145P HOMOCYSTEINE INHIBITS ENDOTHELIUM-DEPENDENT RELAXATION IN ISOLATED RABBIT AORTIC RINGS

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Elevated plasma homocysteine levels are associated with inborn errors in its metabolism (Green & Jacobsen, 1995). Patients with these disorders are prone to premature cardiovascular disease (Scott & Weir, 1996). However, the mechanism of the vascular injury seen in homocystinaemia is not known. In the present study we have therefore investigated the effects of homocysteine on endothelial function in isolated aortic rings from New Zealand White rabbits. 2-3mm wide endothelium-intact (+E) or -denuded (-E) rings were prepared and mounted in 8ml tissue baths containing Krebs buffer (with 10μM indomethacin) and gassed with 95% CO_2/ 5% O_2 at 37°C for isometric tension recording. A resting tension of 2g was used. Data are expressed as mean±s.e.mean (n≥4). All tissues were preconstricted with a submaximal concentration of phenylephrine (PE, 1μM) which produced a mean constriction of 5.2±0.2g. Rings were then exposed to increasing concentrations of either acetylcholine (ACH, 1nM to 10μM, +E) or sodium nitroprusside (SNP, 1nM to 10μM, -E). The tissues were then washed, reconstricted with PE (1μM) and incubated for 3h with either vehicle or homocysteine (1mM, as prepared by Van Der Molen *et al.*, 1996). After this time the concentration responses to ACH or SNP were repeated. Relaxation responses are expressed as a percentage of the PE-induced constriction. Incubation with vehicle caused a small but significant ($P<0.05$) change in the maximum relaxation-response (Rmax) to ACH or SNP compared to the initial responses (65.34±0.89 cf. 71.93±0.84% respectively for ACH; 101.85±0.78 cf. 106.95±1.27% respectively for SNP) . Similar changes were also observed in the absence of vehicle. Incubation with homocysteine caused a highly significant ($P<0.0001$) inhibition of the Rmax to ACH compared to the initial ACH exposure (39.68±0.76 cf. 72.29±0.96% respectively). A small but significant ($P<0.05$) change in the Rmax to SNP was also observed following incubation with homocysteine (93.65±1.23 cf. 107.32±1.12% for the initial SNP exposure). Also in endothelium-intact rings, preincubation with homocysteine significantly inhibited the relaxation-response to increasing concentrations of the calcium ionophore A23187 (1nM to 10μM, Rmax 41.19±2.38%) compared to vehicle (Rmax 76.62±1.67%). These data thus demonstrate that exposure to homocysteine for 3h inhibits endothelium-dependent relaxation to ACH and A23187, though the mechanism of this effect remains unknown.

Green, R. & Jacobsen, D.W. (1995) in *Folate in health and disease* ed. Bailey, L. pp75-122. New York: Marcel Decker.
Scott, J. & Weir, D. (1996) Q. J. Med. 89, 561-563.
Van Der Molen, E.F., Van Den Heuvel, L.P.W.J., Te Poele Pothoff, M.T.W.B. *et al.* (1996) E. J. Clin. Invest. 26, 304-309.

146P FUNCTIONAL EVIDENCE FOR HETEROGENEITY OF RENAL ANGIOTENSIN RECEPTORS

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Angiotensin II (AII), a potent vasoconstrictor, also modulates glomerular filtration rate (GFR), sodium excretion ($U_{Na}V$) and urine flow (UV), producing natriuresis/diuresis at higher doses (Navar and Langford, 1974). Stimulation of AT_1 receptors mediates most of the effects of AII, including vasoconstriction. However, receptors for other renal actions of AII have not been characterized. We, therefore, examined the effects of losartan, a selective AT_1 antagonist and Sar^1Thr^8-AII, a non-selective antagonist on the renal response to AII. Isolated rat kidneys were perfused at 95mmHg for six, 10min clearance periods with buffer containing oncotic agents and amino acids (Bell-Quilley et al., 1993). AII was infused at 1pmol/ml from the start of the second period; antagonists were added to the perfusate 3 minutes earlier at a concentration (1mM) that abolished the AII-induced increase in renal vascular resistance (RVR). Data for the last clearance period are shown in the table (n=6/grp). The increase in RVR was prevented by both antagonists but increased GFR and excretion rates were blocked by Sar^1Thr^8-AII, and not by the AT_1 selective antagonist, losartan. As fractional excretion rates (FEH_20, FEN_a) were not different, changes in GFR can account for the natriuresis/diuresis.

	Control	AII	AII+losartan	AII+SarThr
RVR mmHg/ml/min	3.2±0.1	4.8±0.3*	3.1±0.1	3.2±0.2
GFR ml/min	0.6±0.1	1.0+0.1*	1.2±0.2*	0.5±0.1
UV μl/min	18±2	47±2*	54+6*	14±2
$U_{Na}V$ Meq/min	0.9±0.1	1.9±0.3*	2.6±0.6*	0.9±0.1
FEH_20 %	3.7±0.8	3.4±0.5	3.9±0.5	2.7±0.3
FE_{Na} %	1.5±0.4	1.5±0.3	1.5±0.5	1.7±0.5

* Significantly different from control.

The AII receptor mediating increases in GFR and the associated natriuresis/diruresis can be separated from the receptor(s) for increased overall RVR, providing functional evidence for AII receptor subtypes in the kidney.

Navar and Langford (1974) In: Angiotensin, eds. Page and Bumpus. Springer-Verlag 455-474.

Bell-Quilley et al. (1993) J. Pharmacol. Exp. Ther. 267: 676-682.

147P HUMAN MEGAKARYOBLASTIC CELL LINES AND MARROW PLATELET PRECURSORS EXPRESS ENDOTHELIN CONVERTING ENZYME AND RELEASE ENDOTHELIN-1

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In a previous study (Hamroun *et al.*, 1995), we have shown that the human megakaryoblastic cell line MEG-01 expressed preproendothelin-1 mRNA and cytoplasmic ET-1. The present investigation was designed to know whether: 1- These cells could release ET-1, 2- The expression of ET-1 might be a general phenomenon among leukemic megakaryoblastic cell lines, 3- Normal megakaryoblasts could also express ET-1. Therefore, we examined the expression of endothelin-converting enzyme (ECE) mRNA and ET-1 release in the three megakaryoblastic malignant cell lines MEG-01, DAMI and HEL and in normal platelet precursors. In addition, ECE activity was measured in membrane and cytosolic fractions of the three cell lines.

