zero in 1 and 3 h respectively. Their CSF levels were increased significantly (DOPAC:12.78±1.81, HVA:4.83±0.59; $p<0.05$) some 30-45 min after DA. If the same dose of L-DOPA was administered 20 min after benserazide (20mg/Kg; n=5), the levels of dopa were significantly higher ($p<0.05$) for 3h in both plasma (peak:333±21) and CSF (peak:1.6±0.3). After benserazide DA was not found in plasma and did not appear in CSF and although very little DOPAC and HVA was found in plasma they appeared in CSF at the levels as seen after L-DOPA alone but 20,40 min later (DOPAC:11.01±1.38, HVA:6.94±1.32; $p<0.05$). Since plasma DA (formed from dopa in the absence of benserazide) appeared to cross the BBB we administered DA intravenously (10mg/Kg; n=5) to give a plasma concentration similar to that after L-DOPA alone. This also produced a significant increase in CSF DA (0.25±0.05 vs 0.09±0.02, $p<0.05$).

The appearance of DOPAC and HVA (without any DA) in CSF and their absence in plasma, after L-DOPA with benserazide, indicates that DA has been formed centrally from DOPA crossing the BBB. However these results also show that DA formed peripherally from L-DOPA (without benserazide), or injected intravenously, can itself also enter the brain.

Brannan, T., Knott, P., Kaufmann, H., Leung, L. & Yahr, M. (1989) *J. Neural. Transm.* **75**, 149-157.
Kempster, P.A., Frankel, J.P., Bovingdon, M., Webster, R., Lees, A.J. & Stern, G.M. (1989) *J. Neurol. Neurosurg. Psychiatry* **52**, 718-723.

279P MICRODIALYSIS STUDY OF [^{3}H]NORADRENALINE RELEASE IN A2 TERMINAL REGIONS OF THE HYPOTHALAMUS AND BED NUCLEI: COMPARISONS ACROSS THE OESTRUS CYCLE

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A1 and A2 cell groups in the brainstem provide the major noradrenaline (NA) innervation to the hypothalamus. Perikarya of these neurones concentrate ^{3}H-oestradiol and contain oestrogen receptor mRNA (Simerley *et al.*, 1990). Both A1 and A2 neurones are contacted by oxytocin (OT)-containing neurones of the hypothalamic paraventricular nucleus (PVN)(Saiud *et al.*, 1989). Within the A2 region, OT regulates autonomic functions, including cardiovascular, and may play a role in modulating ascending innervation of the hypothalamus (Buijs *et al.*, 1990). We have shown that neurones in the region of A2 change their sensitivity to OT over the oestrous cycle and after oestrogen treatment (Tolchard & Ingram, 1993). Thus, it is likely that oestrogen modulates OT responses of A2 neurones and, in turn, regulates their release of NA at projections sites. We have examined this possibility by studying ^{3}H-NA release from pre-loaded brain regions (Fernandez-Galaz *et al.*, 1991) combined with OT microinfusions and electrical stimulation of the A2 region.

Studies were performed in dioestrus and oestrus virgin Wistar rats anaesthetised with urethane (1.25 g/kg). Forebrain regions (medial preoptic area (MPOA), bed nucleus of stria terminalis (BST), PVN and control sites outside these regions) were pre-loaded with ^{3}H-NA (1.15 uM; 43.7 Ci/mmol) using a dialysis probe (diam.: 250 um, length: 2 mm, recovery = 14%). Using a second probe introduced into the pre-loaded region, the effects of OT infusions (50 ng/10 min) and electrical stimulation (ES; 100 x 100 uA, 20 Hz, 10 s on/10 s off) in the A2 region and, K^+ depolarisation of the dialysed area (155 mM KCl through probe), were investigated and ^{3}H-NA release measured by liquid scintillation in 10 min dialysate samples. Mean arterial pressure (mAP) was continuously monitored from the saphinous artery.

The most powerful stimulant of ^{3}H-NA release in all regions examined was K^+ depolarisation which resulted in a 210-360% increase in release ($P < 0.01$, paired t-test). ES of the A2 region also increased ^{3}H-NA release in the MPOA, BST and PVN ($P < 0.05$, paired t-test) but only by 6-9% compared to pre-stimulus release. Such small responses to stimulation suggest that the contribution of A2 terminals to the ^{3}H-NA uptake and its subsequent release is relatively weak. No ES-stimulated increase occurred in control areas. Comparing the effects of K^+ and ES on ^{3}H-NA release in dioestrus and oestrus animals showed there to be no differences in the magnitude of these responses in any region. This was also the case when the response to ES was expressed as a fraction of the K^+-evoked release (an indication of the total releasable pool of ^{3}H-NA). Infusions of OT into the A2 region failed to affect basal ^{3}H-NA release in all regions. However, the OT infusions did cause a significant elevation in mAP of most (24/30) animals, although, no significant difference in the magnitude of these responses were observed between oestrus (32 ± 4 mm Hg, n = 15) and dioestrus (21 ± 4 mm Hg, n = 9; P = 0.066).

These data suggest that the magnitude of electrically-evoked release of NA from A2 terminals does not change at different stages of the oestrous cycle. The failure of the OT infusions to affect release of NA in the projection sites of A2 neurones may be due to the insensitivity of the method employed, or that OT does not play a role in the control of A2 neurones. Furthermore, it would appear that the steroid-dependent changes in sensitivity of neurones in this region to OT are not concerned with the regulation of blood pressure.

Buijs, R.M., Vanderbeek, E.M., Renaud, L.P., Day, T.A. & Jhamandas, J.H. (1990) Neurosci. **39**, 717-725.
Fernandez-Galaz, C., Herbison, A. & Dyer, R.G. (1992) J.Physiol. **446**, 65P.
Simerly, R.B., Chang, C., Muramatsu, M. & Swanson, L.W. (1990) J.Comp.Neurol. **294**, 76-95.
Saiud, P., Denoroy, L., Assenmacher, I. & Alonso, G. (1989) J.Comp.Neurol. **290**, 323-33
Tolchard, S. & Ingram, C.D. (1993) Brain Res. *in press*.

280P REGULATION OF ENDOGENOUS NORADRENALINE RELEASE IN RAT HIPPOCAMPUS AND FRONTAL CORTEX: *IN VIVO* MICRODIALYSIS

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Presynaptic α2-adrenoceptor activation and noradrenaline (NA) uptake mechanisms regulate extracellular NA content in brain. In vivo microdialysis studies with the α2-adrenoceptor antagonist idazoxan (IDX) and the NA uptake inhibitor desmethylimipramine (DMI) suggest that such regulation in the frontal cortex is due mainly to activation of the presynaptic receptor; whereas, in hippocampus reuptake is the primary effector mechanism (Thomas & Holman, 1991). However, IDX is known to have a high affinity for the non-adrenoceptor idazoxan binding site (NAIBS; Michel & Insel, 1989) as well as the α2-adrenoceptor. RX811059 (2-ethoxy-IDX) has high affinity for the α2-adrenoceptor at concentrations where there is minimal NAIBS affinity (Mallard et al, 1991). The present microdialysis experiments were undertaken to examine RX811059's effects on the regulation of NA release from rat frontal cortex versus hippocampus.

Rats were anaesthetised with chloral hydrate (400 mgkg-1 + 90 mgkg-1, ip as required) and concentric dialysis probes were stereotaxically implanted into the frontal cortex or hippocampus. RX811059 (3 mgkg-1, ip) was administered when a stable baseline was established in 3 consecutive 15 minute samples and sampling continued for a further 120 min. DMI (5 μM) present in the dialysis medium was continuously infused via the dialysis probe. NA content was determined immediately after each sample was collected by HPLC-electrochemical detection.

	Basal	RX811059¥	Basal + DMI	RX811059 + DMI¥
Frontal Cortex (n = 3/group)	5.06±0.26	9.98±1.30*	-	-
Hippocampus (n = 3/group)	4.25±0.50	5.74±1.47	34.22±3.88**	77.49±18.0†

* P<0.05, **P<0.001 as compared to basal control (Dunnett's t-test); † P<0.05 as compared to basal+DMI (Dunnett's t-test). ¥t = 30-45 minutes post RX811059 administration.

Basal NA release in pg/sample in frontal cortex and hippocampus is shown in the Table. After administration of RX811059, NA release was significantly increased in the frontal cortex only. DMI added to the perfusion media increased the extracellular content of NA in the hippocampus. RX811059 injection significantly increased hippocampal NA release only under these conditions.

These results support the suggestion that autoreceptor activation regulates synaptic NA concentrations to a greater extent in the frontal cortex than in the hippocampus (Thomas & Holman, 1991). In hippocampus an effect of presynaptic α2-adrenoceptor blockade was only evident when uptake was inhibited. Furthermore, the ability of RX811059 and IDX to increase endogenous NA release in cortex and in hippocampus appears to be due to α2-adrenoceptor antagonism and not an action at NAIBS.

Mallard, N.J., Tyacke, R., Hudson, A.L. and Nutt, D.J. (1991) Br. J. Pharmacol. **102**, 221P.
Thomas, D.N. & R.B. Holman. (1991) Br. J. Pharmacol. **102**, 5P.
Michel, M.C. & Insel, P.A. (1989) Trends in Pharmacol. Sci. **10**, 342-344.

281P THE EFFECT OF ISOLATION-REARING ON α_2 ADRENOCEPTOR-MEDIATED RESPONSES AND RECEPTOR BINDING IN THE RAT BRAIN

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Rats reared in isolation from weaning (at 21 days) demonstrate marked behavioural and neurochemical differences compared to group-reared rats. These include increased dopamine release and responsiveness to amphetamine (Jones *et al.*, 1990) but decreased 5-HT release with enhanced post-synaptic 5-HT_{1A} receptor function (Wright *et al.*, 1991). The present study investigated the functional state of pre- and post-synaptic alpha2-adrenoceptor responsiveness and central alpha2-adrenoceptor binding in isolation- compared to group-reared rats.

Male, Lister hooded rats were either housed in isolation or in social groups of five per cage. At 4 weeks post-weaning, clonidine was given (10µg/kg i.p.) and pupil diameter (p.d.) measured at 10, 20, 30 and 60 minutes after drug administration (table 1; *p<0.05, n=8). One week later, clonidine (100µg/kg i.p.) was readministered and the degree of hypoactivity assessed at 10 minute intervals for 60 minutes (table 1; *p<0.05, n=8). Behaviour was scored using passivity, tactile responsiveness, posture and gait as scoring parameters (Heal *et al.*, 1983). 4 weeks later the rats were killed and alpha2-adrenoceptor binding characteristics measured in the frontal cortex and hippocampus using [^{3}H]-Idazoxan as the radioligand (table 2; *p<0.05, n=9). Mydriasis and binding data were analysed using the unpaired t test. Hypoactivity data were analysed using the Mann Whitney U test.

Table 1: Alpha2-Adrenoceptor Responses

	Mydriasis (change in p.d at 10min (mm))	Hypoactivity (total score at 60min)
Isolation	0.55 ±0.07*	38 ±3.0*
Group	0.30 ±0.07	27 ±3.0

Table 2: [^{3}H]-Idazoxan Binding

	Frontal Cortex		Hippocampus	
	Kd (nM)	Bmax(fmol/mg)	Kd	Bmax
Isolation	0.75 ±0.08	226.1 ±5.8	0.59 ±0.05	251.1 ±13.9*
Group	0.73 ±0.06	224.6 ±5.82	0.49 ±0.02	211.4 ±8.05

These results suggest that rats reared in isolation have increased central pre-synaptic (hypoactivity) and post-synaptic (mydriasis) alpha2-adrenoceptor function compared to group-reared rats which is supported by evidence of increased alpha2-adrenoceptor binding in the hippocampus. The role of the alpha2-adrenoceptor changes in the behavioural effects of isolation-rearing remains to be determined.

A.J.F is supported by a S.E.R.C. studentship.
Jones, G.H., Marsden, C.A. and Robbins, T.W. (1990) Psychopharmacol., **102**, 364-372.
Wright, I.K., Ismail, H., Upton, N. and Marsden, C.A. (1991) Psychopharmacol., **105**, 254-263.
Heal, D.J., Lister, S.L., Smith, S.L., Davies, C.L., Molyneux, S.G. and Green, A.R. (1983) Neuropharmacol., **22**, 983-992.

282P EFFECT OF SYSTEMIC CLONIDINE ON THE ACTIVITY OF IDENTIFIED VASOPRESSIN NEURONES IN THE ANAESTHETISED LACTATING RAT

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Systemically administered alpha-2 adrenoceptor agonists, such as clonidine, cause a diuresis in anaesthetised rats. This increase in production of dilute urine could result from a reduction in antidiuretic hormone (vasopressin) secretion or by an action directly on the kidney. A reduction in vasopressin was reported by Sved, (1985), but subsequent studies suggested that alpha-2 adrenoceptor agonists do not alter vasopressin levels but act primarily at the kidney (Gellai & Edwards 1988). It is known that vasopressin-synthesising cells in the hypothalamus receive dense noradrenergic projections from several brainstem regions and these are significantly involved in controlling vasopressin cell activity (Renaud & Bourque, 1991). In this study we have therefore examined the effects of systemically administered clonidine on the activity of vasopressin-secreting neurones in the supraoptic nucleus of the anaesthetised rat.

Experiments were performed on urethane-anaesthetised lactating rats prepared for electrophysiological recording as described previoulsy (Lincoln & Wakerley, 1974). The short-lasting opioid analgesic, fentanyl (30ug/kg), was given during surgery. Supraoptic vasopressin cells were identified by their constant antidromic invasion when the pituitary stalk was electrically stimulated and their lack of bursting activity prior to milk-ejection during suckling (Lincoln & Wakerley, 1974).

Intravenously administered clonidine (0.05mg/kg) had an exclusively inhibitory effect on all vasopressin cells tested. The mean duration of the inhibition was 12.21$\pm$1.5 minutes with the firing rate falling from 3.21$\pm$0.41 Hz to 0.63$\pm$0.17 Hz (n=10). Prior administration of an alpha-2 adrenoceptor antagonist, idazoxan (0.5mg/kg i.v.) prevented the clonidine-induced inhibition (4.10$\pm$1.00 Hz to 4.86$\pm$0.96 Hz (n=6)) but had no consistent effects on the ongoing activity of the vasopressin neurones. The response to clonidine had returned after 30 min for all 5 neurones in which a second dose of the agonist was administered. Clonidine and idazoxan had no apparent effects on the activity of neurones in the adjacent lateral hypothalamus; these neurones did not generate antidromic potentials when the stalk was stimulated.

Thus vasopressin cells are consistently and profoundly inhibited by clonidine but the action is short-lived. The resulting reduction in circulating vasopressin might contribute to the early phase of diuresis but would not account for the total response. These results further emphasise the role of noradrenergic systems in the regulation of vasopressin secretion, and explain the apparent contradiction in the literature.

