374P COMPARISON OF THE EFFECTS OF 5-HT IN BASILAR ARTERIES FROM BOTH SPRAGUE-DAWLEY AND WISTAR RATS

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It is well documented that 5-hydroxytryptamine (5-HT) is a potent vasoconstrictor of the basilar artery of Sprague-Dawley (S-D) rats, acting on 5-HT_2 receptors (Deckert & Angus, 1992). However, in other strains of rat, 5-HT is not as effective as a vasoconstrictor (Yokota *et al*, 1994). Here we present an investigation into differences in vasoreactivity to 5-HT in two strains, the Wistar and the Sprague-Dawley rat.

Basilar arteries (2.0 mm long; normalised internal diameter 200-400 μm) from male Wistar or S-D rats (250-450 g) were mounted on 40 μm wire in a myograph (JP Trading, Aarhus, Denmark) for isometric tension recording. Vessels were bathed in physiological salt solution (composition, mM: NaCl 115.3, KCl 4.6, $MgSO_4$ 1.1, $NaHCO_3$ 22.1, KH_2PO_4 1.1, $CaCl_2$ 2.5, glucose 11.1) equilibrated with 95% O_2/5% CO_2 at 37°C. They were equilibrated for 60 min before normalisation (Mulvany & Halpern, 1977). After a further 30 min, cumulative concentration-effect curves were constructed to 5-HT. A submaximal concentration of either 5-HT (0.3 μM) or KCl (40 mM) was used to contract the vessels before constructing a concentration-relaxation curve to carbachol (CCh). When required, the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) was added 30 min before construction of relaxation curves. Responses were measured as increases in tension (contraction) or expressed as percentage relaxations of either 5-HT- or KCl-induced tone. Statistical comparison was by Student's unpaired t test.

The maximal responses (R_{max}) to 5-HT were significantly different ($P < 0.001$) between Wistar (2.15±0.32 mN; $n = 5$) and S-D (10.3±0.3 mN; $n = 8$) rats but the EC_{50} values were not significantly different (Wistar, 127±61 nM, $n = 5$; S-D, 71.8±4.4 nM, $n = 8$). In S-D rats, relaxations to CCh were seen after adding a submaximal concentration of 5-HT (0.3 μM) with a maximum relaxation of 24.6±0.9 % (EC_{50} = 2.63±0.43 μM; $n = 4$). These relaxations were completely blocked by the presence of L-NAME (100 μM) and this inhibition was reversed by addition of 1 mM L-arginine ($n = 4$). Similarly, relaxations to CCh on KCl-preconstricted vessels in Wistar rats were abolished by L-NAME and the inhibition was reversed by L-arginine ($n = 4$). KCl was used to precontract vessels instead of 5-HT due to the low degree of tone induced by 5-HT in Wistar rats. A lesser degree of CCh relaxation was seen in Wistar than in S-D rats (Wistar R_{max} = 16.7±0.5 %; $n = 18$) with no significant difference in the potency (Wistar EC_{50} = 1.31±0.15 μM).

Removal of the endothelium in Wistar rats or addition of L-NAME augmented the 5-HT-induced contractions significantly ($P < 0.01$ for both), with R_{max} values of 7.44±0.44 mN (L-NAME; $n = 9$) and 9.13±0.56 mN (endothelium denuded; $n = 4$); the potency of 5-HT was not significantly different from that of the Wistar control (L-NAME, EC_{50} = 96.0±12.2 nM; endothelium denuded, EC_{50} = 144±30 nM).

In contrast, in S-D rats there was no significant change in the R_{max} for 5-HT in the presence of L-NAME (10.8±0.2 mN; $n = 5$) relative to control but endothelial denudation significantly ($P < 0.05$) increased the R_{max} to 12.1±0.2 mN ($n = 7$). However, both L-NAME (EC_{50} = 27.9±5.5 nM) and endothelial destruction (EC_{50} = 42.2±3.6 nM) significantly ($P < 0.01$) increased the potency of 5-HT.

These results show a significant difference in the reactivity of the basilar artery of the Wistar rat to 5-HT compared to that of the S-D rat. From the studies using L-NAME, it would appear that nitric oxide may be involved in reducing the contractile effects seen to 5-HT in the Wistar rat basilar artery.

HJD is a Medical Research Council Research Student.

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375P PHOSPHODIESTERASE INHIBITORS ON PULMONARY ARTERIES FROM RATS WITH HYPOXIC PULMONARY HYPERTENSION: COMPARISON OF 1 AND 4 WEEKS OF HYPOXIA

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Phosphodiesterase (PDE) isoenzymes III and V are present in human pulmonary vascular smooth muscle (Rabe *et al.*, 1994). Inhibitors of these PDEs may therefore be useful as pulmonary vasodilators in the treatment of pulmonary hypertension (PH). The aims of this study were (i) to compare the vasorelaxant effects of a PDE V inhibitor, zaprinast (ZAP), with those of two PDE III inhibitors, milrinone (MIL) and SCA40 (6-bromo-8-methylamino-imidazo [1,2α]pyrazine-2-carbonitrile; gift from P-A. Bonnet; University of Montpellier), in rat pulmonary arteries and (ii) to determine whether these drugs remain effective in pulmonary arteries from rats with experimental PH.

Data were obtained in main pulmonary artery rings (modified Krebs solution; 37°C; 95% O_2/5% CO_2; pre-contracted submaximally with 0.1 μM phenylephrine) taken from control male Wistar rats and from rats exposed to hypoxia (10% O_2) for 1 or 4 weeks in order to induce PH. In control arteries, relaxant responses to ZAP, but not MIL or SCA40, were reduced, but not abolished, by removal of the endothelium or by the presence of 100 μM N^G-nitro-L-arginine methyl ester. Hypoxic rats had right ventricular hypertrophy (indicative of PH); this was most pronounced after 4 weeks of hypoxia (right ventricle/(left ventricle + septum): 4 weeks PH, 0.66±0.02, n=16; 1 week PH, 0.39±0.02, n=14; control, 0.29±0.01, n=20; $P<0.05$, PH vs. control and 1 week vs. 4 weeks; 1 way ANOVA and post hoc t test). Arteries from PH rats were hypertrophied and had inherent tone which was greater after 1 week (11.9±0.8 mN mm^{-2}, n=14) than 4 weeks (3.2±0.7 mN mm^{-2}, n=16; $P<0.05$) of hypoxia. For ZAP and MIL, potency (negative log EC50) values in arteries from 1 week PH rats (ZAP 5.02±0.27, n=5; MIL 5.05±0.24, n=6) were significantly lower than control values (ZAP 6.05±0.06, n=8; MIL 5.88±0.06, n=6; $P<0.05$) but potency values in 4 week PH rats (ZAP 5.60±0.09, n=4; MIL 5.64±0.18, n=4) were not different from controls. For SCA40 there was no significant difference between the 3 rat groups (control 6.65±0.14, n=7; 1 week PH 6.11±0.12, n=5; 4 weeks PH 6.41±0.22; n=4). The adenylate cyclase activator, forskolin, was also examined and the potency was found to be lower in both 1 week (6.73±0.06, n=3) and 4 week (7.22±0.17, n=6) PH rats than in controls (7.80±0.07, n=5; $P<0.05$).

This study has shown that, in rat pulmonary arteries, (i) the PDE V inhibitor, ZAP, requires a functional endothelial nitric oxide pathway for optimal vasorelaxation, whereas PDE III inhibitors do not, (ii) the potencies of PDE V or PDE III inhibitors are not compromised in well established PH (i.e. after 4 weeks of hypoxia) despite structural changes to the vessels (vascular hypertrophy) and reduced sensitivity to the adenylate cyclase activator, forskolin, and (iii) not all changes in the functional properties of pulmonary arteries seen in early PH (after 1 week of hypoxia) necessarily persist when hypoxia continues and PH becomes more established.

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376P THE EFFECT OF INCUBATION WITH ENDOTOXIN OR CYTOKINES ON THE RESPONSES OF THE OVINE DIGITAL ARTERY TO PHENYLEPHRINE AND BRADYKININ

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It is generally agreed that laminitis, an important cause of lameness in domestic ungulates, occurs as a result of altered peripheral circulation. Clinically, the condition is often associated with endotoxaemic animals and it has been suggested that endotoxin acting directly or indirectly, through the induction of cytokines, may contribute to the vascular derangements observed (Hunt 1991). The aim of this study was to investigate the effect of endotoxin/cytokines on the vascular responses of sheep digital arteries *in vitro*.

Arteries were obtained from a local abattoir, where they were cannulated and flushed with ice-cold, oxygenated (95% O_2 & 5% CO_2) Krebs solution. In the laboratory, digital artery rings, 2mm long, were mounted between stainless steel wires in 10ml tissue baths containing warmed (30°C), oxygenated Krebs solution of the following composition (mM): NaCl 118, KCl 4.57, $CaCl_2$ 1.27, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25 and glucose 11.1. Endotoxin (3µg/ml) or a cytokine mixture, containing interferon-γ (IFN-γ; 10ng/ml) and tumour necrosis factor-α (TNF-α; 5ng/ml), was added to half of the arterial rings. No additions were made to the remaining rings, which acted as time-based controls. Vessels were maintained at a baseline tension of 2g, measured using an isometric force transducer (Grass FT03), for 6 hr before the addition of vasoactive drugs. Every 30 min vessels were washed with fresh Krebs and new additions of endotoxin/cytokines were made. Cumulative concentration-response curves to phenylephrine (PHE; 10^{-9} - $3x10^{-4}$M) and non-cumulative concentration-response curves to bradykinin (BK; 10^{-10} - 10^{-7}M) were constructed in control and endotoxin/cytokine incubated digital arterial rings. To study BK-induced relaxation the vessels were precontracted with a standard dose of PHE ($6x10^{-7}$M).

PHE responses were expressed as tension in g/g tissue wet weight, BK responses as percentage change in pre-contracted tension. Values for the log EC_{50}(M) and E_{max} were calculated using a sigmoid curve-fitting procedure (Microcal Origin 4.1) and were compared by one-way analysis of variance (Minitab for Windows 10.2). P-values < 0.05 were taken to indicate statistical significance. A summary of the results is presented in Table 1.

TABLE 1: Dose-response curve parameters to PHE and BK in ovine digital arteries, following 6 hr incubation with endotoxin (3µg/ml) or cytokines (IFN-γ 10ng/ml & TNF-α 5ng/ml)

	PHENYLEPHRINE			BRADYKININ		
	n	EC_{50} ± SEM log [PHE]	E_{max} ± SEM g/g	n	EC_{50} ± SEM log [BK]	E_{max} ± SEM %
CONTROL	7	-6.35 ± 0.07	2272 ± 83	6	-8.51 ± 0.09	-55.3 ± 3.1
ENDOTOXIN	7	-6.38 ± 0.08	2527 ± 119	6	-8.69 ± 0.11	-44.5 ± 6.6
CONTROL	6	-6.41 ± 0.08	2119 ± 119	6	-8.44 ± 0.17	-32.2 ± 3.5
CYTOKINES	6	-6.27 ± 0.08	1942 ± 146	6	-8.68 ± 0.16	-54.2 ± 4.6*

* indicates $p < 0.05$ when compared to control group

Incubation with endotoxin produced no significant effect on PHE or BK responses under the conditions of the study. Neither did the PHE response significantly change following incubation with IFN-γ & TNF-α. However, cytokine incubation significantly increased the E_{max} of BK. In ovine digital arteries, BK-induced relaxation is mediated via a combination of nitric oxide and prostanoids (Pawson *et al.* 1997), therefore a cytokine-induced increase in either agent could account for the enhanced BK response. In contrast, similar studies, in which equine digital (Baxter 1994) and bovine pulmonary arteries (Greenburg *et al.* 1993) were incubated with TNF-α demonstrated a decrease in both acetycholine and bradykinin-induced relaxation, suggesting a decrease in nitric oxide synthesis/release. Clearly, further investigation is required.

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377P INSULIN SENSITIVITY IN THE LYON HYPERTENSIVE RAT

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Genetically hypertensive (LH) rats of the Lyon strains compared to their normotensive (LN) counterparts exhibit hypertension associated with an increased body weight, elevated plasma lipids and insulin/glucose plasma ratio (Vincent & Sassard, 1994). In addition, among a large population of F2 hybrids generated from a LH x LN cross the aortic blood pressure measured in the freely moving rat correlated with plasma cholesterol and more weakly with the insulin / glucose plasma ratio. It could therefore be postulated that LH rats could fully mimic the "syndrome X" described in humans (Reaven & Hoffman, 1990). The aim of the present study was to investigate the development of insulin resistance with age in LH and LN fasted male rats.

An intravenous glucose tolerance test (IVGTT) blood sample was obtained via the jugular vein under anesthesia (Pentobarbital sodium 40 mg/kg IP) to measure triglycerides and cholesterol levels. The rats were then starved (18 h) and an IVGTT was performed the next morning by glucose injection: 500 mg/kg or 250 mg/kg to 22 and 52 week-old rats respectively followed by serial blood sampling. The rate of glucose disappearance (K_G= $K.10^{-2}$-min^{-1}) was calculated by mean square regression. Insulin sensitivity was calculated as the ratio of K_G/insulin AUC (area under the curve). Data were analysed using a Bartlett-test followed by an ANOVA or a Mann and Whitney-test.

LH rats compared to the LN rats exhibited spontaneous increases in body weight and plasma lipids (Table 1). Insulin sensitivity coefficient decreased with age in all the animals : from 62.6 +/- 3.3 and 69.1 +/- 4 at 22 weeks to 42.1 +/- 4.4 and 49.5 +/- 12.8 at 52 weeks for LH and LN rats respectively without significant differences between groups.

Table 1		n	22 weeks		n	52 weeks
Body weight (g)	LH	(9)	460±7**		(7)	547±4**
	LN	(7)	300±6	=	(7)	361±2
Total cholesterol	LH	(9)	0.88±0.03**		(7)	1.99±0.09**
(g/l)	LN	(7)	0.42±0.01		(7)	0.44±0.02
Triglycerides (g/l)	LH	(9)	1.37±0.11**		(7)	3.2±0.24**
	LN	(7)	0.25±0.02		(7)	0.35±0.03

**p<0.001 vs LN ; n = number of animals. Data: mean ±S.E.M.

It is concluded that 1) elevated plasma insulin/glucose ratio does not mean insulin resistance and 2) hypertension can develop without being associated, even in aged rats, with a true insulin resistance.

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378P EPOXYEICOSATRIENOIC ACIDS AND RENAL ALTERATIONS IN THE LYON HYPERTENSIVE RAT

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The present study aimed to assess the role of epoxyeicosatrienoic acids (EETs) in the renal vasoconstriction and the blunted pressure natriuresis exhibited by Lyon hypertensive (LH) rats compared to their low blood pressure (LL) controls.

Eight week-old uninephrectomized LH and LL anesthetized rats (Inactin, 100 mg kg^{-1}, i.p.) were used. According to Roman & Cowley (1985), renal functions were studied after elevation of the renal perfusion pressure (RPP) using inflatable cuffs placed around the aorta. RPP and renal blood flow were continuously monitored. Glomerular filtration rate was measured as the inulin clearance. Miconazole, an inhibitor of EETs synthesis, was intrarenally infused (1 µM final concentration) in 8 LH and 8 LL rats. Two other groups of 8 rats received miconazole vehicle and served as controls. The influence of an activation of thromboxane A2-prostaglandin H2 (TP) receptor by EETs was examined by infusing GR 32191B (0.1 mg kg^{-1} min^{-1} i.v.), a TP receptor antagonist in two groups of 6 LH rats infused with miconazole or its vehicle. Data are mean ± s.e.m. Statistical analysis used a two-way ANOVA.

Table 1. Renal vascular resistance (RVR, mmHg ml^{-1} min^{-1} g^{-1}), glomerular filtration rate (GFR, ml min^{-1} g^{-1}), urinary sodium excretion (UNaV, µmol min^{-1} g^{-1}) in LL and LH rats receiving miconazole (M) or its vehicle (C) at a RPP of 140 mmHg. * $p < 0.05$ *vs.* controls.

Strains	RVR	GFR	UNaV
LL C	18.5 ± 1.5	0.98 ± 0.15	8.7 ± 2.0
M	17.1 ± 1.5	0.94 ± 0.05	11.2 ± 3.2
LH C	26.3 ± 1.5	0.70 ± 0.63	4.2 ± 0.8
M	17.9 ± 1.1*	0.61 ± 0.07	6.4 ± 1.2*

Table 1 shows that miconazole decreased RVR and increased UNaV in LH rats only. After TP receptor blockade, the natriuretic effect of miconazole remained unchanged (3,48 ± 0,42 *vs.* 1,76 ± 0,47 µmol. min^{-1} g^{-1} in LH M and LH C respectively) while its dilator effect disappeared (20,33 ± 2,57 *vs.* 18,6 ± 1,19 mmHg ml^{-1} min^{-1} g^{-1} in LH M and LH C respectively).

In conclusion, in kidneys of LH rats, EETs contribute to the increased vascular resistance and the decreased natriuresis. The first but not the second effect involves a TP receptor activation.

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379P KETOPROFEN INHIBITION OF BACTERIAL ENDOTOXIN AND INTERLEUKIN-1-INDUCED HYPOREACTIVITY OF BLOOD VESSELS TO NORADRENALINE

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It is well established that bacterial lipopolysaccharide (LPS) induced vasodilation and hyporeactivity toward catecholamines involves endothelium-derived nitric oxide. In addition, LPS-induces cytokine release especially interleukin-1 (IL-1) which appears to mediate many of the actions of LPS. The vascular effects of IL-1 include hypotension (Okusawa *et.al.*, 1988) and the induction of prostaglandins such as prostacyclin (PGI_2) which have relaxant actions on blood vessels (Arner and Hogestatt, 1991). The present study investigates the role of prostaglandins in the LPS and IL-1-induced vasorelaxation and hyporeactivity toward noradrenaline (NA).

The thoracic aortae of Dunkin-Hartley guinea pigs (350-600g) were used in this study. The blood vessels were cannulated and transferred to an organ bath containing Krebs-Hensliet solution, at 37°C and gassed with 95%O_2/5%CO_2. The vessels were then perfused continuously (6ml.min^{-1}) with Krebs solution via a peristaltic pump. LPS, IL-1, ketoprofen (KP, 1µM), L-N^G-monomethyl arginine (L-NMMA, 5 µM) or iloprost were added to the perfusion reservoir and pumped through the lumen of the tissue. The contractile responses of the blood vessels were measured by a pressure transducer proximal to the cannula. The pressure transducer was attached to a Grass polygraph chart recorder. NA was injected as a bolus (400 nmol) into the lumen of blood vessels via the tubing. This NA dose produced a pressor response (25.2 mm Hg ± 2.6, n = 12) in the order of a 70 % maximal response to NA .

LPS (1pg.ml^{-1} - 1 µg.ml^{-1}) and IL-1ß (20-200 pM) induced a concentration-dependent suppression of the pressor response to noradrenaline. LPS (1 µg.ml^{-1}) and IL-1 (200 pM) reduced the effect of NA to 69.9% ± 7.3 and 58.7% ± 13.2 respectively (both $P < 0.01$) of the response prior to LPS or IL-1 (100% ± 4.1 and 100% ± 3.7 respectively). Pretreatment (30 min) with the cyclooxygenase inhibitor KP prevented both the LPS and IL-1-induced hyporeactivity to NA and appeared to unmask an enhanced pressor response (115.3% ± 8.8 and 119.6% ± 5.7 respectively, both $P < 0.05$). L-NMMA pretreatment (30 min) also prevented both the LPS and IL-1-induced hyporeactivity to NA (110.3% ± 16.3 and 106.4 % ± 10.7 respectively). The PGI_2 analogue, iloprost, inhibited the pressor response to NA in a concentration-dependent manner with a reduction from 100% ± 5.3 to 68.8% ± 8.3 with 1 µg.ml^{-1} iloprost ($P < 0.01$).

These data indicate that both prostaglandin and nitric oxide biosynthesis are involved in the mechanism by which LPS and IL-1ß induce hyporeactivity to NA. In addition, nitric oxide production appears to be involved in the hyporeactivity to NA induced by iloprost.

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380P REDUCTION OF LDL EFFLUX FROM MOUSE MESENTERY VASCULATURE BY DIHYDROPYRIDINE (DHP) COMPOUNDS IS INDEPENDENT OF THEIR CALCIUM CHANNEL BLOCKING ACTIVITY

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Nifedipine reduces the accumulation of atherogenic plasma proteins, viz. low density lipoproteins (LDL) and fibrinogen, by rabbit aorta (Görög and Born, 1993), and also LDL and albumin efflux from mouse mesenteric vasculature, both *in vivo* (Görög and Born, 1995). We now present evidence that the same effect is brought about by DHPs without calcium blocking activity.

Male T.O. mice (20-30g) were anaesthetised (0.05 ml Hypnorm and 0.1 ml Diazepam), injected i.v. with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-LDL (DiI is a fluorescent marker) and 60 min later s.c. with compounds to be tested. After 30 min the abdomen was opened, the mesentery gently extended on a perspex microscope stage and superfused continuously with Tyrode's solution at 37°C. Superfusate was collected over two 40 min periods (the first sample rejected in case of blood contamination), and DiI quantified in chloroform extracts.

BAY R 1224 (nisoldipine) is 30-100 times more active in blocking L-type calcium channels than its enantiomer BAY R 1223 (personal communication from Dr. S. Wohlfeil, Bayer AG), but only twice as effective in reducing LDL efflux (Table 1). BAY N 3628 is 100-1000 times more active on L-type calcium channels than its enantiomer BAY N 3629 (personal communication from Dr. S. Wohlfeil, Bayer AG), but again only twice as active in lowering LDL efflux. Furthermore, the enantiomers of BAY K 8644, one a calcium antagonist and the other an agonist (Hamilton *et al*, 1987), as well as the drug racemate all reduce LDL efflux. In contrast, the non-DHP calcium channel blockers verapamil and diltiazem did not affect efflux significantly.

Table 1. The effect of DHP and non-DHP compounds on LDL efflux from the mouse mesentery *in vivo* (statistics were performed using a t-test).

Compound	Concentration (mg/kg)	n	Reduction in LDL efflux (%)	p
BAY R 1223	4	6	19	<0.01
BAY R 1224	4	6	46	<0.001
BAY N 3628	3	8	66	<0.001
BAY N 3629	3	7	33	<0.001
(±) BAY K 8644	3	4	45	<0.001
(+) BAY K 8644	1.5	5	73	<0.001
(-) BAY K 8644	1.5	8	22	<0.05
Verapamil	3	7	0	ns
Diltiazem	3	7	0	ns

The results indicate that the observed reduction in efflux of atherogenic plasma proteins is a function of the DHP molecular structure and is not attributable to calcium channel blockade.

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381P BINDING PROPERTIES OF A POTENT, LONG-ACTING NEW THROMBOXANE RECEPTOR ANTAGONIST, [3H]S 18886, ON HUMAN, RAT AND DOG PLATELET MEMBRANE

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Thromboxane A_2 causes its powerful pro-aggregatory and vasoconstrictor effects implicated in the pathogenesis of thrombotic disorders by activation of specific membrane receptors called TP-receptors. We have previously reported the long duration of action of a new TP-receptor antagonist S 18886, 3-[(6-(4-chlorophenyl sulfonylamino)-2-methyl-5,6,7,8-tetrahydronapht]-1-yl) propionic acid, in the dog. At 10 µg/kg *per os* the compound completely prevented the ex-vivo platelet response to U 46619 during 72 hours (Verbeuren et al., 1995). The present study reports the kinetic binding properties of [^{3}H]S 18886 on human, dog and rat platelet membranes, in order to correlate the long duration of action of the product with its binding to platelet TP receptors.

Human, dog and rat plasma samples were obtained from citrated whole blood. Platelet membranes were prepared from washed platelets as described by Hedberg et al. (1988). Binding experiments were carried out at 37°C in presence of Tris/$MgCl_2$ buffer (50 mM, pH 7.4), in 200 µl aliquots containing 100 µg protein. Kinetic and competition studies were realised with 0.5-1 nM of [^{3}H]S 18886. Saturation and competition studies were carried out for 90 min. In all experiments, non specific binding was obtained in presence of unlabelled S 18886 at 10 µM. Data were analysed according to Weiland and Molinoff (1981).

Binding reached equilibrium within 90 min for the human, rat and dog platelet membranes with similar association rate constants. The dissociation was complete after 4 h for human and rat platelet membranes whereas more than 24 h were needed to achieve a nearly complete dissociation for the dog. The dissociation rate constants and the calculated Kd values (table 1) were in the nM range for human and rat tissues whereas the binding for the dog platelets displayed a 10 fold higher affinity. In saturation studies of [^{3}H]S 18886, Bmax values were not significantly different for human and rat platelet membranes (table 1) whereas this parameter was lower for the dog. The affinities displayed by the radioligand (Kd value) were in agreement with those calculated by the kinetic method (table 1). The specific binding of [^{3}H]S 18886 to human platelet membranes was inhibited completely and in a concentration-dependent manner by TP receptor antagonists and agonists. The inhibition curves were monophasic, and the rank order of inhibition (Ki values) of [^{3}H]S 18886 binding was S 18886 > ICI 192605 > BayU 3405 ≥ S 18885 > IBOP > SQ 29548 > U 46619. The inhibition by other prostaglandins such as $PGF_{2\alpha}$, PGI_2 or PGD_2 occurred at concentrations higher than 30 µM, showing the specificity of the binding. The specific binding of [^{3}H]S 18886 to rat and dog platelet membranes was inhibited completely in a concentration-dependent and monophasic manner by S 18886 and BayU 3405.

Table 1. Binding characteristics of [^{3}H]S 18886 (mean ± s.e. mean, (n)).

Platelet Membranes		Human	Rat	Dog
Kinetic	Kd, nM	0.96 ± 0.22 (6)	0.49 ± 0.08 (5)	0.087±0.016 (4)
Satur.	Kd, nM	1.47 ± 0.21 (6)	2.66 ± 0.28 (7)	0.18 ± 0.03 (12)
	nH	0.96 ± 0.04	0.96 ± 0.07	1.48 ± 0.12
	Bmax,fmol/mg	47.8 ± 4.3	54.5 ± 5.3	24.1 ± 1.9

The results obtained may explain the long *ex vivo* duration of the antiplatelet action of S 18886 observed specifically in the dog. Indeed, the time necessary to dissociate [^{3}H]S 18886 from the dog platelet TP receptor and its subsequent affinity is 10 fold higher than in the other two species. These data may help to predict the duration of action of the drug in a clinical context.

Hedberg, A. et al. (1988) *J. Parmacol.Exp.Ther.* 245,786-792
Verbeuren,T.J. et al. (1995) *Thromb. Haemost.* 73,1324
Weiland, G.A. & Molinoff, P.B. (1981) *Life Sci.* 29,313-330

382P A PRESSURE-INDEPENDENT IMPROVEMENT IN AORTIC ELASTICITY IN SENESCENT HYPERTENSIVE RATS

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Several authors have shown that antihypertensive treatment lowers aortic ridigity (Levy *et al*, 1968) a contributing factor to cardiovascular morbidity. It is less certain whether this is due to a fall in transmural distending pressure or whether pressure-independent effects on aortic wall geometry and structure are also involved. Furthermore, little data is available from senescent animals which constitute a better model of the human pathology. In this experiment we investigated whether antihypertensive treatment could improve aortic elasticity in senescent animals in a pressure-independent fashion.

Normotensive (WKY) and spontaneously hypertensive (SHR) rats were treated from 3 months onwards with the angiotensin 1 converting enzyme inhibitor, captopril, (38 mg kg^{-1} 24 h^{-1}) and the diuretic, hydrochlorothiazide (19 mg kg^{-1} 24 h^{-1}) mixed in food. After 6 and 12 months' treatment, rats were fitted with polyethylene cannula in the thoracic and abdominal aorta and the abdominal vena cava under halothane (2%)-oxygen anaesthesia. Twenty-four hours later, aortic cannula were connected to pressure transducers and central mean aortic blood pressure (MAP, mmHg) and aortic pulse wave velocity (PWV, cm s^{-1}) measured in the non-anaesthetized, non-restrained rat. Animals then received an i.v. infusion of the nitrovasodilator, sodium nitroprusside (115 ± 7 nmol kg^{-1} min^{-1}, for 28 ± 5 min) which lowered MAP to half its starting value. Isobaric elasticity (IE, cm s^{-1} $mmHg^{-1}$) was taken as the slope of the linear regression of PWV on MAP during the nitroprusside-induced fall in MAP. This is an index of pressure-independent changes in aortic elasticity. Results are expressed as means ± s.e.mean (Table 1, n = 5 to 12 per group); means were compared using ANOVA and the Bonferroni test.