Platelet precursors were purified from normal bone marrow using the method described by Miyazaki *et al.* (1992) which specifically sorts the cells through their affinity for glycoprotein IIb/IIIa, a specific marker of the megakaryocyte-platelet lineage. ECE mRNA expression was appreciated using reverse transcription-polymerase chain reaction (RT-PCR) on total RNA. ET-1 concentration was measured in the culture medium by radioimmunoassay after extraction on C18 cartridges. ECE activities were measured in membrane and cytosolic fractions, at pH 6.5, in presence of pepstatin, chymostatin, thiorphan and PMSF, using ^{125}I-big-ET-1 as substrate, in absence or with 1 µM phosphoramidon. The products and substrate were separated by HPLC on C18.

RT-PCR applied to RNA isolated from the cell lines and platelet precursors amplified fragments of the expected size (567bp). The amplified cDNA of MEG-01 was submitted to restriction enzymes which generated, as expected, 130 & 437 (EcoO109 I) and 282 & 285 (Xho I) bp subfragments. Membrane ECE activities (pg ET-1 formed/mg protein/h) were 355 ± 42, 490 ± 25 & 297 ± 35 in MEG-01, DAMI and HEL, respectively. Phosphoramidon inhibited these activities by 60, 92 & 95 %. Cytosolic fractions also exhibited an enzyme activity capable of producing ET-1 from big ET-1 (216 ± 19, 146 ± 32 & 80 ± 43, for MEG-01, DAMI and HEL, respectively). These activities were non-sensitive to phosphoramidon. The cell lines and marrow platelet precursors produced ET-1 in a time-dependent fashion (Table 1).

Our results show that human megakaryoblastic cell lines and normal platelet progenitors express ECE and produce ET-1.

Hamroun, D., Mathieu, M.N., Clain, E. *et al.* (1995) *J. Cardiovasc. Pharmacol.* 26 (Suppl.3), S156-S158.
Miyazaki, H., Hinoue, H., Yanagida, M. *et al.* (1992) *Exp. Hematol.* 20, 855-861.

Table 1. Concentrations of ET-1 released from the cells (pg/10^6 cells, m ± s.e.mean, n = 4 to 6)

Time	HEL	MEG-01	DAMI	Marrow platelet precursors
2h	0.4 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
8h	0.7 ± 0.1	1.1 ± 0.1	0.6 ± 0.1	1.5 ± 0.2
24h	1.5 ± 0.3	4.3 ± 0.4	1.1 ± 0.2	2.8 ± 0.3

148P FURTHER CHARACTERISATION OF VASOCONSTRICTOR ENDOTHELIN RECEPTORS IN HUMAN SMALL CORONARY ARTERIES

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Endothelin (ET) is a potent spasmogen of the human coronary vasculature. Studies using large muscular coronary arteries suggest that ET-induced constriction is mediated via the ET_A receptor subtype with a small and variable ET_B component (Maguire and Davenport, 1995). Small coronary arteries (internal diameter of 100-400 µm) are likely to contribute substantially to precapillary resistance and are therefore of considerable interest.

Our preliminary studies (Pierre and Davenport, 1996) suggested that ET_A receptors also mediate the vasoconstricton to endothelin in these small arteries. To investigate this further, we have used the novel, non-peptide ET_A receptor antagonist PD156707 (Reynolds *et al.*, 1995).

Twenty-four heart apices were obtained from patients (17 male, 7 female; mean age 47 ± 3 years) undergoing cardiac transplantation for ischaemic heart disease or cardiomyopathy.

Autoradiography: To investigate intramyocardial vessels, slide mounted cryostat sections (10µm) of apex were incubated for 2h with 0.1nM [^{125}I]ET-1 (2000 Ci.mmol^{-1}) which labels both ET_A and ET_B receptors, in the presence or absence of the selective ET_A receptor antagonist PD156707 (100 nM).

In vitro pharmacology: Epicardial arteries were dissected from the apex and 1-2 mm arterial rings were set up in a wire-myograph containing oxygenated Krebs-Henseleit solution (37°C). Each preparation was set to 0.9 of the internal diameter (i.d.) the vessel would have if relaxed and under a transmural pressure of 100mmHg. Following this normalisation procedure the vessels were grouped according to i.d. group A >400µm (604.7±41.3µm); group B <400µm (303.3±11.7µm) (mean±s.e.mean). Following two stimulations with potassium rich solution (95 mM) a robust contraction to U46619 (thromboxane A_2 mimetic; 300nM) was obtained. On plateau of the contraction bradykinin (100nM) was administered to test for a functional endothelium. The effect of PD156707 (10; 30; 100 nM) on cumulative concentration response curves (CRC) to ET-1 was investigated. Antagonist potency (pK_B) was determined by Schild regression.

High density binding of [^{125}I]ET-1 was observed on small intramyocardial vessels. However, little binding remained in the presence of PD156707 (n=5).

All vessels had an intact endothelium. ET-1 potently contracted vessels in both groups. PD156707 caused parallel rightward shift of the CRC to ET-1 with no resistant portion (see Table).

	ET-1 EC_{50}	n	pK_B	slope
Group A	1.4 (0.6-3.2)nM	9	8.48 ± 0.16	0.42 ±0.35
Group B	2.0 (0.9-4.1)nM	10	8.38 ± 0.17	1.48 ±0.41

Table: Slopes of the Schild regressions were not significantly different from one (Student's t-test; p> 0.05) and were therefore constrained to unity to determine pK_B values for PD156707 (n=3-7).

PD156707 effectively blocked ET-1 vasoconstriction and binding, confirming the predominance of ET_A receptors on smooth muscle of small coronary arteries. These data suggest the constrictor ET receptor profile is comparable in small and large diameter human coronary arteries.

Maguire,J. & Davenport,A. (1995) *Br. J. Pharmacol.*, 115, 191-197.
Pierre, L.N. & Davenport, A.P. (1996) *Br. J. Pharmacol.*, 119, 103P
Reynolds, E.E, Keiser, J.A., Haleen, S.J. *et al.* (1995) *J. Pharmacol. Exp. Therap.* 273(3), 1410-1417.

149P U46619 AND ENDOTHELIN-1 INHIBIT THE RELAXANT RESPONSES VIA cGMP PATHWAY IN PIGLET PULMONARY ARTERIES

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Impaired nitric oxide/cyclic GMP (NO/cGMP) pathway and/or increased activities of thromboxane A_2 (TXA_2) and endothelin-1 (ET-1) have been associated with several forms of pulmonary hypertension (Barnes and Liu, 1995). We have evaluated the interactions of the vasoconstrictors noradrenaline (NA), the TXA_2 analogue U46619 and ET-1 with the NO/cGMP pathway as well as the mechanisms involved in NO/cGMP-induced relaxation in isolated piglet (2 week old) intrapulmonary arteries as compared to mesenteric and coronary arteries.