Gellai, M. & Edwards, R.M. (1988) Am. J. Physiol. **255**, F317-F323
Lincoln, D.W & Wakerley, J.B. (1974) J. Physiol. **242**, 533-544
Renaud, L.P. & Bourque, C.W. (1991) Prog. Neurobiol. **36**, 131-169
Sved, A.F. (1985) Eur. J. Pharmacol. **109**, 111-116

283P CARBACHOL- AND POTASSIUM-EVOKED RELEASE OF [^{3}H]NORADRENALINE ARE INHIBITED DOSE-DEPENDENTLY BY FENTANYL IN SH-SY5Y CELLS

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The cellular mechanisms by which opioids produce their clinical effects are unclear. Opioid receptor occupancy leads to a diverse array of cellular events including inhibition of adenylate cyclase (Childers, 1991), increased potassium conductance (North et al., 1987) and the closing of voltage sensitive calcium channels (Seward et al., 1991). Opioids may also modulate neurotransmitter secretion. We have utilised a human neuroblastoma cell line SH-SY5Y, which expresses μ and δ opioid receptors, to examine fentanyl (an opioid related to pethidine) modulation of carbachol- and potassium-evoked [^{3}H]noradrenaline ([^{3}H]NA) release.

[^{3}H]NA release was measured in 6 day differentiated (retinoic acid 10μM), monolayer cultured cells as described by Murphy et al. (1991). Briefly, cells were incubated with [^{3}H]NA 40nM in Krebs/HEPES buffer (pH 7.4) for 1h. After 3 x 15 min washes the monolayers were challenged with carbachol 1mM or potassium 100mM in the absence or presence of fentanyl for 3 min at 37°C. Fentanyl was preincubated for 45 min. Unreleased [^{3}H]NA in the monolayer was extracted with perchloric acid 0.4M. Data were analysed using Student's t-test. Both carbachol and potassium stimulate the release of [^{3}H]NA.

The absolute values for the increase in evoked release are variable and appear to depend on initial seeding density. However, both carbachol- and potassium-evoked release were clearly inhibited by fentanyl in a dose-dependent manner (Table 1).

Table 1. Fentanyl inhibition of [^{3}H]NA release - Data are mean (s.e. mean) n=5-11 except where range* (n=2)

	Inhibition of Release (%)	
[Fentanyl μM]	Carbachol 1mM	Potassium 100mM
0.1	10.29 (3.63)	0.93 (0.62)
0.3	17.66 (3.43)	
1.0	21.83 (2.17)	3.24 (1.26)
10.0	61.24 (2.32)	41.20 (2.18)
100.0	84.00 (5.95)*	84.20 (2.58)

Significant inhibition of carbachol- and potassium-evoked release occurs at concentrations of fentanyl of 0.3μM ($p<0.05$) and 10μM ($p<0.01$) respectively. Fentanyl 0.3μM is the upper limit of serum concentrations seen during clinical anaesthesia; studies to determine whether this response is opioid receptor mediated and clinically relevant are currently underway.

R. Atcheson is a British Journal of Anaesthesia Research Fellow.

Childers, S.R. (1991) Life Sciences, **48**, 1991-2003.
Murphy, N.P., Ball, S.G., Vaughan, P.T.F. (1991) Journal of Neurochemistry, **56**, 1810-1815.
North, R.A., Williams, J., Surprenant, A., MacDonald, J.C. (1987) Proc. Natl. Acad. Sci. USA, **84**, S487-S491
Seward, E., Hammond, C., Henderson, G. (1991) Proc. R. Soc. Lond. B. **244**, 129-135.

284P DIFFERENTIAL EFFECTS OF THE AT_1 RECEPTOR ANTAGONISTS GR117289 AND GR138950 ON AT_1 RECEPTOR-MEDIATED DIPSOGENIC RESPONSES AND IN A BRAIN *EX VIVO* BINDING ASSAY IN THE RAT

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We have previously shown that dipsogenic responses to intraventricular (icv) angiotensin II (AII) and subcutaneous (sc) captopril (capt) can be inhibited by the AT_1 receptor antagonist losartan (Dennes et al., 1992a,b). We now report differential effects of two novel AT_1 receptor antagonists GR117289 (Robertson et al., 1991) and GR138950 (Hilditch et al., 1992) in these models. We have also used an *ex vivo* binding assay to measure the ability of GR138950 to penetrate into the brain. Similar *ex vivo* studies have already been reported for GR117289 (Marshall et al., 1992)

In the dipsogenic studies, LH rats (300-350g) were treated with GR138950, GR117289 (0.1, 1 or 10mgkg^{-1}sc) or saline (1mlkg^{-1} sc), 2h before AII (30pmol icv) or 1h before capt (3mgkg^{-1} sc). Immediately after AII or 1h after capt, rats were allowed access to water for 20min. The volume of water consumed (ml) was measured. GR117289 dose-dependently inhibited dipsogenic responses to AII (AII alone: 9.3±0.7ml; AII + GR117289: 0.1mgkg^{-1} 7.5±2.4ml; 1mgkg^{-1} 6.6±1.2ml; 10mgkg^{-1} 4.8±1.1ml, $P<0.05$). In contrast, GR138950 (10mgkg^{-1} sc) failed to inhibit responses to AII (AII alone: 12.0±2.9ml; AII + GR138950: 9.0±1.2ml, $P>0.05$). Dipsogenic responses to capt were dose-dependently inhibited by both GR117289 (capt alone: 3.5±0.3ml; capt + GR117289: 0.1mgkg^{-1} 2.0±0.3ml, $P<0.05$; 1mgkg^{-1} 1.2±0.2ml, $P<0.05$; 10mgkg^{-1} 0.4±0.1ml, $P<0.05$) and GR138950 (capt alone: 3.1±0.4ml, capt + GR138950: 0.1mgkg^{-1} 3.4±0.4ml; 1mgkg^{-1} 1.7±0.5ml, $P<0.05$; 10mgkg^{-1} 1.2±0.4ml, $P<0.05$).

In the *ex vivo* binding assay, GR138950 (10mgkg^{-1} sc) or saline (1mgkg^{-1} sc) were administered and 2h later, specific binding of [^{125}I]-Sar^{1}Ile^{8}angiotensin II ([^{125}I- Sarile) was measured *ex vivo* in liver and cortex/hippocampus (Cx/H) as previously described (Marshall et al., 1992). Results are expressed as percentage of binding (mean ± S.E.M) in vehicle-treated rats. GR138950 (10mgkg^{-1} sc) significantly reduced [^{125}I]-Sarile binding in liver to 4.0±1.1% of control ($P<0.05$). However, binding in Cx/H was reduced to only 83.4±11.5% ($P>0.05$). This contrasts with the effect of GR117289, which reduced [^{125}I]-Sarile binding in Cx/H to 30±5% (Marshall et al., 1992)

In conclusion, the inability of sc GR138950 (in contrast to GR117289) to inhibit dipsogenic responses to AII and to inhibit [^{125}I]-Sarile binding to rat Cx/H *ex vivo* suggest that this antagonist, at the doses used, does not gain access to the CNS. The ability of both GR117289 and GR138950 to inhibit capt - induced dipsogenic responses suggests that capt induces its dipsogenic actions via AT_1 receptors outside the blood brain barrier.

Dennes, R.P. Barnes, J.C. Michel, A.D. & Tyers, M.B (1992a) Br. J. Pharmacol. **105**, 88P.
Dennes, R.P., Irlam-Hughes, J.J. & Barnes, J.C. (1992b) Br. J. Pharmacol. **105**, 262P.
Hilditch, A., Hunt, A.A.E., Travers, A., Drew, G.M., Robertson, M.J. et al. (1992) This meeting.
Marshall, F.H., Barnes, J.C., Clark, S.A. & Michel, A.D. (1992) Br. J. Pharmacol. **106**, 136P.
Robertson, M.J., Middlemiss, D., Ross, B.C., Drew, G.M., Scopes, D.I.C. & Dowle, M.D. (1991) Br. J. Pharmacol. **104**, 300P.

285P ANGIOTENSIN II STIMULATES NORADRENALINE RELEASE FROM RAT FRONTAL CORTEX SLICES VIA THE AT_1 RECEPTOR

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Angiotensin II (AT) has previously been shown to enhance the release of potassium-stimulated noradrenaline (NA) release from rat parietal cortex in vitro (Huang et al., 1987) and endogenous NA release in the hypothalamus in vivo (Stadler et al., 1992). The former study was performed before the availability of AT receptor subtype selective ligands. However, in the latter study, the response was probably mediated via the AT_1 receptor since it was antagonised by the selective AT_1 receptor antagonist, losartan (Timmermans et al., 1991). In the present study, we assess the ability of AT to modulate the spontaneous release of NA from slices of rat frontal cortex.

Female Wistar rats (70-120 g, Birmingham bred) were killed by cervical dislocation and the frontal cortex removed. Slices of frontal cortex were cut with a McIlwain tissue chopper (400 x 400 μm x thickness of the cortical ribbon). Cut tissue was loaded into perfusion chambers and perfused with gassed (95/5 O_2/CO_2) Krebs buffer (mM; NaCl 120, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11) plus tyrosine (50 μM), nomifensine (0.1 μM) and yohimbine (0.1 μM) for 100 min before the continuous collection of 4 min perfusate fractions. NA in the perfusate was quantified by high performance liquid chromatography with electrochemical detection. Protein content was estimated by the Commassie Blue method using bovine serum albumin as standard.

AT (0.1 μM) enhanced the release of NA release from the cortical slices (Fig. 1) and this response was antagonised by losartan (1.0 μM; Fig.1).

The present studies indicate that AT modulates the release of NA from rat frontal cortex slices via the AT_1 receptor subtype.

This work was supported by the MRC.

Huang, Y., Rogers, J. & Henderson, G. (1987) J. Neurochem. 49, 1541-1549.
Stadler, T., Veltmar, A., Qadri, F. & Unger, T. (1992) Brain Res. 569, 117-122
Timmermans, P.B.M.W.M., Wong, P.C., Chiu, A.T. & Herblin, W.F. (1991) Trends Pharmacol. Sci. 12, 55-62.

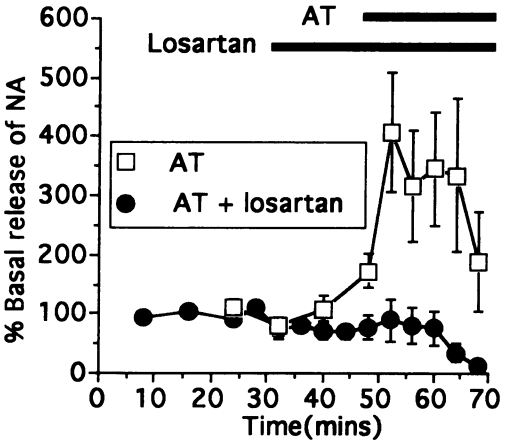


Figure 1. Ability of AT (0.1 μM) and losartan (1.0 μM) to modulate spontaneous NA release from rat frontal cortex slices. Basal NA release = 0.36 pmol/min/mg protein. Data represent mean±SEM, n = 3.

286P THE EFFECTS OF INTRACEREBROVENTRICULAR (icv) ADMINISTRATION OF PENTAGASTRIN ON FEEDING AND CORTISOL RELEASE IN THE SHEEP

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Recent evidence has suggested that CCK, acting on central CCK_B receptors, plays a pivotal role in the mediation of panic attacks and anxiety disorders (Bradwejn et al., 1992). As the sheep is by nature a very nervous animal, we argued that if central CCK_B receptors are involved in the mediation of panic, then activation of these receptors by the CCK_B receptor agonist pentagastrin should (i) suppress feeding in hungry sheep, and (ii) increase plasma concentrations of the stress hormone cortisol. Della Fera and Baile (1979) have previously reported that a very high dose of pentragastrin (approx. 50 μg icv infused over 1h) produced abnormal behaviours and reduced food intake in sheep. Although it is possible that these effects were produced by the induction of a panic-like state in these animals by the activation of CCK_B receptors, it is more likely that they were due to non-specific aversive effects of the drug. In the present study we tested our hypothesis using lower doses of icv administered pentagastrin. Six adult Clun Forest sheep (3F, 3M) were implanted under halothane anaesthesia with cannulae directed towards the lateral ventricle for chronic icv administration of drugs. Success of the canulae placements were confirmed by the drinking responses induced in the sheep after icv injection of angiotensin (250 nmol). The animals were also fitted with jugular catheters for withdrawal of blood samples. In the 1st experiment, the sheep were deprived of food for 6h prior to each session, and offered 100g of pelleted sheep nuts 5, 35, 65 and 95 min after icv administration of vehicle or pentagastrin (4 and 10 μg). The amount of food eaten was measured 10 min after each presentation. In the 2nd experiment, a series of 10 ml blood samples were taken before (-10 min) and after (10, 20, 30 and 60 min) icv injection of vehicle or pentagastrin (4 and 10 μg), and plasma concentrations of cortisol were analysed by radioimmunoassay. The results obtained in the 1st experiment show that pentagastrin had no inhibitory effects on food intake at the doses used. The sheep treated with vehicle or pentagastrin (4 and 10 μg) consumed all their food pellets (i.e.100g) during the 10 min period after each presentation. Interestingly, the sheep did not retreat when we approached them with the food containers which we would have expected if pentagastrin induced panic-like behaviour in these animals. Moreover, we observed no other consistent behavioural changes in the sheep (e.g. vocalization or foot-stamping) after icv injections of pentagastrin. The results from the 2nd experiment showed that icv administration of pentagastrin (4 and 10 μg) did not significantly affect the secretion of cortisol. Thus, for example, the plasma levels of cortisol for vehicle and pentagastrin (10 μg) were 25.09 ± 6.21 and 22.12 ± 3.53 nmol L^{-1} respectively at 10 min, and 17.53 ± 1.52 and 16.14 ± 3.96 nmol L^{-1} respectively at 30 min. The present results are in agreement with those obtained in other species. Thus, it has been shown that icv pentagastrin has no effects on feeding in pigs (Parrott, unpublished results) , and on the release of corticosterone in rats (Itoh et al., 1979). In conclusion, the results of this study indicate that central administration of the CCK_B receptor agonist pentagastrin does not induce behavioural and endocrine changes that are consistent with a panic-like state in sheep.