Table 1: Effects of captopril + hydrochlorothiazide treatment on central pressure and aortic elasticity in SHR and WKY rats

Strain	SHR		WKY	
Age (months)	9	15	9	15
MAP(mmHg)				
Placebo	158 ± 5	152 ± 6	123 ± 2	114 ± 3
Treated	130 ± 4	112 ± 4	107 ± 2	107 ± 6
PWV (cm s^{-1})				
Placebo	744 ± 38	891 ± 59	542 ± 25	535 ± 32
Treated	553 ± 28	433 ± 68	422 ± 27	494 ± 32
IE (cm s^{-1} $mmHg^{-1}$)				
Placebo	4.0 ± 0.3	6.3 ± 0.4	2.7 ± 0.2	2.3 ± 0.3
Treated	1.8 ± 0.2	2.9 ± 0.3	1.8 ± 0.2	2.3 ± 0.4

ANOVA	MAP	PWV	IE
Treatment	0.0001	0.0001	0.0001
Strain	0.0001	0.0001	0.0001
Age	0.0023	0.0155	0.0001
Treatment x strain	0.0141	0.0003	0.0001
Treatment x age	0.4731	0.0288	0.3777
Strain x age	0.9216	0.2110	0.0152

Ageing in WKY (median lifespan 27 months) revealed no change in cardiovascular parameters. Treatment improved aortic elasticity (PWV) at 9 but not at 15 months; improvement was due to a fall in MAP and IE. With senescence, SHR (median lifespan 17 months) showed an increase in aortic rigidity due to an increase in IE (with no further increase in MAP). Treatment normalized PWV at 9 and 15 months in SHR, with falls in MAP and IE. As treatment normalized IE in SHR we suggest that it had a pressure-independent effect on aortic elasticity; this effect was proportionately greater in senescent SHR.

Levy B.I., Stefas L., Babalis D., Benetos A. (1992). Vascular endothelium, mechanical properties on the arterial wall and local angiotensin converting enzyme inhibition. J. Hypertension, 10, S21-S27.

A grant from RPR-Bellon, Paris is acknowledged.

383P MELATONIN POTENTIATES THE CONTRACTILE RESPONSE TO NORADRENALINE WITHOUT MODIFYING INTRACELLULAR CALCIUM MOBILISATION IN THE RAT PERFUSED TAIL ARTERY

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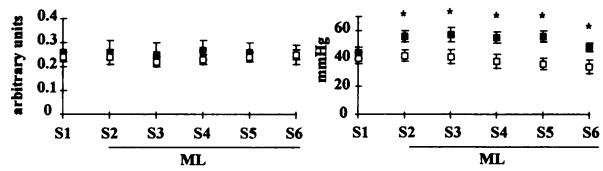
It has been reported that melatonin (ML) potentiates the noradrenaline (NA)-induced contraction of the rat tail artery (Viswanathan *et al.*, 1990). However, the intracellular mechanisms underlying this potentiating effect are unknown. In this study, we investigated the impact of ML on the NA-evoked intracellular calcium ($[Ca^{2+}]_i$) mobilisation and vasoconstriction.

The tail artery was dissected out from adult male Wistar rats (544±17 g, n = 7 per group) under sodium pentobarbitone anaesthesia (60 $mg.kg^{-1}$, i.p.). A 1-cm segment was cannulated, mounted in a perfusion/cuvette system placed in a dual wavelength spectrofluorometer and perfused at a constant rate (1.5 $ml.min^{-1}$) with physiological salt solution. Endothelium was disrupted by coperfusion of air (0.4 $ml.min^{-1}$ for 10 min). Arteries were loaded with Fura 2/AM (5 µM, 90 min; Capdeville-Atkinson *et al.*, 1993). They were then stimulated 6 times (S1 to S6) at 9 min intervals by perfusion with NA (1 µM, 2 min) in the absence or presence (S2 to S6) of increasing concentrations of ML (10^{-12}; 10^{-10}; 10^{-8}; 10^{-6}; 10^{-4} M) added 1 min before each stimulation. Basal $[Ca^{2+}]_i$ (arbitrary units, a.u.; Capdeville-Atkinson *et al.*, 1995) and perfusion pressure (mmHg) and NA-induced increases in $[Ca^{2+}]_i$ and perfusion pressure were measured. Values are means±SEM. Significant differences ($P < 0.05$; in absence *vs* in presence of ML) were determined by one-way ANOVA and the Bonferroni test.

ML produced no change in baseline $[Ca^{2+}]_i$ (S1: 0.24±0.01 *vs* S6: 0.25±0.01 a.u.) or perfusion pressure (S1: 17±3 *vs* S6: 18±3 mmHg).

Figure 1 shows that ML potentiated the vasoconstrictor response to NA, but did not modify $[Ca^{2+}]_i$ mobilisation.

Figure 1: Increases in $[Ca^{2+}]_i$ and in perfusion pressure evoked by NA (1 µM, 2 min) in absence (□) or in presence (■) of ML. (*: $P < 0.05$)



Our results suggest that potentiation of noradrenergic contractile responses by ML occurs downstream of $[Ca^{2+}]_i$ mobilisation.

Capdeville-Atkinson, C., Oster, L., Thorin-Trescases, N., *et al.* (1993) *Am. J. Physiol.*, 265 : C1689-C1702.

Capdeville-Atkinson, C., Oster, L., Thorin-Trescases, N., *et al.* (1995) *Am. J. Physiol.*, 268 : R1394-R1400.

Viswanathan, M., Laitinen, J.T., Saavedra, J.M. (1990) *Proc. Natl. Acad. Sci. USA*, 87 : 6200-6203.

384P ROLE OF OXYGEN-DERIVED FREE RADICALS IN THE SYNTHESIS OF INFLAMMATORY CYTOKINES AFTER HAEMORRHAGIC SHOCK

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We have shown previously that intestinal ischemia/reperfusion during haemorrhage and resuscitation may be a major trigger for cytokine expression (Tamion et al., 1997). Free radicals are produced upon tissue reperfusion, and may play a role in the inflammatory response after haemorrhage. Thus, the present study was designed to assess whether the free radical scavenger N2-mercaptopropionyl glycine (MPG) affects the production of inflammatory cytokines in a rat model of haemorrhagic shock.

Haemorrhage was induced in anaesthetised rats by bleeding the animal to achieve a mean arterial blood pressure of 45 mmHg for 60 min. Rats were then resuscitated over a 3 h period by injecting shed blood, followed by NaCl 0.9 %, in order to maintain arterial blood pressure to control values. Treated rats received MPG (20mg/kg i.v. bolus 30 min before resuscitation followed by 20 mg/kg/h). At the end of the experiment, carotid and mesenteric blood samples were collected in order to measure plasma concentrations of TNFα (ELISA) and IL-6 (bioassay). Peritoneal macrophages were also collected in order to assess TNFα and IL-6 mRNA expression (RT-PCR). Finally, peritoneal macrophages isolated from normal rats and subjected to 24 hypoxia followed by reoxygenation, after which TNFα and IL-6 mRNA expression.

MPG reduced the volume of saline necessary to restore blood pressure during resuscitation (untreated 80±8; MPG 30±9 ml/kg; $p<0.05$)

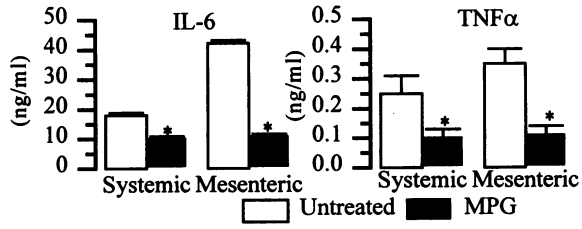

Figure 1. plasma concentrations of cytokines

In macrophages isolated from shocked rats, MPG also reduced the cytokines mRNA expression (IL-6: untreated 52±7; MPG 21±8; TNFα: untreated 66±6; MPG 12±2; both $p<0.05$).

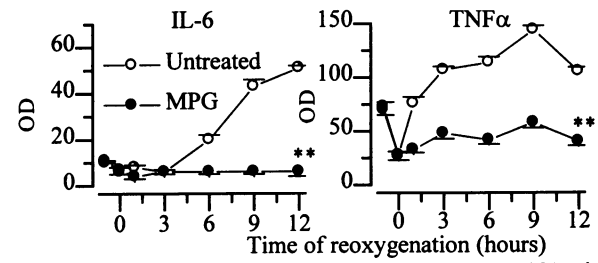

Figure 2. expression of IL-6 and TNFα mRNA in macrophages after hypoxia and reoxygenation.

Thus, free radicals are responsible for the production of proinflammatory cytokines during post-haemorrhage resuscitation, possibly at least in part through stimulation of cytokine expression in reoxygenated macrophages.

Tamion, F. *et al.* (1997). *Am. J. Physiol.*, in press.

385P MELATONIN HAS A DOSE-DEPENDENT CEREBROVASCULAR CONSTRICTOR EFFECT *IN VIVO*

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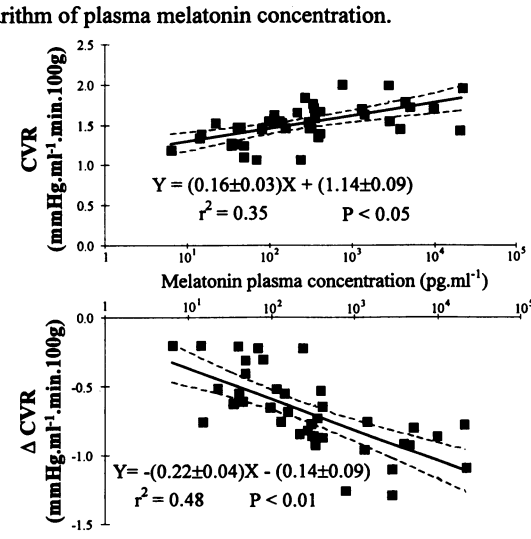
Melatonin has a direct vasoconstrictor effect on large cerebral arteries (Geary *et al.*, 1997), but its effect on small cerebral arterioles is unknown. The present study was performed to evaluate the effect of melatonin on the regulation of cerebral vascular resistance (CVR). CVR was measured at baseline and following hypercapnia, a challenge which induces cerebral vasodilatation capacity (Wei *et al.*, 1980).

Adult, male, outbred Wistar rats (Ico:Wi, IOPS AF/Han, body weight = 516±16 g, n = 9 per group) were chronically implanted under sodium pentobarbitone anaesthesia (60 $mg.kg^{-1}$, i.p.) with cortical electrodes for the measurement of cerebral blood flow (CBF, $ml.min^{-1}.100g^{-1}$). Twelve days later, rats were fitted under halothane (2%)-oxygen anaesthesia with a polyethylene cannula in the abdominal aorta for measurement of mean arterial blood pressure (MAP), and with a silicone cannula in the abdominal vena cava for infusion of melatonin. Two days latter, animals were randomised to no infusion, NaCl 0.9% plus 1% ethanol, or melatonin (60, 600, or 60000 $ng.kg^{-1}.h^{-1}$). After 60 minutes equilibration period, baseline CVR ($mmHg.ml^{-1}.min^{-1}.100g^{-1}$; = MAP/CBF) was measured as the ratio of MAP to CBF. Fifteen minutes later, the CVR response to hypercapnia (ΔCVR, $mmHg.ml^{-1}.min^{-1}.100g^{-1}$) was calculated as the difference between CVR under hypercapnia and baseline CVR, following 10 minutes inhalation of 10% CO_2. Blood samples (1 ml) were collected for the determination of the plasma concentrations of melatonin by radio-immunoassay.

Figure 1 : Relation between baseline CVR or ΔCVR and the logarithm of plasma melatonin concentration.



Melatonin increased baseline CVR and ΔCVR in a dose-dependent way. The increase in ΔCVR under hypercapnia reflects a greater microvascular vasodilatory capacity following an increase in baseline cerebral vasoconstrictor tone. We conclude that melatonin has a cerebral vasoconstrictor effect *in vivo*.

Geary, G.G., Duckles, S.P., Krause, D.N., (1997) *FASEB J.* 11 (3) : 2811 (abs).
Wei, E.P., Kontos, H., Patterson, J.L., (1980) *Am. J. Physiol.* 238 : H697-H703.

386P DIVERGING LONG TERM EFFECTS OF CALCIUM ANTAGONISTS IN A RAT MODEL OF CHRONIC HEART FAILURE

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Calcium antagonists are widely used in the treatment of hypertension and angina pectoris because of their vasorelaxing properties and their favourable effects on myocardial oxygen supply and demand. However, their use in chronic heart failure (CHF) is still controversial. We have shown previously that the calcium antagonist mibefradil increases survival in a rat model of CHF (Mulder *et al.*, 1997).

To test whether this property is shared with other types of calcium antagonists, we compared the effects of mibefradil to those of amlodipine in rats with CHF, induced by coronary artery ligation. Seven days after ligation rats were randomized into three groups (n=50 per group), treated with placebo, mibefradil (15 mg/kg/day) or amlodipine (10 mg/kg/day). Survival was assessed over a 9 month period (Figure 1). At the end of the study, systolic blood pressure (SBP; mm Hg) and heart rate (HR; beats/min) were measured in conscious animals, while left ventricular (LV) end diastolic pressure (LVEDP; mm Hg), LV dP/dt (10^3 mm Hg/sec) and central venous pressure (CVP; mm Hg) were determined in anaesthetised (pentobarbital, 50 mg/kg i.p.) rats. (Table 1).

Table 1. Hemodynamic data in anaesthetised rats.

	SBP	HR	LVEDP	dP/dt	CVP
Control	122±1	444±14	8.1±3.1	7.1±0.4	5.1±0.7
Mibefradil	98±1*	390±6*	3.4±1.3*	7.5±0.7	3.6±0.5*
Amlodipine	100±1*	426±9†	7.0±1.4†	7.0±0.6	7.2±1.0

(Values are mean ± sem;*: $p<0.05$ vs control; †: $p<0.05$ vs mibefradil; ANOVA).

Thus, at equihypotensive doses, only mibefradil reduced heart rate, left ventricular end diastolic pressure and central venous pressure. Neither drugs affected left ventricular dP/dt.

During the first 130 days, both mibefradil and amlodipine improved survival (% survival after 130 days: control: 69; mibefradil: 88; amlodipine: 86 %, both $p<0.05$ vs control). However, after 270 days only mibefradil significantly improved survival (control: 48; mibefradil: 78%, $p<0.05$ vs control; amlodipine: 53 %, NS vs control). Infarct size was similar in the three groups.

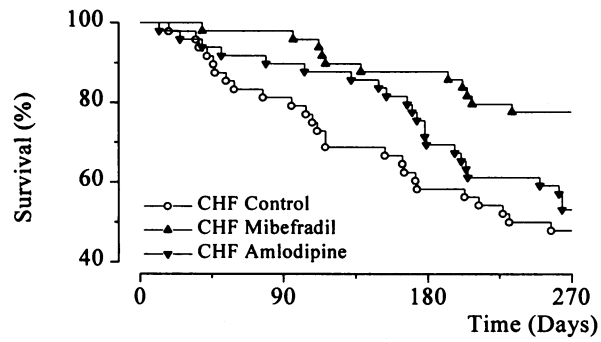

Figure 1. Percent survival in the three groups.

Thus, at equihypotensive doses, the effect of mibefradil on long term survival was more marked than that of amlodipine, possibly because of the different effects of the two drug on heart rate and cardiac hemodynamics.

Mulder, P., et al. (1997) *J. Am. Coll. Card.* **29**: 416-421.

387P RELATIONSHIP BETWEEN VASOCONSTRICTION, MYOSIN LIGHT CHAIN PHOSPHORYLATION AND INTRACELLULAR CALCIUM MOBILISATION IN THE RAT PERFUSED TAIL ARTERY

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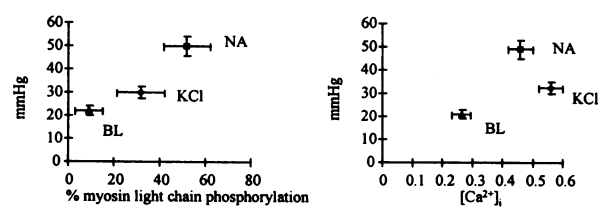
Although intracellular calcium ($[Ca^{2+}]_i$) - vasoconstriction coupling is amplified in the case of pharmacomechanical stimulation (Capdeville-Atkinson *et al.*, 1995), it has been proposed that phosphorylation of the 20-kDa regulatory myosin light chains (MLC) is more directly related to contraction. The aim of this study was to determine the relationship between $[Ca^{2+}]_i$ mobilisation, MLC phosphorylation and vasoconstriction during electro- or pharmacomechanical stimulation.

The tail artery was dissected out from adult male Wistar rats (518±10 g, n = 4-6 per group) under sodium pentobarbitone anaesthesia (60 $mg.kg^{-1}$, ip). In a 1st series of experiments, a 1-cm segment was cannulated, mounted in a perfusion/cuvette system placed in a dual wavelength spectrofluorometer and perfused at a constant rate with PSS. Arteries were loaded with Fura 2/AM (Capdeville-Atkinson *et al.*, 1995). They were then stimulated either with high KCl depolarising solution (80 mM, 10 min perfusion and bath) or with noradrenaline (1 µM, 10 min perfusion; NA). Baseline (BL) $[Ca^{2+}]_i$ (arbitrary units; Capdeville-Atkinson *et al.*, 1995) and perfusion pressure (mmHg), and KCl- or NA-evoked increases in $[Ca^{2+}]_i$ and perfusion pressure were measured. In a 2nd series, arteries were perfused but not loaded with Fura-2/AM. At BL or after 10 min of KCl- or NA-induced stimulation, vessels were quickly frozen and then homogenized. The homogenate was centrifuged and the pellet suspended in a urea-glycerol buffer for electrophoretic analysis of MLC phosphorylation by immunoblotting, according to the method of Word *et al.* (1991) modified by C.L. Buus (personal communication), and using a specific monoclonal antibody (Monical *et al.*, 1993). The level of MLC phosphorylation was estimated from the ratio of phosphorylated to total (phosphorylated and non-phosphorylated) MLC multiplied by 100. Values are means±SEM.

In conclusion as seen in Figure 1, perfusion pressure was proportional to MLC phosphorylation, but not to $[Ca^{2+}]_i$.

Figure 1 : Perfusion pressure, MLC phosphorylation and $[Ca^{2+}]_i$ in the perfused rat tail artery.



The authors thank C.L. Buus (Dept. of Pharmacology, University of Aarhus, Denmark) for his help in the development of this technique.

Capdeville-Atkinson, C., Oster, L., Thorin-Trescases, N., *et al.* (1995) *Am. J. Physiol.*, 268 : R1394-R1400.

Monical, P.L., Owens, G.K., & Murphy, R.A. (1993) *Am. J. Physiol.*, 264 : C1466-R1472.

Word, R.A., Casey, M.L., Kamm, K.E., *et al.* (1991) *Am. J. Physiol.*, 260 : C861-C867.

388P EFFECTS OF 5-$HT_{1B/D}$ RECEPTOR AGONISTS ON FUNCTION IN THE GUINEA-PIG ISOLATED PERFUSED HEART

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The effects of the 5-$HT_{1B/D}$ receptor agonists, dihydroergotamine (DHE), sumatriptan (SUM) and zolmitriptan (ZOL) upon coronary flow and left ventricular function in the isolated perfused guinea-pig heart were investigated in the presence and absence of nitric oxide (NO) synthase inhibition (SI). Guinea-pigs (SPF, Hartley, Charles River, France, 500-600 g) were euthanized with sodium pentobarbitone (250 mg/kg i.p.), and the heart was rapidly excised. Hearts were perfused under constant pressure (80 cm H_2O) with oxygenated (95%O_2-5%CO_2) Krebs-bicarbonate buffer (pH 7.4) containing atenolol (1 μM), and were driven at 4 Hz. A water-filled latex balloon was inserted into the left ventricle (LV) to measure LV developed pressure (DP) and LV end diastolic pressure (EDP). Coronary flow (CF) was measured by a doppler flow probe placed around the aortic cannula. Results are expressed as means ± s.e.mean. Intergroup analysis was performed using ANOVA followed by Dunnett's test. In the absence of NOSI, SUM (0.1-32 μM, n=6-11 per group) failed to statistically significantly affect LVDP, LVEDP, or CF (max. changes: -8.1±1.8%, NS; +10.4±9.8%, NS; -12.2±1.4%, NS compared to vehicle; respectively), whereas DHE from 3.2 μM significantly increased LVDP and CF (at 32 μM; +49.2±5.6%, $p<0.001$ and 72.3±13.1%, $p<0.001$ compared to vehicle, respectively). In hearts perfused with Nω-nitro-L-arginine methyl ester (10 μM) in order to inhibit NOS, SUM (0.1-32 μM, n=6-17 per group) failed to significantly affect CF (max. change 0.2±5.7%, NS), but induced concentration-dependent increases in LVEDP(max. increase 89.0±30.3%, $p<0.05$, EC_{50} 3.6(2.9-5.7) μM) which were resistant to antagonism by the 5-$HT_{1B/D}$ receptor antagonist GR 127935 (Clitherow *et al*, 1994; 0.1 μM; max. increase 51.8±11.1%, $p<0.001$ compared to vehicle and NS compared to SUM alone). Under NOSI, neither DHE (0.1-32 μM) or ZOL (32 μM) significantly affected the parameters measured. In conclusion, the 5-$HT_{1B/D}$ receptor agonists investigated failed to significantly affect CF or LV function, possibly suggesting the absence or weak functional coupling of these receptors in the present model, in agreement with the findings of Paterna *et al.* (1995) in the rabbit but not with those of Ellwood & Curtis (1997). During NOSI, SUM, but not DHE or ZOL induced diastolic contracture which does not appear to be mediated by 5-$HT_{1B/D}$ receptors.

Clitherow, J.W., Scopes, D.I.C., Skingle, M. *et al* (1994) J. Med. Chem. 37, 2253-2257.
Paterna, S., Di Pasquale, P., Antona, A. *et al.* (1995) Drugs Exptl. Clin. Res.(1995) 21,37-40.
Ellwood, A.J. & Curtis, M.J. Br. J. Pharmacol. (1997) 120,1039-1048.

389P DIRECT RELAXING EFFECTS OF PIRETANIDE AND FUROSEMIDE IN RAT AND GUINEA-PIG MESENTERIC RESISTANCE ARTERIES

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It has been previously shown that hydrochlorothiazide could exert direct vasorelaxing effects in guinea-pig mesenteric and human subcutaneous resistance arteries (Calder *et al.*, 1992). The aim of this study was to investigate if these effects were also shared by two loop diuretics -piretanide (Pire) and furosemide (Furo)- in rat and guinea-pig mesenteric resistance arteries.
For this purpose, rat (male, Wistar, 300-350 g) and guinea-pig (male, 300-350 g) mesenteric arteries were removed from non-drug treated animals and mounted on a two-wire myograph (Mulvany and Halpern, 1977). Basal tension was determined using a passive extension protocol (mean diameter at 0.9 L 100 = 233 ± 11 µm from 7 rats and 331 ± 16 µm from 20 guinea-pigs). Arteries were bathed in physiological solution (composition, mM: NaCl 119, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 5.5) and equilibrated with 95 % O_2/ 5 % CO_2 at 37° C.
Relaxing effects were studied following depolarization with K^+-PSS (124 mM) or preconstriction with noradrenaline (NA, 50 µM). In the latter case, the effects of incubation (20 min) with K^+ channels inhibitors: iberiotoxin (a blocker of calcium-activated K^+ channels, 100 nM) and glibenclamide (a blocker of ATP-sensitive K^+ channels, 100 µM) were studied. The effects of endothelium rubbing or incubation with L-NAME (10^{-4} M) or indomethacin (10^{-5} M) were also assessed.
In rat arteries, no vasorelaxing effect was observed in either depolarized or NA-activated preparations (n=7), as was also the case for guinea-pig depolarized arteries (n=10). In contrast, both diuretics have induced concentration-dependent relaxations in guinea-pig-NA-activated arteries, with EC_{50} expressed as minus log EC_{50} : 8.3 ± 0.2 (n=16) and 8.3 ± 0.2 (n=11) for Pire and Furo respectively. Effects of incubations with inhibitors or endothelium suppression are displayed in Table 1.

Table 1 Maximal relaxation (as % of NA-induced tone) of guinea-pig mesenteric resistance artery to loop diuretics in control conditions and after incubation with iberiotoxin, glibenclamide, L-NAME or indomethacin.

	Piretanide		Furosemide	
	%	n	%	n
Control	95 ± 1	16	93 ± 2	11
Iberiotoxin	-5.5 ± 3.0 ***	5	-7.5 ± 2.5 ***	7
Glibenclamide	52 ± 10	4	79 ± 7	6
-E	-1.6 ± 4.0 ***	5	11.5 ± 3.3 ***	5
L-NAME	90 ± 1	8	71 ± 12	4
Indomethacin	ND		93 ± 2	9

Values (mean ± SEM) different from control : *** : $p < 0.001$ vs control (Student's t test for paired groups); -E : preparations without endothelium; ND: non determined.

Iberiotoxin or destruction of endothelium significantly inhibited or even reversed the relaxation induced by Pire and Furo, whereas glibenclamide, L-NAME and indomethacin had no significant effect.
These results show that a vasorelaxing effect, initially reported by Calder et al. (1992) for hydrochlorothiazide, can be also described for loop diuretics such as piretanide and furosemide. This effect appears to be mediated through an activation of calcium-activated-K^+-channels. It appears to be strongly dependent on the presence of an intact endothelium but not on the synthesis of nitric oxide and prostaglandins. Since no relaxation was observed in depolarized preparations, our data suggest that endothelium-derived hyperpolarizing factor could be involved in this direct relaxing effect of piretanide and furosemide in guinea-pig mesenteric resistance arteries.

Calder *et al.*, Eur. J. Pharmacol., 1992, 220 : 19-26.
Mulvany et Halpern, Circ. Res., 1977, 43 : 854-864.

390P ELECTROPHYSIOLOGICAL EFFECTS OF TERFENADINE IN THE GUINEA-PIG LANGENDORFF HEART AND ISOLATED PAPILLARY MUSCLE PREPARATIONS

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Attention has recently focused on the potential of some antihistamines (e.g., terfenadine) to precipitate torsades de pointes, a life-threatening ventricular arrhythmia that occurs in the context of a prolonged QT interval and cardiac action potential duration (Woosley, 1996). In the present study the electrophysiological effects of racemic terfenadine in two guinea-pig heart preparations have been investigated.

Langendorff heart. Epicardial left ventricular monophasic action potentials and ECGs were recorded from electrically paced (3-3.6 Hz) hearts taken from male guinea-pigs weighing about 650 g. The hearts were submerged in oxygenated Tyrode's solution and retrogradely perfused via the aorta at 37°C. Drug or vehicle was administered in the perfusant. The action potential duration measured at 90% repolarization (APD_{90}) and QT interval were measured at 10 min intervals throughout the experiment. Data were analysed by repeated measures ANOVA and Dunnett's test. Prior to treatment with vehicle (0.02% v/v ethanol) or terfenadine (2 μM) the APD_{90} values were, respectively, 161.7 ± 3.5 ms (mean ± s.e. mean; 6 hearts) and 174.5 ± 3.8 ms (5 hearts), while the QT intervals in the two groups were 175.4 ± 5.4 ms and 184.6 ± 3.6 ms, respectively. APD_{90} values after 40 min perfusion were 159.2 ± 3.9 ms in the vehicle group and 177.0 ± 5.8 ms in the terfenadine group (both $P > 0.05$). Although QT interval was unchanged in the vehicle group (172.9 ± 5.2 ms after 40 min perfusion; $P > 0.05$), after 40 min perfusion with terfenadine the QT interval was increased to 200.4 ± 3.1 ms ($P < 0.01$), and this was accompanied by a broadening of the early fast component of the ECG waveform (analogous to the QRS complex). Perfusion with terfenadine for greater than 40 min consistently induced chaotic electrical activity which rendered measurement of APD_{90} and QT interval impossible.

Isolated papillary muscle. Electrically paced (1 Hz) right ventricular papillary muscles, excised from hearts of male guinea-pigs weighing about 350 g, were superfused at 37°C. The muscles were impaled with KCl-filled microelectrodes to monitor the action potential. The effects of terfenadine, applied cumulatively at 0.1 and 1 μM (30 min at each concentration) were examined on APD_{90} in 6 muscles. The mean pretreatment APD_{90} was 196.9 ± 6.6 ms, while in the presence of 0.1 and 1 μM terfenadine the APD_{90} values were 182.6 ± 7.4 and 170.6 ± 16.0 ms, respectively (both $P > 0.05$ compared to control; repeated measures ANOVA). In 3 muscles exposed to 10 μM terfenadine for 30 min, the APD_{90} was 143.2 ± 25.8 ms (compared to a pretreatment average of 188.0 ± 5.9 ms).

These results demonstrate that in two guinea-pig heart preparations terfenadine failed to increase APD. The modest (16 ms) prolongation of QT interval seen in the Langendorff preparation, in the absence of a parallel increase in APD, could conceivably have resulted from a non-specific effect of terfenadine on cardiac conduction by virtue of an inhibitory action of the drug on multiple ion channels (Ming & Nordin, 1995).

The guinea-pig Langendorff heart and papillary muscle preparations may therefore be inappropriate test systems in which to evaluate APD and QT prolonging actions of terfenadine and related agents.