Isolated arterial rings (2-3 mm in length, 0.5-2 mm in diameter) were mounted for isometric tension recording in Krebs solution Pérez-Vizcaíno et al., 1995). Except for the experiments with acetylcholine (Ach), endothelium was mechanically removed. Cumulative concentration-response curves were carried out for vasodilators in arteries contracted by NA, U46619 or ET-1. cGMP was assayed by an acetylated ^{125}I-cGMP radioimmunoassay kit.

Pulmonary vessels contracted by U46619 (10^{-7}M and 10^{-6}M) and ET-1 (3 x 10^{-9}M) showed reduced relaxant responses to Ach (10^{-8}M-10^{-5}M), sodium nitroprusside (SNP, 10^{-8}M-10^{-4}M), atrial natriuretic peptide (ANP, 10^{-10}M-10^{-8}M) and 8-bromo-cyclic-GMP (8-Br-cGMP, 10^{-5}M-3x10^{-4}M) (but not to forskolin, 10^{-9}M-10^{-6}M) as compared to 10^{-5}M NA-contracted arteries (Table 1). In contrast, SNP fully relaxed mesenteric and coronary arteries precontracted byU46619 and ET-1. SNP (10^{-5}M) increased three fold the cGMP content in pulmonary arteries (from 13 ± 1 to 37 ± 8 pmol/g wet tissue) and this increase was not modified by either NA, U46619 or ET-1 (34 ± 6, 47 ± 5 and 40 ± 6 pmol/g wet tissue, respectively, n = 7-8). The relaxant response to SNP in pulmonary arteries was inhibited by the guanylate cyclase inhibitor ODQ (10^{-6}M), the sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin (2 x 10^{-6}M) and phorbol 12-myristate,13-acetate (3 x 10^{-8}M) but not by 80 mM KCl, the Ca^{2+}-activated K^+ channel blocker charybdotoxin (10^{-7} M) or K^+ removal. The inhibitory action of U46619 (but not that of ET-1) on the relaxant responses to SNP was reversed by the protein kinase C (PKC) inhibitor staurosporine (10^{-8}M and 10^{-7}M).

In conclusion, SNP-induced relaxation in piglet pulmonary arteries seems to be mediated by increased Ca^{2+} uptake by the sarcoplasmic reticulum. U46619 and ET-1 inhibited the relaxant responses of the NO/cGMP-pathway in piglet pulmonary but not in mesenteric or coronary arteries. This effect was not associated with changes in cGMP content and was reversed by inhibition of PKC suggesting that PKC may be a part of the signal transduction in U46619-induced inhibition.

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Pérez-Vizcaíno, F., Villamor, E., Moro, M. & Tamargo, J. (1996) *J. Vasc. Res.* 33,249-257.
Barnes, P.J. & Liu, S.F. (1995) *Pharmacol. Rev.* 47, 87-131.

Table 1. Maximal relaxant effects (%) of Ach, SNP, ANP, 8-Br-cGMP and forskolin in piglet pulmonary arteries (* $p < 0.01$ vs NA, n = 6-14).

	Ach	SNP	ANP	8-Br-cGMP	Forskolin
NA (10^{-5}M)	101 ± 2	104 ± 2	82 ± 9	105 ± 2	99 ± 7
U46619 (10^{-6}M)	53 ± 5*	68 ± 4*	26 ± 5*	62 ± 8*	95 ± 3
ET-1 (3 x 10^{-9}M)	49 ± 12*	75 ± 6*	54 ± 8*	53 ± 19*	99 ± 4

150P ROLE OF POTASSIUM CHANNELS IN OESTROGEN-INDUCED VASCULAR RELAXATION

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Previous work showed that oestrogen-induced relaxation of rat aorta is independent of the endothelium and extracellular calcium and is unlikely to be solely due to inhibition of protein kinase C (Babaei *et al.*, 1995, 1996). Since it was reported that opening of large conductance, calcium-sensitive potassium (BK_{Ca}) channels can account for the relaxant effect of oestrogen in porcine vessels (White *et al.*, 1995), we studied the possibility that potassium channel opening contributes to oestrogen-induced relaxation in rat vascular muscle.

Portal veins from male Hooded Lister rats (250-300g) were mounted in Krebs' solution containing 10μM indomethacin under 0.5g tension (37°C; 95% O_2, 5% CO_2). A control concentration response curve to KCl (10-100mM) was constructed. This was repeated after incubation with either cromakalim (CROM, 0.1-1μM), a K_{ATP} channel opener, or 17ß-oestradiol (ß-EST, 1-3μM) for 20min alone and in the presence of glibenclamide (GBC, 1.0-10μM), a blocker of K_{ATP} channels, or charybdotoxin (ChTX, 0.1μM) a blocker of BK_{Ca} channels. Iberiotoxin (IbTX, 10-100nM), a highly selective blocker of BK_{Ca} channels was tested only in the presence of ß-EST. No time or vehicle effects were observed. Relaxant effects are expressed in terms of geometric mean EC_{20} (the KCl concentration required to elicit 20% maximum contraction) with 95% CL. and mean reduction of E_{max} ± s.e.mean (%). Results were analysed by Students' paired or unpaired t-test, n=6-8, except where indicated.

Both CROM and ß-EST reduced KCl responses, CROM (1μM) reduced responses to low KCl concentrations (Table 1) but not E_{max} whereas ß-EST (3μM) depressed E_{max} to 62.7±5.6%, P<0.001. GBC inhibited relaxant effects of CROM but appeared to enhance oestrogen-induced relaxation (Table 1), E_{max} being reduced to 55.8±3.1%. ChTX and IbTX increased spontaneous activity in some tissues but did not affect control KCl responses or inhibit oestrogen-induced relaxation. Unexpectedly ChTX appeared to reduce relaxant effects of CROM on responses to low KCl concentrations (Table 1), E_{max} being unaffected.

Table 1. Effect of glibenclamide (10μM) and charybdotoxin (0.1μM) on relaxation of KCl-induced contraction by cromakalim (1μM) or 17ß-oestradiol (3μM) expressed as mean EC_{20} concentration (μM); 95% confidence limits. n=6-8, except ‡where n=3 and ¶ where n=4.