Bradwejn, J. et al. (1992) J. Psychopharmacol. 6, 345 - 351
Della-Fera, M.A. and Baile, C.A. (1979) Science, 206, 471 - 473
Itoh, S. et al. (1979) Endocrinol. Jpn. 26, 741 - 744

287P EFFECT OF THE CCK_B ANTAGONIST L-365,260 ON BENZODIAZEPINE WITHDRAWAL-INDUCED WEIGHT LOSS IN RATS

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The anxiolytic CCK_B antagonist CI-988 suppresses "anxiogenic" (Hughes et al. 1990) and proconvulsant (Singh et al. 1992) benzodiazepine (BZ) withdrawal signs. We therefore assessed the effect of the CCK_B antagonist L-365,260 on BZ withdrawal-induced weight loss. The effects on weight of L-365,260, *or* chlordiazepoxide (CDP) *or* vehicle were assessed over 8 days (experimental days 22-29) in groups (n = 9 or 10) of female rats (246-373 g) pretreated b.i.d. (i.p.) on days 1-21 with saline or CDP at doses escalated from 10 to 30 mg/kg. L-365,260 was administered b.i.d. (i.p.) on days 22-29 at 1, 3 or 10 mg/kg. Weights were expressed as percentages of day 22 values. CDP withdrawal caused significant weight loss (animals withdrawn from CDP and treated with vehicle were compared with animals maintained on CDP [groups $F(1,17) = 9.83$, $p < 0.006$ - repeated measures ANOVA]). This weight loss peaked on day 24 at 3% of the prewithdrawal baseline. By day 29 withdrawal-induced weight loss had dissipated. The effect of L-365,260 on withdrawal-induced weight loss was assessed by comparing weights of subjects pretreated with CDP and switched to vehicle with weights of subjects pretreated with CDP switched to L-365,260. Comparison of the vehicle group with the 10 mg/kg L-365,260 group showed a marginally nonsignificant groups effect [$F(1,16) = 3.79$ $p < 0.07$]. At this dose L-365 260 treated animals lost *more* weight than vehicle-treated controls (the maximal difference was equal to 3.5% weight loss on day 29). At lower doses of L-365,260 there no evidence for modulation of withdrawal-induced weight loss. L-365,260 caused dose-related weight *gain* in saline-pretreated animals. The *maximal* effect was seen at 10 mg/kg. This involved a 2% stimulation of weight on day 29 relative to vehicle treated controls [$F(1,17) = 4.46$, $p < 0.05$]. Despite this finding, the drug failed to alleviate withdrawal-induced weight loss. The failure of L-365,260 to alleviate BZ withdrawal contrasts with the findings of Hughes et al. (1990) and Singh et al. (1992). Possible explanations for the "negative" results obtained with L-365,260 may relate:- i) To the BZ withdrawal index used in this study, which may differ qualitatively from other BZ withdrawal signs (Leathley and Goudie, 1992), or ii) To differences in the actions of CI-988 and L-365,260, which are structurally different types of CCK B antagonists (Woodruff and Hughes, 1991).

Acknowledgment:-. Supported by Merck, Sharp & Dohme Research Labs, Harlow, U.K.

Hughes, J. et al. (1990) Proc. Natl. Acad. Sci. USA. **87,** 6728-6732.
Leathley, M.J. & Goudie, A.J. (1992) Behav. Pharmacol. **3,** 51-56.
Singh, L. et al. (1992) Brit. J. Pharmacol. **105,** 8-10.
Woodruff, G.N. & Hughes, J. (1991) Ann. Rev. Pharmacol. Toxicol. **31,** 469-501.

288P CONDITIONED SUPPRESSION OF OPERANT FEEDING INDUCED IN PIGS BY CHOLECYSTOKININ: EFFECTS OF LORAZEPAM AND THE CCK_B ANTAGONIST L365,260

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Bolus intravenous (iv) injection of cholecystokinin octapeptide (CCK) produces an abrupt, temporary, cessation of operant feeding in pigs at doses that stimulate vasopressin and cortisol release (Parrott *et al.* 1991). This raises the possibility that CCK might induce conditioned suppression and, thereby, provide an experimental model of anxiety that does not involve the use of electric shock. Also, since iv CCK does not cross the blood/brain barrier (Passaro *et al.* 1982), such a technique might be useful for investigating the action of anxiolytics. The present study examined the feasibility of this experimental approach.

Prepubertal pigs (12♀, 2♂), surgically prepared with venous catheters, were trained to work for food on a fixed ratio (FR10). They were housed in adjacent metabolism cages and given two daily feeding sessions (9-9.45h, 14.45-15.15h) signalled by a tone (T1, 120 secs). CCK injections (1μg/kg iv) were given in the afternoons at the end of a second, different frequency, tone (T2, 300 secs) that commenced 5 min. after the start of feeding. CCK inhibited subsequent feeding and partial, or complete, suppression of operant responding during T2 was also observed in 9/14 pigs after 22.4 $\pm$ 1.9 ($\bar{x} \pm$ sem) daily pairings.

Conditioned animals were given the benzodiazepine lorazepam (0.025 or 0.5 mg/kg) or the putative anxiolytic L-365,260 (Ravard *et al.* 1990; 0.1 mg/kg in ethanol/propylene glycol vehicle, 1:4)* 30 min. before T1; a preliminary study indicated that these doses had no effect on operant food intake under normal conditions. In the conditioning tests, 4/9 animals increased their feeding activity during T2, compared to the preceding day, when treated with the low dose of lorazepam (1.0 $\pm$ 1.0 to 6.3 $\pm$ 0.5 reinforcements, $\bar{x} \pm$ sem) and two pigs also given the higher dose both increased their response rate (1.5 to 6.0 $\bar{x}$). Similarly, 3/5 animals treated with L365,260 increased their food intake during T2 (1.0 $\pm$ 0.6 to 5.7 $\pm$ 0.3, $\bar{x} \pm$ sem).

These results indicate that CCK can condition anxiety in pigs which may be attenuated by lorazepam or L365,260. However, the animals learn rather slowly; this may be because of the high feeding motivation of growing pigs. In addition, learning may be impeded by social facilitation of feeding which can occur when pigs are housed together. This could be avoided in future studies by testing animals in isolation.

Parrott, R.F., Ebenezer, I.S., Baldwin, B.A. & Forsling, M.L. (1991) *Exp. Physiol.* 76, 525-531
Passaro, E., Debas, H., Oldendorf, W. & Yamada, T. (1982) *Brain Res.* 241, 335-340
Ravard, S., Dourish, C.T. & Iversen, S.D. (1990) *Br. J. Pharmacol.* 101, 576p

*L365,260 was donated by Dr M.M. Traub, Merck, Sharp & Dohme Research Laboratories, Harlow, U.K.

289P EFFECTS OF NEUROKININ RECEPTOR ANTAGONISTS ON CARRAGEENAN-INDUCED EXPRESSION OF PREPRODYNORPHIN mRNA IN RAT DORSAL HORN LAMINA I NEURONS

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Sustained activation of nociceptive afferents for example by experimental inflammation or peripheral application of chemical irritants, has been shown to lead to prolonged changes in responsiveness and receptive field properties of dorsal horn neurons (Hylden *et al,* 1989; Woolf & King, 1990). Increases in the levels of preprodynorphin (PPD) mRNA lamina I neurons are elicited by peripheral inflammatory stimuli (Ruda *et al,* 1988) and these neurons include those with long ascending projections to higher levels of the central nervous system (Nahin, 1988). The neurotransmitters responsible for bringing about the increased expression of preprodynorphin mRNA are unknown and the present experiments were carried out to address any role of NK_1 or NK_2 receptors.

Using *in situ* hybridisation histochemistry (ISHH), we investigated the expression of PPD mRNA in the superficial dorsal horn of male Wistar rats (280 - 380 g) anaesthetised with α-chloralose/urethane and thereafter subjected to bilateral intraplantar injection of carrageenan (150 µl of 1 - 4% solution in sterile saline per hindpaw). Neurokinin receptor antagonists (Fleetwood-Walker *et al,* 1991; Hagan *et al,* 1991) or vehicle (0.15 - 1 mM, pH 4.5 - 5.0 in water with or without 0.3% dimethylformamide) were locally administered unilaterally into the spinal cord by continuous ionophoresis (50 nA) for 1 hour prior to induction of inflammation and for a further duration of 6 hours. Spinal segments encompassing drug injection sites (L_{3-4}) were sectioned at 10 µm on a cryostat for ISHH and histology. The probe was a 48-base oligomer complementary to the base sequence 862 - 909 of the PPD mRNA (Lightman *et al,* 1987) 3'-labelled with dATP[α-^{35}S] using terminal deoxynucleotidyl transferase. The corresponding sense probe or RNAse A-treated sections showed no specific hybridisation above background.

Unilateral injection of carrageenan in laminectomised, sham preparations elicited a 2 - 4 fold increase in the number of ipsilateral lamina I cells expressing silver grain counts more than 5 fold greater than background. Typical values were 10 - 25 and 3 - 6 cells per dorsal horn (for carrageenan and control respectively); largely maintained through segments L_{3-4}. In 3 animals treated with the NK_2 antagonist L-659874 there was a marked inhibition of carrageenan-stimulated PPD mRNA expression in lamina I cells (to 59 ± 1% of contralateral controls; mean ± s.e. mean) extending consistently over a rostro-caudal range of 200 - 400 µm. In animals tested with the NK_1 antagonists L-668169 or GR-82334 (or with equivalent ionophoresis of vehicle) there was no evidence for any consistent change in PPD mRNA expression. These results indicate that dorsal horn NK_2 receptors play a role in the delayed expression of a neuropeptide gene in lamina I cells that was elicited by a peripheral inflammatory stimulus. In support of this conclusion, both responses of dorsal horn neurons to brief thermal noxious stimuli (Fleetwood-Walker *et al,* 1991) and their medium term activation by the chemical irritant mustard oil (Munro *et al,* this meeting) also appear to rely on transmission though dorsal horn NK_2 receptors.

Fleetwood-Walker, S.M., Hope, P.J., Parker, R.M.C. & Mitchell, R. (1991) Br. J. Pharmacol. **104**, 472P.
Hagan, R.M., Ireland, S.J., Bailey, F., McBride, C., Jordan, C.C. & Ward, P. (1991) Br. J. Pharmacol. **102**, 622-626.
Hylden, J.L.K., Nahin, R.L., Traub, R.J., Dubner, R. (1989) Pain **37**, 229-243.
Lightman, S.L. & Scott Young, W. III. (1987) J. Physiol. **394**, 23-39.
Munro, F.E., Fleetwood-Walker, S.M., Parker, R.M.C. & Mitchell, R., this meeting.
Nahin, R.L. (1988) Brain Res. **443**, 345-349.
Ruda, M.A., Iadorola, M.J., Cohen, L.V. & Scott Young III, W. (1988) Proc. Natl. Acad. Sci. (USA) **85**, 622-626.
Woolf, C.J. & King, A.E. (1990) J. Neurosci. **10**, 2717-2726.

290P NEUROPEPTIDE-INDUCED CHANGES IN MICROVASCULAR CEREBRAL BLOOD FLOW IN THE ANAESTHETISED GUINEA-PIG

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Calcitonin gene-related peptide (CGRP) and substance P are stored in and released from trigeminal afferent nerve fibres and may be involved in the regulation of microvascular cerebral blood flow (CBF) (Suzuki *et al.*, 1990). The present study examined whether CGRP, substance P or the selective neurokinin (NK) receptor agonists, GR73632 and GR64349 (NK_1 and NK_2 respectively; Hagan *et al.*, 1991a), were able to affect CBF, following systemic administration. Adult male guinea-pigs were anaesthetized and the trachea cannulated to allow artificial respiration of the animals. The left carotid artery was cannulated caudally for blood pressure measurement and collection of blood for acid/base analysis and rostrally for drug administration. A craniotomy was then made over the left parietal cortex. The dura was removed and a laser-Doppler flow probe, coupled to a flowmeter, positioned above the cortex to record CBF.

Cannulation of the carotid artery or vehicle administration had no effect on CBF. Substance P (0.01-1nmole/kg), CGRP (0.01-0.3nmole/kg), GR73632 (0.1 and 0.3nmole/kg) and GR64349 (0.3nmole/kg) each injected intra-arterially, reduced blood pressure and produced an accompanying transient (<1min duration) fall in CBF, occurring presumably as a consequence of the hypotensive action. Upon recovery of blood pressure; GR73632 and GR64349, but not substance P or CGRP, produced a longer lasting (duration 10-20min) significant increase in CBF of 82 ± 20% (n=7) and 37 ± 7% (n=4), respectively, at 0.3nmole/kg. This increase in CBF was accompanied by a rise in arterial $PaCO_2$ and fall in PaO_2 levels (53 ± 9% increase and 55 ± 9% decrease (n=3), respectively, for GR73632 (0.3nmole/kg) and 41 ± 6% and 55 ± 13% (n=4), respectively, for GR64349 (0.3nmole/kg)). The GR73632 (0.3nmole/kg)-induced increase in CBF was significantly attenuated by GR82334 (0.5µmole/kg, 10min pretreatment), a selective NK_1 receptor antagonist (Hagan *et al.*, 1991b). Following a rapid bolus injection of urea (0.25ml, 5.0M, i.a.) to disrupt the blood brain barrier, CGRP (0.1nmole/kg), significantly increased CBF (13 ± 4% rise, n=5) following the initial transient reductions in both blood pressure and CBF. Substance P (0.1nmole/kg), however, failed to increase CBF following urea pretreatment.

The results suggest that upon systemic administration, CGRP increases CBF only when the blood brain barrier is disrupted and access to its receptors on the vascular smooth muscle is possible. GR73632 and GR64349, selective NK_1 and NK_2 receptor agonists respectively, increase CBF possibly as a consequence of their effects on blood $PaCO_2$ and PaO_2 levels, which were due presumably to their recognised bronchoconstrictor activity in the guinea-pig (Hagan *et al.*, 1991a), rather than a direct action on the cerebrovasculature. The attenuation by GR82334 of the GR73632-induced increase in CBF confirms that GR73632 acted via NK_1 receptor activation. The failure of substance P to increase CBF may be due to its rapid metabolism by peptidases, a property not shared by GR73632 and GR64349.

Hagan, R.M., Ireland, S.J., Jordan, C.C., Beresford, I.J.M., Deal, M.J. & Ward, P. (1991a). Neuropeptides, 19, 127-135.
Hagan, R.M., Ireland, S.J., Bailey, F., McBride, C., Jordan, C.C. & Ward, P. (1991b). Br. J. Pharmacol., 102, 168P.
Suzuki, N., Hardebo, J.E., Kahrstrom, J. & Owman, C. (1990). Acta Physiol. Scand., 138, 307-315.

291P COMPARISON OF ANTICONVULSANT EFFECTS OF INTRAVENOUS PROPOFOL, THIOPENTONE AND METHOHEXITONE IN BICUCULLINE-INDUCED CONVULSIONS IN MICE

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Propofol has become popular as a maintenance agent for long-term anaesthesia and intensive care sedation. Originally it was reported to have no excitatory side effects but subsequent animal and clinical reports have been contradictory. Lowson *et al* (1990) demonstrated that propofol displayed marked anticonvulsant properties in mice. against electroshock and PTZ seizures. If as suggested by Concas *et al* (1990). propofol allosterically modulates the GABA recognition site. a $GABA_A$ antagonist may be used to investigate the method of anticonvulsant/proconvulsant action. The aim of this study was to examine the effects of propofol. thiopentone and methohexitone in bicuculline-induced convulsions.

Bicuculline (0.5 mg/ml) was infused into groups of female MF1 mice (n=10) 2 minutes after treatment with intravenous vehicle or anaesthetic. Intralipid or a dilute solution of NaOH. pH 10.4 was used as vehicle control for propofol and thiopentone/methohexitone respectively. The seizure threshold was defined as the amount of bicuculline (mg/kg) required to induce a tonic/clonic seizure. expressed as Mean +/- SEM. The anticonvulsant effect of anaesthesia per se was examined over time by measuring activity of each agent (10 mg/kg). at 0.5. 2. 4. 6 and 30 minutes.

Vehicle controls had no effect on seizure threshold. Methohexitone alone produced involuntary body movements. The seizure threshold rose after addition of each agent in a dose dependent manner. suggesting an anticonvulsant action. Propofol showed the highest degree of protection against seizure which was still evident 30 minutes after initial dosing. Although thiopentone offered a similar level of protection up to 6 minutes, the seizure threshold then returned to control value. Methohexitone at no time increased seizure threshold to a comparable level and lost any activity at 4 minutes. Recovery of righting reflex occurred at 6. 20 and 8 minutes for propofol. thiopentone and methohexitone. All mice had fully recovered 30 minutes after anaesthesia. indicated by return of normal exploratory behaviour (Lowson *et al*. 1991).