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391P COMPARISON OF THE VASODILATOR AND HYPOTENSIVE ACTIONS OF CORTICOTROPHIN-RELEASING FACTOR, SAUVAGINE, UROCORTIN AND D-AMINO ACID SUBSTITUTED ANALOGUES

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The corticotrophin-releasing factor (CRF) superfamily, including the non-mammalian peptides sauvagine (SVG), urotensin I (UI), and the more recently identified mammalian peptide urocortin (UCN; Vaughan *et al.* 1995), induce a prolonged fall in mean arterial blood pressure (MAP) on i.v. administration. Two CRF receptors have been cloned, of which the CRF 2β subtype has been localised to peripheral tissues such as the heart and skeletal muscle (Chang *et al.*, 1993; Kishimoto *et al.*, 1995). Here we have compared the vasoactive properties of this family of peptides *in vivo* and *in vitro*.

Male Wistar rats (230-275 g) were anaesthetised with thiopentone (120 $mg.kg^{-1}$ i.p.). MAP (mmHg) was measured via a carotid artery cannula and a jugular vein cannula was used for injection of peptides. Dose response curves were constructed using cumulative bolus doses of SVG, CRF, UCN and the analogue [D-Pro^4]UCN (30 pmol - 3 nmol per rat, n=6 for each peptide). The perfused rat superior mesenteric arterial bed and rings of rat thoracic aorta were prepared as described previously (Warner *et al.* 1993), with the exception that the de-endothelialised aortic rings were placed under a 2.5 g tension and preconstricted with 50 μM methoxamine. In addition to the above peptides, the analogues [D-Pro^4]SVG and [D-Glu^{20}]CRF were also used *in vitro*. In the perfused mesentery dose response curves to the peptides were constructed using 5 μl bolus administration of 10-300 pmol (n=6). For the thoracic aorta removal of endothelium was demonstrated with acetylcholine (0.1 - 10 μM) and followed by construction of cumulative concentration response curves (0.1 - 100 nM, n=6 except CRF and [D-Glu^{20}]CRF n=4).

We have previously shown that the CRF response in the perfused superior mesenteric arterial bed can be divided into two phases, with the second longer phase being endothelium-dependent (Barker & Corder, 1995). Here the rank order of potency *in vivo* was SVG> UCN=CRF=[D-Pro^4]UCN, whilst in the perfused mesentery it was SVG>UCN≥CRF=[D-Pro^4]SVG=[D-Pro^4]UCN>[D-Glu^{20}]CRF and on the thoracic aorta it was SVG>UCN=[D-Pro^4]UCN>[D-Pro^4]SVG=CRF>>[D-Glu^{20}]CRF (Table 1). Hence this study demonstrates that the hypotensive actions of this family of peptides correlate with their vasodilator activity but shows receptors on smooth muscle and endothelium have differing agonist specificity.

Table 1. Comparison of EC30 values in rings of rat thoracic aorta (nM), and ED40 values in the rat mesenteric vasculature (nmol) and anaesthetised rat ($nmol.rat^{-1}$). Results are derived from % changes and given as mean ± sem. * significantly less than native peptide ($p<0.05$), † significantly more active than CRF ($p<0.05$)

Peptide	*Aorta*	*Mesentery*	*In Vivo*
CRF	53±23	0.7±1.2	1.0±0.2
SVG	0.7±0.2†	0.05±0.03†	0.05±0.01†
UCN	11±6.5†	0.2±0.05†	1.7±0.9†
[D-Glu^{20}]CRF	no response	response too small	not tested
[D-Pro^4]SVG	41±10*	0.6±0.1*	not tested
[D-Pro^4]UCN	4.9±1.9	0.7±0.1*	3.0±0.4

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392P EFFECTS OF GLIBENCLAMIDE ON REGIONAL HAEMODYNAMIC RESPONSES TO LIPOPOLYSACCHARIDE (LPS) IN CONSCIOUS RATS

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Although there is evidence that glibenclamide (GB) reverses the hypotensive effect of bolus injection of LPS in anaesthetised rats (Wu *et al*, 1995), it is not known what the regional haemodynamic correlates of this effect are, or if GB behaves similarly in other experimental models of endotoxaemia. Therefore, we studied conscious, male, Long Evans rats (350-450g) chronically instrumented with pulsed Doppler probes, for monitoring renal, mesenteric and hindquarters haemodynamics, and with intravascular catheters to record mean arterial blood pressure and heart rate (all surgery was carried out under sodium methohexitone anaesthesia, 40-60 $mgkg^{-1}$ i.p., supplemented as required). In separate groups of animals we assessed responses to GB (20 $mgkg^{-1}$ infused i.v. in 1% 2-hydroxy-propyl-β-cyclodextrin/saline over 15 min) before continuous infusion of saline for 5h (Group a, n = 8), responses to vehicle before continuous infusion of LPS (150 $\mu gkg^{-1}h^{-1}$; *E coli* serotype 0127 B8, Sigma) for 5h (Group b, n = 8), and responses to GB before LPS (as above) (Group c, n = 7). Some of the results are summarised in Table 1.

There were no significant differences in resting cardiovascular variables (heart rate (beats min^{-1}) Group a, 322 ± 7; Group b, 318 ± 10, Group c, 331 ± 8; mean arterial blood pressure (mmHg) Group a, 106 ± 2; Group b, 105 ± 2; Group c, 102 ± 2; vascular conductance ([kHz $mmHg^{-1}$]10^3) renal: Group a, 47 ± 3; Group b, 54 ± 4; Group c, 56 ± 4; mesenteric: Group a, 54 ± 5; Group b, 55 ± 6; Group c, 68 ± 7; hindquarters: Group a, 37 ± 5; Group b, 35 ± 3, Group c, 35 ± 2). GB had a slight pressor effect with variable regional vasoconstriction. In the presence of vehicle, LPS caused transient hypotension and hindquarters vasodilatation, but sustained renal vasodilatation. In the presence of GB, the initial hypotensive effect of LPS was not apparent, although this was not due to inhibition of renal or hindquarters vasodilatation. Five h after the onset of LPS infusion following GB there was a substantial increase in mean arterial blood pressure, accompanied by mesenteric and, particularly, hindquarters vasoconstriction (Table 1).

Table 1. Changes in mean arterial blood pressure (MAP, mmHg) and renal, mesenteric and hindquarters vascular conductance (RVC, MVC, HVC, respectively, %) in conscious rats. Values are mean ± s.e. mean; *P<0.05 versus baseline (Wilcoxon test). The 15 min values are the changes after GB or vehicle; the 1h and 5h values are the changes during infusion of saline or LPS.

		15 min	1h	5h
MAP	GB/saline	4 ± 1*	7 ± 1*	10 ± 2*
	Vehicle/LPS	1 ± 2	-11 ± 3*	-2 ± 2
	GB/LPS	3 ± 1	-2 ± 4	18 ± 3*
RVC	GB/saline	-14 ± 3*	0 ± 5	7 ± 5
	Vehicle/LPS	-8 ± 3*	21 ± 6*	26 ± 8*
	GB/LPS	-15 ± 5*	37 ± 7*	22 ± 7*
MVC	GB/saline	-15 ± 2*	1 ± 5	4 ± 6
	Vehicle/LPS	-7 ± 4	8 ± 4	-4 ± 7
	GB/LPS	-10 ± 4*	-6 ± 6	-22 ± 9*
HVC	GB/saline	-10 ± 5	6 ± 9	-18 ± 8
	Vehicle/LPS	-6 ± 5	28 ± 9*	-7 ± 8
	GB/LPS	-10 ± 7	28 ± 9*	-63 ± 2*

It is not possible from these findings to say if the effects of GB on the responses to LPS were due to inhibition of K_{ATP}-channels and/or to suppression of inducible nitric oxide synthase (iNOS) (Wu *et al*, 1995). However, the effects of GB on responses to LPS differ in some respects from those of the iNOS inhibitor, aminoguanidine (Gardiner *et al*, 1996). Moreover, the delayed and particularly marked hindquarters vasoconstrictor response to LPS following GB might indicate interference with K_{ATP}-channel-mediated muscle vasodilatation, consequent upon metabolic disturbance in that tissue.

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393P EVIDENCE FOR Y_1 AND Y_2 SUBTYPES OF NEUROPEPTIDE Y RECEPTORS LINKED TO OPPOSING POSTJUNCTIONAL EFFECTS OBSERVED IN RAT CARDIAC MYOCYTES

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Neuropeptide Y (NPY) and the related peptide YY (PYY) can activate at least six different receptor populations in a wide array of tissues and cells (Playford & Cox, 1996). In the cardiovascular system, there exists at least three of the subtypes of NPY receptors, including mainly Y_1 and some Y_2 receptors on smooth muscle cells, Y_2 and Y_3 receptors in ventricular tissue, and possibly Y_2 receptors located prejunctionally on nerve endings (McDermott *et al.*, 1993). Multiple subtypes of cardiac NPY receptors may be inferred from previous studies in rat cardiomyocytes (Millar *et al.*, 1991), in which we showed that a negative effect of NPY on the contractile response, mediated by stimulation of the transient outward current, and a positive effect elicited through L-type calcium channels, are antagonised differentially by NPY(18-36).

The aim of this study was to identify the receptor subtypes for NPY that are present postjunctionally in myocardium, by establishing the rank order of agonist potency and by application of a novel Y_1 receptor antagonist (Balasubramaniam *et al.*, 1996) in the myocyte bioassay. The effects of the selective agonists, [Leu^{31},Pro^{34}] NPY (Y_1 receptors), NPY(13-36) and PYY(3-36) (Y_2 receptors), and NPY and PYY, which have differential action at Y_3 receptors, on amplitudes of contraction of electrically-stimulated adult rat ventricular cardiomyocytes were studied. Also, the effect of the Y_1-selective antagonist, bis(31/31'){[Cys^{31}, Trp^{32}, Nva^{34}] NPY(31-36)} on NPY-mediated changes in myocyte contraction was investigated.

NPY, PYY, NPY(13-36) and PYY(3-36) attenuated the isoprenaline (10^{-7} M)-stimulated contractile response, and the EC_{50} values were 9.0×10^{-9} M, 4.3×10^{-10} M, 3.1×10^{-11} M and 8.5×10^{-11} M, respectively. [Leu^{31}, Pro^{34}] NPY increased the contractile response of cardiomyocytes, and the EC_{50} values were 8.1×10^{-9} M and 1.5×10^{-9} M, in the absence and presence of isoprenaline, respectively. Since [Leu^{31}, Pro^{34}] NPY caused a positive effect on ventricular myocyte contraction and NPY(13-36) and PYY(3-36) produced the most potent negative effects, it is proposed that both Y_1 and Y_2 receptors, linked respectively to the positive and negative responses, are expressed in cardiomyocytes. As there was no significant discrepancy between the potencies of PYY and NPY to attenuate the contractile response, it appears that Y_3-like receptors are not linked principally to contractile function in rat cardiomyocytes. Bis(31/31'){[Cys^{31}, Trp^{32}, Nva^{34}] NPY(31-36)} antagonised the NPY-mediated stimulation of contractile activity through Y_1 receptors, but the compound also inhibited the attenuation of isoprenaline-stimulated contraction, apparently by acting as a partial agonist at the Y_2 receptors.

In conclusion, these findings indicate that on cardiomyocytes there are two populations of receptors for NPY, which have characteristics related to Y_1 and Y_2 receptors. The finding of Y_2 receptors on heart muscle cells represents a further example of a postjunctional location for this subtype. It is probable that the population of Y_2 receptors on cardiomyocytes is atypical in comparison with previously identified prejunctional NPY receptors.

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394P INVOLVEMENT OF THE HEME OXYGENASE/CARBON MONOXIDE PATHWAY IN THE SUPPRESSION OF ACUTE PRESSOR RESPONSES IN RAT AORTIC RINGS

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The enzyme heme oxygenase, which exists in inducible (HO-1) and constitutive (HO-2) isoforms, catalyses the conversion of heme to biliverdin and carbon monoxide (CO). HO-1 has been shown to be up-regulated in vascular cells following exposure to NO donors (Durante *et al*, 1997; Foresti *et al*, 1997; Motterlini *et al*, 1996), and CO has been demonstrated to act as a vasorelaxant analogous to nitric oxide (NO) (Suematsu *et al*, 1995). In this study we hypothesised that pre-incubation of isolated rat aortic rings with the NO donor *S*-nitroso-*N*-acetyl-penicillamine (SNAP) up-regulates the heme oxygenase pathway thereby affecting the phenylephrine (PE)-induced pressor responses.

Thoracic aortas from male Lewis rats (300-350 g) were cut into 3 mm ring segments. Each ring was mounted in a 9 ml organ bath under an applied tension of 2 g, bathed with Krebs Henseleit buffer (37°C) and gassed with 95% O_2/5% CO_2. Rings were contracted using a standard dose of KCl (100 mM) in order to provide a reference contraction. The preparations were washed and incubated with SNAP (500 μM) for 1 hr, then repetitively washed and maintained for a further 4 hr prior to producing a concentration-response curve to PE (1-3000 nM). PE elicited a concentration-dependent increase in the vascular tone of control aortic rings. Following SNAP treatment, however, the aortic contractile response to PE (100-3000 nM) was significantly ($p < 0.05$) reduced (Figure 1A). Incubation of aortic rings with the NO synthase inhibitor N^G-Monomethyl-L-arginine (L-NMMA; 100 μM) significantly ($p < 0.05$) increased the contractile response to PE in controls but failed to restore the PE pressor responses in SNAP-treated rings. Tin protoporphyrin IX (SnPPIX; 10 μM), a selective inhibitor of heme oxygenase, did not have any significant effect on PE-induced pressor responses in controls (Figure 1B). However, in the presence of SnPPIX the pressor response to PE was significantly ($p < 0.05$) increased in SNAP-treated rings to 68.7 % of the control response. Co-incubation with L-NMMA and SnPPIX further increased the response to PE (30-3000 nM) in rings treated with SNAP.

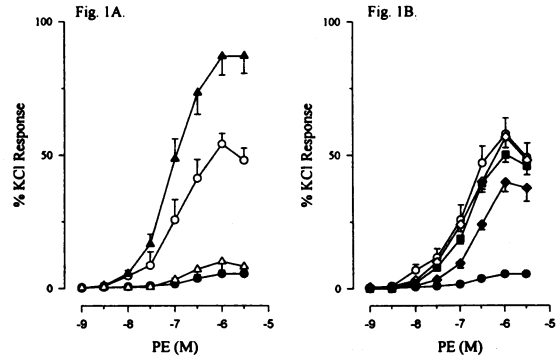

Figure 1. PE concentration-dependent curves in control rings (○) and in rings treated with SNAP (●), SNAP + L-NMMA (△), L-NMMA (▲), SNAP + SnPPIX (◆), SnPPIX (◇), SNAP + L-NMMA + SnPPIX (■); (n=5).

The present findings show that pre-treatment of aortic rings with SNAP suppressed the vasoconstrictor effect of PE and that this effect was partially reversed by a potent inhibitor of heme oxygenase. L-NMMA did not restore the PE-mediated vasoconstriction in SNAP-treated rings suggesting that endogenous NO was not involved in the control of pressor responses. The results indicate that the heme oxygenase/CO pathway may be an important determinant in the prolonged vaso-hyporeactivity observed following treatment with NO donors.

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395P INFLUENCE OF L-NAME AND REMOVAL OF ENDOTHELIUM ON NORADRENALINE CONTRACTION AND ISOPRENALINE RELAXATION IN RAT ISOLATED THORACIC AORTA

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There are conflicting reports regarding the role of endothelium in β-adrenoceptor-mediated vasorelaxation. For example, Gray & Marshall (1992) found that endothelium removal abolished isoprenaline-induced relaxation in noradrenaline-contracted rat aorta and proposed that endothelial β-adrenoceptors stimulate release of nitric oxide (NO) to cause relaxation. Eckly *et al.* (1994) also found that endothelium removal reduced the response to isoprenaline, but discounted an effect of isoprenaline on the endothelium, attributing the reduction to the greater contraction obtained with noradrenaline after endothelium removal. The present study compares isoprenaline-induced relaxation and noradrenaline-induced contraction in rat aorta after removal of endothelium or treatment with the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME).

Male Wistar rats were stunned and killed by cervical dislocation. The thoracic aorta was removed, carefully cleaned of fat and connective tissue and cut into 4 mm ring preparations. The endothelium was removed from some preparations by gently abrading the intimal surface of the rings with the tip of small steel forceps. The aortic rings were suspended in Krebs physiological salt solution (PSS) under 1g of tension. The PSS was maintained at 37°C, gassed with 95% O2/5% CO2 and contained EDTA (30 μM) and ascorbic acid (30 μM) to prevent oxidation of isoprenaline. After an equilibration period of 1 h the artery rings were constricted with noradrenaline (1 μM) and the contraction allowed to stabilise over a period of 10 min. The integrity of the endothelium was tested with acetylcholine (1 and 10 μM). Preparations with intact endothelium produced greater than 50% relaxation while successful endothelial denudation was confirmed by lack of acetylcholine-induced relaxation. After washout, some tissues were incubated with L-NAME (10 or 100 μM) for 30 minutes with control tissues receiving no treatment. The rings were then contracted again with noradrenaline and a cumulative concentration-response curve to isoprenaline carried out. After washing, tissues were contracted with noradrenaline for a third time before challenging with acetylcholine to check endothelial function.

In the presence of L-NAME (100 μM), relaxation (expressed as mean % relaxation of noradrenaline pre-contraction ± s.e.mean) to isoprenaline was significantly reduced (maximal relaxation to 100 μM isoprenaline reduced from 94±3, n=8, to 42±6, n=8, $P<0.001$). Endothelium removal had a similar effect to L-NAME (maximal relaxation reduced to 56±7, n=12, $P<0.001$). The combination of L-NAME and endothelium removal produced no further reduction in isoprenaline-induced relaxation (maximal relaxation 59±9, n=9, $P<0.01$). Contractions to noradrenaline (mean g tension ± s.e.mean) were increased from control values (0.38±0.04, n=8) after L-NAME treatment (0.73±0.07, n=8, $P<0.01$) but not after endothelium removal (0.41±0.04, n=12, $P>0.05$), or after a combination of L-NAME and endothelium removal (0.45±0.08, n=9, $P>0.05$).

The present results confirm that treatment with L-NAME or removal of endothelium reduces isoprenaline-induced relaxation in rat thoracic aorta. However, the reduction in response to isoprenaline is not simply explained by an increased contraction to noradrenaline.

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396P MULTIPLE PATHWAYS TO NITRITE RELEASE IN MOUSE PERITONEAL MACROPHAGES

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Nitric oxide (NO) is produced from L-arginine by activation of NO synthase. NO acts as multipurpose messenger molecule and plays important role in blood vessel relaxation, modulation of synaptic transmission and phagocytic activity of macrophages (Sowa & Przewlocki, 1994; Muhl *et al.* 1994). Mouse macrophages contain inducible form of NO synthase. This study was conducted to examine the effects of bacterial endotoxin (lipopolysaccharide; LPS), cholera toxin, cAMP and Ca^{++} on the release of nitrite in mouse peritoneal macrophages.

The macrophages were collected from mouse peritoneum as follows: mice were anaesthetized, the skin was cut, and the peritoneal cavity was washed several times with sterile ice-cold phosphate-buffered saline (PBS). The lavages were centrifuged for 5 min (1000 rpm), the supernatants removed, and the pellets resuspended in small amounts of PBS. Finally the cells were washed in Hank's buffer and cultured in RPMI-1640 medium in 24 well plates. Nitrite release was measured after 24 h of drug treatment using Griess reaction (Di Rosa *et al.* 1990).

Incubation of macrophages with cholera toxin (100 ng-1 µg/ml) and LPS (1-10 µg/ml) for 24 h led to significant increase in nitrite release. To examine the second messenger(s) involved in this cascade, macrophages were treated with agents that elevate intracellular cAMP e.g., isoproterenol (a β2-adrenoceptor agonist), forskolin (an activator of adenylyl cyclase) and a cAMP analogue (dibutyryl cAMP). Results show that forskolin (10 µM), isoproterenol (10 µM) and dibutyryl cAMP (1 mM) caused 92±13, 52±6, and 44±5% increase (mean±SD, n=4) in the nitrite release, respectively. Nitrite release induced by these agents was inhibited when macrophages were pretreated with NO synthase inhibitor (L-NAME; 10 µM) indicating an effect mediated at the level of NO synthesis.

Treatment of macrophages with Ca^{++}-ionophore, A-23187 (5 µM) caused 200±17% increase in nitrite release. Thapsigargin (10 µM), an inhibitor of endoplasmic reticular Ca^{++}-ATPase, mimicked the effect of A-23187 suggesting the activation of nitrite production by increased cytosolic Ca^{++} levels. Treatment of macrophages with phorbol myristate acetate (PMA; 10 µM), an activator of protein kinase C (PKC) decreased nitrite release (45 ±6 %) compared to untreated controls. These studies suggest that nitrite release from mouse peritoneal macrophages can be modulated by a diverse array of stimuli including activation of Gs/adenylyl cyclase and PKC pathway, and Ca^{++} influx.

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397P EFFECT OF INSULIN ON VASCULAR REACTIVITY IN THE RAT PERFUSED HINDLIMB *IN SITU*

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Insulin has been reported to elicit nitric oxide (NO)-dependent vasodilation in the human forearm (Steinberg *et al.*, 1994; Scherrer *et al.*, 1994). It has been suggested that this vascular action may facilitate glucose disposal (Petrie *et al.*, 1996). To further investigate the vasoactivity of insulin in insulin-sensitive tissue, we have assessed its effects on both nitrovasodilator reactivity and vasoconstrictor reactivity to a selective α_1-adrenoceptor agonist in the skeletal muscle vascular bed of the rat perfused hindlimb *in situ*.

Male Wistar rats (250-350 g) were anaesthetised with pentobarbitone sodium (60 mg kg^{-1} i.p.) and exsanguinated after heparinisation (200 iu kg^{-1} i.v.). Hindlimbs were perfused via the aorta at 7 ml min^{-1} with modified Krebs-Henseleit solution (composition in mM: NaCl 133; KCl 4.7; NaH_2PO_4 1.35; $NaHCO_3$ 16.3; $MgSO_4$ 0.61; CaCl 2.52; D-glucose 7.8), gassed carbogen and warmed to 37° C. Hindlimbs received an infusion of human insulin (100 nM) or its vehicle (0.1 % bovine serum albumin (BSA) in phosphate buffered saline (PBS)) 20 min before and during preconstriction with phenylephrine infusion (Phe, 300 nmol min^{-1}) (Laight *et al.*, 1996). Vasodilation to bolus dose acetylcholine (ACh, 0.001-30 nmols) or sodium nitroprusside (SNP, 0.001-100 nmols) was then assessed. In separate preparations, vasoconstrictions to Phe infusion (0.3-1000 nmols min^{-1}) was examined 20 min after the start of insulin (100 nM) or BSA vehicle infusion with or without a co-infusion of N^G-nitro-L-arginine methyl ester (L-NAME, 300 µM). Data are mean±s.e. mean.

Basal perfusion pressure (PP) was 27.0±1.4 mm Hg (n=52). Basal PP 20 min after insulin (34.2±3.2 mm Hg, n=19), BSA vehicle (27.5±1.4 mm Hg, n=17), insulin+L-NAME (26.2±4.4 mm Hg, n=9) or BSA+L-NAME (27.2±2.5 mm Hg, n=7) were comparable (P>0.05). Phe (300 nmols min^{-1}) raised PP to 183.7±11.5 mm Hg (n=9) and 162.9±14.9 mm Hg (n=10) in BSA vehicle- and insulin-treated groups, respectively (P>0.05). Vasodilator reactivity to ACh (pD_2=10.46±0.02, E_{max}=44.0±4.9 %, n=5) was significantly but modestly, enhanced by insulin (pD_2=10.77±0.03*; E_{max}=46.2±4.0 %, n=6) (*P<0.05) while vasodilation to SNP pD_2=8.89±0.04; E_{max}=45.7±2.9 %, n=4) was not significantly affected by insulin (pD_2=8.79±0.02; E_{max}=38.9±3.2 %, n=4) (P>0.05). Furthermore, vasoconstrictor reactivity to Phe (0.3-1000 nmols min^{-1}) (pD_2, 7.16±0.02; E_{max}=160.2±0.02 mm Hg, n=8) was slightly enhanced by insulin (pD_2=7.34±0.02*; E_{max}=189.7±13.3 mmHg, n=9) (*P<0.05). This small effect of insulin persisted in the presence of L-NAME (BSA vehicle group: Phe pD_2=8.10±0.01†; E_{max}=169.1±22.4 mm Hg, n=7 and insulin group: Phe pD_2=8.37 ±0.05†*; E_{max}=170.2±22.7 mm Hg, n=9) (*P<0.05), which elicited an increase in vasoconstrictor reactivity *per se* (†P<0.05 with respect to the absence of L-NAME).

In conclusion, while effects on both NO-dependent and -independent vascular function can be demonstrated in response to a high concentration of insulin in the rat perfused hindlimb, their limited magnitude is not consistent with a direct action of this peptide in the acute, insulin-mediated, NO-dependent vasodilation previously reported in human insulin-sensitive tissue *in vivo*.

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398P COMPARISON OF THE VASORELAXANT EFFECTS OF ANANDAMIDE AND EDHF IN RAT MESENTERIC AND CAROTID ARTERIES

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Investigations into endothelium-induced vasorelaxation that have lead to the identification of NO and prostacyclin as endothelial relaxant agents, have failed to identify the third agent or agents, endothelium-derived hyperpolarising factor (EDHF). Recently, it was reported that EDHF-induced relaxation of the rat isolated mesenteric bed could be inhibited by the CB_1 cannabinoid receptor antagonist SR141716A, and that anandamide (ANA), an endogenous cannabinoid, may be an EDHF in mesenteric arteries (Randall et al, 1996).

In this study we have compared the effects of ANA and EDHF on isolated rings of 3rd order mesenteric arteries from the rat, with respect to membrane potential and relaxation, and on relaxation of carotid artery rings. Arterial rings were mounted in a myograph in the presence of 2.8μM indomethacin and 100μM L-NAME for the measurement of tone and membrane potential. Prior to the construction of ANA concentration-effect curves tissues were pre-constricted with 3μM phenylephrine.

In mesenteric rings ANA produced a biphasic relaxant response. The initial, peak relaxation rapidly returned, either to baseline at concentrations of <100nM or to a maintained plateau relaxation at higher concentrations. The pD_2 value for the initial peak was 8.0 ± 0.04 (n=26) and for the plateau 6.8 ± 0.1 (n=20). ANA also caused repolarisation of the tissue (pD_2 8.0 ± 0.1, n=4). Both of these effects showed pronounced desensitisation to ANA. Increasing the extracellular $[K^+]$ to 25mM resulted in a small but significant reduction in the maximum relaxation induced by ANA (93% control; 70% 25mM K^+) while at the same time virtually abolishing the repolarisation due to ANA. Increasing extracellular $[K^+]$ to 60mM abolished the remaining ANA-induced relaxation while pre-treatment with the guanylyl cyclase inhibitor ODQ (10μM) had no effect. As we have already reported SR141716A had no significant effect on ANA-induced relaxation (Plane et al, 1997). ANA also failed to cause relaxation of rat (n=10) or rabbit (n=8) carotid artery rings.

ACh or A23187 caused concentration-dependent relaxation (pD_2s 7.4±0.1 & 7.5±0.2 respectively) and hyperpolarisation (pD_2s 7.3± 0.1 & 7.5±0.2 respectively) (n>4) of mesenteric arterial segments that was abolished by raising the extracellular $[K^+]$ to 25mM. SR141716A (5μM) had no effect on the relaxation induced by either agent but did antagonise the small relaxation induced by the CB_1 agonist HU210. ACh and A23187 both induced a relaxation of the carotid artery rings that was unaffected by SR141716A.

These, and previously published data (Plane et al, 1997), show that ANA, like EDHF, causes both relaxation and repolarisation of mesenteric artery. However, hyperpolarisation does not seem to be crucial for the relaxant actions of ANA and there are many other apparent differences between ANA and EDHF, e.g. toxin sensitivity, sensitivity to extracellular $[K^+]$ and tissue specificity. Taken together these data suggest that ANA and EDHF are not the same molecule and additionally CB_1 receptors do not seem to be involved in EDHF-induced relaxation.

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399P NITROTYROSINE FORMATION BY NITRIC OXIDE DONORS IN RABBIT AORTIC RINGS *IN VITRO*: INTERACTION BETWEEN NITRIC OXIDE AND ENDOGENOUS SUPEROXIDE

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The vascular endothelium continuously releases nitric oxide (NO) from a constitutive NO synthase (NOS). Once formed, NO can react with other radical species such as superoxide (O_2^-) which may be released from blood vessels. This reaction may result in the formation of peroxynitrite, a highly oxidizing species that interacts specifically with proteins via the nitration of tyrosine residues (Beckman & Koppenol, 1996). In the present study we have investigated whether NO donors can cause nitrotyrosine formation by the interaction between exogenous NO and endogenous O_2^- in isolated rabbit aorta.