Control	Treatments	
KCl alone	**+CROM**	**+ß-EST**
2.6 (1.9-3.6)	29.5 (22.9-37.3)**	10.8 (6.3-17.7)**
KCl+GBC	**+CROM+GBC**	**+ß-EST+GBC**
2.8 (2.0-3.8)	11.3 (6.6-20.0)**	21.4 (17.0-26.6)***
KCl+ChTX	**‡+CROM+ChTX**	**¶+ ß-EST+ChTX**
2.2 (1.9-2.7)	15.9 (7.0-25.3)**	10.7 (5.7-18.7)**

**P<0.01, ***P<0.001; Treatment *vs* Control.

We conclude that relaxation of portal vein by ß-EST does not involve opening of K_{ATP} channels. Since ChTX and IbTX, in concentrations found to close BK_{Ca} channels (White *et al.*, 1995) did not reverse the relaxant effects of ß-EST, opening of BK_{Ca} channels is unlikely to contribute significantly to oestrogen-induced relaxation in portal vein.

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Babaei H. *et al.* (1995) *Br.J. Pharmacol.*, 115, 152P.
Babaei H. *et al.* (1996) *Br.J. Pharmacol.*, 118, 135P.
White R.E. *et al.* (1995) *Circ. Res.*, 77, 936-942.

151P MYOCARDIAL ISCHAEMIC TOLERANCE FOLLOWING HEAT STRESS IS ABOLISHED BY K_{ATP} CHANNEL BLOCKADE

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Heat stress induces delayed myocardial protection against ischaemia (Yellon & Marber, 1994), however, the mechanism is unclear. We examined the role of ATP-sensitive potassium channels (K_{ATP}), in heat stress protection, using two structurally dissimilar K_{ATP} channel blockers, sodium 5-hydroxydecanoate (5HD) and glibenclamide (Gli) in a rabbit model of myocardial infarction.

Male New Zealand White rabbits underwent 15 min of heat stress, under pentobarbitone anaesthesia, at a core temperature of 42±0.2°C; sham controls were anaesthetised only. 24 h later the rabbits were reanaesthetised and a midline sternotomy and pericardiotomy were performed. 10 min prior to occlusion, of an anterior branch of the circumflex coronary artery, the rabbits received either vehicle (control), 5 mg/kg 5HD (Hide *et al.*, 1995) or 0.3 mg/kg Gli (Toombs *et al.*, 1993). The animals were then subjected to 30 min ischaemia and 2 h reperfusion. Risk area (R) was determined by fluorescent microspheres and infarct zone (I) by tetrazolium staining. Infarct size was calculated as the ratio of infarct zone to risk area (I/R.)

Western blotting confirmed the increased expression of the inducible 70 kDa heat stress protein, 24 h following heat stress, in left ventricular tissue. No significant difference in temperature or haemodynamic data during infarction was observed. Prior heat stress resulted in significant infarct size reduction which was abolished in the presence of either 5HD or Gli [Table 1.] These results suggest K_{ATP} channels are involved in the delayed protection observed, against ischaemia, as a consequence of prior heat stress.

T.J. Pell holds a Glaxo-Wellcome studentship.

Yellon, D.M. & Marber M.S. (1994) *Experientia* 50, 1075-1084
Hide, E.J., Piper, J. & Thiemermann, C. (1995) *Br. J. Pharmacol.* 116, 2597-2602
Toombs, C.F., Moore, T. L. & Shebuski, R.B. (1993) *Cardiovascular Research* 27, 617-622

Table1. Risk area and infarct size data

	Sham	HS	Sham+Gli	HS+Gli	Sham + 5HD	HS + 5HD
Number	10	9	10	9	6	5
R(cm3)	0.9±0.07	1.0±0.05	0.9±0.08	1.0±0.07	1.1±0.12	1.1±0.07
I/R (%)	41.3±4.0	*24.1±5.0	42.3±5.1	45.2±6.4	51.9±2.2	41.5±5.0

Data presented as mean ± s.e.mean. * = $p < 0.05$ versus controls by one-way ANOVA; HS = heat stress.

152P GLIBENCLAMIDE FAILS TO BLOCK *IN VITRO* INDUCTION OF NITRIC OXIDE SYNTHASE OR VASORELAXATION INDUCED BY BACTERIAL LIPOPOLYSACCHARIDE IN RAT AORTA

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In a variety of *in vivo* and *in vitro* animal models, administration of bacterial lipopolysaccharide (LPS) produces vasodilation and hyporeactivity to catecholamines which is related in part to the induction of a Ca-independent nitric oxide synthase (iNOS; Thiemermann, 1994). Moreover, glibenclamide (Glib), an inhibitor of ATP-sensitive K^+ channels, substantially reverses changes in vascular reactivity induced by LPS *in vivo* (Landry & Oliver, 1992; Wu *et al.* 1995) although evidence from the latter study suggests that inhibition of iNOS induction may be an important mechanism of action. We sought therefore to investigate whether Glib could inhibit *in vitro* induction of iNOS and vasorelaxation induced by *LPS* in isolated rat aorta.

Thoracic aortae from male Wistar rats (250 g) were incubated at 37 °C for 7.5 hr in physiological salt solution (PSS) containing 2% (v/v) foetal calf serum in the absence and presence of LPS (*Salmonella typhosa*; 0.1 μg ml^{-1}). Drugs or PEG/DMSO (0.1% v/v) were added 30 min prior to LPS and remained throughout the incubation. Aortae were then homogenised and assayed for iNOS activity by measuring the NADPH-dependent conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline in a Ca-free buffer (Szabo *et al.* 1993). In other experiments isometric tension was measured in endothelium-denuded helical strips pre-contracted with 10 μM phenylephrine (PE).

Results in Table 1 show that pre-treatment with Glib failed to inhibit induction of iNOS by LPS, whereas dexamethasone or pyrrolidine dithiocarbamate (an inhibitor of nuclear transcription factor kappa B) were effective at inhibiting induction. In muscle strips treated with LPS (0.1 μg ml^{-1}) for 4 hr, PE contractions were reduced to a similar extent with (60.7±9.0%, n=8) or without (58.9±4.6%, n=6) Glib, though contractions could be restored if the iNOS inhibitor, 4-aminoguanidine (0.4 mM) was applied at 4.5hr (95±3.8%, n=6).