Table 1 Values for seizure threshold with intravenous anaesthetics (10 mg/kg). Vehicle assessed 2 minutes post-dosing.

	Control	Vehicle	0.5 mins	2 mins	4 mins	6 mins	30 mins
Propofol	0.71±0.02	0.91±0.01	1.70±0.04 **	1.55±0.03 **	1.33±0.04 **	1.21±0.02 **	1.13±0.02 **
Thiopentone	0.83±0.02	0.79±0.03	1.61±0.09 **	1.42±0.07 **	1.28±0.04 **	1.21±0.07 **	0.90±0.03
Methohexitone	0.60±0.02	0.65±0.02	1.22±0.03 **	1.16±0.04 **	0.76±0.06	0.63±0.03	0.63±0.02

$p<0.01$ (**) denotes that group is significantly different to own control. (ANOVA and Dunnets test).

These data suggest that propofol. thiopentone and methohexitone are anticonvulsant in bicuculline-induced convulsions. In addition the anticonvulsant action of propofol is still evident when mice are fully recovered from anaesthesia.

The financial support of ICI is gratefully acknowledged.

Concas A., Santoro G., Mascia M. P., Serra M., Sanna E., Biggio G., (1990). *Journal Neurochemistry*, **55**, 2135-8.
Lowson S., Gent J. P., Goodchild C. S., (1990). *British Journal Anaesthesia*, **64**, 59-63.
Lowson S., Gent J. P., Goodchild C. S., (1991). *British Journal Pharmacology*, **102**, 879-882.

292P DIAZEPAM TREATMENT MODULATES mRNA FOR GAD AND CCK IN SEVERAL RAT BRAIN REGIONS

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We examined mRNA levels of GAD (glutamate decarboxylase) and CCK (cholecystokinin) in several brain regions in 4 groups of 6 male hooded Lister rats: control; acute diazepam (DZ 2 mg/kg); chronic diazepam (2 mg/kg/day for 21 days); diazepam withdrawal (24h after last of 21 daily injections). Thirty mins after the last injection, animals were killed by cervical dislocation, brains removed and rapidly frozen. 25μm sections were cut onto subbed microscope slides, fixed in formaldehyde, acetylated, delipidated and dehydrated. Two oligonucleotides, one complementary to rat GAD (TCC TTG TGA GGT TCC AGG TGA CTG AGT TGG CCC TTT CTAT), the other to rat preprocholecystokinin (CCA GCC CAT GTA GTC CGG TCA CTT ATC CTG TGG CTAG) were radiolabelled with ^{35}S-dATP to a specific activity of 3000 Ci/mmol and used at a concentration of 2nM. After overnight hybridization at 37°C, sections were washed for 1h in 165 mM Na^+ at 55°C to remove non-specific binding. They were placed against x-ray film with autoradiographic standards for 7 days. Relative optical densities were measured using an image analysis system. The results were analysed with a 2x2 ANOVA, and Table 1 shows the regions for which there was a significant acute x chronic treatment interaction ($p<0.05$). There were no significant changes in either message in hippocampal regions CA_2-CA_4, no change in mRNA for GAD in the molecular layer of the hippocampus (SLM) or in mRNA for CCK in the ventral tegmentum or thalamus.

Table 1 Mean (±sem) mRNA levels of GAD and CCK (* p<0.05, ** p<0.01 vs control, $^+$ p<0.05 vs acute DZ, Duncan's tests)

GAD	Control	Acute DZ	Chronic DZ	Withdrawal	CCK	Control	Acute DZ	Chronic DZ	Withdrawal
CA_1	2.6 ±0.8	4.6 ±0.9	1.6$^+$ ±0.4	3.2 ±0.6	CA_1	11.4 ±1.0	14.4* ±0.9	12.0 ±0.7	13.5 ±0.5
Dentate	3.3 ±0.7	5.4 ±1.0	2.9 ±0.7	4.5 ±0.7	SLM	8.3 ±0.4	11.1* ±0.5	8.5 ±0.6	10.2* ±0.9
Reticular Thalamus	8.4 ±0.5	10.5 ±1.0	8.0$^+$ ±0.7	9.9 ±0.7	Cortex (inner)	7.8 ±0.7	11.1** ±0.6	9.1 ±0.5	10.6* ±0.8
Cerebral Cortex	1.8 ±1.0	2.7 ±1.3	0.6$^+$ ±0.3	3.3 ±0.8	Cortex (outer)	8.2 ±0.6	12.1** ±1.1	9.2 ±0.8	11.4* ±0.9

The only individual groups to differ significantly for GAD were the acute and chronic diazepam treatments, the acute treatment raising and the chronic lowering, the levels. The withdrawal group also had elevated levels, but these were not significantly different from

293P MODULATION OF BENZODIAZEPINE BINDING BY γ_2 SUBUNIT ANTIBODIES

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The $GABA_A$/chloride channel is a multimeric protein complex which can be allosterically modulated by a variety of compounds including the benzodiazepines. A number of subunits and isoforms have been identified (α1-6, β1-4, γ1-3, δ and ρ) by molecular biological approaches but the functional role of many of these is at present unknown. We have investigated the role of the γ2 subunit by studying the effect of an antibody raised against this subunit on radioligand binding to various modulatory sites on the $GABA_A$ receptor complex.

A peptide ($\gamma 2_{1\text{-}16}$) corresponding to the N-terminal amino acid sequence of the rat γ2 subunit (Shivers et al., 1989) was synthesised and then coupled to keyhole limpet haemocyanin for antibody production. Antibodies were raised in New Zealand white rabbits, with the production of the immune response being monitored by enzyme-linked immunoadsorbent assay (ELISA) with the synthetic peptide as the antigen. The polyclonal antibodies in serum and a control rabbit serum were investigated for their effects on the binding of [^{35}S]-TBPS (NEN Du Pont; Gee 1988), [^{3}H]-flunitrazepam (Amersham; Martin & Doble 1983) and [^{3}H]-muscimol (Amersham; Peters et al. 1988) to extensively washed rat cortical/hippocampal membranes.

The γ2 subunit antibody did not significantly affect [^{3}H]-muscimol binding over a wide range of serum dilutions (10 to 300,000 fold). In contrast, the γ2 antibody serum potentiated [^{35}S]-TBPS binding at high concentrations. However, since this was also observed with the control serum, this effect could not be attributed to the γ2 antibody. [^{3}H]-Flunitrazepam binding was inhibited in a concentration dependent manner by the γ2 antibody with 50% inhibition occuring at approximately a 1000 fold serum dilution (whilst control serum had no effect). Scatchard analysis of saturation binding data for [^{3}H]-flunitrazepam (using a 1000 fold serum dilution) revealed a significant decrease in binding affinity (Kd 1.45 $\pm$ 0.21 and 3.35 $\pm$ 0.23nM for control and γ2 antibody respectively, mean $\pm$ s.e.mean [n=3], $p<0.005$ Student's t-test compared to control) with no change in Bmax (1.37 $\pm$ 0.04 and 1.37 $\pm$ 0.06 pmoles/mg protein for control and γ2 antibody respectively, mean $\pm$ s.e.mean [n=3]).

In conclusion the γ2 subunit antibody selectively inhibits benzodiazepine binding to the $GABA_A$/chloride channel complex by reducing the affinity. This extends the finding of Stephenson et al. (1990) that the γ2 subunit is important in benzodiazepine binding.

Gee, K.W. (1988) Mol. Neurobiol. 2(4), 291-317.
Martin, I.L. & Doble, A. (1983) J. Neurochem. 40(6), 1613-1619.
Peters, J.A., Kirkness, E.F., Callachan, H., Lambert, J.J. & Turner, A.J. (1988) Br. J. Pharmacol. 94, 1257-1269.
Shivers, B.D., Killisch, I., Sprengel, R., Sontheimer, H., Kohler, M., Schofield, P.R. & Seeburg, P.H. (1989) Neuron 3, 327-337.
Stephenson, F.A., Duggan, M.J. & Pollard, S. (1990) J. Biol. Chem. 265(34), 21160-21165.

294P EXPRESSION AND PROPERTIES OF RECOMBINANT $\alpha_1\beta_2\gamma_2$ AND $\alpha_5\beta_2\gamma_2$ FORMS OF THE $GABA_A$ RECEPTOR

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Studies on recombinantly-expressed $GABA_A$ receptors have revealed that an $\alpha_x\beta_x\gamma_x$ combination (where x represents any isomeric subunit form) represents a minimal requirement for conventional $GABA_A$ receptor pharmacology (i.e. allosteric modulation by omega (ω (BZ)) site drugs, steroids and barbiturates; Burt and Kamatchi, 1991). For the present report we have specifically studied, using ^{3}H-flumazenil as a probe, the interaction of ω (BZ) modulators with 2 different recombinant forms of the rat $GABA_A$ receptor, namely $\alpha_1\beta_2\gamma_2$ and $\alpha_5\beta_2\gamma_2$, that were transiently expressed in human embryonic kidney 293 cells.

cDNAs encoding the α_1, α_5, β_2 and γ_2-subunits of rat $GABA_A$ receptor were combined either in $\alpha_1\beta_2\gamma_2$ or $\alpha_5\beta_2\gamma_2$ plasmid recombinations to transfect exponentially-growing adenovirus-transformed human embryonic kidney 293 cells (Pritchett and Seeburg, 1990). Transfected cells were harvested 48 h after addition of DNA. ^{3}H-Flumazenil binding to membrane preparations of transfected cells was performed as described by Pritchett and Seeburg, 1990).

Equilibrium saturation binding of ^{3}H-flumazenil to either the $\alpha_1\beta_2\gamma_2$ or $\alpha_5\beta_2\gamma_2$ constructs revealed that in each case the binding occurred to a single class of high-affinity binding sites (K_d values of 0.59 and 0.50 nM for α_1 and α_5 constructs, respectively). The pharmacological profile of ^{3}H-flumazenil binding to each construct was evaluated in equilibrium competition inhibition experiments using a number of selected ω (BZ) modulatory site drugs. The potencies of diazepam, zopiclone, triazolam, bretazenil and flunitrazepam to inhibit ^{3}H-flumazenil binding to the α_1-construct as opposed to the α_5-construct did not differ significantly. However, the imidazopyridines, alpidem and zolpidem, exhibited pronounced selectivity for the α_1-recombinant form (α_5/α_1 IC_{50} value ratios of >200 and >580 for zolpidem and alpidem, respectively). In addition, the potencies of various drugs to inhibit ^{3}H-flumazenil binding to the α_1-construct in the presence and absence of 10 μM GABA revealed distinct differences. In particular, GABA increased 4-5 fold the potencies of the full ω (BZ) agonists diazepam, zolpidem and flunitrazepam to inhibit ^{3}H-flumazenil binding but decreased by a factor of 2.5 the potency of the negative allosteric ω (BZ) modulator, the β-carboline DMCM.

In conclusion, the pharmacological profile of ^{3}H-flumazenil binding in the absence and presence of GABA to predetermined hetero-oligomeric recombinant $GABA_A$ receptors offers an attractive model to evaluate receptor subtype affinity for ω (BZ) modulatory ligands as well as to determine the intrinsic activities of these ligands.

Pritchett, D.B. & Seeburg, P.H. (1990) *J. Neurochem.* **54**, 1802-1804.
Burt, D.R. & Kamatchi, G.L. (1991) *FASEB J.* **5**, 2916-2923.

295P [^{3}H]FLUMAZENIL MAY LABEL THE NATIVE α_2-CONTAINING ω(BZ) RECEPTOR SUBTYPE IN THE NEONATAL RAT CEREBRAL CORTEX

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The imidazopyridine and $BZ_1(\omega_1)$ selective ligand zolpidem reveals a heterogeneity in [^{3}H]flunitrazepam binding to cerebral cortical membranes from the adult as well as the neonatal rat (Ruano et al. 1992). While this heterogeneity corresponds to the $BZ_1(\omega_1)$ and $BZ_2(\omega_2)$ receptor subtypes in the adult animal, this question remains to be studied for the neonatal brain. We studied the identity of BZ(ω) receptor subtypes in the neonatal rat cerebral cortex using [^{3}H]flumazenil, a radioligand that presents the advantage of being insensitive to GABA.

Well washed membranes obtained from the cerebral cortex of the neonatal Sprague-Dawley rat (IFFA CREDO, FRANCE) were incubated in 50 mM Tris-HCl (pH = 7.4) for 45 min at 0° C with 0.9 nM [^{3}H]flumazenil (87 Ci/mmol, NEN). Non-specific binding was determined in the presence of 1 µM flunitrazepam.

[^{3}H]Flumazenil binds with high affinity (Kd = 0.82 ± 0.05 nM, Bmax = 198 ± 10 fmol/mg protein, n=3) to cerebral cortical membranes from the 4 day-old rat. Specific [^{3}H]flumazenil binding was displaced with Hill slopes close to unity, by flumazenil, flunitrazepam and oxoquazepam. Zolpidem, alpidem, CL 218872 and βCCE displacement curves were significantly (p) better described by a 2-site model (table 1) with a majority of sites being recognized with high affinity (% R_h).

Table 1 Pharmacological profile of [^{3}H]flumazenil binding to cerebral cortex membranes from the 4 day-old rat

Drug	[^{3}H]flumazenil					[^{3}H]flumazenil + 2 µM zolpidem		
	Ki_h (nM)	Ki_l (nM)	% R_h	n	p	Ki (nM)	nH	n
flumazenil	1.54 ± 0.10	-	-	3	-	1.68 ± 0.18	0.84 ± 0.03	3
flunitrazepam	3.59 ± 0.21	-	-	4	-	5.43 ± 0.85	0.90 ± 0.05	3
oxoquazepam	190 ± 17	-	-	3	-	362 ± 22	1.07 ± 0.10	3
βCCE	2.30 ± 0.41	67.7 ± 12.5	61.1 ± 4.5	3	< 0.01	51.3 ± 0.1	0.91 ± 0.08	3
CL 218872	420 ± 60	13600 ± 5780	68.8 ± 3.4	3	< 0.05	1210 ± 15	0.88 ± 0.05	3
alpidem	202 ± 79	27600 ± 18700	54.0 ± 4.8	3	< 0.01	8890 ± 2460	0.85 ± 0.05	3
zolpidem	128 ± 23	11800 ± 1050	58.7 ± 3.3	3	< 0.01	8190 ± 1430	0.90 ± 0.01	3

In the presence of 2 µM zolpidem to mask [^{3}H]flumazenil binding sites recognized with high affinity by zolpidem, [^{3}H]flumazenil binding (Kd = 0.88 ± 0.06 nM, Bmax = 93 ± 9 fmol/mg protein, n = 3) was displaced with a pharmacological profile (table 1) similar to that described using [^{3}H]flumazenil binding to the BZ(ω) receptor subtype obtained by coexpression of the α_5 β_3 γ_2 subunits of the $GABA_A$ receptor complex (Pritchett & Seeburg, 1990).