Aortae were obtained from male New Zealand white rabbits (3-4 kg). Aortic rings (5 mm) were equilibrated in bicarbonate buffer (95% O_2 / 5% CO_2, 37°C) for 30 min. Rings were rinsed with HEPES-buffered Krebs and incubated with lucigenin (250 μM) in a luminometer at 37°C and chemiluminescence signal measured. Certain rings were pre-treated with the superoxide dismutase (SOD) inhibitor, diethyldithiocarbamate (10 mM, DDC) to unmask the signal. To elicit O_2^- production, rings were stimulated with NADH (1×10^{-5} - 1×10^{-2} M) or NADPH (1×10^{-5} - 3×10^{-2} M). The ability of NO donors, including S-nitrosoglutathione (GSNO; 1×10^{-6} - 3×10^{-3} M) and sodium nitroprusside (SNP; 1×10^{-8} - 1×10^{-3} M) to quench O_2^- chemiluminescence was also investigated. Release of NO from GSNO was measured by monitoring the conversion of oxyhaemoglobin to methaemoglobin. Nitrotyrosine formation was detected by western blotting using a polyclonal rabbit anti-nitrotyrosine antibody.

Under basal conditions O_2^- production was not detected from aortic rings. Addition of DDC, revealed significant basal O_2^- release that reached $2.4 \pm 0.3 \times 10^{-12}$ mol min^{-1} mg $tissue^{-1}$ (n=4). Incubation with NADH or NADPH resulted in a significant ($P<0.05$), concentration-dependent increase in O_2^- production with EC_{50} values of $1.98 \pm 0.5 \times 10^{-4}$ M and $4.01 \pm 0.95 \times 10^{-3}$ M respectively (n=4). NADH-stimulated O_2^- production was significantly ($P < 0.05$) inhibited in the presence of SOD (IC_{50} =18.5 ± 4.9 U ml^{-1}). Incubation of rabbit aortic rings with NADH (3×10^{-4} M) and increasing concentrations of GSNO or SNP resulted in significant ($P < 0.01$) quenching of O_2^- chemiluminescence (n=4). In other experiments we examined the effects of GSNO on the O_2^- chemiluminescence produced by reaction of xanthine and xanthine oxidase (X/XO) and compared this with the ability of GSNO to release NO. Release of NO from GSNO occurred over a similar concentration range that quenched O_2^- chemiluminescence. Incubation of X/XO with reduced glutathione did not affect O_2^- detection at concentrations up to 1×10^{-3} M.

Under basal conditions or in the presence of NADH (3×10^{-4} M) nitrotyrosine residues were not detected in aortic rings. Incubation with GSNO (1×10^{-3} M, 10 min) alone or in combination with NADH (3×10^{-4} M) resulted in nitrotyrosine formation which was not inhibited in the presence of SOD (1×10^5 U ml^{-1}) or tiron (1×10^{-2} M). The requirements for tyrosine nitration were further examined using bovine serum albumin (BSA). Nitrotyrosine residues were not detected on BSA incubated in Krebs buffer, xanthine/xanthine oxidase or GSNO alone. Incubation of BSA with SIN-1 (1×10^{-3} M), a donor of NO and superoxide resulted in nitrotyrosine formation suggesting that the presence of O_2^- is essential for tyrosine nitration.

Our results suggests that NO donors causes nitrotyrosine formation in aortic rings *in vitro*. This is likely to result from the reaction of NO and endogenous O_2^- resulting in peroxynitrite formation. These findings might have implications for unwanted effects of NO donors in long term use.

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400P EFFECT OF HYPOXIA ON VASODILATATION MEDIATED BY EDHF AND NO IN THE GUINEA-PIG BASILAR ARTERY

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Both EDHF (endothelium-derived hyperpolarizing factor) and NO may mediate endothelium-dependent relaxation in the guinea-pig basilar artery (Nishiye *et al.*, 1989; Petersson *et al.*, 1997). In the present study we have further characterised these responses under control (95% O_2 and 5% CO_2) and hypoxic (95% N_2 and 5% CO_2; pO_2 = 5.9 ± 1.0 mmHg; n = 7) conditions. pO_2 values were measured with a Clark-type electrode. Ring segments of guinea-pig basilar arteries were contracted with prostaglandin $F_{2\alpha}$ (0.1 - 1 μM) or endothelin-1 (1.0 - 30 nM) in the presence of indomethacin (10 μM). cGMP was measured as previously described (Zygmunt *et al.*, 1994) in artery segments. Values are presented as mean ± s.e.mean. Statistical significance (*) was accepted for $P < 0.05$ (ANOVA/Bonferroni-Dunn's post hoc test).

The relaxations induced by acetylcholine (ACh) were not significantly affected by the NO synthase inhibitor N^G-nitro-L-arginine (L-NOARG; 0.3 mM), the guanylate cyclase inhibitor ODQ (1 μM; Garthwaite *et al.*, 1995) or by these inhibitors combined (Table 1). Treatment with a combination of the K channel inhibitors charybdotoxin (ChTx; 0.1 μM) plus apamin (0.1 μM), which prevents the action of EDHF (Petersson *et al.*, 1997), reduced the maximal relaxation (E_{max}) in response to ACh whereas the sensitivity (EC_{50}) to ACh was unaffected (Table 1). When ChTx *plus* apamin were combined with L-NOARG (n = 6) or ODQ (n = 4), ACh-induced relaxations were completely abolished. Exposure of vessel segments to ACh (10 μM) for 2 min significantly increased the tissue cGMP content from 0.43 ± 0.07 pmol/ml to 2.97 ± 0.65 pmol/ml (n = 6). Such an effect of ACh was not observed in vessels pre-treated with L-NOARG (0.3 mM) for 30 min (0.11 ± 0.05 pmol/ml, test; 0.07 ± 0.02 pmol/ml, control: n = 6). In the presence of ChTx (0.1 μM) *plus* apamin (0.1 μM), to study NO-mediated responses, exposure to hypoxia for 30 min abolished ACh-induced relaxations (Figure 1). In contrast, hypoxia only marginally affected ACh-induced relaxations mediated by EDHF, ie in the presence of 0.3 mM L-NOARG (Figure 1).

Table 1 Contribution of EDHF and NO to acetylcholine-induced relaxations

	n	pEC_{50}		E_{max} (%)	
		control	treated	control	treated
L-NOARG	10	6.87 ± 0.14	6.58 ± 0.08	99 ± 0	98 ± 1
ODQ	5	6.77 ± 0.18	6.47 ± 0.17	99 ± 1	93 ± 2
L-NOARG + ODQ	5	6.45 ± 0.10‡	6.42 ± 0.06	95 ± 2‡	97 ± 1
ChTx+apamin	8	6.87 ± 0.14	6.45 ± 0.21	99 ± 0	75 ± 6*

‡ in the presence of L-NOARG.

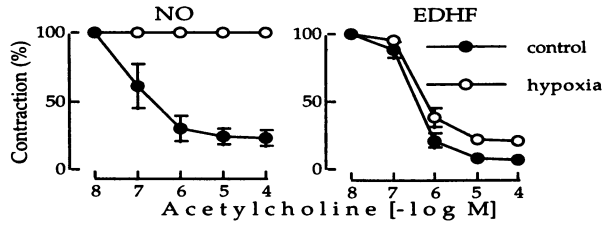

Figure 1 Effects of hypoxia on ACh-induced relaxations in the presence of ChTx + apamin (NO-mediated; left panel; n = 6) or L-NOARG (EDHF-mediated; right panel; n = 10).

The present study shows that EDHF and NO are different mediators of endothelium-dependent relaxations in the guinea-pig basilar artery. In contrast to NO, EDHF caused vasodilatation also under hypoxic conditions. These results also suggest that EDHF is not formed by an oxygen-dependent enzyme such as cytochrome P450 oxygenase in this artery.

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401P INVOLVEMENT OF DERIVATIVES OF ARACHIDONIC ACID IN ENDOTHELIUM-DEPENDENT RELAXATIONS MEDIATED BY EDHF IN THE GUINEA-PIG BASILAR ARTERY

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An endothelium-derived hyperpolarising and relaxing factor (EDHF) distinct from nitric oxide and prostacyclin has been proposed to be released by acetylcholine in the guinea-pig basilar artery (Nishiye *et al.*, 1989; Petersson *et al.*, 1997). Although its chemical identity is unknown, EDHF is believed to produce hyperpolarisation and vasodilatation through potassium channel activation in the smooth muscle cells (Petersson *et al.*, 1997). The epoxyeicosatrienoic acids, which are epoxy-derivatives of arachidonic acid formed by cytochrome P450-dependent mono-oxygenase activity, behave similar to EDHF in some vascular tissues (Campbell *et al.*, 1996; Popp *et al.*, 1996). Recently, Randall *et al.* (1996) suggested that EDHF is an endocannabinoid, possibly anandamide (arachidonylethanolamide), acting at cannabinoid receptors (CB1) in the rat isolated perfused mesenteric bed, since the selective CB1 receptor antagonist SR141716A was able to inhibit vasodilator responses mediated by both anandamide and EDHF in this preparation (Randall *et al.* 1996).

In isolated ring segments of the guinea-pig basilar artery, which were set up in conventional organ baths and contracted with prostaglandin $F_{2\alpha}$ in the presence of indomethacin (10 μM) and L-N^G-nitro-L-arginine (L-NOARG, 0.3 mM), acetylcholine and anandamide induced concentration-dependent relaxations (Table 1), whereas 11,12-epoxyecosatrienoic acid (0.3 - 3 μM) was inactive (n = 6). 17-octadecynoic acid (17-ODYA; 50 μM), a selective cytochrome P450 mono-oxygenase inhibitor (Zou *et al.*, 1994), and 5,8,11,14-eicosatetraynoic acid (ETYA; 10 μM), a non-selective inhibitor of arachidonic acid metabolism (McGiff, 1991), as well as SR141716A (3 μM) were unable to antagonise the acetylcholine-induced relaxation (Table 1). However, SR141716A (3 μM) significantly inhibited anandamide-induced relaxations (Table 1).

The results of the present study suggest that EDHF is not an arachidonic acid metabolite such as epoxyeicosatrienoic acid or anandamide in the guinea-pig basilar artery. The nature of EDHF in this artery remains elusive. However, endogenous cannabinoids may represent an interesting group of cerebral vasodilators.

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Table 1 Effects of 3 μM SR141716A (CB1 receptor antagonist), 50 μM 17-ODYA (cytochrome P450 mono-oxygenase inhibitor) or 10 μM ETYA (non-specific inhibitor of arachidonic acid metabolism) on acetylcholine-induced relaxations mediated by EDHF or on anandamide-induced relaxations in the guinea-pig basilar artery.

	n	pEC_{50}		E_{max} (%)	
		control	treated	control	treated
Acetylcholine					
17-ODYA	5	6.37 ± 0.11	6.31 ± 0.20	96 ± 2	97 ± 1
ETYA	4	6.40 ± 0.14	6.16 ± 0.27	96 ± 3	95 ± 1
SR141716A	6	6.34 ± 0.13	6.14 ± 0.16	98 ± 1	94 ± 2
Anandamide					
SR141716A	5	5.75 ± 0.16	5.53 ± 0.02	94 ± 1	43 ± 16*

*$P < 0.01$ compared to control (Students t-test, unpaired).

402P EFFECTS OF ANANDAMIDE IN THE RAT HEPATIC ARTERY

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Randall *et al.* (1996) have proposed that EDHF (endothelium-derived hyperpolarizing factor) is the arachidonic acid derivative anandamide, since both EDHF- and anandamide-mediated relaxations in the rat perfused mesenteric bed were antagonised (i) by the selective cannabinoid receptor antagonist SR141716A and (ii) by an increased extracellular potassium concentration. However, it is not known whether the action of anandamide involved activation of potassium currents. The objective of the present study was to characterise the effects of anandamide in the rat hepatic artery.

Relaxant effects of anandamide were studied in ring segments of rat hepatic arteries contracted with phenylephrine (0.1-1 μM), in the presence of the NO synthase inhibitor N^G-nitro-L-arginine (L-NOARG; 0.3 mM) and indomethacin (10 μM), conditions under which acetylcholine elicits EDHF responses in this preparation (Zygmunt *et al.*, 1994). Whereas SR141716A (3 μM) significantly (ANOVA/Bonferroni) reduced the sensitivity but not the maximal relaxation to anandamide (pEC_{50}: 6.1±0.1, control; 5.4±0.2, test; $P<0.05$ and E_{max}: 97±1%, control; 84±10%, test: n=10), EDHF responses evoked by ACh were unaffected (pEC_{50}: 7.3±0.2, control; 7.4±0.1, test and E_{max}: 98±1%, control; 96±2%, test: n=6). Relaxations mediated by anandamide were attenuated in the presence of 30 mM KCl (pEC_{50}: 6.2±0.2, control; 5.5±0.1, test; and E_{max}: 96±2%, control; 47±12%, test: $P<0.05$, n=10), conditions which almost abolish EDHF responses (Zygmunt *et al.*, 1994), but were unchanged in the presence of a combination of 0.3 μM charybdotoxin (ChTX) *plus* 0.3 μM apamin (Figure 1), which totally prevents the action of EDHF (Figure 1; Zygmunt & Högestätt, 1996).

Whole-cell potassium currents were measured in hepatic artery myocytes by the use of the patch-clamp technique as previously described (Zygmunt *et al.*, 1997). In order to detect any increase in potassium currents produced by anandamide (10 μM), cells were held at a potential of 0 mV in a calcium-containing physiological salt solution. Spontaneous transient outward currents (STOCs), which are believed to reflect activation of large conductance calcium-sensitive potassium channels (BK_{Ca}) as a result of release of calcium from intracellular stores, were frequently observed under these conditions. Exposure to anandamide (10 μM) for 5-10 min did not produce any outward currents, but completely inhibited STOCs (n=9). Application of caffeine (10 mM) for 1 s induced an iberiotoxin-sensitive potassium current (755±61 pA) which was totally abolished after 10 min exposure to 10 μM anandamide (n=5). However, $I_{BK(Ca)}$ generated by NS1619, an opener of BK_{Ca}, was unaffected by 10 min exposure to 10 μM anandamide (n=5).

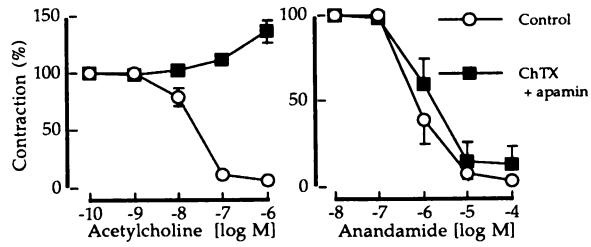

Figure 1. Effects of ChTX *plus* apamin on relaxations mediated by EDHF (liberated by ACh) and anandamide in arteries contracted by phenylephrine.

The present study provides no evidence that anandamide is EDHF in the rat hepatic artery. The data suggest that anandamide inhibits contractions produced by agonists such as phenylephrine by interfering with calcium mobilisation.

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403P THE RELATIONSHIP BETWEEN ENDOTHELIN AND THE PROGRESSION OF CONGESTIVE HEART FAILURE IN CARDIOMYOPATHIC HAMSTERS

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In patients with congestive heart failure (CHF) plasma endothelin-1 (ET-1) correlates with disease severity and its inhibition improves haemodynamic measurements (Kiowski *et al*, 1995). Therefore, we investigated the relationship between ET and the progression of congestive heart failure in cardiomyopathic hamsters. This experimental model was selected as hypertrophic or dilated CHF develops spontaneously and presents clinical features not found in other animal models (Gertz, 1972).

The progression of CHF was monitored histologically and ET was evaluated by measuring plasma and ventricular ET-1 in ventricular tissue. All parameters were studied in normal (CHF 148), hypertrophic (CHF 146) and dilated (CHF 147) hamsters aged 1, 3, 6 and 9 months (n = 6 per group). Cardiac function was measured in 9 month old CHF 148, 147 and 146 hamsters (n = 6 per group). Following terminal pentobarbitone anaesthesia, blood was obtained via cardiac puncture and plasma prepared. A Sagittal section of the heart was removed and fixed in formalin and the remaining ventricular tissue was separated, snap frozen and freeze dried. Histological sections were stained with haematoxylin-eosin or modified Martius Scarlet Blue and lesions were quantified by computer assisted image analysis. ET-1 was extracted and measured using a R & D Systems human ET-1 immunoassay kit (Rolinski *et al.*, 1994). Following Pentobarbitone anaesthesia (60 mg kg^{-1}) left ventricular developed pressure (LVDP) was measured in isolated perfused hearts as described by Sargent *et al*, (1993). All results are expressed as mean ± s.e.mean.

Histological assessment of CHF 146 and 147 showed histopathological features characteristic of CHF (Table 1). The CHF 147 strain showed no evidence of dilated cardiomyopathy, but both CHF 146 and 147 strains demonstrated similar hypertrophic cardiomyopathy. No difference in plasma ET-1 levels were found over time or between strains (mean = 1.6 ± 0.1 pg/ml). Significant increases in ventricular ET-1 levels in CHF 146 and 147 versus normal animals were found at 6 and 9 months (Table 1). When compared to normal animals, a significant reduction in cardiac function was observed in both CHF 146 and CHF 147 hamsters (Table 1).

In conclusion, CHF 146 and 147 hamsters demonstrate histopathology similar to patients with hypertrophic cardiomyopathy. Cardiomyopathy is associated with elevated levels of ET-1 in ventricular tissue at a time point where cardiac function is compromised. Endothelin may therefore play a role in the latter stages of this animal model of congestive heart failure.

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Table 1 Histological features, ventricular ET-1 levels and LVDP in normal, CHF 146 and 147 hamsters. (Different from controls * = $p<0.05$ Dunnetts test)

Age month	Ventricular histological analysis of normal (CHF 148) versus CHF 146 and CHF 147	Right ventricular ET-1 (pg/mg)			LVDP (mm Hg)		
		normal	CHF 146	CHF 147	normal	CHF146	CHF147
1	Moderate dilation, normal histology	5.7±0.4	6.6±0.4	5.8±0.2	-	-	-
3	Cardiomyopathy : calcification, focal myolysis, mild cell infiltration and mild fibrosis	5.9±0.9	6.1±0.7	7.4±0.7	-	-	-
6	Severe cardiomyopathy : marked calcification, marked fibrosis and focal necrosis	6.1±1.0	9.9±1.3 *	11.2±0.9*	-	-	-
9	Severe cardiomyopathy : interstitial fibrosis, marked calcification and focal necrosis	6.1±0.9	12.9±2.6 *	11.7±1.1*	27±5	9.5±0.7*	6.1±0.7*

404P *IN VIVO* CHARACTERISATION OF THE BLOOD PRESSURE RESPONSE TO ENDOTHELIN-1 IN THE ANAESTHETISED MINIPIG

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Endothelin-1 (ET-1) is a potent vasoactive peptide which exerts its effects via actions on at least two distinct endothelin (ET) receptors, ET_A and ET_B. Our aim was to evaluate the blood pressure (BP) response to ET-1 in the minipig, a species whose cardiovascular system bears close similarities to that of humans. We have determined the dose-dependent effect of PD-156,707, a selective ET_A receptor antagonist, on the BP response to ET-1 in the presence and absence of BQ-788, a selective ET_B receptor antagonist.

Female Göttingen minipigs were anaesthetised with sodium pentobarbitone and artifically ventilated with medicinal air. The left femoral artery and vein were cannulated for measurement of mean arterial pressure (MAP) and administration of drugs respectively. PD-156,707 (0.35 - 10 $\mu gkg^{-1}min^{-1}$) was given cumulatively as an i.v. infusion 30 min prior and continuing for 40 min after an i.v. bolus of ET-1 (40 $pmolkg^{-1}$). In a separate group of animals, BQ-788 (1 $mgkg^{-1}$) was given as an i.v. bolus 10 min prior to an i.v. bolus of ET-1 (40 $pmolkg^{-1}$). This was followed, 40 min later, with an i.v. infusion of PD-156,707 (3.5 $\mu gkg^{-1}min^{-1}$) and an i.v. bolus of ET-1 (40 $pmolkg^{-1}$) given 30 min into the infusion. A paired Student t-test was used for statistical analysis ($p<0.05$ denotes statistical significance).

ET-1 (40 $pmolkg^{-1}$, n=4) produced a biphasic increase in MAP, an initial increase which peaked within 1 min (9 ± 2 mmHg) lasting for 2-3 min, followed by a secondary increase which peaked (16 ± 2 mmHg, n=4) within 6 min post-dose, returning to control levels within 45 min. The secondary pressor effect was decreased by 38% (n=3), 63% (n=3), 75% (n=4, $p<0.05$) and 75% (n=4, $p<0.05$), by 0.35, 1, 3.5 and 10 $\mu gkg^{-1}min^{-1}$ PD-156,707 respectively, however the initial transient increase in MAP was resistant to PD-156,707. In the second group of animals (n=4), ET-1 (40 $pmolkg^{-1}$) produced a similar biphasic increase in MAP which reached a maximum within 1 min (12 ± 2 mmHg) and 6 min (14 ± 3 mmHg). Pretreatment with BQ-788 (1 mg/kg) significantly potentiated (43 ± 4 mmHg, $p<0.05$) the sustained secondary pressor response to ET-1. Addition of PD-156,707 (3.5 $\mu gkg^{-1}min^{-1}$) resulted in complete abolition of the secondary pressor response to ET-1, however the initial transient increase in MAP was still evident.

These data show (i) as in man (Maguire & Davenport, 1995), ET-1 is a potent vasoconstrictor in the minipig vasculature, mediated predominantly via the ET_A receptor, (ii) an ET_B vasodilatory component is masked by the pressor response and revealed by BQ788 and (iii) a transient increase in MAP in response to ET-1 which is resistant to BQ-788 and PD-156,707 blockade. The latter finding has previously been reported in the pig (Cirino *et al.*, 1997) and requires further investigation.

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405P N-SUBSTITUTED ANALOGUES OF S-NITROSO-N-ACETYLPENICILLAMINE: CHEMICAL STABILITY AND SELECTIVE RETENTION IN RAT ENDOTHELIUM-DENUDED FEMORAL ARTERIES

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S-Nitroso-N-acetylpenicillamine (SNAP; 2 carbon side-chain) is an S-nitrosothiol that decomposes spontaneously in solution to its corresponding disulphide and nitric oxide (NO). The rate of decomposition is heavily influenced by Cu(I) catalysis, a factor which is largely responsible for the therapeutically undesirable variability in SNAP decomposition. Here we investigated the chemical stability and vasodilator properties of the propanyl (SNPP; 3C), valeryl (SNVP; 5C) and heptanyl (SNHP; 7C) N-substituted analogues of SNAP. It was expected that increasing carbon chain length would sterically hinder Cu(I)-catalysed decomposition and would impart a degree of non-polarity to the compounds, which might alter their vasodilator properties.

Rate of S-nitrosothiol decomposition was determined spectrophotometrically by monitoring the characteristic S-nitrosothiol absorption peak (339-342 nm). Our results indicate that SNVP was the most stable analogue in Krebs buffer solution at 24 °C (<1% decomposition. h^{-1}). Decomposition of all four compounds was accelerated by Cu(II) (1 µM) and cysteine (1 mM). Addition of neocuproine, a specific Cu(I) chelator, significantly slowed decomposition of the compounds.

Vasodilator studies were carried out on isolated lengths of rat femoral artery (7.5 mm long) taken from adult male Wistar rats (400-500g) killed by cervical dislocation. Vessels were perfused (0.6 ml min^{-1}) and superfused (1 ml min^{-1}) with oxygenated Krebs solution and perfusion pressure monitored continuously (Flitney *et al.* 1992). Bolus injections of SNAP (10 µl; 10^{-8}-10^{-3} M) into the perfusate of phenylephrine-precontracted (4-8 µM; 77-93 mmHg) vessels caused dose-dependent, transient vasodilatations ($EC_{50} = 2.8 \times 10^{-6}$ M; n = 16) which recovered to pre-injection pressure, irrespective of endothelial integrity. Vasodilatations in response to analogues of SNAP were transient in endothelium-intact vessels (EC_{50} = 3.1 - 10.1×10^{-6} M) but failed to recover to pre-injection pressures at high doses (10^{-5}-10^{-3} M) in those denuded of endothelium by perfusing with air. Vessels were confirmed as being denuded when they failed to constrict on perfusion with the NO scavenger, ferrohaemoglobin (Hb; 10 µM). This sustained effect was most prevalent with SNHP, where responses to a 10^{-3} M dose only recovered to 43.1±3.5 % of pre-injection pressure after 1 h washout. Sustained responses were consistantly reversed by Hb.

Our results indicate that increasing the carbon chain length of the N-substituted side-chain of SNAP generally increased the stability of the compounds in solution, largely through steric hindrance of Cu(I) catalysed decomposition. Bolus injections of the three novel analoges of SNAP caused transient vasodilatation in endothelium-intact vessels, due largely to NO generated from spontaneous decomposition of S-nitrosothiols in the lumen. However, compounds with longer side-chains, and SNHP in particular, caused sustained vasodilator responses in vessels denuded of endothelium. We suggest that elongation of the N-substituted side-chain facilitates retention of analogues of SNAP by vessels denuded of endothelium. Subsequent slow decompoition within the tissue generates sufficient NO to maintain significant vasodilatation for >1 h after bolus washout. This feature may have important therapeutic implications with respect to targeting of NO donor drugs at areas of the vascular where the endothelium is damaged and endogenous NO generation impaired.

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406P PEROXYNITRITE-INDUCED DEPRESSION OF CONTRACTILITY IN RAT AORTA: THE EFFECTS OF L-CYSTEINE

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Peroxynitrite (PN) is a potent vasorelaxant species (Liu *et al.*, 1994). In addition, pre-exposure to PN depresses subsequent contractility of blood vessels (Jothianandan & Furchgott, 1996). We have investigated the nature of this depression of contractility and examined the ability of thiol compounds and their analogues to modulate it.

PN was synthesised according to the method of Beckman *et al.* (1990), and its vasodepressant actions investigated on endothelium-denuded rings of rat aorta. PN or neutralised (pH 7.4) PN (1 mM) were added to rings for 10 min. The tissues were then washed for 10 min and contracted with phenylephrine (30 or 300 nM). When tone had stabilised L-cysteine (1 mM) or one of its analogues (see below) were added and the effects recorded. Data are expressed as mean ± s.e. mean, n≥6 and differences determined by ANOVA (Bonferroni post-test).

Incubation of aortic rings with PN resulted in profound depression of subsequent phenylephrine-induced contraction. This depression was marked at 30 nM phenylephrine (0.6 ± 0.1 g tension; control 1.6 ± 0.2 g tension, $p<0.001$), but not significant at the near maximal concentration of 300 nM (1.7 ± 0.2 g tension; control 2.1 ± 0.2 g tension). In contrast, neutralised PN had no effect on subsequent contraction to phenylephrine (30 or 300 nM). In control rings or rings exposed to neutralised PN, the addition of L-cysteine (1 mM) to phenylephrine (300 nM)-contracted tissues had no effect on the level of tone. However, in rings exposed to PN, the addition of L-cysteine to phenylephrine (300 nM)-contracted tissues resulted in rapid relaxation (62 ± 6 %, $p<0.001$), which was maximal after 1-2 min, followed by a contraction. This contraction was sufficient not only to reverse the initial L-cysteine-induced relaxation but also to restore tone to the level seen in rings which had not been exposed to PN (1.9 ± 0.3 g tension). To investigate which functional group of the L-cysteine molecule was responsible for the observed vasorelaxation of phenylephrine-induced tone following exposure to PN, analogues of L-cysteine (all at 1 mM) were assayed in a similar manner. Compounds with a free thiol group induced relaxation which was not significantly different from that induced by L-cysteine. These were D-cysteine (68 ± 7%), L-cysteine ethyl ester (56 ± 15%), N-acetyl-L-cysteine (42 ± 13%) and reduced glutathione (50 ± 6%). In contrast, analogues which lack a free thiol group, i.e. oxidised glutathione, S-methyl-L-cysteine and L-alanine, induced no relaxation of phenylephrine-induced tone following exposure to PN.

These results suggest that PN acts in the tissue bath to form a long-lasting NO-donor which depresses contractility. L-cysteine appears to enhance the liberation of NO from this compound, thus initially depressing contraction further, then abolishing the depression as the 'store' of NO becomes exhausted. The enhanced liberation of NO by L-cysteine results solely from the presence of its thiol functional group.

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407P COMPONENTS INVOLVED IN THE FLOW-INDUCED DILATION OF RAT CORONARY ARTERY WITH SPONTANEOUS TONE

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In a previous study (Véquaud & Freslon, 1997), we have investigated the endothelial factors involved in the flow dependent dilation of a rat perfused coronary artery which was initially preconstricted by serotonin. In the present study, we have assessed the flow-dependent dilation in preparations which exhibited a spontaneous tone when submitted to a 90 mm Hg pressure. Furthermore, we intended to determine the involvement of the various endothelial factors (NO, PGI_2, EDHF) in this phenomenon. For this purpose, segments of the right interventricular coronary artery were taken from 10-15 week-old male Wistar rats (250-300 g). Arteries were mounted in an arteriograph where internal diameter was continuously monitored while intraluminal pressure was controlled. They were bathed in physiological solution (composition, mM : NaCl 119, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 5.5) and equilibrated with 95 % O_2/ 5 % CO_2 at 37° C.