Table 1. Measurement of iNOS activity in rat aorta

Treatment	iNOS activity ($pmol.min^{-1}.mg^{-1}prot$)
PSS	1.50±0.50(4)
LPS (0.1 μg ml^{-1})	13.58±2.99(4)*
LPS + glibenclamide (10 μM)	11.65±2.93(4)*
LPS + PEG/DMSO (0.1% v/v)	11.92±2.61(4)*
LPS + dexamethasone (10 μM)	0.92±0.16(4)
LPS + pyrrolidine dithiocarbamate (100μM)	1.84±1.09(6)

Results expressed mean ± s.e. mean. *$p < 0.05$ (ANOVA, with Bonferroni correction) when compared to PSS

We conclude that Glib does not regulate vascular iNOS *in vitro*, and whilst our results may conflict with earlier results (Wu *et al.* 1995), they could highlight differences in the mechanisms controlling iNOS induction *in vivo* or suggest that Glib inhibits the release of circulating factors controlling the expression of iNOS.

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Landry, D.W. & Oliver, J. A. (1992) *J. Clin. Invest.* 89, 2071-2074.
Szabo, C., Wu, C.C., Gross, S.S. *et al.* (1993) *Eur.J. Pharmacol.* 250, 157-160.
Thiemermann, C. (1994) *Adv. in Pharmacol.* 28, 45-79.
Wu, C.C., Thiemermann, C. & Vane, J.R. (1995). *Br. J. Pharmacol.* 114, 1273-1281.

153P FUNCTIONAL ASSESSMENT OF RABBIT THORACIC AORTA STORED IN UNIVERSITY OF WISCONSIN, ST THOMAS' HOSPITAL AND KREBS SOLUTIONS

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The purpose of this study was to determine the effects of storage in University of Wisconsin (UW), St. Thomas' Hospital (ST) and Krebs-Ringer (Krebs) solutions on vascular endothelium and smooth muscle function. Rings (3-5 mm width) of male New Zealand White rabbit thoracic aorta were stored either in UW, ST or Krebs solution at 4°C for 24,48 or 72 hours. Table shows composition of these solutions (Belzer & Southard, 1988; Lidingham *et al.*, 1990).

TABLE. Composition of University of Wisconsin (UW), St. Thomas' Hospital (ST) and Krebs Solutions (mmol/L)

UW*		ST		Krebs
KH_2PO_4	25.0	NaCl	110.0	118.5
$MgSO_4$	5.0	KCl	16.0	4.7
Adenosine	5.0	$MgCl_2$	16.0	–
Glutathione	3.0	$CaCl_2$	1.2	1.8
Raffinose	30.0	$NaHCO_3$	10.0	25.0
Allupurinol	1.0	$MgSO_4$	–	1.2
K lactobionate	100.0	KH_2PO_4	–	1.2
Pentastrach	5%	Glucose	–	11.0
Osmolarity	320		300	300
pH	7.4**		7.8	7.4

*Bought it as Belzer UW-CSS from Dupont-Canada.
**pH adjusted with NaOH

Following storage, tissues were equilibrated in Krebs solution for 2 h, maintained under 2 g tension and cumulative concentration-response curves to various agents were recorded using a Grass Force Transducer (FTO3) on a Grass Polygraph 7P. Two-way ANOVA, with Fisher's Protected LSD Multicomparison Test, was used to compare the differences among the groups (Chedrawy *et al.*, 1995).

No significant difference in the contractile response to potassium chloride (KCl) or noradrenaline (NA) or in the maximum relaxation to sodium nitroprusside was observed among endothelium-denuded (ED) rings stored in UW, ST or Krebs solution. On the other hand, the maximum contractile response of endothelium-intact (EI) rings to KCl increased at 72 h, compared to 0 h, in rings stored in UW or Krebs solution (8.0±0.5 vs 5.8±0.4 and 8.0±0.6 vs 6.4±0.5g tension respectively, n=16, P<0.05) but not in those stored in ST solution (8.8±0.6 vs 8.2±0.5g). However, the contractile response of EI rings to NA significantly increased at 72 h compared to 0 h in rings stored in Krebs, UW or ST solution (8.5±0.4 vs 6.3±0.4; 8.6±6.3 vs 7.9±0.3; 8.5±0.5 vs 7.2±0.6 respectively; n=16). The maximum relaxations elicited by acetylcholine (Ach), in KCl-precontracted rings, were reduced at 72 h, compared to 0 h, in EI rings stored in UW or Krebs solution (20.2±2.5vs27.8±2.8 and 24.6±4.1vs 49.6±4.8% relaxation respectively, n=16, P<0.05) but not ST solution (42.0±4.4 vs 49.6±2.0%). The maximum relaxations elicited by Ach in NA precontracted rings were reduced at 72 h, compared to at 0 h, in EI rings stored in Krebs (45.3±5.9 vs 80.4±2.1%) but not UW or ST. These findings show little or no significant alterations in the contraction or relaxation processes of vascular smooth muscle but do indicate a greater reduction in the endothelium-dependent relaxation process of rings stored in UW or Krebs solution, as compared to those stored in ST solution. Based on these findings, it is concluded that storage of rabbit thoracic aorta at 4°C for up to 72 h, in ST solution is better than storage in UW or Krebs solution for preservation of vascular endothelium and smooth muscle function.

This investigation was supported by the Heart and Stroke Foundation of Nova Scotia, Canada. E.G.C. is a recipient of a Dalhousie MRF Studentship.

Belzer, F.O. & Southard, J.H. (1988) *Transplantation* 45, 673-676.

Chedrawy, E.G., Sullivan, J.A., Li, G. and Vohra, M.M. (1995) *Br.J.Pharmacol.* 116, 197P.

Lidingham, S.J.M., Katayama, O., Lachno, D.R., and Yacoub, M. (1990) *Circulation* 82 (suppl.IV), 351-358.

154P PULMONARY VASCULAR RESISTANCE AND PULMONARY REACTIVITY IN RATS EXPOSED PERINATALLY TO EITHER CHRONIC HYPOXIA, L-NAME OR INDOMETHACIN

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Adverse conditions *in utero* predispose to cardio-pulmonary disease in later life (Barker, 1991). We studied the effect of 3 interventions *in utero* on the pulmonary vasculature of the young adult male rat; 1) chronic hypoxia, 2) blockade of nitric oxide synthase with L-NAME, and 3) blockade of dilator prostanoids with indomethacin. Pregnant dams were either exposed to 10% O_2, or drank 300μg/ml L-NAME ad libitum for 1 week pre and 1 week post partum, or were injected subcutaneously with 1 mg/kg indomethacin daily for 3 days pre-partum. Pups were weaned as normal and at 10 weeks old their pulmonary vascular resistance (PVR) in isolated blood perfused lungs was tested. In addition to this the vascular reactivity of isolated pulmonary arteries was also measured. 2 vessels from each animal were mounted in a wire myograph and constrictor responses to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 1-100μM) and potassium chloride (KCl; 1-100mM) were recorded. Dilator responses to acetyl choline (ACh; 0.1-100μM) and sodium nitroprusside (SNP; 2-600nM) were also recorded after the vessels had been maximally preconstricted with $PGF_{2\alpha}$ (100μM). Normal age matched rats were used as controls and results are expressed as mean±standard deviation. No significant differences were seen in PVR between rats exposed to any treatment and their controls.