In conclusion, the present study shows that in membranes from the neonatal rat cerebral cortex [^{3}H]flumazenil labels a heterogenous population of binding sites as previously demonstrated by autoradiographic studies (Benavides et al., 1992) or using [^{3}H]flunitrazepam as the radioligand (Ruano et al., 1992). In the presence of 2 µM zolpidem, [^{3}H]flumazenil labels a homogenous population of sites that may represent the native α_5-containing BZ(ω) receptor subtype.

Ruano, D., Vizuete, M., Cano, J., Machado, A. & Vitorica, J. (1992) J. Neurochem. 58, 485-493
Pritchett, D.B. & Seeburg, P.H. (1990) J. Neurochem. 54, 1802-1804
Benavides, J., Peny, B., Ruano, D., Vitorica, J. & Scatton, B. (1992) Brain Res. in press

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296P EFFECTS OF LORECLEZOLE ON CONDITIONED SUPPRESSION OF LICKING IN THE RAT

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Loreclezole is a novel 'broad spectrum anticonvulsant' which inhibits seizure spread and increases seizure threshold. The anticonvulsant effect of loreclezole is unusual since it shows a differential sensitivity to the competitive benzodiazepine receptor antagonists flumazenil and CGS 8216 suggesting that interacts in a unique way with the benzodiazepine receptor (Wauquier *et al.*, 1990). In the present report the potential anxiolytic properties of loreclezole are described.

Thirsty male hooded-Lister rats (Bantin and Kingman, Hull, U.K.) were trained on a conditioned suppression of licking task. On days 1, 2, 4, 6 and 7 of the experiment the animals were placed in dark operant chambers for 30 minutes and allowed to drink freely for water rewards. They were rested on the day 3. On day 5, the conditioning day, the animals were placed the chambers for one hour and allowed to drink freely. Three times during this session the houselight in the operant chamber ('conditioning stimulus' or CS) was illuminated for 60s and the termination of each of these presentations was paired with the delivery of a 0.4mA footshock for 1s. As a consequence lick rates declined rapidly on presentation of the light, in the absence of shocks, on day 8. This suppression was quantified using the equation: Suppression ratio=CS rate/(CS rate + Pre CS rate) where CS rate=number of licks during the light period, and Pre CS rate=number of licks during the one minute before the light was illuminated. On day 8, the animals (n=8-10 per group) were administered loreclezole (30-50 mg/kg, i.p.) or drug vehicle (0.5% methocell-0.2% Tween 80) and 30 minutes later placed in the test boxes. The benzodiazepine receptor partial agonist FG 8205 (10mg/kg, p.o.) was included as a positive control. In a second experiment flumazenil (10mg/kg i.p.) was given 15 minutes after either FG 8205 (10mg/kg p.o.) or loreclezole (50mg/kg i.p.) and 15 minutes before the start of the test session.

The suppression ratios following loreclezole and FG 8205 (mean ±s.e. mean, 0.53 ±0.07, and 0.41 ±0.07 respectively) were significantly higher than that of the vehicle treated control group (mean ±s.e.mean, 0.07 ± 0.04) suggesting that loreclezole and FG 8205 have an anxiolytic- like action ($p<0.05$ *post hoc* Newman Keuls tests). Flumazenil completely reversed the increase in the suppression ratio induced by FG 8205 (mean ±s.e. mean, FG 8205 alone= 0.47, ±0.09; FG 8205 + flumazenil = 0.01 ±0.01) but was without effect on the increase induced by loreclezole (mean ±s.e. mean, loreclezole alone= 0.38±0.12; loreclezole + flumazenil = 0.51 ±0.14).

Thus in addition to its anticonvulsant profile, loreclezole has anxiolytic-like effects that are resistant to blockade by flumazenil. It remains to be seen whether these effects are similarly mediated via an interaction with the GABA-A/benzodiazepine receptor complex.

Wauquier, A. Fransen, J. Melis, W. *et al.* (1990) *Drug Development Research* **19**, 375-392.

297P A POSSIBLE ROLE FOR $GABA_B$ RECEPTORS IN ETHANOL WITHDRAWAL HYPEREXCITABILITY

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Chronic ethanol intake causes an increase in hyperexcitability upon withdrawal. Baclofen inhibited hyperexcitability caused by epileptiform activity in the hippocampal slice without affecting normal synaptic transmission (Swartzwelder *et al.*, 1986). We have examined the effects of baclofen on ethanol withdrawal-induced hyperexcitability *in vitro* .

Male C57 mice (30-40g) received 24% ethanol v/v (10-14 g/kg/day) for 18 weeks. In extracellular recordings from hippocampal slices, area CA1, with stimulation of the Schaffer collateral commissural pathway, the population spike thresholds and EPSP slopes were measured every 15 minutes, and input/output measurements every hour, for 7 hours following withdrawal. In two separate experiments, (±)baclofen, 250nM, 500nM (experiment 1) or 2μM (experiment 2), was present throughout recording. Results over several time intervals were analysed using parametric or nonparametric (as appropriate) 2-way analysis of variance.

Slices from ethanol-treated animals showed decreases in single and multiple spike thresholds compared with controls ($p<0.05$, 4-7 h) 500nM baclofen significantly reduced single ($p<0.05$) and multiple ($p<0.001$) spike thresholds in control slices (5-7h) but not in ethanol-withdrawn slices, while 2μM baclofen increased single spike thresholds ($p<0.05$) and decreased multiple spike thresholds ($p<0.05$) in slices from ethanol-treated animals (2-7h), with no significant effect on controls. 250nM baclofen decreased multiple spike thresholds in control slices ($p<0.001$). The input/output curves showed a shift to the left for ethanol-withdrawn slices compared with controls ($p<0.05$, 3-7h, for stimulation, S_{50}, to produce half-maximal responses); 500nM baclofen caused a shift to the left in the curves from control slices (S_{50}'s, $p<0.01$ 2-7h) but no change was observed in ethanol-withdrawn slices. The 2μM baclofen curve was shifted to the right after ethanol treatment (S_{50}'s, $p<0.005$, 2-7h). Results below are at time-point 7h, mean ± s.e.m., $n \geq 5$ (Table 1).

Table 1.

CONTROLS	ACSF (Exp.1)	250nM Baclofen	500nM Baclofen	ACSF (Exp. 2)	2μM Baclofen
Spike threshold - single	18.5 ± 1.4	15.0 ± 1.0	13.6 ± 1.61	14.2 ± 1.0	20.2 ± 2.8
(μA x10) - multiple	>100	87.0 ± 13.0	66.6 ± 20.42	>100	>100
EPSP slope (V/s)	0.88 ± 0.33	0.91 ± 0.18	0.76 ± 0.30	0.66 ± 0.3	1.22 ± 0.33
ETHANOLS					
Spike threshold - single	14.7 ± 1.33	15.0 ± 2.3	15.6 ± 1.8	13.3 ± 0.7	20.0 ± 2.4
(μA x10) - multiple	68.5 ± 13.1	83.8 ± 16.2	56.38 ± 25.19	85.3 ± 13.4	60.0 ± 9.0
EPSP slope (V/s)	0.79 ± 0.1	0.98 ± 0.29	0.90 ± 0.38	1.08 ± 0.26	1.07 ± 0.14

The selective reduction in hyperexcitability by 2μM baclofen in slices from ethanol-treated animals suggests a possible role for the $GABA_B$ receptor in some of the changes associated with ethanol withdrawal hyperexcitability.

Swartzwelder, S., Bragdon, A.C., Sutch, C.P., Ault, B. and Wilson, W.A. 1986 J. Pharm. Exp. Ther., 237, 881-887.

298P EFFECT OF S12024-2 ON THE PERFORMANCE OF MARMOSETS IN AN OBJECT DISCRIMINATION REVERSAL TASK

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S12024-2 (R.S methyl-1(morpholinyl-2-methoxy)-8 tetrahydro-1,2,3,4 quinoline methane sulphonate) is a novel compound being investigated for cognitive enhancing potential. The following studies were designed to investigate the ability of S12024-2 to modify performance of marmosets in an object discrimination reversal paradigm.

The studies used adult marmosets (325-370g, 5 male and 1 female). All animals had received extensive training in a Wisconsin General Test Apparatus (WGTA) and were experienced in object discrimination reversal learning. Throughout testing animals were presented with a series of trials in the WGTA in which the screen was raised to reveal two plastic junk objects covering two food wells. The task for the marmoset was to select the food-rewarded object, the left/right position of which was altered throughout each test session according to a pseudorandom schedule (Gellerman, 1933). The intertrial interval was 15s and each trial lasted until the marmoset made a response. On completing 6 consecutive correct responses on the first object (initial discrimination task) the reward paradigm was changed such that the marmoset was required to select the second, previously unrewarded object (reversal task) to the same criterion. Drug treatment (daily over a 5 day period) was carried out according to a blind crossover design with vehicle, animals being randomly assigned to treatment groups.

S12024-2 (4-16mg/kg orally daily) significantly reduced the mean number of trials required by animals to reach criterion on the initial discrimination task, whilst a wider dose range (1-16mg/kg orally daily) was effective to improve performance of the reversal task. For example trials to criterion were reduced after treatment with S12024-2 in comparison with vehicle treatment for the initial discrimination task from 11.69±0.61 to 7.70±0.91 (4mg/kg) and 11.96±0.63 to 9.03±0.69 (16mg/kg) and for the reversal task from 15.76±0.51 to 12.90±0.64 (1mg/kg), 15.34±0.48 to 12.03±0.64 (4mg/kg) and 16.11±0.70 to 10.43±0.77. The ability of S12024-2 to improve the performance of marmosets was also measured on an additional parameter of mean task errors where reductions in the number of errors made by the animals before reaching criterion were observed for the reversal task. Thus, mean task errors were reduced after treatment with S12024-2 in comparison with vehicle from 6.31±0.26 to 5.20±0.34 (1mg/kg), 6.0±0.23 to 5.00±0.33 (4mg/kg) and 6.75±0.29 to 4.77±0.39 (16mg/kg). Differences significant at $P<0.05$ level (paired t test).

These data indicate that S12024-2 is able to improve the performance of marmosets in an object discrimination reversal learning paradigm and this may provide evidence for a cognitive enhancing potential of this compound.

Gellerman, L.W. (1933) *J. Genet. Psychol.*, 42, 206-208

299P LACK OF EFFECT OF THE K^+ CHANNEL OPENERS LEVCROMAKALIM (BRL 38227) OR PINACIDIL ON ^{86}Rb EFFLUX OR [^{3}H]-GABA RELEASE FROM BRAIN SLICES

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The pharmacological characteristics of neuronal K^+ channels activated by K^+ channel openers (KCOs) such as levcromakalim (BRL 38227) and pinacidil are not clear. Most studies in which CNS effects of such drugs were observed have used very high concentrations (100-500μM) of KCOs (Hausser et al, 1991; Politi & Rogawski, 1991). However, it has been reported that in substantia nigra BRL 38227 can potently (EC_{50} 10nM) stimulate ^{86}Rb efflux and inhibit ^{3}H-GABA release, with these effects being reversible by glibenclamide (Schmid-Antomarchi et al 1990). To find out whether KCOs have similar actions in other brain areas, we have investigated the effects of BRL 38227 and pinacidil on ^{86}Rb efflux from rat cortical and nigral slices, and on ^{3}H-GABA release from cortical and hippocampal slices.

Cross-chopped (350x350μm) slices of rat cortex or hippocampus or 700μm slices of substantia nigra were labelled by incubation with either ^{86}Rb or ^{3}H-GABA. They were then transfered to a multiwell box and ^{86}Rb efflux or ^{3}H-GABA release assessed in a manner similar to that described by Schmid-Antomarchi et al (1990). In some experiments, ^{3}H-GABA release was assessed from superfused slices using a modification of the method of Herdon & Nahorski (1989). Hypoglycaemic conditions (HG) were produced by exposure to buffer without added glucose.

In cortical slices, exposure to HG for up to 60 min had little effect on ^{86}Rb efflux. Neither BRL 38227 (10μM) nor pinacidil (10μM) produced any significant effect on ^{86}Rb efflux under either normal or HG conditions. In nigral slices, ^{86}Rb efflux was doubled under HG conditions, and this effect could be reversed by readdition of glucose. However, BRL 38227 (10 but not 3μM) produced only a small (16±7%, n=6; results are means±s.e.) increase in ^{86}Rb efflux, whilst pinacidil (20μM) had no significant effect. In hippocampal slices, BRL 38227 (100μM) caused only a small inhibition (21±7%, n=7) of ^{3}H-GABA release evoked by K^+ (25mM, 2 min), whilst pinacidil (100μM) had no significant effect. Exposure to HG for 30 min produced a 46±3% increase in basal ^{3}H-GABA release (n=20). Glibenclamide had no effect under normal conditions, but produced a concentration-dependent potentiation of the HG-induced increase in release (to 87±5% at 30μM, n=20). However, BRL 38227 (30 or 100μM) had no significant effect on HG-induced release or its potentiation by glibenclamide. A similar pattern of findings was observed in cortical slices.

Our studies indicate that BRL 38227 or pinacidil have little effect on cortical ^{86}Rb efflux, or on cortical or hippocampal ^{3}H-GABA release under a variety of conditions. One explanation for this could be that ATP-sensitive K^+ channels are present only at low density in these brain areas, although this would be inconsistent with the high levels of sulphonylurea binding in these regions (Mourre et al 1990). However, we were also unable to replicate findings of a large and potent effect of BRL 38227 on ^{86}Rb efflux from substantia nigra (Schmid-Antomarchi et al 1990). Overall, therefore, our results do not provide any evidence in favour of widespread actions of these KCOs in the CNS.

Herdon, H. & Nahorski, S.R. (1989) Naunyn-Schmiedeberg's Arch. Pharmacol. **340**, 36-40.
Hausser, M.A., de Weille, J.R. & Lazdunski, M. (1991) Biochem. Biophys. Res. Commun. **174**, 909-914.
Mourre C., Widmann C. & Lazdunski M. (1990), Brain Res. **519**, 29-43.
Politi, D.M. & Rogawski, M.A. (1991) Mol. Pharmacol. **40**, 308-315.
Schmid-Antomarchi H., Amoroso S., Fosset M. & Lazdunski M. (1990), Proc. Natl. Acad. Sci. **87**, 3489-3492.

300P KS-505a, AN ISOFORM-SELECTIVE INHIBITOR OF CALMODULIN-DEPENDENT CYCLIC NUCLEOTIDE PHOSPHODIESTERASE

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Ca^{2+}-calmodulin-dependent cyclic nucleotide phosphodiesterases (CaM-PDEs) play a critical role in intracellular signaling in that they respond to calcium by decreasing the concentration of cAMP and cGMP. The CaM-PDE family consists of tissue-specific and immunologically distinct isozymes, subtly different in their kinetic and regulatory properties. Among the well-characterized forms are the 59-kDa isozyme isolated from bovine heart and the 61kDa- and 63kDa- isozymes isolated from bovine brain (Beavo & Reifsnyder, 1990). Although a number of compounds are capable of inhibiting CaM-PDE, most of them lack overall selectivity and to date no inhibitor distinguishing between CaM-PDE isozymes has been described.