Firstly the effect of an increase in perfusion flow (0-800 µl/min) was studied in preparations submitted to a 90 mm Hg intraluminal pressure. This dilator effect was tested in control conditions, after incubation with L-Name (100 µM-30 min),with indomethacin (100 µM-30 min), or with TEA (5 mM-30 min) and after mechanical destruction of the endothelium (-E).

Dilations were expressed as percent of the pressure-induced constriction, and wall shear-stress τ due to the physical forces exerted on the wall of vessels was calculated using the following formula: $\tau = 4\eta Q/\pi r^3$, τ is shear stress in dyn/cm^2, η is viscosity in Poise (dyn.s.cm^{-2}), Q is flow in ml/s, r is radius in cm. Results obtained with n=6 preparations (mean diameter: 308±4 µm) are given in the Table 1 and Figure 1. Differences between two means were determined using a paired Student's *t* -test

In control conditions, the increase in flow led to a progressive dilation of the vessel associated with a progressive increase in shear stress. With L-NAME and TEA, this regulation was greatly altered, with the loss of the plateau of shear stress values. Therefore, the flow-induced dilation observed in this preparation appears to be dependent on both the synthesis and release of NO and on the involvement of K^+ channels. In contrast, with indomethacin, our results did not differ from those obtained in control conditions: they do not support a significant involvement of a prostanoid vasodilating substance in this phenomenon. When endothelium was removed, the flow-induced dilation was still present but greatly altered. The mechanism(s) involved in this endothelium-independent component remain(s) to be determined.

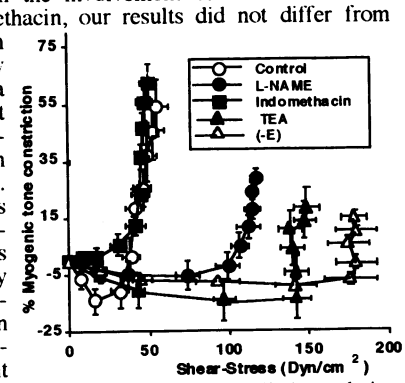

Figure 1 Shear stress-Dilation relation ship

Table 1 Relaxation of coronary artery to flow in control conditions and after L-NAME, indomethacin, TEA and suppression of endothelium (-E).

	Maximal dilation	Maximal shear stress
Control	54 ± 10	55 ± 7
+ L-NAME	28 ± 4 **	117 ± 4***
+ Indomethacin	62 ± 7	50 ± 5
+ TEA	18 ± 7 **	149 ± 8 ***
(-E)	15 ± 2 **	178 ± 8 ***

Values are mean ± s.c.m.- ** : $p < 0.01$ and *** : $p < 0.001$ vs control.

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408P ACTIVATION OF MYOCARDIAL NO/CGMP PATHWAY ENHANCES FUNCTIONAL RECOVERY OF ISOLATED WORKING RAT HEARTS DURING REPERFUSION FOLLOWING 8 HOURS CARDIOPLEGIC ARREST

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Impaired recovery of left ventricular (LV) mechanical function during reperfusion of hearts subjected to cardioplegic arrest may be due to a reduced production of nitric oxide (NO). In this study, we determined whether the cardioprotective action of the NO donor, sodium nitroprusside (SNP) involves activation of the NO/cGMP pathway.

Hearts from pentobarbitone-anaesthetised rats (250 to 300g) were perfused in the Langendorff mode (L) for 10 min, and then arrested with St. Thomas' II cardioplegia and stored at 4°C (S). After 8 hr, they were reperfused at 37°C in Langendorff mode (LR) for 10 min, and then paced (5 Hz) in working mode (W) with Krebs' solution containing glucose (11 mM) and palmitate (1.2 mM) for 60 min. In one series, stored hearts were either untreated (n=8) or were exposed to SNP (200 μM) only during LR either in the absence (n=8) or presence of the specific soluble guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 3 μM, n=8) or the specific cGMP-dependent phosphodiesterase inhibitor, zaprinast (ZAP, 20 μM, n=8). In a second series, hearts were exposed continuously to SNP (200 μM, n=8) or to SNP (50 μM), either in the absence (n=8) or presence of ZAP (20 μM, n=8). Freshly excised hearts served as non-stored controls (n=6). Additional hearts (n=4 per group) were frozen at the end of LR for analysis of total myocardial cGMP (pmol.mg protein^{-1}) by enzyme immunoassay. All values are means ± s.e.mean; * indicates $P<0.05$ by ANOVA.

At the end of reperfusion, LV work (l.min^{-1}.mmHg) was depressed in untreated hearts (1.6 ± 0.5*), relative to fresh hearts (6.6 ± 0.6). SNP, when present only during LR, improved recovery of LV work to 4.2 ± 0.4*, an effect that was blocked by ODQ. The content of cGMP after LR was lower in untreated stored hearts (0.5 ± 0.1*) than in fresh hearts (1.0 ± 0.1). SNP, given only during LR, increased cGMP content in stored hearts to 2.2 ± 0.3*, an effect that was again blocked by ODQ. While ZAP potentiated the SNP-induced elevation of cGMP (3.3 ± 0.4*), it reduced the cardioprotective effect of SNP (2.5 ± 0.4*). ZAP or ODQ *per se* did not affect LV work or cGMP content.
When SNP was present during all phases, recovery of LV work (5.4 ± 0.6*) and cGMP content (2.1 ± 0.3*) were enhanced by 200 μM, but not by 50 μM SNP. The combination of ZAP and SNP (50 μM) elevated cGMP (2.0 ± 0.1*) and also improved recovery of LV work (5.0 ± 0.5*) to levels equivalent to those observed with SNP 200 μM alone.

Since ODQ antagonised, and ZAP potentiated the effects of SNP on cGMP and on mechanical function, we propose that the activation of myocardial NO/cGMP pathway is a key determinant for the extent of functional recovery following cardioplegic arrest and prolonged hypothermic storage.

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409P CONTINUOUS TELEMETRIC MONITORING OF HEART RATE, ARTERIAL BLOOD PRESSURE AND ACTIVITY DURING CHRONIC ORAL INGESTION OF NG-NITRO-L-ARGININE METHYLESTER (L-NAME) IN CONSCIOUS RATS

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Since our original reports of the hypertensive and differential regional vasoconstrictor effects of oral ingestion of the nitric oxide synthase inhibitors, N^G-monomethyl-L-arginine and L-NAME (Gardiner *et al* 1990, 1992, 1993a,b) in polydipsic Brattleboro (*ie* vasopressin-deficient) rats, many workers have reproduced our findings in normal rats. However, in the majority of the published studies, no information is provided about the detail of the profile of the changes in arterial blood pressure and heart rate. An exception is the study of Witte *et al* (1995), in which these variables were monitored telemetrically, and it was reported that L-NAME at a daily dose of 10mgkg^{-1} caused hypertension and bradycardia, although the effects on blood pressure waned over 8 days.

Here we report findings on the cardiovascular effects of L-NAME hydrochloride (~10 mgkg^{-1}day^{-1}, Sigma) over 56 days in conscious, Long Evans rats. Under sodium methohexitone anaesthesia (40-60 mgkg^{-1} i.p., supplemented) a non-occlusive aortic catheter attached to a radiotransmitter (Datasciences Inc) was implanted. Following surgery animals (n = 2) were returned to their home cages which were standing on receiver modules (Datasciences Inc). Thereafter, data (systolic, diastolic and mean arterial blood pressures, heart rate and activity counts) were captured (using a computer-based data acquisition system; Datasciences Inc.) continuously for 21 days before and for 56 days after addition of L-NAME (14 mg 100 ml^{-1}) to the drinking water. Table 1 summarises some of the results .

To date, our results show L-NAME ingestion causes an immediate and sustained elevation in systolic, diastolic and mean arterial blood pressure in Long Evans rats, accompanied by bradycardia and an apparent reduction in activity. The extent to which the latter two variables are related, and whether or not the effects of L-NAME change over more prolonged periods, remain to be determined. However, in the animals studied so far, we have not detected a diminution of the hypertensive effect of L-NAME over the first 8 days of exposure, contrary to the findings of Witte *et al* (1995).

Table 1. Heart rate (HR, beats min^{-1}), mean arterial pressure (MP, mmHg), and activity counts (AC, units) averaged over 24h (289 data points) on the day before (control) and 1, 10 and 56 days after the onset of oral ingestion of L-NAME in 2 Long Evans rats. Values are mean ± s.d.

		Control	1	10	56 days
Rat 1	HR	354 ± 46	329 ± 46	309 ± 49	323 ± 53
	MP	109 ± 8	125 ± 8	124 ± 8	132 ± 10
	AC	39 ± 52	36 ± 62	36 ± 54	35 ± 50
Rat 2	HR	356 ± 46	335 ± 40	313 ± 49	331 ± 49
	MP	107 ± 7	125 ± 7	120 ± 7	127 ± 9
	AC	58 ± 86	43 ± 74	43 ± 68	48 ± 67

Grateful thanks to Gill Brooker and Dr John Mullins for their help in setting up the telemetry system, and to Dr Ray Hatton (Zeneca) for donating some of the equipment

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410P EFFECTS OF LIPOPOLYSACCHARIDE (LPS *E. Coli* SEROTYPE 0127:B8) ON HAEMODYNAMIC PARAMETERS AND ERYTHROCYTE MEMBRANE FATTY ACIDS: LACK OF PROTECTION BY α-PHENYL N-TERT-BUTYL NITRONE (PBN)

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The role of free radicals in the pathogenesis of LPS-induced septic shock, and the benefits of using free radical scavengers in this disorder are controversial (Hamburger & McCay, 1989). The present study assesses the effects of α-phenyl N-tert-butyl nitrone (PBN) on the haemodynamic profile and lipid peroxidation associated with LPS-induced septic shock.

Male Wistar rats (434±9 g; n=34) were anaesthetised with pentobarbitone (30 $mg.kg^{-1}$, i.p.), heparinised (50 iu) and ventilated (Palmer Ideal Pump). The rats were randomly assigned to receive either, saline (1 $ml.kg^{-1}$, i.p.) or LPS (5 $mg.kg^{-1}$, i.p.) alone or following 30 min pre-treatment with PBN (150 $mg.kg^{-1}$, i.p.). Following haemodynamic stabilisation (30 min) baseline recordings were made (time zero). Mean arterial blood pressure (MAP) and heart rate (HR) were recorded on paper (Gould 3400) every 10 min over the first 60 min and then every 20 min over the subsequent 180 min. At the end of the experiment arterial blood was collected for analysis of erythrocyte membrane (EMB) fatty acid composition by gas chromatography with electron capture detection (Gylldhal & Ehrsson, 1975) and plasma levels of thiobarbituric acid-reactive substance (TBARS) by HPLC with fluorescence detection.

Data were analysed by two-way ANOVA and differences between groups at each time point were analysed by one-way ANOVA and Duncan's multiple comparison test. Data are expressed as mean ± s.e. mean. A value of $P<0.05$ was taken as being statistical significant.

There was a 40% mortality ($P<0.05$) in the LPS+PBN group while no deaths were noted for the other groups. The baseline MAP and HR were not different between groups. PBN in the presence of saline did not affect MAP or HR. LPS decreased ($P<0.05$) MAP during the first 80 min before returning towards normal and finally falling ($P<0.05$) again after 160 min (220 min: 93±8 vs 132±4 mmHg with saline). LPS had no effect on HR. In the presence of PBN, LPS had a similar effect on MAP, but increased HR (240 min: 490±30 vs 416±14 bpm with saline; $P<0.05$). The EMB fatty acid ratio of 18:2/16:0 in the LPS (0.114±0.004), PBN+saline (0.113±0.014) and PBN+LPS (0.118±0.022) groups were not different from that in the saline treated group (0.113±0.006). However, the EMB fatty acid ratio of 22:4/16:0 was decreased ($P<0.05$) in the LPS (0.009±0.003), PBN+saline (0.011±0.002) and PBN+LPS (0.012±0.003) groups from a saline control value of 0.028±0.009. Serum TBARS were unaffected by LPS (0.72±0.05 $ng.ml^{-1}$), PBN+saline (0.73±0.15 $ng.ml^{-1}$) or PBN+ LPS (0.86±0.17 $ng.ml^{-1}$) compared with the saline control group (0.73±0.09 $ng.ml^{-1}$).

In this rat model of LPS-induced septic shock PBN increases mortality and does not offer protection against LPS-induced free radical damage of erythrocyte membrane fatty acids (decreased ratio of 22:4/16:0).

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411P CANNABINOID RECEPTOR BLOCKADE DOES NOT MODIFY THE CONSTRICTOR RESPONSE TO NORADRENALINE IN RAT ISOLATED, PERFUSED MESENTERIC BED

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Recent evidence has indicated that the vascular endothelium releases an endogenous cannabinoid (CB), anandamide, that may be the endothelium-derived hyperpolarizing factor (EDHF, Randall *et al.*, 1996). In isolated, endothelium-intact blood vessels responses to contractile agents are enhanced when release of the vasodilator, nitric oxide (N0) is inhibited. Inhibition of NO release is also believed to cause upregulation of EDHF activity (Kilpatrick & Cocks, 1994). The aims of this study were to confirm the presence of CB receptors that mediate relaxation in the rat mesenteric bed, to investigate whether release of an endogenous CB relaxant factor modifies contractile responses to noradrenaline (NA) and to investigate whether CB release modifies potentiation of NA responsiveness when NO synthesis is inhibited.

Male Wistar rats (300-400g) were anaesthetised (pentobarbitone, 60 $mg.kg^{-1}$ i.p.) and the mesenteric arterial bed was cannulated and perfused with Krebs-Henseleit buffer at 2ml/min (McGregor, 1965). After equilibration, dose-response curves (DRC) were constructed to NA (10^{-7}-10^{-4} M) in the presence and absence of the NO synthase inhibitor, L-NAME (10^{-4}M), the CB_1 receptor antagonist SR141716A (10^{-6}M) or both. After a further equilibration period perfusion pressure was raised (from 25±4 mmHg to 96±8 mmHg) by infusion of NA (10^{-6}M) prior to injection of anandamide (10^{-9}M) or the endothelium-dependent relaxant, acetylcholine (ACh, 10^{-10}-10^{-3} M) in the continuous presence of antagonist as above. Responses were compared by one-way ANOVA.

NA caused dose-dependent vasoconstriction of the mesenteric bed (EC_{50} 4.68±0.76 x 10^{-6}M, n=6). In the presence of L-NAME the NA DRC was significantly shifted to the right (EC_{50} 1.55 ± 0.16 x 10^{-6}M, $P<0.05$, n=6). SR141716A had no effect of response to NA when administered alone or in the presence of L-NAME (EC_{50} 2.3±0.5 x 10^{-6}M, n=6). Anandamide caused a relaxation (10.1±3.5% reduction in NA-induced tone, n = 7) that was significantly inhibited by SR141716A (4.0± 1.2%, $p<0.05$, n=6) but unaffected by L-NAME (8.5±2.4%, n=4). L-NAME only slightly inhibited the relaxant response to ACh, while SR141716A caused significant ($P<0.05$) inhibition of responses to low but not to high concentrations of ACh.

These results suggest the presence of CB_1 receptors that mediate relaxation in the rat mesenteric bed and that ACh can release an endogenous activator of these receptors. Furthermore they demonstrate that release of an endogenous CB does not modify contractile responses to NA either in the presence or absence of endogenous NO.

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412P LACK OF EFFECT OF A BK B_2 RECEPTOR ANTAGONIST, CP 0597, ON AIRWAY RESPONSES IN ALLERGIC RABBITS

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Asthma is characterised by chronic inflammation. Bradykinin (BK), a potent inflammatory mediator causes bronchoconstriction (BC) in asthmatics, but not in non-asthmatics (Fuller *et al.*, 1987). We examined the effect of a novel BK B_2 receptor antagonist, CP 0597 (Hanson *et al.*, 1996) on airway hyperresponsiveness (AHR) and pulmonary eosinophilia in an allergic rabbit model.

New Zealand white rabbits were immunised within 24h of birth and until 12 weeks of age with *Alternaria tenuis* antigen (Ag) in $Al(OH)_3$ gel (i.p.) as described previously (El-Hashim *et al.*, 1997). On day 1, rabbits (2.5-3.5 kg) were injected with ketamine hydrochloride (35 $mg \cdot kg^{-1}$, i.m.) and xylazine (5 $mg \cdot kg^{-1}$, i.m.) to induce and maintain neuroleptanalgesia, and then challenged with doubling concentrations of aerosolised histamine (1.25-80 $mg \cdot ml^{-1}$). Concentration-response curves were established to determine the provocative concentration of histamine which produced a 50% increase in lung resistance (RL PC_{50}) and that which caused a 35% fall in dynamic compliance (Cdyn PC_{35}). Bronchoalveolar lavage (BAL) was performed after challenge. On day 2, rabbits were treated either with CP 0597 (30 or 300 $\mu g \cdot kg^{-1}$, i.v.) or with the saline vehicle as a control. 5 min after treatment rabbits were challenged with Ag aerosol (20,000 $PNU \cdot ml^{-1}$) for 20 min and lung function monitored. On day 3, rabbits were challenged again as on day 1 and BAL performed.

Pre-treatment with CP 0597 (30 $\mu g \cdot kg^{-1}$ and 300 $\mu g \cdot kg^{-1}$) did not alter basal lung function (data not shown). Ag-induced acute BC was reduced, but not significantly, by treatment with CP 0597 (30 $\mu g \cdot kg^{-1}$) (%increase in RL 11.00 vs vehicle 23.85%; decrease in Cdyn 7.89 *vs.* vehicle 13.75%). CP 0597 (300 $\mu g \cdot kg^{-1}$) had no effect on Ag-induced BC (%increase in RL 32.95 vs vehicle 30.02%; decrease in Cdyn 16.38 *vs.* vehicle 15.57%). Ag-induced AHR was evident as reduced RL PC_{50} and Cdyn PC_{35} values to inhaled histamine 24h post-challenge in vehicle treated rabbits (Table 1). This AHR was unaffected by treatment with CP 0597.

Table 1. Histamine PC_{50} and PC_{35} values Pre and Post Ag-challenge

Treatment	N	$\log_{10}$ RL PC_{50}		$\log_{10}$ Cdyn PC_{35}	
		Pre	Post	Pre	Post
vehicle	11	1.55±0.15	1.18±0.09*	1.38±0.15	1.09±0.08
CP 0597 30$\mu g \cdot kg^{-1}$	10	1.79±0.12	1.32±0.2*	1.57±0.15	1.00±0.14*
vehicle	10	0.93±0.16	0.61±0.11†	0.88±0.18	0.42±0.1†
CP 0597 300$\mu g \cdot kg^{-1}$	10	0.99±0.13	0.45±0.05†	0.83±0.1	0.43±0.06†

*P<0.05, †P<0.01 *vs.*Pre (paired t-test)

An increase in median number of eosinophils was observed post challenge in the vehicle treated group. This increase was unaffected by treatment with CP 0597 (30 and 300 $\mu g \cdot kg^{-1}$) (Table 2).

Table 2. Eosinophil count in BAL Pre and Post Ag-challenge

Treatment	N	Eosinophils (x 10^4 cell ml^{-1}) Pre	Post
vehicle	7	0.00(0.00-0.52)	2.78(0.05-6.47)*
CP 0597 30$\mu g \cdot kg^{-1}$	6	0.00(0.00-0.83)	0.95(0.00-5.35)*
vehicle	10	0.00(0.00-0.30)	2.14(0.10-6.42)†
CP 0597 300$\mu g \cdot kg^{-1}$	10	0.00(0.00-0.21)	1.96(0.75-12.7)†

*P<0.05, †P<0.01 *vs.*Pre (Wilcoxon test)

We conclude that BK B_2 receptor mediated responses do not contribute to AHR and eosinophilia in an allergic rabbit model.

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413P EFFECT OF ADENOSINE 5′-TRIPHOSPHATE (ATP) AND AN ATP ANALOGUE ON PROSTAGLANDIN PRODUCTION BY THE GUINEA-PIG UTERUS

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Adenosine 5′-triphosphate (ATP) and 2-methylthio-ATP contract the guinea-pig uterus, with the latter compound being the more potent (Piper & Hollingsworth, 1996). These spasmogenic actions of ATP and 2-methylthio-ATP are inhibited by indomethacin, suggesting that prostaglandins (PGs) mediate this contractile effect. Removal of the endometrium and circular muscle from the uterus also reduces the spasmogenic action of ATP and its analogue (Piper & Hollingsworth, 1996). The purpose of this study has been to investigate the effect of ATP and 2-methylthio-ATP on PG output from the guinea-pig uterus on day 7 of the cycle during an 80 min period of superfusion of the whole uterus (with an ATP contact time of 20 min), and during a 24 h tissue culture period of the separated endometrium and myometrium. PGs were measured by radioimmunoassay. The results (expressed as mean ± s.e. mean, n = 5) were analyzed by Duncan's multiple range test (superfusion experiment) or the paired t test (culture experiment).

In the superfusion experiment, ATP (100 µm) significantly ($P < 0.05$) increased the uterine outputs (pg/100 mg tissue/min) of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ from 6.2 ± 1.3 to 18.8 ± 1.9 and 10.9 ± 2.5 to 29.5 ± 7.6, respectively. Similarly, 2-methylthio-ATP (25 µm) significantly ($P < 0.05$) increased the outputs of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ from 10.2 ± 1.1 to 28.0 ± 1.7 and 20.4 ± 2.6 to 40.6 ± 5.2, respectively. Neither ATP nor 2-methylthio-ATP had any effect on PGE_2 output. In tissue culture experiments, after 24 h of culture ATP (10 to 100 µM) and 2-methylthio-ATP (5 to 25 µM) acting on the endometrium significantly ($P < 0.05$) increased $PGF_{2\alpha}$ output between 5.9- and 9.6-fold, while they significantly ($P < 0.05$) decreased 6-keto-$PGF_{1\alpha}$ output by 41 to 67%. They had no effect on PGE_2 output from the endometrium. On the myometrium, all concentrations of ATP and 2-methylthio-ATP significantly ($P < 0.05$) decreased the output of 6-keto-$PGF_{1\alpha}$ by 55 to 74%, but only 2-methylthio-ATP (25 µM) significantly ($P < 0.05$) increased $PGF_{2\alpha}$ output (by 2.9-fold). ATP (10 µM) and 2-methylthio-ATP (10 µM) significantly ($P < 0.05$) inhibited PGE_2 output from the myometrium by 33%. The actions of ATP and 2-methylthio-ATP on PG output did not show a concentration-response relationship.

This study has shown that ATP and 2-methylthio-ATP stimulate $PGF_{2\alpha}$ production by the guinea-pig uterus, particularly by the endometrium. Their effect on the production of PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) is more variable showing stimulation in the short-term and inhibition of production by both the endometrium and myometrium in the long-term. Since $PGF_{2\alpha}$ contracts guinea-pig myometrium (Whalley & White, 1980), the present findings support the view that ATP and its analogues contract the guinea-pig uterus by stimulating PG output (particularly $PGF_{2\alpha}$) from the endometrium.

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414P EFFECTS OF L-NOARG AND SOD ON VASCULAR PARAMETERS IN ISOLATED LUNGS FROM MONOCROTALINE-TREATED RATS

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Monocrotaline (MCT) is known to cause right ventricular hypertrophy and pulmonary hypertension (PH) (Bruner *et al.*, 1983). It has been postulated that endothelial injury may play a crucial role in the development of PH in this model (Wilson *et al.*, 1992). We have carried out experiments to determine whether altered production of nitric oxide (NO) in the lung contributes to the pulmonary effects of MCT.

MCT (60 mg/kg; i.p) or vehicle were administered to male Wistar rats (230-270g). 3 wk after MCT injection lungs were isolated, ventilated with room air and perfused *via* the pulmonary artery (Lal *et al.*, 1994) using Krebs solution gassed with $20\%O_2/5\%CO_2$ /$75\%N_2$. Pulmonary perfusion pressure (PPP), lung weight (LW) and pulmonary inflation pressure (PIP) were recorded simultaneously. Drugs were added to the perfusate 20 min after the initial stabilisation.

MCT was found to cause a marked increase in the ratio of right ventricular weight compared to total ventricular weight in MCT 0.28 ± 0.01g, n=34 ($p< 0.01$) vs. control animals 0.21 ± 0.01g, n=12. This is indicative of right ventricular hypertrophy and is a consequence of pulmonary hypertension. In addition dry lung weight / % body weight ratio was also significantly increased in MCT 0.13 ± 0.01 ($p< 0.001$) vs. control animals 0.07 ± 0.01. In isolated perfused lungs basal PPP (10.66 ± 0.56 mmHg, n=17, $p< 0.001$) was significantly higher in MCT treated animals compared with control 6.22 ± 0.57 mmHg, n=7. Inclusion of L-NOARG (100 μM) caused marked increase over the basal PPP (5.58 ± 2.26 mmHg, n=5, $p< 0.05$) after 120 min of perfusion in lungs from MCT treated animals vs. MCT lungs without L-NOARG (1.2 ± 0.43 mmHg, n=7). D-NOARG (100 μM) had no effect on basal PPP in perfused lungs from MCT animals. Increase over basal PPP after 120 min of perfusion in the presence of D-NOARG was 0.7 ± 0.1 mmHg , n=4.

LW increased significantly ($p< 0.05$) after 120 min of perfusion in lungs from MCT 5.16 ± 1.45g, n=7; vs. control animals 0.6 ± 0.18g, n=3. L-NOARG prevented this increase in LW in MCT treated rat lungs. LW in the presence of L-NOARG after 120 min of perfusion was reduced significantly (1.1 ± 0.59g, n=5, $p< 0.05$) to a level comparable to control. D-NOARG was ineffective in protecting LW increase in MCT lungs (7.6± 2.4g, n=4).

Interestingly inclusion of the free radical scavenger superoxide dismutase (SOD) (20 units ml^{-1}) in the perfusate also attenuated the increase in LW in MCT treated rat lungs (1.58 ± 0.4g, n=3; $p< 0.05$).

The fact that basal PPP was higher in lungs from MCT treated animals and increased significantly in the presence of L-NOARG would suggest that the enhanced vasoconstrictor tone was not due to reduced NO production. The finding that L-NOARG and SOD were both beneficial in opposing the onset of oedema in the perfused lung strongly suggests a role for peroxynitrite in this effect. The source of this radical requires further studies.

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415P INFLUENCE OF β-ADRENOCEPTOR AGONISTS ON HYPOXIC VASOCONSTRICTIONS IN THE ISOLATED PERFUSED LUNG OF RAT: EVIDENCE OF ATYPICAL β-ADRENOCEPTORS

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The recent introduction of the first β_3- selective adrenoceptor antagonist, SR 59230A (Manara *et al*, 1996) prompted us to determine whether functionaly relevant atypical β-adrenoceptors would also be present in the pulmonary vasculature.

The aims of this study were to compare in the rat isolated perfused lung preparation, the effects of graded concentrations of isoprenaline (0.001-1 μM) and of three β_3-adrenoceptors agonists, SR 59104A, SR 59119A and SR 58611A (0.3-100 μM) (Croci *et al*, 1995; Manara *et al*, 1995) on hypoxia-induced pulmonary vasoconstriction, and to investigate the existence and determine the functional role of potential atypical β-adrenoceptors in these effects.

Isolated lungs from male Wistar rats (280-340 g) were ventilated with 21%O2+5%CO2+74%N2 (normoxia) or 5%CO2+95%N2 (hypoxia) and perfused with a salt solution supplemented with ficoll (Dumas *et al*, 1997). Propranolol (0.1 μM) was used to antagonize β_1- and β_2-adrenoceptors whereas SR 59230A (0.3 μM) was used to block β_3-adrenoceptors.

Isoprenaline, SR 59104A, SR 59119A and SR 58611A caused concentration-dependent relaxations during the hypoxic pulmonary pressure response. The order of potency was: isoprenaline > SR 59104A = SR 59119A = SR 58611A (pD_2 values Table 1). Propranolol and SR 59230A inhibited the relaxant effects of isoprenaline. SR 59230A but not propranolol produced a significant rightward shift of the concentration response curve to SR 59104A. Finally propranolol and SR 59230A, when used alone or combined, failed to oppose SR 59119A- and SR 58611A-induced relaxant effects. In concentrations ≥ 1 μM, SR 59230A caused per se a relaxation of the hypoxic vasoconstricted lung.

It is concluded that in the rat pulmonary vessels submitted to hypoxic vasoconstriction, there is a strong functional evidence for the existence of atypical β-adrenoceptors. Furthermore our results suggest that the selective β_3-adrenoceptor antagonist, SR 59230A exerts additional intrinsic relaxant properties in high concentrations.

Table 1 Potencies (pD_2) of isoprenaline, SR 59104A, SR 59119A and SR 58611A in pulmonary vessels. Influence of SR 59230A (0.3 μM), propranolol (0.1 μM) and both.