PVR in rats (n=3) exposed to chronic hypoxia was 0.67±0.04 mmHg/ml/min compared to 0.67±0.09 mmHg/ml/min in age matched controls (n=3) (P>0.05), PVR in rats (n=3) exposed to L-NAME was 0.80±0.13 mmHg/ml/min compared to 0.66±0.04mmHg/ml/min in controls (n=4) (P>0.05), and PVR in rats (n=9) exposed to indomethacin was 0.81±0.09mmHg/ml/min compared to 0.81±0.17mmHg/ml/min in controls (n=8) (P>0.05). Isolated pulmonary arteries from rats (n=5) exposed perinatally to chronic hypoxia had a significantly lower -logEC_{50} to ACh; 6.00±0.14 compared to 6.50+0.34 in vessels from controls (n=5) (P<0.05). Isolated pulmonary arteries from rats (n=4) exposed perinatally to L-NAME had a significantly higher -logEC_{50} to SNP; 9.31±0.42 compared to 8.46±0.21 in vessels from controls (n=4) (P<0.05). No difference was seen in either V_{max} or -logEC_{50} between rats (n=7) treated perinatally with indomethacin and their controls (n=7).

In summary, perinatal exposure to hypoxia resulted in a reduction in ACh potency and perinatal exposure to L-NAME resulted in an increase in SNP potency. There was no evidence of a change in PVR or a change in vasoreactivity to vasoconstrictors with any intervention.

Barker D.J.P. (1991) *J.Roy.Coll.Physicians London.* 25, 129-133

155P THE EFFECTS OF LEVCROMAKALIM ON NITRIC OXIDE- AND EDHF-MEDIATED RELAXATIONS IN THE RAT ISOLATED MESENTERIC ARTERIAL BED

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Recent evidence has indicated that in the absence of basal nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF; now thought to be anandamide or related substance, Randall *et al.*, 1996) can compensate for the loss of NO (Kilpatrick & Cocks, 1994; McCulloch & Randall, 1996a). This interaction is thought to be mediated through an interaction at potassium channels via cyclic guanosine monophosphate (McCulloch & Randall, 1996a). We have now investigated whether hyperpolarization, through activation of ATP-sensitive potassium (K_{ATP}) channels with levcromakalim, may influence either NO- or EDHF-mediated relaxations.

Male Wistar rats (320-480g) were anaesthetised with sodium pentobarbitone (60mg kg^{-1}, i.p.) and the mesenteric arterial bed was cannulated and perfused with Krebs-Henseleit buffer at 5ml min^{-1} (McCulloch & Randall, 1996b). After equilibration, perfusion pressure was raised (ca. 80mmHg) by methoxamine (7-30μM). Dose-response curves were then constructed to the endothelium-dependent relaxant carbachol and the endothelium-independent agent verapamil in the absence and presence of levcromakalim (at the EC_{50} concentration for each tissue; 25-600nM) or the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 100μM). Dose-response curves were also constructed in the combined presence of both of these agents. The addition of levcromakalim resulted in a reduction of tone (46.2±3.2%, n=14) and so tone was established at comparable levels in the absence and presence of levcromakalim.

Basal perfusion pressure was 15.9±0.8mmHg (mean±s.e.mean, n=14) and was increased by 73.1±3.7mmHg following addition of methoxamine. Carbachol relaxed established tone (ED_{50}= 130±19pmol; R_{max}=86.6±1.9%). Levcromakalim alone had no effect on relaxation to carbachol (ED_{50}=356±190pmol; R_{max}=80.5±3.3%). L-NAME significantly ($P<0.001$, Student's t-test) reduced the potency of carbachol (ED_{50}=776±169pmol) with no change in R_{max} (R_{max}=84.6±0.9%). In the presence of both levcromakalim and L-NAME, the potency of carbachol was further reduced ($P<0.05$) compared with L-NAME alone (ED_{50}=2156±473pmol v 776±169pmol), with R_{max} unchanged (77.2±4.0% v 84.6±0.9%). The dose-response curve to verapamil was unaffected by levcromakalim, L-NAME, or a combination of the agents.

The present investigation clearly shows that hyperpolarization with levcromakalim does not influence endothelium-dependent relaxations when both NO and EDHF are present. However, when the effects were examined against the EDHF-mediated component alone, levcromakalim shifted the dose-response curve to carbachol to the right. These results indicate that K_{ATP} channel activation, and therefore hyperolarization, may influence the EDHF component of endothelium-dependent relaxations. Since this interaction was only observed on the loss of NO, this finding may further support the concept that EDHF activity is up-regulated on the loss of basal NO.

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Kilpatrick, E.V. & Cocks, T.M. (1994) *Br. J. Pharmacol.*, 112, 557-565.
McCulloch, A.I. & Randall, M.D. (1996a) *Br. J. Pharmacol.*, 119, 135P.
McCulloch, A.I. & Randall, M.D. (1996b) *Br. J. Pharmacol.*, 117, 859-866.
Randall, M.D. *et al.*, (1996). *Biochem. Biophys. Res. Comm.* 229, 114-120