We found that a new microbial metabolite KS-505a selectively inhibited the CaM-PDEs partially purified from bovine brain (Nakanishi et al.,1992, Kase et al.,1992). In order to test whether KS-505a could differentially recognize the CaM-PDE isozymes, we employed the 61kDa- and 59kDa-isozymes purified from bovine brain cortex and heart, respectively. KS-505a potently inhibited the 61kDa-isozyme with an IC_{50} value of 0.17μM, but required at least 80 times this dose to inhibit the 59kDa-isozyme and other families of phosphodiesterase. KS-505a apparently acted by selectively binding the regulatory domain of the enzyme. The results demonstrate that KS-505a is a highly selective inhibitor distinguishing between members within the CaM-PDE family.

The 61kDa- and 63kDa-isozymes are found predominantly in nerve cells. Immunocytochemical studies in rat brain have shown the CaM-PDEs to be prominent in large pyramidal cells of the neocortex, the hippocampus, and Purkinje cells in the cerebellum, where the isozymes might play an important role in synaptic integration in these neurons(Kincaid et al., 1987). KS-505a showed a potent ameliorative effect on amnesia induced in rodents by electroconvulsive shock. Future studies on the regulation of specific CaM-PDE isozymes by KS-505a may provide clues not only to the role of the isozyme in neuronal function and differentiation but also the potential therapeutic use of the compound.

Beavo, J.A & Reifsnyder, D.H. (1990) Trends Pharmacol. Sci. 11, 150-155
Kase, H., Yoshizaki, R., Ichimura, M., Osawa, K. Nakanishi, S. & Matsuda, Y. (1992) FASEB J. 6, A-1846
Kincaid, R.L., Balaban, C.D., Billingsley, M.L. (1987) Proc. Natl. Acad. Sci. USA 84, 1118-1122
Nakanishi, S., Osawa, K., Saito,Y., Kawamoto, I., Kuroda, K.& Kase, H. (1992) J. Antibiotics 45, 341-347

301P DOSE-RELATED AND OPPOSITE EFFECTS OF HALOTHANE AND LIGNOCAINE ON CYCLIC ADENOSINE 3',5' MONOPHOSPHATE (cAMP) FORMATION IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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Local anaesthetics, e.g. lignocaine are thought to exert their effects by sodium channel blockade (Strichartz and Ritchie, 1987) whereas volatile anaesthetics, e.g. halothane have an unknown site of action. We have examined the effects of both halothane and lignocaine on cAMP formation in SH-SY5Y human neuronal cells, as this second messenger may be important in neurotransmitter secretion.

cAMP was measured in suspensions of intact cells in Krebs/HEPES buffer pH7.4. The incubation cocktail (300μl) contained the phosphodiesterase inhibitor 3-isobutyl-1-methyl xanthine (1mM) and forskolin (10μM) to dierctly activate adenylate cycalse. Lignocaine was added to produce final concentrations of 2, 0.2, 0.02% w/v. Halothane, 1,2 or 3% was delivered to the reaction via a calibrated fluotec 3 vaporiser, with humidified air as the carrier gas; for control incubations humidified air alone was used. After 15 mins reactions were terminated and cAMP extracted as described previously (Brown et al., 1971). Data were analysed using Students t-test.

Table 1: Effects of Halothane and Lignocaine on Forskolin Stimulated cAMP Formation

Halothane % v/v	Effect on Forskolin Stimulated cAMP (% enhancement)	Lignocaine % w/v	Effects of Forskolin Stimulated cAMP (% inhibition)
1	+ 54.8 ± 6.0*	0.02	- 5.9 ± 3.8
2	+ 84.0 ± 4.2*	0.2	- 18.4 ± 6.5*
3	+ 139.7 ± 12.0*	2.0	- 67.0 ± 6.4*

All data are shown as % change from the control (forskolin no lignocaine or forskolin + air) and are expressed as mean ± s.e. mean (n=5-6). *p<0.05 compared with appropriate control.

Halothane (1-3%) produced a significant enhancement of forskolin stimulated cAMP production ($p<0.05$) in contrast, lignocaine (0.2 - 2% w/v) caused a significant inhibition ($p<0.05$). Whilst both halothane and lignocaine at clinical concentrations reduce evoked neurotransmitter secretion (Charlesworth et al., 1992 and Pocock and Richards, 1988), the above results suggest that changes in cAMP concentrations may not mediate this effect in SH-SY5Y cells. However, these findings may still have an important, but as yet unresolved, role to play in the production of anaesthesia.

R. Atcheson is a British Journal of Anaesthesia Research Fellow.

Brown, B.L., Albano, J.D.M., Ekins, R.P., Sgherzi, A.M., (1971) Biochem. J. **121**, 516-552.
Charlesworth, P., Jacobsen, I., Pocock, G., Richards, C.D. (1992) Brit. J. Pharmacol. **106**, 802-812.
Pocock, G. and Richards, C.D. (1988) Brit,. J. Pharmacol. **95**, 209-217.
Strichartz, G.R. and Ritchie, J.M. (1987) Handbook of Experimental Pharmacology, **V81**, 21-52.

302P BLOCK OF THE DELAYED-RECTIFIER POTASSIUM CURRENT IN ISOLATED RAT SYMPATHETIC NEURONS BY IMIPRAMINE AND OTHER TRICYCLIC COMPOUNDS

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Recently, tricyclic antidepressant drugs (TCAs) such as imipramine have been shown to block certain potassium channels in cardiac muscle (e.g. Delpón *et al.* 1992). It is not known, however, whether TCAs have a similar blocking action in neurons. Bennett & Middleton (1975) reported that the TCA, desipramine, can prolong the preterminal action potential of rat sympathetic neurons by a mechanism that does not involve the block of noradrenaline uptake. This action could be explained by a block of K^+ currents and the aim of this study was to test directly for such a block in these neurons.

Sympathetic neurons, isolated from 7-8 day old rats, were voltage-clamped using whole-cell patch-clamp recording and the membrane potential was stepped from -50 mV to +10 mV for 150 ms once every 6 s. This evoked an outward current of 1147 ± 74 pA (n= 41 cells; mean ± s.e.mean, whole-cell capacitance = 17 ± 1 pF) measured as the average current over 25 ms, 116.5 ms following the step. The tail current seen on stepping back to -50 mV represents the slow deactivation of the delayed rectifier current (IK_V) which allows this current to be studied in relative isolation. When the pipette solution contained 10 mM BAPTA to minimise contribution of calcium-activated K^+ currents to the outward current, imipramine (10 µM) inhibited the outward current by 63 ± 5% (n=5) and the IK_V tail current by 79 ± 7%. With 0.1 mM BAPTA in the pipette, 10 µM imipramine only inhibited the outward current by 30 ± 7% (n = 4). Inhibition of the IK_V tail current was unaltered. The block of outward current by imipramine in the concentration range 1 to 100 µM was fitted using the equation: $y = max / (1 + (K_D / x)^S)$. This gave a K_D of 7.0 ± 1.7 µM, a maximum inhibition (max) of 92.0 ± 6.4% and a slope (s) of 1.05 ± 0.19 (n = 21 cells). Block of the IK_V tail current gave a similar K_D of 4.3 ± 1.0 µM suggesting that most of the outward current is carried by IK_V under these conditions.

Amitriptyline (10 µM) was slightly less potent than imipramine, blocking the outward current by 48 ± 4% (n = 11). Tacrine (9-amino-1,2,3,4-tetrahydroacridine) a tricyclic anticholinesterase, lacking the N-atom side chain of the TCAs, also blocked IK_V but was about ten fold less potent; 50 µM tacrine produced a 44 ± 7% (n=3) inhibition. Initial experiments on the transient outward current I_A of rat sympathetic neurons suggest that TCAs are less effective at inhibiting this current than IK_V. It is concluded that TCAs are potent inhibitors of IK_V in rat sympathetic neurons and that this action may explain the prolongation of the preterminal action potential seen with TCAs in sympathetic neurons (Bennett & Middleton 1975).

Bennett, M.R. & Middleton, J. (1975) *Br. J. Pharmacol.* 55, 87-95
Delpón, E., Tamargo, J. & Sánchez-Chapula, J. (1992) *Br. J. Pharmacol.* 106, 464-469

303P DISTRIBUTION OF *trkB* IN RAT BRAIN BY *IN SITU* HYBRIDIZATION

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Brain derived neurotrophic factor (BDNF) is one of a family of neurotrophins, of which nerve growth factor is the best characterized. BDNF has been shown to promote the survival and neurite outgrowth of certain placodal and neural-crest derived sensory neurons. Recently this protein has been found to be highly expressed in CNS tissue, especially within the principal targets of the ascending cholinergic projection system of the forebrain, particularly the hippocampus, and to support the survival of these forebrain cells in culture and increase their cholinergic function. The recent findings that mRNA for BDNF is reduced in Alzheimer's disease (Philips et al., 1991), a neurodegenerative disease characterised by cholinergic dysfunction; and the finding that BDNF protects substantia nigra neurons from MPTP toxicity has led to the suggestion that BDNF may have a therapeutic role in Alzheimer's and Parkinson's diseases. The distribution of the mRNA for high affinity receptor for BDNF, $p145^{trkB}$, was examined in rat brain.

In situ hybridization was carried out on cryostat cut sections (20μm) from three adult rat brains. cDNA probes (1311bp) to the domain coding for the extracellular region of *trkB* were constructed from the gene for rat *trkB* (a gift from Regeneron) by PCR. The sequence was verified by dideoxynucleotide sequencing. Probes were labelled (^{35}S dATP) using the random primer method. Brain sections were hybridised overnight at 42°C with denatured probe in hybridization buffer containing 50% formamide. Slides were washed overnight at room temperature in buffer containing 50% formamide. Sections were dehydrated in ethanol and placed on autoradiography film or dipped in emulsion (Amersham, LM-1).

mRNA for *trkB* was found to be heavily expressed in hippocampus, particularly in the pyramidal cell layer, the primary olfactory cortex, the hypothalamic nuclei and the cerebellar cortex. Other areas found to contain mRNA were the neocortex, cells lining the ventricles and dorsal root ganglia.

We wish to acknowledge the Medical Research Council, the Wellcome Foundation, Bristol Research into Alzheimer's disease and Care of the Elderly (BRACE), Sandoz Foundation for Gerontological Research, the Royal Society and the Bristol Molecular Recognition Centre.

Philips, H.S., Hains, J.M., Armanini, M., Laramee, G.R., Johnson, S.A., Winslow, J.W. (1991) *Neuron* 7, 695-702

304P CONSTRUCTION OF β-NGF/BDNF CHIMERIC GENES AND EXPRESSION OF RECOMBINANT PROTEINS

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One of the most apparent symptoms of Alzheimer's disease (AD) is loss of memory function which has been associated with a reduction in basal forebrain cholinergic function. β-nerve growth factor (β-NGF) acts as a trophic factor for these neurons. Administration of β-NGF has been shown to increase choline acetyltransferase (ChAT) activity of basal forebrain neurons and to prevent their degeneration following fimbria-fornix transections. In addition spatial memory impairments in aged rats have been reversed by infusion of β-NGF. These findings suggest that β-NGF may have a therapeutic role in the treatment of AD.

β-NGF is a member of the neurotrophin family, a group of structurally homologous neurotrophic proteins (brain derived neurotrophic factor, BDNF; neurotrophin-3, NT-3 & neurotrophin-4, NT-4). Comparison of their protein sequences reveals the existence of four variable regions (V1-V4) that show little homology between the four neurotrophins. All of the neurotrophins bind to $p75^{NGFR}$, and each to a specific high affinity tyrosine kinase receptor. β-NGF binds to $p140^{trkA}$ and BDNF binds to the related receptor $p145^{trkB}$.

The human β-NGF gene was cloned by PCR from genomic DNA, the human BDNF gene was cloned from a λgt10 hippocampal cDNA library. Pairs of oligonucleotides were constructed containing BDNF tails corresponding to each of the four variable domains. These were used to produce four mutant protein DNA sequences by PCR homologue mutagenesis, each consisting of β-NGF with one of the four variable domains replaced with the equivalent domain from BDNF. The sequences of the four β-NGF/BDNF chimeric proteins and the wild type genes were verified by dideoxy sequencing and each was subcloned into the baculovirus transfer vector (pEVmXIV) (Wang *et al.*, 1991). These constructs were co-transfected into Sf21 insect cells with linearized baculovirus DNA (Baculogold™, AMS Biotechnology). Positive plaques containing recombinant virus were identified and selected for the generation of high titre viral stocks which were used to transfect spinner flasks of Sf21 cells grown in serum free medium (Excell 401). Recombinant proteins were secreted into the medium and quantified using a two site ELISA. The levels of expression of recombinant proteins were between 65 and 300ng/10^6 cells. These chimeric proteins will be used to understand the structure/activity relationships of the neurotrophins.

We wish to acknowledge the Medical Research Council, the Wellcome Trust, BUPA Medical Foundation, BRACE, the Sandoz Foundation for Gerontological Research, the Royal Society and the Bristol Molecular Recognition Centre.

Wang, X., Guat Ooi, B. & Miller, L.K. (1991) *Gene* 100, 131-137.

305P ISOLATION AND SEQUENCE OF cDNAs ENCODING A NOVEL VOLTAGE-SENSITIVE CALCIUM CHANNEL, α_{1x}, EXPRESSED IN HIPPOCAMPAL NEURONS

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To date the cDNAs of five mammalian Ca^{2+} channel α_1 subunits have been reported, including skeletal muscle (Tanabe et al., 1987) and the four neuronal subunits $\alpha_{1A,B,C,D}$ (Snutch et al., 1990). In addition, some have areas of alternative splicing creating further variability. Whether this constitutes the complete set of such genes is uncertain. Using PCR amplification with degenerate primers designed to amplify a well conserved region in the fourth repeat domain of Ca^{2+} channel α_1 subunits, we have cloned a 320 bp fragment of a putative novel α_1 subunit channel (α_{1X}) from rat brain cDNA. Subsequent screening of a mouse brain cDNA library (Clonetech) with the 320 bp fragment as probe yielded a 1.1 kb fragment which contained a single open reading frame. Further screening yielded several overlapping clones totaling 2 kb. Northern blot analysis of polyA+-enriched mouse brain RNA revealed that this α_1 subunit mRNA is expressed as a single size class of approximately 8 kb. Nucleotide sequence comparisons in the fourth repeat domain show it to be 72% identical to the A type Ca^{2+} channel and 65% identical to the C type. The deduced amino acid sequence shows the IV H5-H6 region to be approximately 85% identical to both the A and B type α_1 subunits. Investigation of mRNA expression patterns in adult mouse brain by *in situ* hybridization using a specific cRNA probe showed abundant expression in hippocampal neurons, a diffuse pattern in the visual cortex, caudate, putamen, superior and inferior colliculi, and medulla and a lower level of staining in the cerebellum. This cDNA has also been found in rat superior cervical ganglion neurons in culture, using PCR amplification of cDNA with primers designed specifically for this channel. Investigations to date have not revealed the α_{1X} transcript in PC-12 cells (differentiated and undifferentiated), NG108-15 cells, nor IMR32 cells. It is possible that this α_{1x} subunit is responsible for some dihydropyridine and ω-conotoxin GVIA resistant Ca^{2+} channel activity found in several neuronal types including hippocampal pyramidal cells (Regan et al., 1991) and sympathetic neurons (Elmslie et al., 1992).

Elmslie, K.S., et al. (1991) *J. Physiol.* **456**, 107-123.
Regen, L.J., et al. (1991) *Neuron* **6**, 269-280.
Snutch, T.P., et al. (1990) *Proc.Natl.Acad.Sci. U.S.A.* **87**, 3391-3395.
Tanabe, T. et al. *Nature* **328**, 313-318 (1987).