Drugs	*Control*	*SR 59230A*	*Propranolol*	*SR 59230A + propranolol*
Isoprenaline	7.36 ± 0.12	6.30 ± 0.13*	5.36 ± 0.26**	5.01 ± 0.24**
SR 59104A	5.26 ± 0.10††	4.71 ± 0.08*††	4.84 ± 0.06*	4.75 ± 0.09*
SR 59119A	5.32 ± 0.13††	5.41 ± 0.11†	5.27 ± 0.02	5.24 ± 0.04
SR 58611A	4.93 ± 0.11††	5.11 ± 0.25†	5.01 ± 0.13	5.20 ± 0.06

Significant (* $P<0.05$, ** $P<0.01$) against corresponding value obtained in control experiments. Significant († $P<0.05$, †† $P<0.01$)) against corresponding value obtained with isoprenaline (mean ± s.e. m., n=5-8).

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416P A METHOD FOR MEASURING AIRWAYS MECHANICS AND HYPER-RESPONSIVENESS IN SPONTANEOUSLY BREATHING MICE

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Asthma is characterised by an increase in airway hyperresponsiveness (AH) to various bronchoconstrictor stimuli which is associated with eosinophilic airway inflammation. There are several advantages in using mice for animal models of asthma. Firstly, their extensive use in studies of immunological and inflammatory processes has provided us with many tools with which to explore the molecular mechanisms involved in the inflammatory process. Secondly, the availability of transgenic and 'knock-out' mice for many cytokines, adhesion molecules, inducible enzymes and cells which have been implicated in asthma, provides an invaluable opportunity to study the mechanism(s) involved in the airway inflammatory response. Because of their size researchers have only recently been able to develop the technology needed to use mice to characterise the mechanisms involved in AH. Most of the published methods for measuring pulmonary mechanics either involve surgery to facilitate measurement of pleural pressure, the use of neuromuscular blockers or both. We present here a method for measuring airway mechanics in mice together with AH without the use of neuromuscular blockers or puncturing of the pleura.

Male CD-1 mice (40g) were anaesthetised with urethane (2g kg^{-1} i.p.) and connected to a Fleisch tube (5.0) via a tracheal cannula to facilitate measurement of air flow. A water filled, oesophageal cannula was placed such that transpulmonary pressure could be recorded and the animals allowed to respire spontaneously. Intravenous administration of spasmogen was achieved by cannulating the subclavian vein: Resistance (R_L) ($cmH_2O/ml/s$) and compliance (C_{dyn}) (ml/cmH_2O) were continuously computed (Amdur and Mead,1958) on a Buxco LS-20 analyser. Mean base line R_L was 0.79±0.07 (s.e.mean) and mean base line C_{dyn} was 0.031±0.002 (n=16); values were very similar to those described by Martin *et al.* Dose response curves of changes in R_L and C_{dyn} obtained to 5-hydroxytryptamine (5-HT) and methylcholine (MCh) (5 to 320µg kg^{-1}, i.v.) were determined. Maximal increases in R_L were greatest following 5-HT (12 fold) compared to MCh (7 fold) but larger in terms of decreases in C_{dyn} (4 fold) following MCh compared to 5-HT (3 fold).

Mice were sensitised with ovalbumin (OA), (10 µg) with $AL(OH)_3$, (20mg), administered i.p. in 0.2ml saline. This was repeated twice at weekly intervals. Repeat antigen challenge was performed 4 weeks after the initial sensitisation with aerosolised OA (1%) in saline for 1hr every day for 6 days. Challenged animals received an additional i.p. injection of OA, 10µg in saline on days 1 & 5. 48hrs after the last aerosolised challenge there was an influx of inflammatory cells as recoverable by bronchoalveolar lavage. These cells were predominantly eosinophils (0.002±0.001 to 0.416±0.133*x10^6/ml), and neutrophils (0.002±0.009 to 0.401±0.094*x10^6/ml). Macrophages (0.168±0.011 to 0.229±0.041 x10^6/ml) and lymphocytes (0.0 to 0.001±0.0009x10^6/ml) were unaffected. In a parallel group of mice AH to intravenous 5-HT was demonstrated in antigen challenged animals without changing base line R_L and C_{dyn}, see table.

	Δ R_L (+cmH_2O/ml/s)		Δ C_{dyn} (-ml/cmH_2O)	
5-HT. i.v.	Control	Challenged	Control	Challenged
BASAL	0.67±0.11	0.82±0.17	0.027±0.001	0.028±0.002
5µg kg^{-1}	0.36±0.07	0.66±0.16	0.006±0.001	0.009±0.001*
10µg kg^{-1}	0.47±0.08	1.40±0.38*	0.006±0.001	0.010±0.001*
20µg kg^{-1}	0.79±0.16	3.16±0.89*	0.007±0.002	0.012±0.001*
40µg kg^{-1}	2.95±0.53	6.59±1.89*	0.012±0.001	0.014±0.002
80µg kg^{-1}	6.83±0.89	15.31±4.7*	0.018±0.001	0.020±0.003

*indicates p<=0.05 compared to control students t-test n=8-13

In this study we have measured oesophageal pressure which has been shown to correspond closely to changes in intrapleural pressure (Lai & Hildebrandt, 1978). This has provided a means of measuring transpulmonary pressure non-surgically. In addition, the use of urethane as an anaesthetic provides a stable preparation where the animal can breathe spontaneously (Maggi & Meli, 1986). This procedure removes many of the technical problems associated with the use of a respiration pump and neuromuscular blockers.

In conclusion, we have developed a method for measuring airways mechanics, demonstrating AH and correlating this with inflammatory cell influx in the lung. This model should prove a useful tool for evaluating new anti-asthma therapies.

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417P EFFECT OF MEN 11420 ON TACHYKININ-INDUCED BRONCHOCON-STRICTION IN ANAESTHETIZED GUINEA-PIGS

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We have investigated the effect of the NK_2 receptor antagonist MEN 11420 ([Asn(2-AcNH-β-D-Glc)-Asp-Trp-Phe-Dap-Leu]c(2β-5β), a glycosylated water-soluble derivative of MEN 10627, (Maggi et al., 1994) on bronchoconstrictor responses induced by intravenous (i.v.) administration of the selective NK_2 receptor agonist [βAla^8]NKA(4-10) (1 nmol/kg) or capsaicin (20 nmol/kg) and bilateral electrical stimulation of the vagi at cervical level (20Hz for 20 sec, 1 ms pulse width, 10V) in urethane (1.5 g/kg s.c.) anaesthetized guinea-pigs. The animals were mechanically ventilated through a tracheal cannula (rate 60 strokes/min) and treated with gallamine triethiodide (3.4 µmol/kg i.v) to prevent spontaneous respiratory movements. Basal insufflation pressure was kept constant by means of a water valve.

[βAla^8]NKA(4-10)-induced bronchoconstriction: Administration of [βAla^8]NKA(4-10) (1 nmol/kg i.v.) at 30 min intervals induced a reproducible bronchoconstriction averaging 31.7 ± 1.9 mmHg (n = 45). After two reproducible control responses, MEN 11420 (30-300 nmol/kg), SR 48968 (Emonds-Alt et al., 1992)(30-300 nmol/kg), MEN 10627 (100-1000 nmol/kg) or vehicle were administered i.v.. The challenge with the agonists was performed at 5 and 30 min and then every 30 min up to 4 h.

MEN 11420 inhibited in a dose-dependent manner the bronchoconstrictor response to [βAla^8]NKA(4-10), which at 300 nmol/kg was blocked for 2 h (n = 5). In this model MEN 11420 was three times more potent than MEN 10627 (99 ± 1% inhibition at 1 µmol/kg, n = 5) and almost equieffective with SR 48968 (100 ± 0% inhibition at 300 nmol/kg, n = 5). MEN 11420 was more rapid than SR 48968 in inhibiting [βAla^8]NKA(4-10)-induced bronchoconstriction: at 100 nmol/kg the two compounds attained their maximal inhibitory effect at 5 and 90 min from administration, respectively. MEN 11420 showed a longer duration of action than MEN 10627: the inhibition produced by a maximally effective dose of MEN 10627 (1 µmol/kg) and of MEN 11420 (300 nmol/kg) lasted for 1 h and more than 4 h, respectively.

Electrical stimulation of the vagi: NANC bronchoconstrictor responses were obtained by electrical stimulation of the vagi in animals pretreated with atropine (5.5 µmol/kg, i.v.) and SR 140333 (1 µmol/kg, i.v.). The control bronchoconstrictor responses averaged 24 ±1.5 mmHg (n = 65). The stimuli were applied at 5, 30 and 60 min from antagonists administration. MEN 11420 (3-100 nmol/kg) inhibited the NANC bronchoconstriction in a dose-dependent manner, with complete blockade at 100 nmol/kg. The maximal inhibitory effect was reached within 30 min. MEN 11420 showed to be more potent than SR 48968 (100 ± 1% inhibition, n = 5) and MEN 10627 (96 ± 1% inhibition, n = 6) both at 300 nmol/kg.

Capsaicin-induced bronchoconstriction: Intravenous administration of capsaicin (20 nmol/kg) at 30 min intervals, in atropine (5.5 µmol/kg) and SR 140333 (1 µmol/kg)-pretreated animals, induced a reproducible bronchospastic response averaging 15 ± 3 mmHg (n = 6). The challenge with capsaicin was repeated at 5, 30, 60 min after antagonist administration. MEN 11420 (3-30 nmol/kg i.v.) was very potent in inhibiting dose-dependently the bronchoconstrictor response. The maximal inhibitory effect (95 ± 1%, n = 5) was obtained at 30 nmol/kg, within 30 min from administration.

MEN 11420 (300 nmol/kg i.v.) did not modify the bronchospasm induced by histamine (10-3000 nmol/kg i.v., n = 6).

These findings indicate that MEN 11420 is a potent and selective antagonist of the NK_2 receptor-mediated bronchoconstriction in guinea-pig airways with a long duration of action.

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418P IL1-β UPREGULATES BK B_2 RECEPTOR EXPRESSION THROUGH mRNA STABILISATION AND GENE TRANSCRIPTION IN HUMAN BRONCHIAL SMOOTH MUSCLE CELLS

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Bradykinin (BK) has been implicated in the pathogenesis of asthma (Christiansen *et al.*, 1987) and causes bronchoconstriction in asthmatic patients whereas no effect is observed in non-asthmatic subjects (Simmonsson *et al.*, 1973). Biological effects of BK are mediated through two receptor types, B1 and B2. In asthma, an increased release in various cytokines is reported, in particular interleukin-1ß (IL-1ß) (Borish *et al.*, 1992). Intratracheal administration of IL-1ß induces airway hyperresponsiveness to BK (Tsukagoshi *et al.*, 1995). We have investigated the effect of IL-1ß on the number and affinity of BK receptors, on BK B2 mRNA, on mRNA stability and on the B2 transcription rate in human bronchial smooth muscle cells in culture (HBSMCs).

Competition experiments on HBSMCs with selective B1 and B2 ligands indicated an order of potency : NPC 17761 (0.11 ± 0.03 nM) = HOE 140 (0.16 ± 0.01 nM) > BK (1.20 ± 0.15 nM) >> Des $Arg^9[Leu^8]$BK (ineffective). Hence, the BK receptors of HBSMC are of the B2 type. On untreated HBSMCs, saturation experiments with [^{3}H] BK indicated a single population of binding sites with a Kd of 0.36 ± 0.01 nM and a Bmax of 87.8 ± 5.5 fmol/mg proteins. After IL-1ß treatment (1ng/ml), an increase in specific [^{3}H] BK binding was observed after 3 hours (24.5%), reaching a maximum of 151.6 ± 11.15 fmol/mg proteins after 6hrs (72.7%) (Table 1), then decreasing to 30% after 24 hrs of treatment. No alteration in the affinity of [^{3}H] BK was observed (Table 1). After Northern blotting and hybridization with a specific B2 cDNA probe, autoradiography revealed a 5-fold enhancement in B2 mRNA 3 hrs after IL-1ß treatment. Results on mRNA stability and transcriptional rates demonstrated that IL-1ß increase B2 mRNA level through an increase of 5-fold of the transcription rates and a weak mRNA stabilisation.

Our data show the occurrence of an up-regulation of BK B2 receptors in HBSMCs after IL-1ß treatment in agreement with the IL-1ß-induced airway hyperresponsiveness to BK.

Table 1 : Bmax and Kd in control and IL-1ß (6 hours) treated cells.

Exp.	Untreated		Treated	
	Kd (nM)	Bmax (fmol/mg prot)	Kd (nM)	Bmax (fmol/mg prot)
1	0.39	97.12	0.46	126.63
2	0.31	90.25	0.32	180
3	0.37	70.4	0.37	161.2
4	0.38	93.4	0.30	138.7
Mean ± SEM	0.36 ± 0.01	87.8 ± 5.49	0.36 ± 0.03	151.6 ± 11.15

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419P HUMAN BLADDER VERSUS MESENTERIC ARTERY: SELECTIVITY OF LEVCROMAKALIM AND YM934

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There is considerable interest at present in researching new treatments for detrusor instability. One such possibility is potassium channel openers (PCO's). These have been shown to inhibit spontaneous, electrically-stimulated, potassium-induced and muscarinic-receptor-mediated contractions of bladder smooth muscle from several species, including human. It has therefore been suggested that PCO's may be of use in the treatment of bladder instability (Wein, 1991), but these compounds also act on vascular smooth muscle where they produce vasodilation. In this study, we have compared the relaxant effects of two PCO's, levcromakalim (LK) and YM934 (YM), in stable and unstable (hyperreflexic) human bladder and human mesenteric artery.

Stable bladder was obtained from four patients undergoing cystectomy for bladder cancer and from two organ donors. Unstable bladder was obtained from seven patients undergoing clam ileocystoplasty. Mesenteric artery was taken from three organ donors. Full informed patient consent was acquired where necessary.

Strips of detrusor muscle (mucosa and serosa removed) and endothelium-denuded mesenteric artery were mounted under 1g resting tension in organ baths (containing Krebs solution at 37°C, gassed with 95%O_2/5%CO_2 for isometric tension measurements. Following a 60-minute equilibration period, concentration-response curves for carbachol and phenylephrine were constructed in the bladder and mesenteric artery respectively. The agonist concentration producing 80% maximum contraction was determined and, following a 30-minute washout period, was used to contract the preparations prior to the construction of cumulative concentration-response curves for relaxation by the PCO's.

For both potassium channel openers the potency on normal and hyperreflexic bladders was not significantly different (ANOVA). Although for both drugs the EC50 value for relaxation of mesenteric artery was greater than that obtained for either type of bladder, the difference was significant for YM934 only.

	STABLE DETRUSOR	UNSTABLE DETRUSOR	MESENTERIC ARTERY
LK EC_{50} (μM)	0.27 (0.16-0.47)	0.25 (0.16-0.39)	0.62 (0.47-0.82)
LK MAX*	62.07 ± 4.17	69.92 ± 2.84	59.52 ± 5.45
n	12	17	5
YM EC_{50} (μM)	0.14 (0.09-0.22)	0.19 (0.15-0.24)	0.36 (0.27-0.47)Δ
YM MAX*	70.41 ± 4.33	67.71 ± 2.89	63.48 ± 2.94
n	13	15	6

Δ $p \leq 0.05$; * % precontraction

Thus, in contrast to results reported for rabbit tissues (Barras *et al.*, 1996), the potency of PCO's on human mesenteric artery *in vitro* would appear to be less than their potency on normal or hyperreflexic bladder.

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420P STUDIES ON ANTI-HEPATOTOXIC ACTIVITY OF ESCULIN, A FLAVONOID FROM *CICHORUM INTYBUS*

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Esculetin is a phenolic compound found in a popular food plant, *Cichorium intybus* (chicory) which is widely distributed in different parts of the world (Duke, 1992). The plant has been used traditionally for liver damage (Nadkarni, 1976). In an attempt to validate this folkloric use of the plant, we recently found that the crude extract of *Cichorium intybus* seeds and shoots exhibited hepatoprotective activity in rats (Gilani & Janbaz, 1993; 1994). The aim of this study was to see whether esculetin exhibits anti-hepatitis activity which may explain the folkloric use of the plant in liver damage.

Male Wistar rats (200-250 g) were divided into 3 groups of 10 each. Group 1 served as control and received normal saline (10 ml/kg) and vehicle (1% methylcellulose; 13 ml/kg) orally. Group 2 was given 4 doses of saline at 12 h interval and toxic dose of paracetamol (640 mg/kg) was administered orally 1 h post-treatment of the last dose. Group 3 was treated similar to that of group 2, except that esculetin (6 mg/kg) was administered instead of saline. In a parallel study on 3 similar groups, the treatment remained the same except that paracetamol was replaced by CCl_4 and vehicle was changed to olive oil (7.5 ml/kg). Animals were anaesthetized with ketamine (100 mg/kg, i.m.) 24 h after the last treatment and blood (3 ml) was collected by cardiac puncture. Liver function was assessed by measuring serum alkaline phosphatase (AP) and aminotransferases (GOT and GPT). Results are mean ± s.e.mean and were compared using Student's *t*-test.

Oral administration of paracetamol (640 mg/kg) produced liver damage in rats as manifested by significant rises ($P<0.05$) in serum enzyme values of ALP, GPT and GPT to 262 ± 14, 1210 ± 242 and 735 ± 223 iu/l respectively, compared to respective control values of 219 ± 10, 84 ± 12 and 33 ± 7. Pretreatment of rats with esculetin (6 mg/kg) lowered significantly ($P<0.05$) the respective serum ALP, GOT and GPT levels to 204 ± 18, 137 ± 41 and 71 ± 24. The hepatotoxic dose of CCl_4 (1.5 ml/kg; orally) raised serum ALP, GOT and GPT levels to 267 ± 25, 868 ± 249 and 576 ± 159 iu/l respectively, compared to respective control values of 198 ± 13, 103 ± 15 and 48 ± 12. The same dose of esculetin (6 mg/kg) was able to prevent significantly ($P<0.05$) the CCl_4-induced rise in serum enzymes and the estimated values of ALP, GOT and GPT were 207 ± 12, 133 ± 23 and 85 ± 27 respectively. These results indicate that esculetin possesses anti-hepatitis activity and the presence of this compound in *Cichorium intybus* may explain the folkloric use of the plant in liver damage.

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421P Ca^{2+} INHIBITION OF AGONIST-EVOKED CURRENTS IN THE 5-HT_3 RECEPTOR

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The agonist-evoked currents at the 5-HT_3 receptor have been shown to be inhibited by divalent cations in receptors endogenous to, amongst others, murine neuronal cell lines, rat dorsal root ganglion neurones and rat hippocampal dentate gyrus. The same effect has been found in recombinant 5-HT_3 receptors stably expressed in HEK 293 cells (Maricq et al., 1991, Gill et al., 1995), but whilst the latter authors find that the inhibitory effect of calcium and magnesium is concentration-dependent but voltage-independent, Maricq et al. report a voltage-dependent Ca^{2+} inhibition. This work seeks to assess whether or not the site of action for divalent cations resides in the ion channel region of the 5-HT_3 receptor, a possibility strongly suggested by the work of Eiselé et al. (1993) who reported their chimaeric receptor with a nicotinic ligand-binding N-terminal and 5-HT_3 receptor C-terminal domain retained the Ca^{2+} inhibitory effects characteristic of the 5-HT_3 receptor.

HEK 293 cells were stably transfected with 5-HT_3 receptor cDNA cloned from N1E 115 neuroblastoma cells, and 5-HT_3 receptor currents were examined using whole cell voltage clamp as previously described (Hargreaves et al., 1996). EC_{50} values for 5-HT and the 5-HT_3 selective agonist mCPBG were 2.7 ± 0.2 μM and 2.3 ± 0.1 μM (n=5) respectively.

When increasing amounts of Ca^{2+} were added to the external solution (in mM: NaCl 140, KCl 2.8, D-glucose 10, HEPES 10, pH 7.2), the maximal response evoked by 3 μM 5-HT was inhibited with an IC_{50} of 4.9 ± 2.2 mM (n=6).

The 5-HT_3 ion channel proved to be relatively permeable to Ca^{2+}: application of 1 μM mCPBG, in a solution containing 70mM $CaCl_2$ as the sole permeant cation, evoked a current which was 24 ± 3% (n=6) of control (140 mM NaCl). The interaction between Ca^{2+} and Na^+ within the channel was studied by analysing the conductance dependence between the molar fraction of Na^+ and Ca^{2+}. This relationship was not linear, but there was no evidence for an anomalous mole fraction effect. Voltage ramps given during agonist application in the presence or absence of Ca^{2+} revealed Ca^{2+} inhibition was not voltage dependent. However, activation of the channel with increasing amounts of 5-HT in normal saline, in the absence and presence of $CaCl_2$ (10 mM), changed the EC_{50} from 2.35 ± 0.26 to 3.8 ± 0.24 μM (n=4).

Thus, the lack of strong voltage dependence, combined with the high Ca^{2+} permeability of the channel, suggest that that the site of action of Ca^{2+} is unlikely to be within the pore, although it does not rule out an interaction at or near its entrance. However, the shift in potency of 5-HT in the presence of Ca^{2+} suggests a possible alternative mechanism: allosteric modulation of the conformational change produced by the agonist binding to the receptor.

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422P C-TERMINAL VARIATION OF THE HUMAN 5-HT$_7$ RECEPTOR AFFECTS 5-HT-INDUCED DESENSITISATION

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Recently at least three human 5-HT$_7$ (h5-HT$_7$) receptor isoforms have been identified which are generated from a single gene by different splicing events, producing variants which differ in their carboxyl termini (Heidmann et al., 1997). This C-terminal region of G-protein coupled receptors has been implicated in determination of receptor regulatory properties by desensitisation following phosphorylation (Hausdorff et al., 1990). To determine the importance of the distal C-terminal of h5-HT$_7$ in receptor regulation we have investigated the agonist-induced desensitisation in cAMP response of the h5-HT$_{7(b)}$ isoform and of another C-terminal variant (h5-HT$_{7(a)}$.var; Table 1.) stably expressed in CHO-K1 cells.

Table 1. Amino acid sequences of h5-HT$_7$ receptor C-termini.

h5-HT$_{7(a)}$	^{430}FVLQNADYCRKKGHDS
h5-HT$_{7(a)}$.var	^{430}FVLQNADYCRKKRS
h5-HT$_{7(b)}$	^{430}FVL
h5-HT$_{7(d)}$	^{430}FVLRACTRRVLLRPEK$^{446\text{-}479}$

CHO-K1 cells expressing h5-HT$_{7(b)}$ and h5-HT$_{7(a)}$.var were cultured in selection media (Dulbecco's Modified Eagle's Medium supplemented with 10% dialysed foetal calf serum, 3µM mycophenolic acid, 16µM xanthine, 100µM hypoxanthine, 16µM thymidine, 100U/ml penicillin G and 100µg/ml streptomycin) and seeded at a density of 2x10^5 cells into 24 well culture dishes. Cells were grown in serum-free media for 20h prior to assay. For desensitisation experiments cells were pre-treated with 10µM 5-HT in triplicate in Krebs buffer for 1h at 37°C prior to washing and induction of cAMP formation with 10µM 5-HT in the presence of 0.5mM 3-isobutyl-1-methylxanthine. The reaction was terminated after 15min by the addition of 100µl 0.5M perchloric acid. To characterise the mechanisms involved in agonist-induced desensitisation, experiments were performed in the absence and presence of the protein kinase inhibitors staurosporine, H-89 and bisindolylmaleimide I (2, 20 and 2µM respectively). cAMP formation was measured by scintillation proximity assay (Amersham) and values corrected for protein. Basal levels were subtracted and 5-HT pre-treatment data expressed as mean±SEM of % 5-HT-stimulated cAMP accumulation.

5-HT induced 23 and 9 fold increases over basal cAMP levels in h5-HT$_{7(b)}$ and h5-HT$_{7(a)}$.var, respectively. Prior exposure of cells expressing h5-HT$_{7(b)}$ to 10µM 5-HT for 1h had no effect on 5-HT-induced cAMP formation (106.0±8.2%, n=8). However, in cells expressing h5-HT$_{7(a)}$.var, in the absence of protein kinase inhibitors, the initial 5-HT-stimulated increase over basal (38.1±17.8pmol.mg protein^{-1}) was reduced (to 40.5±12.9%, n=3) following 1h pre-treatment with 10µM 5-HT. This desensitisation of the 5-HT-stimulated cAMP response was abolished by pre-treatment with the non-specific protein kinase inhibitor, staurosporine (95.7±16.0%, $p<0.05$) and the selective protein kinase A (PKA) inhibitor, H-89 (106.3±9.0%, $p<0.01$, Duncan's New Multiple Range following ANOVA from no inhibitor). Desensitisation was unaffected by the selective protein kinase C inhibitor bisindolylmaleimide I (56.4±10.6%).

These data suggest that the human 5-HT$_{7(b)}$ receptor isoform is not regulated by agonist-induced desensitisation. However, a modified form of h5-HT$_{7(a)}$ (h5-HT$_{7(a)}$.var), which contains a consensus sequence for PKA phosphorylation involving the terminal serine residue, shows desensitisation which is consistent with a mechanism involving receptor phosphorylation by PKA.

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423P INVOLVEMENT OF THE NPVIY MOTIF IN AGONIST-MEDIATED INTERNALISATION OF THE HUMAN 5-HT$_{1A}$ RECEPTOR

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The human 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) undergoes functional desensitisation in response to short-term association with its specific agonist, 8-OH-DPAT. This response is characterised by abolition of an agonist-induced decrease in adenylate cyclase activity. However, there is a concomitant loss in the number of high affinity binding sites suggesting that the receptor may internalise in an agonist-dependent manner. The molecular mechanisms governing short-term receptor regulation have yet to be fully elucidated but the loss of functional response is mediated at least in part, by phosphorylation of the receptor protein. However, internalisation of some G protein-coupled receptors is known to occur independently of receptor phosphorylation and other motifs, including the NPXXY sequence in the 7th transmembrane domain, have been implicated in the modulation of receptor activity.

In this study, we have attempted to determine whether there is a time-dependent redistribution of the cloned human 5-HT$_{1A}$R in response to the agonist, 8-OH-DPAT. PCR site-directed mutagenesis was used to substitute phenylalanine for tyrosine$_{399}$ in the N$_{395}$PVIY motif to determine the effect of a structurally conservative amino acid substitution on redistribution of the 5-HT$_{1A}$R. Moreover, to track receptor movement using confocal microscopy, an epitope tag (P$_{15}$PEPET$_{20}$) was engineered into the amino terminal domain of the receptor. PCR products were ligated with the expression vector, pcDNA3^R (Invitrogen) and used to transfect CHO-K1 cells using the Lipofectin^R (Gibco-BRL) carrier system. Agonist affinities and expression levels of recombinant receptors have been reported elsewhere (Page *et al*, 1996). Cells were routinely maintained in Hams F-12 medium supplemented with 5% dialysed serum and 0.4 mg/ml G418. Cells expressing unmutated or modified receptors were treated with 1 µM unlabelled 8-OH-DPAT and incubated at 37°C for 0-40 minutes. Reactions were stopped by placing cells on ice followed by washing with ice-cold buffered saline. A membrane fraction was prepared to assess binding of 2.5 nM [^{3}H] 8-OH-DPAT for 30 minutes at 30°C. Non-specific binding was defined by 10 µM unlabelled 5-HT. Data were calculated as % change in specific bound radioligand (mean ± s.e. mean; n=3) relative to control (cells incubated with buffered saline for a comparable incubation time). Mean values were analysed using the Students t-test (unpaired) where $P<0.05$ was considered significant.

Within 5 minutes of agonist incubation, membrane fractions expressing the unmutated 5-HT$_{1A}$R or epitope-tagged receptors showed a significant decrease in specific binding of [^{3}H] 8-OH-DPAT to 51.4 ± 4.6% ($P<0.05$) or 28.8 ± 2.7% of control ($P<0.001$) respectively. These receptors reached a maximum decrease in binding within 15 minutes (19.0 ± 9.3%, $P<0.05$ or 15.3 ± 8.1% of control respectively, $P<0.05$). In contrast, the time taken for binding of the Y$_{399}$F mutant to decrease to 70% of control was approximately 20 minutes. The maximum decrease in specific binding was 35 ± 12.8 % ($P<0.05$) during a 60 minute incubation period. We conclude that the dynamics of agonist-induced movement of the unmutated 5-HT$_{1A}$R are rapid and compare with those reported for other G protein-coupled receptors. Epitope-tagged receptors are viable for the study of 5-HT$_{1A}$R movement in whole cells. Tyrosine$_{399}$ is able to modulate the rate of agonist-mediated receptor redistribution.