156P K^+ CHANNELS AND THE NO-INDEPENDENT VASODILATOR ACTION OF ACETYLCHOLINE (Ach) IN THE RAT ISOLATED, PERFUSED KIDNEY

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NO-independent endothelium-dependent responses to vasodilators such as bradykinin (BK) and Ach have been attributed to release of an hyperpolarizing factor (Taylor and Weston, 1988). Our studies in the rat kidney and heart provide evidence that BK utilizes a cytochrome P450 (P450)-arachidonic acid (AA)-dependent mechanism that involves Ca^{2+}-activated K^+ channels, consistent with a P450-derived AA metabolite as an hyperpolarizing factor (Fulton et al, 1992, 1994, 1995, 1996). However, in the kidney, vasodilator responses to Ach also exhibit a substantial NO-independent component that is unaffected by P450 inhibitors at concentrations that attenuate responses to BK (Fulton et al., 1992), implying a different mediator. This study addressed whether NO-independent responses to Ach involved the same type of K^+ channel as that utilized by BK, using the rat (Male Wistar, weight 350-500g), isolated perfused kidney treated with nitroarginine (50 μM) and indomethacin (2.8 μM) to eliminate NO and prostaglandins. Following elevation of perfusion pressure (PP) to 180-200mmHg from 60-80mmHg with phenylephrine (2-4x10^{-7}M) dilator responses to 10,30 and 100ng Ach were determined in the presence and absence of K^+ channel inhibitors. The non-specific K^+ channel inhibitors, procaine (1mM; n=3) and tetraethylammonium (10mM;n=3), abolished responses to Ach. In control (n=7) 10, 30 and 100ng Ach reduced PP by 6±3, 25±5 and 46±8mmHg, respectively. Glibenclamide (10 μM;n=4) which abolished dilator responses to cromakalim (3μg), did not affect responses to 10, 30, 100ng Ach (11±5, 29±5 and 59±7mmHg, respectively), excluding a role for ATP-sensitive K^+ channels. In contrast, inhibition of Ca^{2+}-activated K^+ channels with charybdotoxin (CTX; 10nM;n=4) reduced dilator responses to Ach to 0, 1±1 and 3±2mmHg, respectively. However, the inhibitor of large conductance Ca^{2+}-activated K^+ channels, iberiotoxin (10-50nM;n=6) was without effect on responses to 30 and 300ng Ach, 30±2 and 67±5 mmHg, respectively, vs control (n=5) responses of 27±2 and 55±5mmHg, respectively. These results show that the renal vasodilator action of Ach, like BK, involves a CTX-sensitive K^+ channel. At present, we cannot distinguish whether the inhibitory effect of CTX relates to an action on endothelial cell K^+ channels to prevent Ca^{2+} mobilization and, hence, prevent release of mediators in response to Ach and BK or an action on a common K^+ channel in vascular smooth muscle that is shared by different mediators, results consistent with the differential effects of inhibitors of P450 on dilator responses to Ach and BK.

Fulton et al. (1992) Br. J. Pharmacol. 107:722-725
Fulton et al. (1994) Br. J. Pharmacol. 113:954-958
Fulton et al. (1995) Br. J. Pharmacol. 114:99-102
Fulton et al. (1996) Br. J. Pharmacol. 278:518-526
Taylor and Weston (1988) TIPS 16:23-30

157P EFFECTS OF CHYLOMICRON REMNANTS ON ENDOTHELIAL FUNCTION IN RAT AORTA

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Atherosclerosis is known to impair endothelium-dependent vascular relaxation. This is thought to be due to cholesterol from lipoprotein accumulating within the artery wall. Low density lipoprotein (LDL) is considered to be the most atherogenic due to its small size and high cholesterol content and it has been shown to impair endothelium-dependent relaxation by interfering with the nitric oxide (NO) pathway (Myers *et al.*, 1994). Although the effects of endogenous lipoproteins such as LDL on endothelial function have been extensively studied, little is known about the influence of chylomicrons and chylomicron remnants (CMRs) which transport dietary lipid. There is increasing evidence that CMRs may be involved in the atherogenic process (Mamo & Wheeler, 1994). The aim of this study was to determine whether CMRs cause inhibition of endothelium-dependent relaxation and to investigate the underlying mechanisms involved.

CMRs were prepared as previously described (Lambert *et al.*, 1995). Male Wistar rats were killed and 3mm rings of aorta prepared (endothelium intact) for isometric tension recording. Rings were contracted with depolarising Krebs solution (118 mM KCl) to ensure their viability. Rings were incubated in Krebs solution with or without CMRs (1.6 μM cholesterol) for 45 min. Cumulative concentration response curves (CRCs) were constructed for contractions to phenylephrine (PE; 1 nM to 10 μM). In vessel segments where tone was raised with PE (0.3 μM) CRCs to the relaxants carbachol (CCh; 10 nM to 0.1 mM) and S-nitroso-N-acetylpenicillamine (SNAP; 0.1 nM to 10 μM) were obtained. In a second series of experiments both endothelium intact and denuded rings were studied. Rings were contracted with PE (10 or 30 nM) to approximately 40 % maximum tension (0.16 ± 0.02 g/mg) before CRCs were constructed to CMRs (0.2 to 1.4 μM cholesterol) in both intact and rubbed vessel segments, and in intact segments in the presence of 0.1 mM L-nitroarginine (L-NOARG). Data are expressed as mean ± s.e.m. (n = 6 to 8).

CMRs significantly increased vessel sensitivity to PE without changing the maximum response (EC_{50} decreased from 77.2 ± 15.4 to 40.6 ± 9.9 nM; $P<0.05$, paired Student's t test). Maximum % relaxation to CCh was significantly decreased by CMRs (from 85.2 ± 5.7 to 62.5 ± 7.7; $P<0.05$, paired Student's t test). Relaxation to SNAP was not significantly affected by CMRs. In the second series of experiments CMRs caused contraction of intact vessels (maximum increase in tone of 0.079 ± 0.026 g/mg). Removal of the endothelium tended to reduce the contraction (maximum increase in tone of 0.045 ± 0.016 g/mg) whereas addition of L-NOARG caused a much larger response to CMRs (maximum increase in tone of 0.209 ± 0.022 g/mg; $P<0.01$ between treatment groups by 3 way ANOVA).

CMRs inhibit CCh-induced relaxation of endothelium in rat aorta without affecting responses to SNAP, suggesting that they interfere with agonist-induced generation of NO. Potentiation of PE-induced contraction appeared to be endothelium-dependent but was increased rather than inhibited by L-NOARG, suggesting this effect may not be due to inhibition of basal NO production. The endothelium-dependent mechanism by which CMRs enhance PE-induced tone is currently under study.

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Lambert, M., Botham, K., Mayes, P.(1995).*Biochem.J.* 310,845-52.
Mamo, J, Wheeler, J. (1994). *Cor.Art.Dis.* 5, 695-705.
Myers, P., Wright, T., Tanner, M. *et al* (1994). *J.Lab.Clin.Med.* 124, 672-83.