306P COMPETITIVE INHIBITION BY CAPSAZEPINE OF [^{3}H]RESINIFERATOXIN BINDING TO THE VANILLOID RECEPTOR

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Capsazepine, a synthetic analogue of capsaicin, has been found to block capsaicin-induced responses *in vitro* with Schild plots suggesting a competitive mechanism of action (Bevan *et al.*, 1992).

Specific binding of [^{3}H]resiniferatoxin (RTX), an ultrapotent analog of capsaicin, is thought to represent the vanilloid (capsaicin) receptor (Szallasi & Blumberg, 1990). In the present report, we have used this binding assay to explore the inhibitory mechanism of capsazepine at the receptor level.

In competition assays, capsazepine inhibited [^{3}H]RTX (25pM) binding by dorsal root ganglion (DRG) and spinal cord membranes of the rat with K_i values of 3.5 μM and 4.0 μM, respectively. In experiments in which the concentration of [^{3}H]RTX was varied, 10 μM capsazepine reduced the affinity of the vanilloid receptor expressed by rat DRG and spinal cord membranes for [^{3}H]RTX from 13 pM to 40 pM, and from 20 pM to 80 pM, respectively, without a measurable change in B_{max} or in Hill number; these shifts in affinity yield K_i values of 5.0 μM and 3.3 μM for DRG and spinal cord membranes, respectively. In parallel experiments, capsaicin inhibited [^{3}H]RTX binding with a 7-10-fold higher potency (the K_i values were 0.5 μM for DRG and 0.4 μM for spinal cord membranes, respectively).

We conclude that (1) the inhibition by capsazepine of specific [^{3}H]RTX binding to the vanilloid receptor is consistent with a competitive mechanism of action, and (2) in contrast to functional studies where capsazepine is similar to capsaicin in potency, capsazepine inhibits RTX binding to the vanilloid receptor with 7-10-fold lower potency. The dissimilar potencies of capsazepine relative to capsaicin in receptor binding and in functional studies might indicate that capsazepine acts on multiple targets as already suggested by Maggi's group (Santicioli *et al.*, 1992).

Bevan S. *et al.* (1992) *Br. J. Pharmacol.* 107, 544-552.
Santicioli P. *et al.* (1992) *Proc. Br. Pharm. Soc.* P136.
Szallasi A. & Blumberg PM. (1990) *Brain Res.* 524, 106-111

307P RESINIFERATOXIN CAUSES TRANSLOCATION OF PROTEIN KINASE C IN DORSAL ROOT GANGLION NEURONS FROM NEONATAL RAT

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The daphnane derivative resiniferatoxin (RTX) is a potent irritant and inflammatory agent. It has structural analogy with other irritants and inflammatory compounds including phorbol esters which exert their effects by activating protein kinase C (PKC). RTX lacks the free OH group at the carbon 20 position, a feature of the most potent phorbol esters, and is thought to be a relatively weak PKC activator. It has been suggested, however, that RTX may activate only certain isoforms of PKC (Ryves *et al.*, 1991). RTX also shows structural similarity to capsaicin, an irritant which exerts selective excitatory effects on a subset of sensory neurons that includes the polymodal nociceptor population. The excitatory effects of both capsaicin and RTX are thought to be mediated by opening a non-selective cation channel found in the membranes of these neurons (Bevan and Szcolcsanyi, 1990). We have investigated the possibility that RTX might activate PKC in sensory neurons from dorsal root ganglion.

RTX (up to 10μM) did not displace [^{3}H]-phorbol dibutyrate ([^{3}H]-PDBu) binding in either the membrane or cytosolic fractions obtained from adult rat dorsal root ganglia (DRG). It did, however, stimulate a kinase activity in the cytosolic fraction from the DRG at 10μM. In primary cultures of DRG neurons, RTX caused translocation of PKC from the cytosol to the membrane indicating that PKC had been activated. The EC_{50} for this effect was 18nM, the extent of the translocation at maximal concentrations of RTX being 65% of that obtained with PDBu. Capsaicin (1μM) also caused translocation of PKC. The responses to both RTX and capsaicin, but not to PDBu, were greatly reduced in the absence of external calcium. Furthermore, raising intracellular levels of calcium with 50mM KCl resulted in a translocation of PKC that was similar in magnitude to the response to RTX. The calcium ionophore, ionomycin, also caused PKC translocation, but to a lesser extent than RTX.

These results suggest that RTX activates PKC in sensory neurons indirectly by stimulating calcium entry into the cell. Experiments in broken cell preparations suggest, however, that RTX may also have a direct effect on a kinase in the DRG neurons.

Bevan S. & Szcolcsanyi J. (1990) *TiPS* 11, 330-333.
Ryves W.J., Evans A.T., Olivier A.R., Parker P.J. & Evans F.J. (1991) *FEBS Letts* 288 (1), 5-9.

308P SPATIAL CONSTRAINTS IN MONITORING NEUROCHEMICAL HETEROGENEITY IN BRAIN TISSUE

C. Davidson, P. Palij & J.A. Stamford. Anæsthetics Unit, London Hospital Medical College, Alexandra Wing, Royal London Hospital, Whitechapel, London E1 1BB.

Various sampling probes (eg; dialysis fibres, voltammetric microelectrodes & push-pull cannulae) are currently used to study extracellular levels of neurotransmitters or other neurochemicals in the brain extracellular fluid (ECF). The spatial resolution of each method is determined by the volume of tissue sampled by the probe which, in turn, governs the size of structure that may be examined. Many important nuclei are small or have been shown to exhibit regional differences, such as the corpus striatum, nucleus accumbens and hippocampus. In the present study, mathematical modelling was used to investigate the relationship between probe size (volume of tissue sampled) and the ability to delineate small neurochemically distinct structures.

In the model, a small spherical structure of radius 250 μm (r_s) was penetrated by a probe sampling a sphere of radius 5-400 μm (r_p). It was assumed that the *actual* ECF level of neurochemical within and outside the structure was 10 and 1 concentration units respectively. Figure 1A shows how the *measured* concentration varies across the structure (horizontal bar) for the sample probe radii (μm) arrowed. Values of r_p greater than r_s mean that true concentration maxima cannot be recorded and interfacial detail can only be observed at values of $r_p << r_s$.

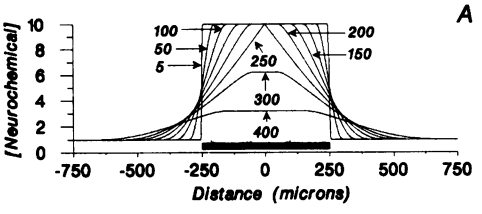
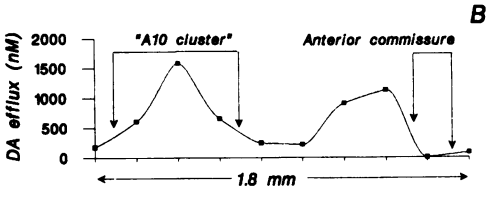


Figure 1B shows an illustrative experiment in a brain slice (+9.2 mm vs interaural line) in which stimulated dopamine (DA) efflux was mapped across the ventral striatum. DA efflux was evoked using trains (20 pulses, 50 Hz) of pulses (0.1 ms duration, 10 mA) applied at 10 min intervals and monitored using fast cyclic voltammetry (FCV) at carbon fibre microelectrodes (4 μm radius) as previously described (Davidson & Stamford, 1992). The graph shows the variation in DA efflux across the substructures of the ventral striatum



The data emphasises the need to use probes with small sampling radii when examining neurochemical events within small or heterogeneous nuclei. The experimental data shown is an example of how the microelectrodes used with FCV can be used to delineate spatial differences in DA efflux within the corpus striatum.

This research was supported by the Royal Society.

Davidson, C. & Stamford, J.A. (1992). *Br. J. Pharmac.*, **107**, 213P.

309P TEMPORAL CONSTRAINTS IN MONITORING RAPID NEUROTRANSMITTER EFFLUX AND UPTAKE EVENTS

P. Palij, C. Davidson & J.A. Stamford. Anæsthetics Unit, London Hospital Medical College, Alexandra Wing, Royal London Hospital, Whitechapel, London E1 1BB.

Various methods are currently used to sample neurotransmitter levels in the brain extracellular space, such as push-pull cannulation, intracerebral dialysis and voltammetry. These methods differ in their temporal resolution. In the present study, mathematical modelling was used to characterise the temporal sampling requirements for monitoring short duration transmitter efflux events.

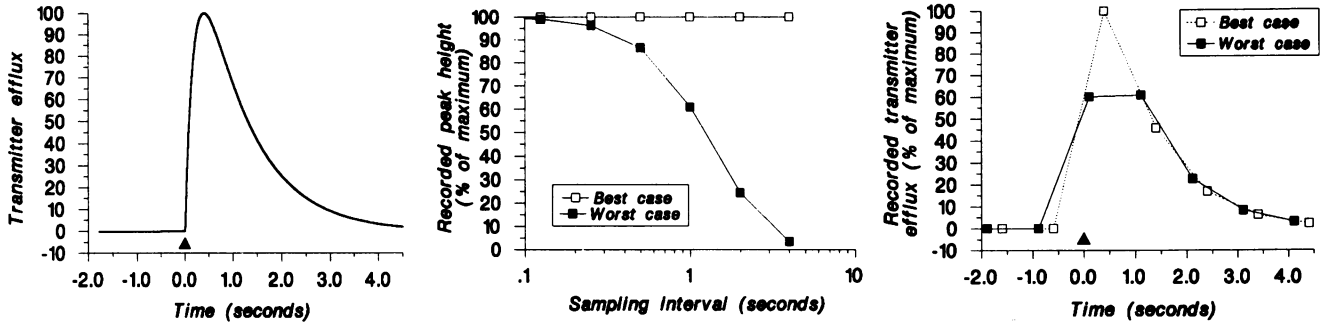


Figure 1 (left panel) shows a model transmitter efflux event following a hypothetical stimulation at t=0.0s, marked by the triangle. The event took 0.4s to reach maximum and had an uptake half-life of 1.3s. The centre panel shows the effect of asynchronous measurements at intervals of 0.125 to 4.0 s on the peak detected transmitter efflux.. The best case occurs with a measurement 0.4s after the stimulus. The worst case is calculated by iteration. With a sampling interval of 4.0s, it is possible to miss the peak entirely, while a sampling interval of 0.25s confers an error range of only 3.7%. The right hand panel shows the best and worst cases when the model event is sampled once per second: Errors of up to 40% in the peak height can be encountered at this rate. The triangle, marking the stimulus, shows also that inaccuracies in the perceived onset time course can be incurred by extrapolation between points. The above model supports previous experimental data (Millar et al, 1992) showing that high and low sampling rates gave different apparent time courses for dopamine efflux. The present study indicates that rapid efflux events should not be sampled at intervals longer than the time to peak.

This research was supported by the Royal Society.

Millar, J. O'Connor, J.J., Trout S.J. & Kruk, Z.L. (1992) *J. Neurosci. Meth.*, 43, 109-18.

310P A COMPUTER SIMULATION FOR USE IN TEACHING BASIC PHARMACOKINETICS TO MEDICAL, DENTAL AND SCIENCE STUDENTS

C.J.Bowmer, D.G.Dewhurst[1] & I.E.Hughes, Dept. of Pharmacology, Worsley Medical & Dental Building, University of Leeds, Leeds LS2 9JT and [1]Faculty of Health and Social Care, Leeds Metropolitan University, Leeds LS1 3HE.

The pharmacokinetic behaviour of a drug in the body can be characterised by a variety of parameters which relate the dose given to the concentration in plasma through various equations. This mathematical basis to the subject tends to discourage student learning of the topic unless they first appreciate the fundamental importance of an understanding of pharmacokinetics to the successful use of drugs in man. The mathematical basis does however allow computer simulations to be constructed which can be used very successfully to illustrate the importance of pharmacokinetics, and to motivate students towards understanding kinetic theory.

The simulation described here is for IBM-compatible PCs (VGA) and is menu driven. Users choose a drug (ampicillin, digoxin, propranolol, phenytoin, diazepam, lignocaine, quinidine, gentamycin or paracetamol) and specify the route, the dose, the period for which plasma concentrations should be followed and, if multiple doses are to be used, the dose interval. Intravenous infusions can also be simulated. The resulting plasma concentrations are then displayed graphically on the screen (scaled automatically) using log/linear or linear/linear axes. Hard copy of these graphic screens is not provided by the program since the traffic generated when the program is used in a network of terminals tends to overload the network and printers but the data can be obtained in numeric form if required.

Various options are available. A loading dose can be used if appropriate. Subjects can be selected who are normal or who have severe kidney or liver failure. An indication of the usual therapeutic level and the level at which unacceptable side effects are likely to be encountered is displayed on the screen. The area under the curve can be obtained automatically using a single key-press; estimates of bioavailability and plasma clearance can therefore be made.

Since the choice of dosage regimen etc. is made by the user, students can use "learn-by-discovery" methods or teachers can provide schedules which lead students through a set of exercises appropriate to the level and type of student and the teaching objectives of the course. Explanatory notes and a set of 9 student-centred exercises is available in machine readable form on the disc with the program and may be used as they stand or modified appropriately. These exercises are designed to illustrate the importance of basic pharmacokinetic concepts using active learning methods. It is also possible for the simulation to be used with screen display projection facilities to illustrate lectures on pharmacokinetics.

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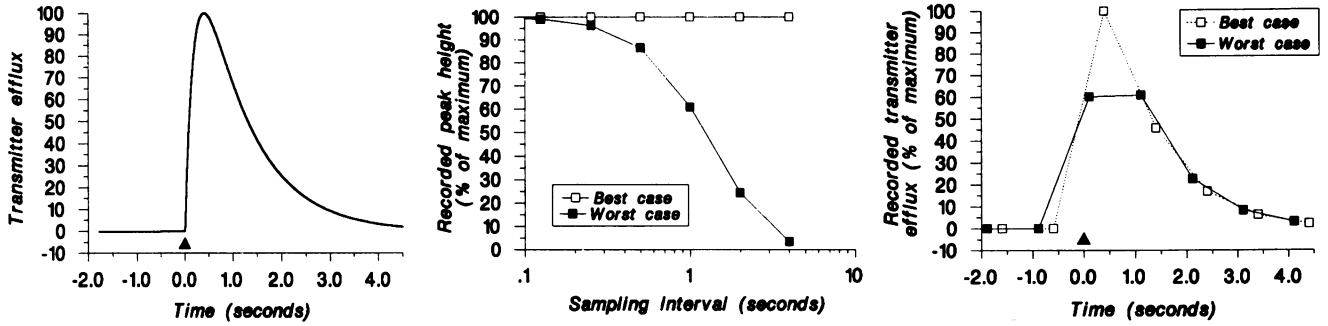


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311P A CAL SYSTEM FOR TEACHING ELEMENTARY DRUG DISPOSITION AND PHARMACOKINETICS

G. D. Ogg & I.H. Stevenson, Dept. of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital, Ninewells, Dundee, DD1 9SY.