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424P CHARACTERIZATION OF CYCLIC NUCLEOTIDE PHOSPHO-DIESTERASE mRNA EXPRESSION IN CORTICOTROPH TUMOUR (AtT20) CELLS: COEXPRESSION OF PDE1C AND PDE4

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In corticotrophs, 41-residue corticotrophin releasing-factor (CRF) is known to signal through the cAMP pathway leading to the activation of protein kinase A (PKA) and subsequently the release of adrenocorticotrophic hormone (ACTH). Previous studies have shown that calyculin A, a protein phosphatase 1/2A inhibitor, activated cyclic nucleotide phosphodiesterase (PDE) activity in AtT20 cells (Antaraki et al.,1997). Analysis of PDE activity in these cells has indicated the presence of both calmodulin (CaM)-stimulated and CaM-independent PDE activities, and the biochemical/pharmacological analyses of these activities indicated the presence of PDE1C and PDE4 (Ang & Antoni,1996) . Here we have used DEAE chromatographic separation and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis to explore which PDEs are expressed in AtT20 cells. 30000g supernatant of AtT20 cell homogenate was loaded onto a 9 mm x 15 cm column packed with Whatman DE-52 cellulose. The column was eluted with a linear sodium acetate gradient of 50 mM to 1 M. Two distinct PDE activity peaks, peak 1 and peak 2, eluting at 0.1-0.2 M and 0.4 - 0.5 M sodium acetate respectively, were obtained. Peak 1 PDE activity was stimulated 5-fold by 100 μM Ca^{2+}/100 nM CaM, while more than 80% of PDE activity in peak 2 was selectively inhibited by 10 μM rolipram (indicating predominantly PDE4 activity). Substrate saturation studies with cAMP indicated the presence of a low K_m component (K_m = 0.75 ± 0.41 μM, V_{max} = 1.8 ± 0.9 nmol/min/mg protein, mean ± S.D., n = 5) and a high K_m component (K_m = 44 ± 33 μM, V_{max} = 3.3 ± 1.4 nmol/min/mg protein, mean ± S.D., n = 5). RT-PCR was carried out using specific primers designed to distinguish the various isozymes/splice variants. PCR products of the expected size were cloned and subsequently sequenced. The sequences obtained matched with mouse PDE1B1 (L01695) and PDE1C4A/5A (L76947/L76946) DNA sequences in GeneBank database with more than 95% identity and that of rat PDE4A5 (L27057) and PDE4D4B (U09457) with more than 90% identity. In particular, the presence of PDE1C4/5 and PDE4D4B expression correlates with the kinetic and cAMP-dependent phosphorylation studies (Ang & Antoni, 1996). The coexistence of PDE1C4/5 and PDE4D4B in AtT20 cells provides the molecular basis whereby degradation of cAMP signals may proceed in a Ca^{2+}/CaM-independent or -dependent manner, as a function of the extent of phosphorylation of the respective PDEs. Such a switch or fine tuning of Ca^{2+}/CaM dependence could be important in determining the amplitude of the cAMP response to agonist stimulation.

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425P GABA$_B$ RECEPTORS IN HUMAN SPINAL CORD: LOCALIZATION OF mRNA BY *IN SITU* HYBRIDIZATION AND RECEPTOR PROTEIN BY AUTORADIOGRAPHY USING [^{3}H]-CGP62349

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GABA$_B$ receptors have been shown to be present in rat (Price *et al.*, 1984) and human spinal cord (Waldvogel *et al.*, 1990). Their location has been shown to be throughout the grey matter, with the highest densities in the superficial laminae of the dorsal horn. Recently, the localization of mRNA for GABA$_B$ receptors has been made possible by the cloning of the cDNA (Kaupmann *et al.*, 1997), so the regional expression of mRNA may be compared with the receptor protein expression. Here we report on the localization of the mRNA encoding these receptors in human spinal cord, making comparison with the localization of GABA$_B$ receptors in the same tissue using a new high affinity antagonist radioligand, [^{3}H]-CGP62349 (Bittiger *et al.*, 1996).

Samples of post mortem spinal cord (5 lumbar, 3 cervical; mean age 76 ± 6.5 years; post mortem delay 10 - 70 hours) were frozen in embedding matrix on dry ice stored at -80°C. Cryostat sections (10 μm) were prepared at -15°C to -20°C, and stored at -80°C until assay. Sections were pre-incubated (20 then 60 minutes) in fresh assay buffer (50 mM TRIS/HCl, pH 7.4, 2.5 mM $CaCl_2$) before incubation for 60 min at 25°C with [^{3}H]-CGP62349 (0.125 nM - 8 nM). Non-specific binding was determined with 10 μM CGP54626 (Bittiger *et al.*, 1992). Slides were washed for 2 x 1 minute in assay buffer at 25 °C, dipped briefly in distilled water, air dried and apposed to [^{3}H]-sensitive film for 3 weeks. Resulting images were analysed on an MCID M4, and optical density converted to fmol/mg using calibrated standards. Values of Kd and Bmax were determined using non linear regression, single site saturation analysis (GraphPad Prism). *In situ* hybridization methodology was adapted from Hand *et al.*, (1997).

[^{3}H]-CGP62349 binding to GABA$_B$ receptors was measured in superficial dorsal, deep dorsal and ventral laminae, giving Bmax and Kd values shown in Table 1. The greatest binding density was clearly seen to be in the superficial dorsal laminae, with much lower levels in the deep dorsal and ventral laminae. The Kd value was constant throughout the laminae.

Table 1. [^{3}H]-CGP62349 binding in human spinal cord.

	Lumbar		Cervical	
Laminae	Bmax (fmol/mg)	Kd (nM)	Bmax (fmol/mg)	Kd (nM)
Superficial	140.54 ± 4.36	0.36 ± 0.05	117.37 ± 12.42	0.33 ± 0.02
Deep	30.78 ± 2.67	0.41 ± 0.12	34.42 ± 1.57	0.33 ± 0.01
Ventral	13.96 ± 1.73	0.43 ± 0.17	14.98 ± 0.64	0.33 ± 0.06

Data are expressed as mean ± s.e.mean

In situ hybridization experiments indicated that the mRNA was uniformly distributed throughout the grey matter with a mean density of 2.95 ± 0.95 attmol ^{35}S/mg tissue in lumbar grey matter, and 2.63 ± 0.67 attmol ^{35}S/mg tissue in cervical grey matter.

The homogeneous expression of GABA$_B$ mRNA throughout the human spinal cord is not consistent with the high levels of receptor expressed in the superficial dorsal laminae. This discrepancy may be due to the cell bodies of the neurones projecting to these laminae being located in the dorsal root ganglia, where the mRNA would be produced. The lower levels of expression of both mRNA and GABA$_B$ receptor protein throughout the deep dorsal and ventral laminae, presumably reflects GABA$_B$ receptor-expressing interneurones.

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426P INTERLEUKIN-2 INHIBITS GLUCOCORTICOID RECEPTOR TRANSCRIPTIONAL ACTIVITY: CONSEQUENCES FOR APOPTOSIS

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In murine interleukin-2 (IL-2) dependent T-lymphocytes CTLL-2 and HT-2, we previously demonstrated that transcription is an absolute requirement in the mechanism of apoptosis induced by glucocorticoids (GC; Perrin-wolff *et al.*, 1996). Furthermore, IL-2 at saturating concentrations completely abolishes GC-induced apoptosis in these cells (Nieto & Lopes-Rivas, 1989). The aim of this work is to elucidate the mechanism of the protective effect of IL-2 against GC-induced apoptosis on CTLL-2 and HT-2 cells. We therefore assessed the influence of IL-2 on glucocorticoid receptor (GR) transcriptional activity by reporter gene assays.

Plasmids: the human Bcl-2 expression vector pSFFV-bcl-2-neo was initially described by Hockenberry *et al.* (1990). pLTR-luc plasmid contains the mouse mammary tumor virus long terminal repeat (MMTV-LTR) GC-responsive promoter driving the luciferase reporter gene. Plasmids p870, p620, p500, p360, p220, p200 and p107 contain the MMTV-LTR lacking its 5' end up to position -870, -620, -500, -360, -220, -200 and -107 respectively. GRE-tk-CAT contains one Glucocorticoid Responsive Element (GRE) under the control of the thymidine kinase minimal promoter. *DNA transfections:* for the transient assays, cells were cultivated 24 hours before transfection in treatment medium (RPMI 1640 supplemented with 10 % charcoal-treated fetal calf serum), and cotransfected with 1 µg of a pCMVβgalactosidase plasmid as a control for transfection efficiency. After transfection, cells were incubated in treatment medium for 20 hours with dexamethasone (DEX; 0.1µM) and/or IL-2 (1ng/ml). Luciferase activity was determined using chemiluminescence and corrected to βgalactosidase activity. Chloramphenicol acetyl transferase activity (CAT) was determined as previously described (Perrin-Wolff *et al.*, 1996).

To mesure the transcriptional activity of the GR in non apoptotic cells, CTLL-2 and HT-2 cells were stably transfected with a plasmid containing the human *bcl-2* cDNA. Upon Bcl-2 expression, we observed a prolonged survival of the cells following IL-2 deprivation and exposure to GC. CTLL-2 Bcl-2 and HT-2 Bcl-2 cells were then transfected with the pLTR-luc or GRE-tk-CAT plasmids. Results show that IL-2 reduces by 70% DEX-induced pLTR-luc transactivation in both cell lines, but has no effect on GRE-tk-CAT activity. This suggests that a sequence within the composite MMTV promoter could play a crucial role in the inhibitory effect of IL-2. To further elucidate the mechanism of inhibition of pLTR-luc transactivation by IL-2, we assessed the influence of deletions within the MMTV promoter on GC-induced luciferase activity. Plasmids p870 to p200 are still inducible by the hormone. In contrast, with p107, which only contains the two proximal half palindromic GREs, induction of luciferase expression by DEX is abolished. Addition of saturating concentrations of IL-2 results in a strong reduction of hormone induction levels with all plasmids tested (50 to 70 %), except p200, suggesting the existence of a regulatory element between positions -220 and -200 of the LTR, which could potentially bind a factor, induced by IL-2, critical for GR transcriptional activity.

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427P INHIBITION OF THE MEK SIGNALLING PATHWAY POTENTIATES FAS-MEDIATED APOPTOSIS IN HUMAN T CELLS

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Ligation of the Fas antigen (CD95) induces apoptosis in Jurkat T cells, but normal activated peripheral blood T cells may be resistant to apoptosis via this pathway (McLeod *et al*, submitted). T cell fate following Fas engagement may therefore depend on the provision of signals from additional surface receptors but the nature of these signals is presently unknown. Current understanding of Fas signal transduction indicates the activation of acidic sphingomyelinase with consequent ceramide generation (Cifone *et al*, 1995) in addition to the triggering of cysteine aspartate-specific proteases (caspases) (Martin & Green, 1995). Since ceramide-induced apoptosis is known to be antagonised by diacylglycerol (Jarvis *et al*, 1994), we therefore investigated the effect of activators and inhibitors of protein kinase C (PKC) on Fas-mediated apoptosis in Jurkat cells. Apoptosis was measured by ^{3}H-thymidine pre-labelling cells and harvesting onto glass fibre mats to assess DNA fragmentation (Matzinger, 1991). Students t test was used to assess statistical difference.

The PKC activating phorbol ester, phorbol myristate acetate (PMA), inhibited apoptosis induced by the anti-Fas antibody CH11 (0.1µg/ml) (54 ± 18% inhibition at 40ng/ml PMA, $p<0.05$)(n=4) whereas treatment with the PKC inhibitor RO-31/8220 (Davis *et al*, 1989) potentiated Fas-mediated apoptosis in Jurkat T cells (50 ± 22% increase in apoptosis at 300nM RO-31/8220, $p<0.05$)(n=3). One effect of PKC activation is the triggering of sphingosine kinase activity leading to the accumulation of sphingosine-1-phosphate which is known to stimulate MAP kinase kinase (MEK) activity in U937 cells (Cuvillier *et al*, 1996). Since PKC inhibition potentiated Fas-mediated apoptosis in Jurkat, we therefore sought to determine whether basal MEK activity was providing a mechanism of protection from Fas-induced cell death. Accordingly, Jurkats were pre-incubated for 1 hour with the MEK inhibitor PD 98059 (Dudley *et al*, 1995) (0.1-5µM) prior to a 4 hour treatment with the anti-Fas antibody CH11 (0.1µg/ml). Whilst MEK inhibition alone did not trigger the apoptotic pathway, it markedly potentiated Fas-mediated apoptosis (121 ± 11% increase in apoptosis at 1µM, $p<0.05$)(n=3). These data suggest that MEK activity can antagonise Fas-mediated apoptosis in T cells and that inhibition of this pathway allows cell death following Fas ligation to proceed.

Since both Fas and Fas-Ligand are upregulated following T cell activation (Boshell *et al*, 1996), controls must therefore exist to regulate the induction of Fas-mediated apoptosis for T cell immune functions to be allowed. This study indicates that one candidate signalling pathway which is capable of modulating the response to Fas engagement is the MEK pathway. Interestingly, ligation of the T Cell Receptor (TCR) is known to be a potent stimulator of this pathway (Li *et al*, 1996). Thus the functional outcome of a Fas-mediated signal may be regulated by signalling through the TCR following antigen engagement.

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428P CENTRALLY ADMINISTERED Ac-YVAD-cmk INHIBITS NEURONAL DAMAGE AND APOPTOSIS IN RATS

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Apoptosis is induced through a complex cascade of caspase proteases, including interleukin-1β converting enzyme (ICE), which are implicated in neuronal injury following hypoxia and experimental ischaemia. Acetyl-tryval-ala-asp chloromethylketone (YVAD), a broad spectrum inhibitor of caspases (Takahashi et al. 1996), prevents K+ deprivation-induced apoptosis in cerebellar granule neurons in vitro (Schulz et al, 1996). Benzyloxycarbonyl val-ala-asp dichlorobenzoate has been shown to reduce cortical and striatal lesion volume in rats, when administered prior to the ischaemic event (Loddick et al, 1996). The aim of the present study was to investigate whether YVAD, was neuroprotective in a model of transient focal ischaemia, administered post ischaemia and whether a reduction in lesion volume could be correlated to a decrease in apoptosis, as measured by TUNEL staining.

Under medetomidine hydrochloride/ fentanyl anaesthesia, intracerebroventricular (icv) cannulae were positioned in the left lateral cerebral ventricle of male Sprague Dawley rats (300-350g, n=9-11/group). A rapid drinking response following angiotensin II challenge (100ng in 5µl saline), 4 days after cranial surgery, confirmed correct placement of the cannulae. One day later, under halothane anaesthesia, left middle cerebral artery transient occlusions (MCAO) were performed using the intraluminal thread model (Longa et al, 1989). Briefly, a 3/0 nylon filament, with a heat blunted tip (0.26-0.30mm diameter), was advanced through an arteriotomy in the external carotid artery until it occluded the MCA at its origin with the Circle of Willis. Reperfusion was instituted under halothane anaesthesia by complete removal of the filament 2h post-MCAO. Vehicle (Ve -2.5µl, 0.5% DMSO in saline) or YVAD in vehicle (Y -0.7nmol) was administered at 30min post-MCAO and 1h post-reperfusion, in the following combinations: Ve/Ve, Y/Ve, Ve/Y, Y/Y, in 4 randomised groups. Twenty four hours post-MCAO rats were neurologically assessed using a subjective 7 point scoring system, euthanased and transcardially perfused with neutral buffered formalin. Fixed brains were sectioned and stained with Cresyl Fast Violet (CFV, all groups) for histological image analysis of lesion volume, or with TUNEL (groups Ve/Ve and Y/Y only) to assess apoptosis. Lesion volume, TUNEL staining and hemispheric swelling (Mackay *et al*, 1996) data were analysed parametrically. Neurological scores were subjected to Kruskall-Wallis analysis.

Following MCAO the treatment combination Y/Y produced a significant reduction in cortical lesion volume compared to Ve/Ve (28±11 vs 74±7 mm^3, mean ± s.e.mean, $P<0.05$). Treatments Y/Ve and Ve/Y had no effect. There were no significant differences between the treatment groups regarding striatal lesion volume ($P>0.05$). Hemispheric swelling was also only significantly reduced in Y/Y (13±2%) compared to Ve/Ve (25±2%). For the neurological scores there were no significant differences between the groups. Cell counting revealed that there was a significant reduction in TUNEL positive cells in the Y/Y cortical lesion compared to Ve/Ve (36±15 vs 131±58 cells/mm^2, mean ± s.e.mean, $P<0.05$). No significant reduction in TUNEL positive cell numbers was observed in the striatum.

These results confirm that a caspase inhibitor administered during the early ischaemic and reperfusion phases of focal ischaemia is neuroprotective and that there is a parallel reduction in apoptosis, as indicated by a reduction in TUNEL staining.

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429P DEVELOPMENT OF H_3-RECEPTOR RADIOLIGAND BINDING ASSAYS IN GUINEA-PIG CEREBRAL CORTEX MEMBRANES FOR THE DETECTION OF AGONIST EFFICACY

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Agonists can express high apparent affinity and complex behaviour in radioligand binding assays presumably due to the inherent complexity of agonist-receptor interactions. A pK_A value of ~7.4 was estimated for the agonist *R*-α-methylhistamine (*R*-α-MH) at H_3.receptors (Watt *et al.*, 1997). In radioligand binding assays the affinity is overestimated (pK_i'~9.5) indicating that the agonist expresses an additional property, possibly efficacy. The binding of [^{3}H]-R-α-MH has been shown to be sensitive to metal ions (Arrang *et al.*, 1990). We confirmed this observation (Harper *et al.*, 1997) and have investigated whether this effect of metal ions correlates with the expression of agonist efficacy.

Cortex membranes were prepared as described previpusly (Harper *et al.*, 1997). For saturation studies, tissue (400µl; 4mg ml^{-1} original wet weight) and [^{3}H]-clobenpropit (50µl) were incubated for 165min in 20mM Hepes buffer containing 3mM metyrapone (pH7.4 at 21±3°C) and final assay concentrations of either A (100mM NaCl); B (70mM $CaCl_2$); C (A and B) and D (C and 100mM KCl). Changing the assay buffer had no significant effect on the estimated Bmax and n_H parameters for [^{3}H]-clobenpropit although the pK_D' estimates in buffers C and D were significantly lower than control (table 1; $p<0.05$ ANOVA).

For competition studies, tissue, [^{3}H]-clobenpropit (0.2nM) and competitors were incubated for 165min in either control buffer or buffer D. Notwithstanding the flat slopes of the competition curves in the presence of metal ions, the pK_i' values were similar to those estimated from analysis of functional bioassay data (Watt *et al.*,1997). Furthermore, the difference in the pK_i' values estimated in the two buffers (ΔpK) appeared to be related to the expression of efficacy in functional assays. If the ΔpK is direcly related to efficacy then the radioligand binding assay can detect lower levels of residual agonist efficacy than the guinea-pig ileum assay. Thus, it would appear that it is necessary to have a ΔpK of at least 1 log unit in order for an agonist to produce a response in the guinea-pig ileum assay. To test these conclusions, other functional assays with more efficient receptor-effector coupling are being investigated.

Table 1 pK_D, n_H and Bmax estimates for [^{3}H]-clobenpropit in buffer containing ions (n=3±s.e.mean) (*p<0.05)

	pK_D'	n_H	Bmax (fmol mg^{-1})
control	10.38±0.08	0.96±0.01	4.35±0.85
A	10.39±0.16	0.94±0.03	3.90±0.46
B	10.20±0.01	0.95±0.03	3.88±0.40
C	10.03±0.04*	0.98±0.02	3.37±0.63
D	9.90±0.07*	0.96±0.02	3.95±0.33

Table 2 pK_i' and n_H parameters for H_3 receptor ligands in the absence and presence of metal ions (±s.e.mean).

	control		ions (buffer D)			
	pK_i	n_H	pK_i'	n_H	ΔpK	n
1	8.68±0.07	0.93±0.06	8.83±0.08	0.92±0.09	+0.15	4
2	10.11±0.07	1.00±0.11	9.45±0.07	1.05±0.08	-0.66	4
3	6.88±0.19	1.15±0.06	6.14±0.03	0.90±0.11	-0.74	3
4	9.33±0.26	0.82±0.05	8.27±0.06	0.78±0.03	-1.06	4
5	8.68±0.09	0.83±0.23	7.51±0.09	0.70±0.09	-1.17	4
6	9.96±0.32	0.67±0.07	8.62±0.14	0.70±0.04	-1.34	5
7	9.68±0.05	0.88±0.14	7.95±0.14	0.74±0.07	-1.73	4
8	7.93±0.13	0.67±0.03	6.12±0.14	0.47±0.03	-1.81	6
9	9.99±0.24	0.63±0.07	8.12±0.27	0.50±0.03	-1.87	7
10	9.10±0.30	1.06±0.03	6.80±0.14	0.53±0.03	-2.30	3
11	10.12±0.16	0.91±0.25	7.65±0.09	0.62±0.07	-2.47	4

Compounds 1, 2 and 3 are classified as antagonists in the guinea-pig ileum functional assay. Compounds 4, 5 and 6 are classified as partial agonists and 7, 8, 9, 10 and 11 are full agonists in the ileum functional assay.

1, thioperamide; 2, iodophenpropit; 3, *trans*-4(5)-(3-benzyloxyprop-1-enyl)-imidazole; 4, (4(5)-[3-(4-chlorobenzyloxypropyl)-imidazole)); 5, proxyfan (4(5)-(3-benzyloxypropyl)-imidazole)); 6, iodoproxyfan; 7, immepip; 8, *S*-α-MH; 9, *N*-α-MH; 10, histamine; 11, *R*-α-MH;

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430P DEVELOPMENT OF HISTAMINE H_3-RECEPTOR RADIOLIGAND BINDING ASSAYS IN GUINEA-PIG CEREBRAL CORTEX MEMBRANES AND ILEAL LONGITUDINAL MUSCLE MYENTERIC PLEXUS

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Although radioligand binding assays have been used to characterise the histamine H_3-receptor in cerebral cortex (e.g. Jansen *et al.*, 1994; Clark & Hill, 1995), so far, functional bioassays provide the only detailed characterisation of this receptor in the ileum (e.g. Coruzzi *et al.*, 1991). There are potential problems with comparing results from assays performed using different assay buffers. Therefore, we have developed new H_3-receptor radioligand binding assays, using [^{3}H]-*R*-α-methylhistamine ([^{3}H]-*R*-α-MH) as label, in guinea-pig cortex and ileal longitudinal muscle myenteric plexus (LMMP) and performed under identical incubation conditions

Cortex and small intestine (SI) were rapidly removed from guinea-pigs (200-300g) and placed in ice-cold 20mM Hepes-NaOH (buffer A) (pH7.4 at 21°C). The SI was immediately washed (buffer A) and cut into ~10cm lengths which were threaded onto a glass pipette and the LMMP peeled away from the circular muscle. The LMMP was placed in Viaspan® (1g 15ml^{-1}), cut finely with scissors and incubated at 4°C. After 24h the tissue was homogenised (Polytron Kinematica AG; PT-DA 3020/2TS), diluted to a final concentration of 50mM Tris-HCl (pH6.9 at 4°C) and centrifuged (39,800 x g; 12min at 4°C). The supernatants were discarded, pellets rehomogenised in buffer A (teflon-in-glass) and the homogenate recentrifuged. The final pellet was resuspended in buffer A (21±3°C) using a polytron (Brinkman PT10). The cortex was homogenised (1g 15ml^{-1}) in buffer A (Polytron Kinematica) and centrifuged (100 x g; 5min at 4°C). The supernatants were stored (4°C) and pellets rehomogenised in buffer A and recentrifuged. The combined supernatants were centrifuged (39,800 x g; 12min at 4°C) and the pellets homogenised, in buffer A (teflon-in-glass).

In the absence of tissue, 0.49±0.03% of the added [^{3}H]-*R*-α-MH bound to GF/B filters (presoaked in 0.1% polyethyleneimine). There was a linear relationship between the specific binding of [^{3}H]-*R*-α-MH and added tissue up to 5mg (cortex) and 28mg (LMMP). When 3mg of cortex tissue and 20mg of LMMP were added 9.6±1.1 and 2.8±0.2% (n=5±s.e.mean) of the radioligand was bound and specific binding was 88.6±1.1 and 69.0±3.6%, respectively.

Mono and divalent cations (NaCl and $CaCl_2$) significantly reduced the binding of 0.1nM [^{3}H]-*R*-α-MH to cortex membranes (100mM NaCl=57.8±11.2; 70mM $CaCl_2$=33.3 ±7.1; NaCl and $CaCl_2$=36.0±1.6% of control).

The specific binding of [^{3}H]-*R*-α-MH was saturable in both tissues. Hill plot slopes were not significantly different from unity in the cortex and LMMP (n_H=1.07±0.03 and 0.97±0.02, respectively; n=5±s.e.mean). Scatchard plots were linear and the estimated apparent affinity (pK_D') of [^{3}H]-*R*-α-MH for H_3-receptors was not significantly different between tissues (LMMP=9.75±0.21; cortex=9.91± 0.07; n=5±s.e.mean). The Bmax in the cortex (3.91±0.37 fmol mg^{-1} original wet weight) was significantly higher than that estimated in LMMP (0.39±0.11fmol mg-1).

[^{3}H]-*R*-α-MH binding in cortex and LMMP reached equilibrium after a 150min incubation and remained constant for >3h. Association data could be fitted to a pseudo-first order rate equation. The bound radioligand could be dissociated by addition of thioperamide (1μM) and these data were best fitted to a double exponential function. The $t_{1/2}$ for the dissociation of [^{3}H]-*R*-α-MH from cortex (35±11min) was not significantly different from that estimated in LMMP (26±4min) (n=3± s.e.mean). The binding of [^{3}H]-*R*-α-MH is consistent with expectations for the binding of an agonist to a homogeneous population of receptors in both tissues. The higher than previously reported affinity of *R*-α-MH (West *et al.*, 1990; Clark & Hill, 1995) may be due in part to the ionic composition of the buffer and assay incubation time (Motulsky & Mahan, 1983).

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431P CHARACTERISATION OF THE BINDING OF HISTAMINE H_3-RECEPTOR LIGANDS IN GUINEA-PIG CORTEX AND ILEAL LONGITUDINAL MUSCLE MYENTERIC PLEXUS

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The release of histamine (Arrang *et al.*, 1983), noradrenaline (Schlicker *et al.*, 1989), 5-hydroxytryptamine (Schlicker *et al.*, 1988) and acetylcholine (Hew *et al.*, 1990) can be modulated by presynaptic histamine H_3-receptors. It has been suggested that there are H_3-receptor sub-types (e.g. Leurs *et al.*, 1996) but the evidence for this has come from comparison of affinity estimates obtained in functional assays (cortical-slice: transmitter release; ileum: inhibition of neurogenic contractions) with those obtained in radioligand binding assays (cortex). In addition, the possibility of species-dependent antagonist affinity cannot be excluded. We have therefore characterised the binding of a chemically-diverse series of H_3-receptor ligands in both cerebral cortex and longitudinal muscle myenteric plexus (LMMP) in order to determine whether radioligand binding assays, using tissues from the same species, provide any evidence for H_3-receptor heterogeneity.

Tissue was prepared as described (Harper *et al.*, 1997) and incubated (400μl; 165min at 21±3°C) (cerebral cortex=7.5mg ml^{-1}; LMMP=50mg ml^{-1}) with 50μl [^{3}H]-*R*-α-methylhistamine (*R*-α-MH) (cortex 1nM; LMMP 3nM) and competing ligand.

In the cortex the mid-point slope parameter estimates (n_H) of competition curves for compounds 2, 3, 4, 6, 7, 8, 9, 13 and 17 were not significantly different from unity (table). Competition curves, for compounds 2, 4, 5, 6, 8, 9, 11, 12, 13, 15 and 17, had n_H values that were not significantly different from unity in the LMMP (table). Compounds 7, 13 and 14 expressed significantly higher affinity (pK_i') for sites in cortex compared with those in LMMP, whilst compound 17 expressed a significantly higher affinity in LMMP. There was a significant correlation between apparent affinity estimates (pK_i') obtained in cortex and those obtained in LMMP (r=0.96).

In conclusion, H_3-receptors, labelled with [^{3}H]-*R*-α-MH, in the cortex and LMMP appear to be pharmacologically indistinguishable with the majority of ligands used in this study. However, the apparent affinity differences obtained for compounds 7, 13 (impentamine), 14 (histamine) and 17 require further investigation.