With the introduction of new interfaces (Windows) for IBM PC's, the computer literacy required to operate computers is reduced considerably. Another advantage to the graphical interface is that applications can be made more attractive and more inviting to the student. There are several packages using a similar system, 'HYPERCARD' on the Apple Macintosh. The main computing resource adopted in this department, however, is the IBM PC, in the main, due to the cost of hardware. This demonstration will illustrate the progress made using the hypermedia authoring system 'GUIDE' in the field of teaching drug disposition and pharmacokinetics to undergraduate classes. One earlier disadvantage this system had was that of the purchase of a 'GUIDE' Reader program for each machine in operation. The most recent release of the program (ver. 3.1), however, allows the distribution of 'royalty free' reader software with the 'Guide line'.

The subject area is one where any help to the student in extending understanding in this, generally difficult, physico-chemical and mathematical area of pharmacology, is thought to be extremely useful. In order to achieve a comprehensive introduction and explanation of the topics introduced it had to be extensively illustrated and interactive in order to capture the students' imagination. The use of graphical, textual expansions and the liberal use of definitions and notes allows the inquisitive student to find out more about their chosen topic.

The package gives a very simple introduction to pharmacokinetics and gradually develops the concepts until a full explanation is achieved. Also included is a series of worked problems which illustrate ways of tackling pharmacokinetic problems and interpretation of the conclusions obtained, thus illustrating the, 'why' as well as the 'how'. The package is a self-contained teaching module and could be used without any lectures being given, it is acknowledged, however, that staff-student interaction is an important part of any students learning process. The lecture-time allocation could, perhaps, be used more productively in tutorial sessions where individual problems may be discussed. Inherent in the hypertext philosophy is the ability to jump to topics in which the student is either interested in or requires further tuition. The package thus allows students the opportunity to learn at their own pace within a university setting or elsewhere for distance learning.

312P MODULATION OF K-CURRENTS BY P1060 AND APRIKALIM IN RAT PORTAL VEIN: INHIBITION BY GLIBENCLAMIDE AND PHENTOLAMINE

T. Ibbotson, G. Edwards & A.H. Weston. Smooth Muscle Research Group, Department of Physiological Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT

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313P EFFECTS OF GLIBENCLAMIDE DURING FATIGUING STIMULATION IN SINGLE MOUSE SKELETAL MUSCLE FIBRES

S. Duty & D.G. Allen, Department of Physiology, University of Sydney, NSW 2006, Australia (Introduced by A.H. Weston)

314P ADENOSINE A_1 AGONISTS STIMULATE PROTEIN KINASE C IN A SMOOTH MUSCLE CELL LINE

P. Gerwins & B. B. Fredholm, Dept. of Pharmacology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden.

315P THE N-TERMINAL THROMBIN RECEPTOR PEPTIDE SFLLRN, BUT NOT CATALYTICALLY INACTIVE THROMBIN DERIVED AGONISTS, ACTIVATE U937 HUMAN MONOCYTIC CELLS

S.Joseph & J.MacDermot, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN, U.K.

316P COMPARISON OF THE POTENCY OF CALCIUM ANTAGONISTS IN HUMAN CORONARY ARTERY (HCA) AND INTERNAL MAMMARY ARTERY (IMA)

T. Godfraind, C. Dessy and S. Salomone, Laboratoire de Pharmacologie, UCL 7350, Université Catholique de Louvain, Avenue E. Mounier, 73, B-1200 Brussels, Belgium

317P INTESTINAL NEURO-EFFECTOR TRANSMISSION UNDER CONDITIONS OF ANOXIA OR STIMULATED ISCHAEMIA

A.D. Corbett & G.M. Lees, Department of Biomedical Sciences, University of Aberdeen, Marischal College, Aberdeen

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318P SALMON CALCITONIN POTENTLY MIMICS AMYLIN ACTIONS ON ISOLATED SOLEUS MUSCLE AND IN INCREASING PLASMA LACTATE AND GLUCOSE IN ANAESTHETIZED RATS

Timothy J. Rink, Kevin Beaumont & Andrew A. Young, Amylin Pharmaceuticals, Inc. San Diego, CA USA 92121

ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

1P **Brady AJB, Warren JB, Harding SE & Poole-Wilson PA** Effect of nitric oxide in aqueous solution, and 8-bromo-guanosine-3':5'-monophosphate, on contraction of isolated guinea-pig cardiac ventricular myocytes

2P **Frew JD, Paisley K & Martin W** Inhibition of basal but not acetylcholine-stimulated production of nitric oxide in rat aorta by N^G-monomethyl L-arginine

3P **Waldron G, McPherson GA & Garland CJ** Acetylcholine-induced relaxation in the rat isolated mesenteric artery: relative importance of smooth muscle hyperpolarization

4P **Bennett T, Gardiner SM, Kemp PA, Palmer RMJ & Moncada S** Regional haemodynamic responses to N^G,N^G-dimethyl-L-arginine (ADMA) or N^G-monomethyl-L-arginine (L-NMMA) in conscious rats

5P **Gardiner SM, Kemp PA, Bennett T, Palmer RMJ & Moncada S** S-nitroso-N-acetyl-penicillamine (SNAP) abolishes the pressor, but not the vasoconstrictor, effects of N^G,N^G-dimethyl-L-arginine (ADMA) in conscious rats

6P **Rubino A, Goss-Sampson MA, Hoyle CHV & Burnstock G** Impaired endothelium-mediated vasodilatation in aorta of rats with vitamin E deficiency

7P **van Gelderen EM & Saxena PR** Nitric oxide synthesis inhibition and arteriovenous anastomoses shunting in the pig

8P **Berman RS & Martin W** Endotoxin-induced endothelial barrier dysfunction assessed by an *in vitro* model

9P **Szabó Cs, Mitchell JA, Gross SS, Thiemermann C & Vane JR** Nifedipine inhibits the induction of nitric oxide synthase by bacterial lipopolysaccharide *in vitro* and *in vivo*

10P **Ialenti A, Moncada S & Di Rosa M** Modulation of adjuvant arthritis by the L-arginine:NO pathway

11P **Perretti M, Flower RJ & Goulding NJ** Murine leukocytes lose lipocortin 1 binding capacity during acute inflammation

12P **Teixeira MM, Williams TJ & Hellewell PG** Locally-injected E-type prostaglandins enhance oedema formation and neutrophil accumulation but suppress eosinophil accumulation in guinea-pig skin

13P **Foster AP, Lees P & Cunningham FM** Inhibitory effects of the PAF receptor antagonist WEB 2086 on antigen-induced responses in equine allergic skin disease

14P **Lobo IB, de las Heras B & Hoult JRS** Differing profiles of inhibition of various secretory phospholipase A_2 enzymes by 'classic' inhibitors and a novel plant-derived labdane

15P **Charalambous K & Hoult JRS** Ionophore-induced calcium toxicity in rat peritoneal leukocytes: suppression of leukotriene generation but thromboxane is spared

16P **Jones RL** Prostacyclin analogues potentiate contraction of the guinea-pig vas deferens to electrical field stimulation

17P **Nagy I, Pabla R, Dray A, Woolf CJ & Urban L** Bradykinin-sensitive neurons in rat dorsal root ganglion *in vitro* identified by uptake of cobalt ions

18P **Perkins MN & Kelly D** Interleukin 1-β (IL-1β), but not tumour necrosis factor-α (TNFα), can induce bradykinin B_1 receptor-mediated thermal hyperalgesia in the rat

19P **Davies A & Perkins MN** Interleukin 1-β, but not tumour necrosis factor–α, can induce desArg9-bradykinin-mediated mechanical hyperalgesia in the rat

20P **Smith GD, Harrison SM & Birch PJ** Supersensitivity to (±)baclofen in an animal model of chronic neuropathic, but not chronic inflammatory, hyperalgesia

21P **Malcangio M & Bowery NG** Effect of (-) baclofen on substance P-like immunoreactivity release from rat spinal cord

22P **Thompson SWN, Urban L & Dray A** NMDA and tachykinin receptor-mediated contributions to the C-fibre-evoked response in the neonatal rat spinal cord *in vitro* are enhanced following peripheral inflammation

23P **Urban L, Naeem S & Dray A** Effects of neurokinins on excitatory amino acid-induced activation in the neonatal rat spinal cord *in vitro*

24P **Boyle SJ, Manley S, Tang K-W, Meecham K, Guard S, Watling KJ, Woodruff GN & McKnight AT** Affinity of the NK_2 antagonist SR 48968 at NK_3-tachykinin and μ-opioid receptors

25P **Smith AJ, Patel S & Freedman SB** Characterisation of CCK_B receptors in GH3 cells

26P **Hendrie CA & Dourish CT** Effects of cholecystokinin tetrapeptide (CCK-4) and the CCK_B antagonist LY-288513 in a putative model of panic in the mouse

27P **Day HEW, Poat JA, McKnight AT & Hughes J** Cholecystokinin increases c-*fos* expression in the rat nucleus tractus solitarius via CCK_A receptors

28P **Rigo M, Lübbert H, Bruns C, Yasuda K, Bell GI & Hoyer D** Distribution of three putative somatostatin (SRIF) receptor mRNA's in rat brain by *in situ* hybridisation

29P **Caulfield MP, Palazzi E, Lazareno SH, Jones S, Popham A & Birdsall NJM** Lack of significant selectivity of "benzyl 4-DAPine" between four muscarinic receptor subtypes: binding and functional studies

30P **Escriba PV, Sastre M & García-Sevilla JA** Increased density of guanine nucleotide-binding (G) proteins in the frontal cortex of heroin addicts

31P **MacDermot J, Donnelly LE, Boyd RS, Williams RJ & Kelly E** The turnover of Gsα may involve ADP-ribosyltransferase activity in NG108-15 cells

32P **Mason R, Delagrange P & Guardiola B** Melatonin and S-20098 mediated resetting of rat suprachiasmatic circadian clock neurones *in vitro*

33P **Greenwood I, Criddle D & Weston AH** Effects of levcromakalim and nifedipine on noradrenaline- and KCl-induced increases in tension and intracellular calcium in rat isolated resistance vessels

34P **Morrison JJ, Smith SK, Khan RN & Ashford MLJ** The effects of potassium channel openers on the human myometrium before and after the onset of labour

35P **Lee K & Ashford MLJ** Mg^{2+}-dependent inhibition of K_{ATP} channel activity by sulphonylureas in CRI G1 insulin secreting cells

36P **Kernohan AFB, Rossiter SP, Wright L, Williams BC, Baird JD & Lindsay RM** The effect of streptozotocin-induced diabetes on vascular reactivity in perfused rat mesenteric arteries

37P **Fernyhough P, Diemel LT, Smith WJ & Tomlinson DR** Reduced mRNA for neurotrophic factors and GAP-43 in peripheral nerve and target tissue of diabetic rats: effects of brain-derived neurotrophic factor

38P **Stevens EJ, Lockett MJ, Carrington AL & Tomlinson DR** Essential fatty acid treatment prevents nerve ischaemia and associated conduction anomalies in experimental diabetes

39P **Dowell FJ, Hamilton CA, Lindop GBM, Jardine E & Reid JL** Functional and structural properties of aortae from heterozygous and homozygous Watanabe heritable hyperlipidaemic rabbits

40P **Cuthbert AW, MacVinish LJ, Colledge WH, Ratcliff R & Evans MJ** Forskolin responses in epithelia of the cystic fibrosis mouse

41P **Al-Laith M, Matthews EK & Cui ZJ** Photodynamic action of sulphonated aluminium phthalocyanine on the release of arachidonic acid and PGE_2 from perifused rat pancreatic acini

42P **Matthews EK, Flaherty C & Smith W** Effect of thiols on the photodynamic action of sulphonated aluminium phthalocyanine on guinea-pig taenia coli

43P **Wardell CF & Cunnane TC** Involvement of protein kinase C in frequency-dependent facilitation of transmitter release from sympathetic nerve terminals in the guinea-pig vas deferens

44P **Michel AD, Brown HC, Sewter C, Kennedy I & Humphrey PPA** Identification of [^{3}H]αβ-methylene-ATP binding sites in rat brain and peripheral tissues

45P **Kirk IP & Richardson PJ** Adenosine A_2 receptor modulation of striatal acetylcholine and GABA release

46P **Facklam M & Bowery NG** Solubilization and characterization of $GABA_B$ receptors from pig brain

47P **Noble PJ, Stephenson FA, Anderson SMP, De Souza RJ & Cross AJ** Identification of the $GABA_A$ receptor α_3 subunit in IMR-32 neuroblastoma cells

48P **Dawson GR, Bayley PJ & Tricklebank MD** The effect of the partial benzodiazepine receptor agonist FG 8205 and diazepam in rodent models of sedation

49P **Sanger DJ, Benavides J, Perrault G & Morel E** Correlation between the potencies of different (Bz)ω receptor ligands to produce a rotorod deficit and inhibition of *in vivo* [^{3}H]-flumazenil binding

50P **Rodgers RJ & Shepherd JK** Prior plus-maze experience abolishes anxiolytic response to diazepam in the murine light/dark test

51P **Chessell IP, Francis PT, Heath PR, Pearson RCA & Bowen DM** Effect of entorhinal injection of volkensin on the structure and neurotransmitter receptors of the hippocampus

52P **Jolkkonen J, Granata R, Jenner P & Marsden CD** Acute and subacute effects of dopamine agonists on neuropeptide gene expression in the striatum of the rat

53P **Pralong E & Jones RSG** Modification of glutamate and GABA-mediated synaptic transmission by dopamine in the rat entorhinal cortex *in vitro*

54P **Daly SA & Waddington JL** Behavioural responses to the putative D_3 dopamine receptor agonist 7-OH-DPAT in comparison with other 'D_2-like' agonists

55P **Bull DR, Hayes AG & Sheehan MJ** A clozapine-insensitive response to dopamine agonists in rat substantia nigra

56P **Sheehan MJ, Hayes AG & Bull DR** Evidence for a clozapine-insensitive response to the dopamine agonist, N,N-dipropylamino-5,6-dihydroxytetralin (dpADTN), on dopaminergic nerve terminals

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The proceedings of the British Pharmacological Society are published as a supplement to the *British Journal of Pharmacology*.

The *British Journal of Pharmacology* is published monthly by the Scientific & Medical Division, Macmillan Press Ltd.

All business correspondence and reprint requests should be addressed to the Scientific & Medical Division, Macmillan Press Ltd., Houndmills, Basingstoke, Hampshire RG21 2XS, UK. Telephone: (0256) 29242; Fax: (0256) 810526.

Annual subscription prices for 1992 EEC £412, elsewhere £445/US$825 (sterling rate is definitive). Orders must be accompanied by remittance. Cheques should be made payable to Macmillan Press, and sent to: Macmillan Press Ltd., Subscription Department, Brunel Road, Houndmills, Basingstoke, Hampshire RG21 2XS, UK.

Overseas subscribers may make payments into UK Post Office Giro Account No. 5192455. Full details must accompany the payment.

Second Class postage paid at Rahway NJ, US Mailing Agent: Mercury Airfreight International Ltd, Inc., 2323 Randolph Avenue, Avenel, New Jersey, NJ 07001, USA.

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British Journal of Pharmacology

Proceedings Supplement

Proceedings of the British Pharmacological Society Meeting

University of Cambridge

5th–7th January, 1993

Dr S J Coker
Department of Pharmacology
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University of Cambridge, 5–7 January 1993

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