1, thioperamide; 2, iodophenpropit ; 3, benpropit (*S*-[3-(4(5)-imidazoyl) propyl]-*N*-benzyl-isothiourea); 4, clobenpropit (VUF9153); 5, proxyfan (4(5)-(3-benzyloxypropyl)-imidazole)); 6, iodoproxyfan; 7= (4(5)-[3-(4-chlorobenzyloxy)propyl]-imidazole); 8, (4(5)-[3-(4-bromobenzyloxy) propyl]-imidazole)); 9, *trans*-4(5)-(3-benzyloxyprop-1-enyl)-imidazole; 10, *trans*-1-[4(5)-imidazolyl]-2-[(4-iodobenzyloxy)methyl] cyclopropane; 11, *N*-[2-(4(5)-imidazoyl)ethyl]-4-phenylbutyramide; 12, burimamide 13, impentamine; 14, histamine; 15, *R*-α-MH; 16, pyrilamine; 17, cimetidine.

pK_i' and n_H estimates (±s.e.mean) *(p<0.05)

	LMMP			cortex		
	pK_i'	(n)	n_H	pK_i'	(n)	n_H
1	8.64±0.06	(16)	0.83±0.05*	9.08±0.13	(17)	0.66±0.03*
2	9.69±0.16	(5)	1.40±0.23	9.96±0.23	(6)	0.94±0.12
3	9.85±0.08	(4)	0.77±0.05*	10.31±0.29	(5)	0.87±0.08
4	10.07±0.13	(7)	1.20±0.10	10.49±0.16	(10)	1.03±0.09
5	8.74±0.10	(5)	1.01±0.18	8.80±0.23	(6)	0.79±0.05*
6	9.73±0.20	(6)	1.02±0.11	10.00±0.09	(10)	0.94±0.08
7	9.45±0.08	(5)	1.34±0.11*	10.01±0.13	(6)	0.87±0.06
8	9.57±0.08	(5)	1.18±0.18	9.91±0.19	(6)	0.94±0.08
9	7.29±0.06	(5)	0.95±0.13	7.32±0.06	(5)	0.86±0.07
10	8.77±0.06	(3)	1.14±0.02*	8.75±0.14	(4)	0.84±0.04*
11	6.62±0.08	(4)	0.91±0.13	6.48±0.24	(5)	0.74±0.05*
12	7.97±0.10	(7)	1.13±0.11	8.12±0.14	(5)	0.74±0.03*
13	8.98±0.14	(6)	0.88±0.06	10.07±0.16	(3)	0.91±0.20
14	7.98±0.16	(7)	0.74±0.07*	9.84±0.14	(6)	0.66±0.04*
15	9.82±0.09	(9)	0.95±0.15	10.07±0.16	(8)	0.91±0.20
16	5.47±0.06	(4)	0.77±0.02*	5.12±0.12	(5)	0.69±0.08*
17	4.82±0.11	(4)	0.80±0.08	4.03±0.10	(3)	0.83±0.04

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432P CHARACTERISATION OF THE BINDING OF THE HISTAMINE H_3-RECEPTOR ANTAGONIST, [3H]-CLOBENPROPIT, TO SITES IN GUINEA-PIG CEREBRAL CORTEX MEMBRANES

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There are no commercially-available selective histamine H_3-receptor antagonist radioligands and therefore the majority of radioligand binding studies that have characterised these receptors have used agonists such as [^{3}H]-*R*-α-methylhistamine ([^{3}H]-*R*-α-MH) (e.g. West *et al.*, 1990a), [^{3}H]-*N*-α-methylhistamine (eg. West *et al.*, 1990b) and [^{125}I]-iodoproxyfan (Ligneau *et al.*, 1994). In this study, we describe the characterisation of the binding of [^{3}H]-clobenpropit to guinea-pig cortex membranes (tissue preparation see Harper *et al.*, 1997a).

The amount (56.7±3.7%, n=6) of added [^{3}H]-clobenpropit (>95% purity by RP-HPLC) bound to GF/B filters was reduced (0.3± 0.1%) by soaking filters in 0.3% polyethyleneimine. Specific binding of [^{3}H]-clobenpropit (0.2nM) increased linearly with added tissue up to 1mg (original wet weight). When 0.7mg (o.w.w) of tissue was added 6.6±1.9% (n=5±s.e.mean) of the radioligand was bound and the 'apparent specific binding' was 52.6 ± 7.7%. The saturation isotherm for the binding of [^{3}H]-clobenpropit appeared biphasic, scatchard plots were curvilinear and the mean Hill plot slope parameter estimate (n_H=0.63±0.03; n=12±s.e.mean) was significantly less than unity (p<0.05). The estimated pK_D' and Bmax were 9.73±0.29 and 29.86±4.72fmol.mg^{-1} . (original wet weight (o.w.w.); n=12±s.e.mean), respectively. Analysis of the data, using a two-receptor model, suggested that [^{3}H]-clobenpropit had a pK_D of 10.91±0.12 (Bmax=5.34±0.85fmol.mg^{-1} o.w.w.) and 9.17±0.16 (Bmax= 23.20±6.70 fmol.mg^{-1} o.w.w.) at the two sites. Metyrapone (3mM) rectified the low n_H (0.91±0.04; control n_H=0.70±0.03; n=6±s.e.mean) and [^{3}H]-clobenpropit appeared to label a homogeneous population of binding sites (Bmax=3.41±0.46fmol.mg^{-1} o.w.w.; n=6±s.e.mean) with an estimated affinity (pK_D) of 10.59±0.17. There was a linear relationship between the specific binding of [^{3}H]-clobenpropit (0.2nM and 3mM metyrapone) and added tissue up to 5mg (o.w.w.). When 1.6mg of tissue (o.w.w) was added 12.0±1.0% (n=5±s.e.mean) of the radioligand was bound and the specific binding was 45.4±2.5%. [^{3}H]-clobenpropit binding reached equilibrium after a 25min incubation (21±3°C) and remained constant for >180min. The binding of [^{3}H]-clobenpropit was reversed by addition of (1μM) thioperamide. Association (k_{+1}) and dissociation (k_{-1}) rate constants were 7.49±1.32x10^8 M^{-1}min^{-1} and 0.044±0.006min^{-1}, respectively (n=3±s.e.mean) and the pK_D (10.27±0.27) was not significantly different to that estimated by saturation analysis. For competition studies, tissue (400μl; 4mg.ml^{-1} o.w.w.), [^{3}H]-clobenpropit (50μl; 2nM) and competing ligand were incubated for 165min in Hepes buffer (20mM and 0.3mM metyrapone). The pK_i' of compounds were comparable to those estimated when [^{3}H]-*R*-α-MH was used to label H_3-receptors in the cortex (Harper *et al.*, 1997b). Competition curves for imetit, iodoproxyfan and thioperamide had mid-point slope parameter estimates (n_H) significantly different from unity (* p<0.05) (Table).

pIC_{50}, pK_i and n_H values for competing ligands (±s.e.mean)

Compound	pIC_{50}	pK_i	n_H	n
R-α-MH	8.41±0.17	9.48±0.16	0.71±0.09	12
histamine	7.45±0.20	8.47±0.21	0.86±0.09	10
imetit	8.40±0.17	9.45±0.17	0.66±0.03*	10
proxyfan	7.27±0.12	8.32±0.13	0.89±0.14	9
iodoproxyfan	8.68±0.23	9.76±0.23	0.76±0.07*	10
thioperamide	7.58±0.06	8.69±0.05	0.86±0.03*	9
iodophenpropit	9.03±0.13	10.08±0.11	0.95±0.06	10

In conclusion, in the cortex, [^{3}H]-clobenpropit appears to label H_3-receptors and at least one other population of binding sites. Binding to the low affinity sites is sensitive to the cytochrome P450 enzyme inhibitor, metyrapone (Halpert *et al.*, 1994), suggesting that they may represent a cytochrome P450 enzyme. This observation is consistent with a radioligand binding study in which the structurally-similar H_3-receptor antagonist, [^{3}H]-thioperamide, was found to bind to metyrapone-sensitive sites (Alves-Rodriguez *et al.*, 1996).

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433P THE MOLECULAR ORIGINS OF AGONISM AT HISTAMINE H_3-RECEPTORS: AN INVESTIGATION OF AGONIST BEHAVIOUR IN HALOGENATED DERIVATIVES OF PROXYFAN 3-(1H-IMIDAZOL-4-YL)PROPYLPHENYLMETHYL ETHER

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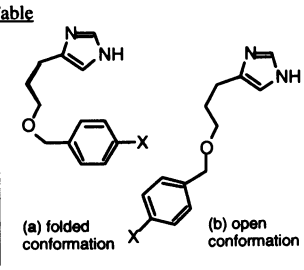
Iodoproxyfan (X=I) was originally described as a "novel potent and selective histamine H_3 receptor antagonist"(Ligneau *et al.*, 1994). However we, in line with others (Schlicker *et al.*, 1996), found that this compound produced an agonist response (inhibition of electrical field stimulation in the presence of H_1-and H_2-receptor blockade; Watt *et al.*, 1997a) in a selective H_3-receptor bioassay of guinea pig ileum. Furthermore, simple derivatives of this compound elicited agonist responses of varying magnitude that appeared to be related to the nature of the substituent in the 4-position of the aromatic ring. Partial irreversible receptor inactivation studies with the agent EEDQ suggested that the underlying ligand affinities were broadly similar and that the behaviour observed in this *in vitro* assay was a reflection of the differing efficacies of individual ligands (Watt *et al.*, 1997b).

Conformational analysis of these compounds using the molecular modelling package COSMIC(Morley *et al.*, 1991) led us to propose that the gradual loss of agonism through the series was associated with an increased preference for adopting folded conformations - bringing the imidazole and aromatic groups close together in space (Table). Furthermore, examination of a set of possible bioactive conformations* showed that the degree of efficacy observed correlated with the relative proportions of "folded" structures. Thus all of the conformations of proxyfan within this energy range were folded, but only 1/3 of those identified for iodoproxyfan fell into this category. Weinstein has proposed that the role of histamine at the histamine H_2 receptor is to mediate proton transfer across the receptor via its imidazole group (Weinstein *et al.*, 1986). In this series the imidazole can activate the histamine H_3 receptor in the open conformation. However, the agonist response is reduced in cases where the majority of the

Table

X	% folded confor-mations	pK_L ±s.e.	% α^a
H	100	7.7±0.5	33
F	85	7.8[b]	15
Cl	56	8.0±0.3	58
Br	26	7.8±0.3	84
I	33	8.2±0.2	82



[a] Relative to *R*-α-MH; [b] p[A_{50}] value

conformations are folded because the π-stacking interaction between the imidazole and the remote aromatic ring reduces the propensity for proton transfer.

*All conformations within 3kcal/mol of the global minimum at 310K were included in this study and weighted equally. The probability of a conformation existing above this threshold is less than 0.5%

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434P ANALYSIS OF H_1-, H_2- AND H_3-RECEPTOR-MEDIATED COMPONENTS OF THE RESPONSE TO HISTAMINE IN GUINEA-PIG ISOLATED ILEUM

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There is evidence that all known histamine (HA) receptor types exist in the guinea-pig ileum (H_1-, Ash & Schild, 1966; H_2-, Barker & Ebersole, 1982; H_3-, Trzeciakowski, 1987). In this study, we investigated the role of each receptor on the isolated ileum assay with the aim of defining the experimental conditions required to achieve a selective H_3-receptor bioassay. In so doing, the operational interaction between H_2- and H_3- receptors was examined. On quiescent tissues activation of H_1-receptors by HA and the selective H_3-receptor agonist, *R*-α-methylhistamine (*R*-α-MH, Arrang *et al.*, 1987) produced mepyramine-sensitive smooth muscle contraction (Table). Cholinergic responses (0.1Hz, 0.5ms, 1.3x maximal voltage) were inhibited by both agonists, although the maximum response to HA was significantly lower than that to *R*-α-MH. The H_2-receptor antagonist, famotidine (0.03-10μM), potentiated and amplified the HA E/[A] curve such that HA and *R*-α-MH maximum responses were indistinguishable in the presence of 10μM famotidine. Under these conditions *R*-α-MH responses were competitively antagonised by the selective H_3-receptor antagonist, thioperamide (Arrang *et al.*, 1987). These data indicate that HA concomitantly activated H_2- and H_3-receptors which mediate opposing effects, and that H_2-receptor-mediated agonism was 10-fold more potent than in atrial assays. The H_2–H_3-receptor interaction was investigated using the selective agonists, amthamine (Coruzzi *et al.*, 1992) and *R*-α-MH. The data obtained were consistent with a two receptor–addition of stimuli model of agonism (Roberts *et al.*, 1996). It was possible to obtain a good fit of the HA-famotidine interaction using an established pK_B value for famotidine at H_2-receptors (Black *et al.*, 1985).

These studies illustrate, through the use of selective ligands, the relationship between histamine receptor types, and highlight the need to develop selective receptor bioassays that avoid erroneous estimation of ligand activity.

Table	H_1-receptor	H_2-receptor	H_3-receptor
Agonists	$p[A]_{50}$±s.e. (n)	$p[A]_{50}$	$p[A]_{50}$±s.e (n)
HA	6.71±0.18 (4)	~7.3[1]	7.94±0.10 (3)
R-α-MH	4.24±0.11 (6)	~6.0[1]	7.15±0.08 (4)
amthamine	n.d.	7.11±0.21	< 3.0
Antagonists	pA_2 ±s.e.	pK_B±s.e.	pK_B±s.e.
mepyramine	8.97±0.21	< 6.0[2]	< 5.5
famotidine	< 4.0	7.74±0.07[3]	< 4.0
thioperamide	< 4.5	< 4.5[2]	8.53±0.08

[1] potency derived from model fitting of H_2–H_3-receptor interaction on g.p. ileum, [2] determined on g.p. right atrium assay, [3] Black *et al.* (1985), n.d. not determined.

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435P ESTIMATION OF AGONIST AFFINITY AND EFFICACY PARAMETERS OF HISTAMINE H_3-RECEPTOR LIGANDS GUINEA-PIG ILEUM

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Tissue-dependent expression of agonist efficacy has been observed for a number of histamine H_3-receptor ligands: iodoproxyfan and its derivatives (Schlicker *et al.*, 1996) and homologues of histamine (Leurs *et al.*, 1996). We attempted to rationalise these differences in behaviour by determining ligand affinity (pK_L) and efficacy (log τ) estimates in a selective H_3-receptor guinea-pig ileum bioassay (Watt *et al.*, 1997). This was achieved using EEDQ, an "irreversible antagonist of histamine H_3-receptors" (Taylor & Kilpatrick, 1992) and then by applying the operational model of agonism (Black & Leff, 1983). EEDQ (0.3 and 1μM for 15min followed by 6 washes at 10min intervals) exhibited concentration-dependent rightward shift and concomitant depression of the *R*-α-methylhistamine (*R*-α-MH) E/[A] curve, which was readily reversed following receptor protection using the competitive antagonist, thioperamide (1μM). Individual pK_L and log τ estimates (Table 1) were determined for *R*-α-MH, histamine and iodoproxyfan and E_m and n values were determined for each experiment. In another study, we investigated the activity of iodoproxyfan and analogues in which the 4-position of the phenyl ring (X – Table 2) was modified. All ligands exhibited concentration-dependent agonist responses, although the maximum response of each ligand varied. The E/[A] curve data were model-fitted using values of E_m and n which were fixed on the basis of the previous analyses (Table 1) to generate pK_L and log τ values (Table 2).

Table 1				
Ligand	pK_L±s.e.	log τ±s.e.	E_m±s.e. (% inhibition)	n±s.e.
Expt. 1:				
R-α-MH	7.28±0.15	0.39±0.16	78.8±8.4	1.03±0.11
histamine	6.37±0.16	0.51±0.18		
Expt. 2:				
R-α-MH	7.48±0.23	0.45±0.21	71.0±9.0	1.43±0.28
iodoproxyfan	8.11±0.21	0.31±0.15		

These results indicate that EEDQ selectively inactivates H_3-receptors and that histamine and *R*-α-MH are relatively low efficacy agonists. The analysis of the derivatives of iodoproxyfan illustrates that the intrinsic efficacy of these ligands, but not their affinity, is dependent on the nature of the substituent, X (Table 2).

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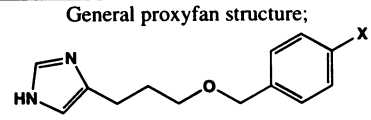
Table 2 General proxyfan structure;



X	$p[A]_{50}$±s.e.	α±s.e. (% of control E/[A] max.)	pK_L±s.e.	log τ±s.e.
I	8.40±0.22	82±7	8.16±0.20	0.24±0.07
Br	8.49±0.04	84±5	7.76±0.33	0.36±0.11
Cl	7.81±0.15	58±12	8.04±0.25	-0.07±0.03
H	7.36±0.06	33±5	7.66±0.49	-0.48±0.12

436P THE ROLE OF THE L-ARGININE-NITRIC OXIDE (NO) PATHWAY IN THE BRADYKININ (BK) RESPONSE ON HUMAN ISOLATED MYOMETRIUM

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In the previous studies carried out in this laboratory (Abbas *et al.*, 1993) BK has been shown to induce a biphasic response (initial excitatory response followed by an inhibitory response) on myometrial strips from non-pregnant (NP) and pregnant (P) donors. It has been reported that the relaxant responses to BK in a number of tissues, e.g. guinea-pig isolated trachea, are mediated by the release of NO or a related substance (Schlemper and Calixto, 1994). According to a study by Izumi *et al.* (1993), in the rat and human myometrium, an L-arginine-NO system has an important role in inhibiting uterine contractility and possibly maintaining pregnancy. The present study was designed to determine whether or not the L-arginine-NO pathway was involved in the BK mediated inhibition of myogenic activity in human isolated myometrial strips by investigating the effects of BK ± two selective NO synthase inhibitors, N^G-nitro-L-arginine (L-NOARG) and N^G-nitro-L-arginine methyl ester (L-NAME) (Schlemper and Calixto, 1994).

Samples of human myometrium were obtained from pre-menopausal patients at hysterectomy or from P donors during Caesarean section (all patients gave written consent). The myometrial strips were set up for superfusion (2g tension) in Krebs' solution with 2.79μM indomethacin (37°C, 95% O_2/5% CO_2) at 2ml min^{-1} as previously described by Senior *et al.* (1991). After equilibration of the tissues bolus doses of BK were injected directly into the flow of the superfusate. As the profile of the spontaneous activity changed throughout the course of the experiments, comparisons were made between preparations in a non-paired manner. Because of the variations in myogenic activity results have been normalised to take this into account (Senior *et al.*, 1991). Briefly, excitatory potency was expressed as an ED_1 value. The inhibitory actions of BK were expressed as ID_4 values (see Senior *et al.*, 1992).

Dose response curves to BK were constructed ± 100μM L-NOARG or L-NAME which were added to the superfusate at least 1h before administration of BK.

The responses to BK on human myometrium from NP or P donors were not significantly affected by the presence of L-NOARG or L-NAME in the superfusate.

Table 1 The ED_1 and ID_4 values of BK ± 100μM L-NOARG or L-NAME on NP and P tissues. ED_1 and ID_4 values are expressed as geometric means (nmol) with 95% CL in parentheses.

Donor	ED_1 (95% CL)			ID_4 (95%CL)		
	control	+L-NOARG	+L-NAME	control	+L-NOARG	+L-NAME
NP	0.003 (0.0005 -0.008)	0.003 (0.0005-0.007)	0.004 (0.0005 -0.008)	4.44 (0.9-9.0)	4.80 (1.0-9.5)	4.60 (0.9-9.0)
P	0.006 (0.002-0.01)	0.005 (0.002-0.008)	0.004 (0.003-0.009)	0.26 (0.05-1.0)	0.30 (0.05-1.0)	0.45 (0.06-1.0)

The results of the present study demonstrate that in human isolated myometrial strips from NP and P donors, BK elicits inhibition of the myogenic activity that is not NO mediated, since L-NOARG and L-NAME failed to significantly reduce any inhibitory responses to BK.

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437P THE EFFECT OF des-Arg^9(leu^8)BRADYKININ (BK) ON PROSTAGLANDIN (PG) RELEASE BY des-Arg^9-BK ON HUMAN ISOLATED MYOMETRIUM

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Previous studies in this laboratory have shown that on the human isolated myometrial strips from non-pregnant (NP) and pregnant (P) donors des-Arg^9-BK evokes a stimulant response which in NP tissues is reduced by the presence of 2.9μM indomethacin (Abbas *et al.*, 1993). In this study PGE_2 and PGI_2 release during the response to des-Arg^9-BK was investigated in the absence and presence of $3x10^{-6}$M des-Arg^9(leu^8)BK (B_1 receptor antagonist) (Regoli *et al.*, 1977). Samples of human myometrium were obtained from NP pre-menopausal patients at hysterectomy or from P donors during Caesarean section (all patients gave written consent).

The myometrial strips were set up for superfusion (2g tension) as described by Senior *et al.* (1991). For the measurement of PG release during a response, des-Arg^9-BK (0.5-50nmol) was injected as a bolus dose into the flow of the superfusate. The superperfusate was collected after passing over the tissue during a response and PG concentration measured by enzyme immunoassay (Amersham International).

The B_1 receptor agonist, des-Arg^9-BK, evoked a stimulant response releasing PGE_2 and PGI_2 from NP and P tissues (table 1). The release of both PGE_2 and PGI_2 from tissues from P donors was significantly less than that from tissues from NP donors ($P<0.001$). The presence of $3x10^{-6}$M des-Arg^9(leu^8)BK resulted in a significant reduction of PG release in NP and P tissues ($P<0.05$-0.001) (table 2) when compared with table 1.

Table 1 Concentration of PG released in response to des-Arg^9-BK

Dose agonist (nmol)	Concentration (pg min^{-1}) released			
	NP (PGE_2)	P (PGE_2)	NP (PGI_2)	P (PGI_2)
0	0	0	0	0
0.5	44.0±4.3	3.4±0.9	5.5±4.0	0.5±0.3
1	56.0±3.0	5.4±0.55	9.0±6.0	1.25±0.75
5	78.0±3.2	12.0±2.3	19.0±10	3.0±0.8
10	92.0±4.0	18.0±2.0	26.0±5	5.2±0.9
50	109.0±7.0	26.0±3.2	37.0±14	10.5±1.3

Table 2 Concentration of PG released in response to des-Arg^9-BK in the presence of $3x10^{-6}$M des-Arg^9(leu^8)BK

Dose agonist (nmol)	Concentration (pg min^{-1}) released			
	NP (PGE_2)	P (PGE_2)	NP (PGI_2)	P (PGI_2)
0	0	0	0	0
0.5	26.0±3.0**	0***	2.0±1.9	0***
1	38.0±2.0**	1.25±0.3***	4.0±3.6	0.25±0.15
5	55.0±3.0**	9.0±4.0	10.5±5.0	0.75±0.48*
10	74.0±3.0*	10.0±1.2**	15.0±2.0*	1.5±0.65***
50	84.0±6.0*	15.0±2.5*	26.0±9.0	2.5±0.70***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, significantly different from PG release in the absence of the antagonist.

These results suggest that PGE_2 and PGI_2 are released during the des-Arg^9-BK response and this release is B_1 receptor mediated.

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438P AN AUTORADIOGRAPHIC STUDY OF α_2-ADRENOCEPTORS AND IMIDAZOLINE-2 (I_2) RECEPTORS IN A RAT MODEL OF PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION

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The α_2-adrenoceptor / I_2 receptor ligand idazoxan protects neurones from ischaemic damage following global or focal transient forebrain ischaemia in rats (Gustafson *et al.*, 1990). These observations suggest that changes in α_2-adrenoceptor mediated noradrenergic neurotransmission or I_2 receptors may play a role in the development of cerebral ischaemia in rats. For this reason we set out to study possible alterations in α_2-adrenoceptors and I_2 receptors in ischaemic rats following occlusion of their left middle cerebral artery (MCA), using an intraluminal filament.

Focal ischaemia was induced in male Sprague-Dawley (300-350g) rats by permanent occlusion of the left MCA using a modification of the intraluminal filament technique, as described by Longa *et al.*, (1989). Sham-treated rats underwent the same surgical procedure except an intraluminal filament was not inserted. Twenty-four hours post-surgery, brains were removed, frozen, sectioned (12 µm) and processed for autoradiography according to the methods of Lione *et al.*, (1995). [^{3}H]RX821002 (1 nM) and [^{3}H]2-BFI (0.5 nM) were used to label α_2-adrenoceptors and I_2 receptors, with the specific components of binding determined with rauwolscine (5 µM) and 2-(4,5-dihydroimidaz-2-yl)-quinoline (BU224; 10µM), respectively.

Quantitative autoradiography revealed no significant alteration in [^{3}H]RX821002 labelled α_2-adrenoceptors in ischaemic rat brain compared with sham-treated controls (Table 1). In contrast a significant decrease in the density of [^{3}H]2-BFI binding to I_2 receptors in the caudate putamen (54 %), entorhinal cortex (27 %), and central grey (42 %) was observed in rats subjected to permanent MCA occlusion, relative to sham-treated controls.

The significant decrease of binding to I_2 receptors following permanent cerebral ischaemia could indicate that some of these receptors are present on the neurones that degenerate in this model of ischaemia. These findings are compatible with those of Reynolds *et al.*, (1996); that a population of I_2 receptors appear to be neuronal.

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Table 1. Quantitative autoradiography of α_2-adrenoceptors and I_2 receptors in selected rat brain areas following sham or MCA occlusion (fmol mg^{-1} tissue, mean ± s.e.mean, n = 3 - 4). * indicates data were significantly different from sham-control (unpaired Student's t-test, $p<0.05$).

	α_2-Adrenoceptors		I_2 Receptors	
Brain Region	Sham	MCA Occluded	Sham	MCA Occluded
frontal cortex	29.1 ± 3.3	24.4 ± 4.0	7.0 ± 1.1	5.1 ± 0.9
entorhinal cortex	88.9 ± 13.4	98.7 ± 10.7	12.2 ± 1.6	8.9 ± 0.5 *
hippocampal fissure	157 ± 9.6	175 ± 11	12.4 ± 1.1	13.0 ± 1.6
caudate putamen	26.1 ± 3.4	15.6 ± 4.7	7.8 ± 1.2	3.6 ± 1.2 *
central grey	77.6 ± 7.1	68.3 ± 5.6	13.9 ± 1.9	8.1 ± 0.2 *
area postrema	82.6 ± 5.1	94.1 ± 2.1	116 ± 19	115 ± 6.7
interpeduncular nucleus	56.7 ± 2.2	67.4 ± 8.5	85.0 ± 8.0	140 ± 68

439P COLONIC LEVELS OF DYNORPHIN A(108) ARE INCREASED IN CROHN'S DISEASE BUT NOT IN ULCERATIVE COLITIS

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Inflammatory Bowel Disease (IBD) is a chronic, relapsing inflammatory condition of unknown aetiology. Two main clinical manifestations are seen, ulcerative colitis (UC) and Crohn's disease. It is well recognised that peptidergic neurons are important in the control of gastrointestinal function, and changes in these neurons have been implicated in the pathophysiology of IBD (Koch *et al.*, 1987). Recent findings from our laboratory support this hypothesis; alterations in the levels of pro-enkephalin-derived opioid peptides were found in the colon from patients with Crohn's disease but not from those with UC (Corbett *et al.*, 1995). In this investigation, we have measured the amounts of three pro-dynorphin-derived opioid peptides and also the opioid binding sites in healthy gut and that from patients with IBD.

Healthy specimens were obtained from patients undergoing surgery for non-obstructive carcinoma of the colon or ileum. Diseased specimens were taken from patients with IBD. Tissues were dissected into mucosa+submucosa and *muscularis externa* within 30 min of resection. Pro-dynorphin-derived opioid peptides were extracted, fractionated by HPLC and quantified by bioassay (Corbett *et al.*, 1988). Opioid binding sites were measured using binding assays as described by Kosterlitz *et al.* (1981).

In the mucosa+submucosa of healthy colon, dynorphin A(1-8) was found at a concentration of 1.7±0.6 pmol g^{-1} (n=10). In Crohn's disease the amount of this peptide was significantly increased ($p<0.05$) to 10.9±5.8 pmol g^{-1} (n=6) whereas no change was seen in UC (1.7±0.9 pmol g^{-1}, n=5). Dynorphin A and dynorphin B were present in amounts less than 1.5 pmol g^{-1}, and found at similar concentrations in health and IBD. In the *muscularis*, dynorphin A(1-8) was the preponderant pro-dynorphin fragment, 1.2±0.56 pmol g^{-1}, with dynorphin A and dynorphin B found at 0.2±0.08 and 0.4±0.19 pmol g^{-1}, respectively. No significant changes in the amounts of pro-dynorphin-derived peptides were seen in the *muscularis* of patients with IBD.

In the *muscularis* from healthy sigmoid colon, the total number of binding sites labelled with the non-selective opioid ligand [^{3}H]-bremazocine was 40.7±13.9 fmol mg^{-1} protein (n=11). This represented binding exclusively to δ-sites as the binding of [^{3}H]-naltrindole, a δ-selective ligand, was 30.8±6.7 fmol mg^{-1} protein (n=10). There was no specific binding of either the µ-selective ligand [^{3}H]-[D-Ala^2, MePhe^4, Gly-ol^5]enkephalin or the κ-selective ligand [^{3}H]-CI-977. Fewer binding sites were seen in healthy mucosa+submucosa where [^{3}H]-bremazocine bound to 5.7±2.2 fmol mg^{-1} protein (n=11). Again, these sites corresponded to δ-binding sites since the binding of [^{3}H]-naltrindole was 18.3±11.3 fmol mg^{-1} protein (n=11) and no specific binding was seen with µ- or κ-selective ligands. In the *muscularis* from patients with IBD, the K_D and the B_{max} were unchanged compared to healthy tissue. Preliminary experiments using ileal specimens, however, indicated that Crohn's disease did not alter the B_{max} of bremazocine but the affinity was apparently decreased, reflected in an increase in K_D from 0.31nM (n=2) to 1.73±0.81nM (n=3).

This study confirms and extends our previous observations that characteristic changes in the levels of endogenous opioid peptides are seen in Crohn's disease but not UC. In addition, initial observations of changes in opioid binding sites in the ileum are also consistent with a role for the opioid system in Crohn's disease.

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