158P CARDIOVASCULAR SAFETY OF INTRAVENOUSLY ADMINISTERED MnDPDP AS COMPARED TO $MnCl_2$ IN THE CONSCIOUS BEAGLE DOG

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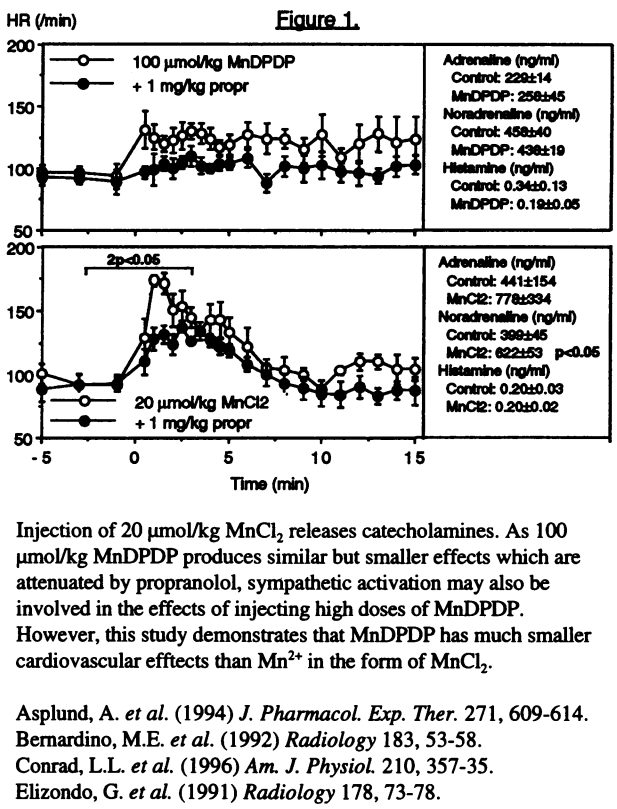
Intravenously administered Mn^{2+} acts as a liver MRI contrast agent, but is considered too toxic (Wolf & Baum, 1983). Chelation of Mn^{2+} by dipyridoxyl diphosphate (DPDP) reduces toxicity 10 fold without affecting efficacy (Elizondo *et al.*, 1991). MnDPDP may induce facial flushing, probably due to a stabilizing effect on endothelial-derived NO (Asplund *et al.*, 1994), and transiently increased BP and HR in humans (Lim *et al.*, 1991; Bernardino *et al.*, 1992). Injection of Mn^{2+} induces positive inotropy in dogs, due to catecholamine release (Conrad *et al.*, 1966). In the present study, we have investigated the cardiovascular effects of MnDPDP as compared to $MnCl_2$ in the conscious beagle dog.

Beagle dogs, implanted with carotid artery and jugular vein catheters for BP and HR measurements and i.v. injections, were given 1 min injections of 100 μmol/kg MnDPDP (n=4) or 20 μmol/kg $MnCl_2$ (n=4), ±1 mg/kg propranolol. Plasma catecholamines and histamine (before and 1 min after treatment) and ECG were measured. Values are given as means ± s.e.m. and tested by Dunnett's ($2p<0.05$) or Student's t-test ($p<0.05$).

At a dose 20 times the clinical dose, MnDPDP caused small increase in HR (Fig. 1) and BP (approx. 20 mmHg). The ECG was little affected. MnDPDP did not significantly change catecholamine levels, whereas $MnCl_2$ caused significant increases in HR and catecholamines (Fig. 1) and affected several ECG parameters. $MnCl_2$ induced an immediate fall in BP followed by a transient small increase. Propranolol attenuated the rises in HR after both MnDPDP and $MnCl_2$ (Fig. 1). Propranolol also potentiated the $MnCl_2$-induced BP increase, probably due to α-adrenoreceptor-mediated vasoconstriction. Neither MnDPDP nor $MnCl_2$ caused significant changes in plasma histamine levels (Fig. 1).

Figure 1.



Injection of 20 μmol/kg $MnCl_2$ releases catecholamines. As 100 μmol/kg MnDPDP produces similar but smaller effects which are attenuated by propranolol, sympathetic activation may also be involved in the effects of injecting high doses of MnDPDP. However, this study demonstrates that MnDPDP has much smaller cardiovascular efftects than Mn^{2+} in the form of $MnCl_2$.

Asplund, A. *et al.* (1994) *J. Pharmacol. Exp. Ther.* 271, 609-614.
Bernardino, M.E. *et al.* (1992) *Radiology* 183, 53-58.
Conrad, L.L. *et al.* (1996) *Am. J. Physiol.* 210, 357-35.
Elizondo, G. *et al.* (1991) *Radiology* 178, 73-78.
Lim, K.O. *et al.* (1991) *Radiology* 178, 79-82.
Wolf, G.L. & Baum, L. (1983) *Am. J. Radiol.* 141, 193-197.

159P PROTECTION OF AGONIST-INDUCED NITRIC OXIDE BY Cu-Zn SUPEROXIDE DISMUTASE IN RABBIT AORTA

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Nitric oxide (NO) is rapidly inactivated by superoxide anion (O_2^-). We have previously shown in rat aorta that endothelium-derived NO is protected from destruction from O_2^- by endogenous Cu-Zn superoxide dismutase (SOD; Mian & Martin, 1995). The aim of this study was to determine if Cu-Zn SOD exerted a similar protective role of endothelium derived NO in rabbit aorta.

Rabbits were killed with an i.v. injection of sodium pentobarbitone (200 mg Kg^{-1}). The thoracic aorta was removed, cleaned of fat and connective tissue, and cut into rings (2.5 mm wide). Aortic rings were then suspended in tissue baths containing oxygenated Krebs solution at 37°C and contracted with phenylephrine (30 nM - 0.3 μM. Relaxation was induced with acetylcholine (ACh; 10 nM - 3 μM. Endogenous Cu-Zn SOD was inactivated by incubating aortic rings for 60 min with diethyldithiocarbamate (DETCA; Mian & Martin, 1995). Hypoxanthine/xanthine oxidase (HX/XO) or LY-83583 were used to generate O_2^-. Data are expressed as mean ± s.e. mean of ≥ 5 observations.

The relaxation induced by maximal concentration of ACh (3 μM) was reduced from 90.7 ± 1.1% to 84.3 ± 1.8, 70.0 ± 2.7, 61.4 ± 3.7, and 32.9 ± 7.8 % following treatment with DETCA at 0.1, 0.3, 1 and 3 mM, respectively. The blockade induced by the highest concentration of DETCA (3 mM) was unaffected following treatment with SOD (250 u ml^{-1}); maximum ACh-induced relaxation was 40.6 ± 8.2 %. HX (0.1 mM)/XO (4.8 mu ml^{-1}) and LY-83583 (0.3 μM) each reduced maximal ACh-induced relaxation (to 65.3 ± 7.0 and 51.0 ± 3.2 %, respectively) and these actions were completely reversed by SOD (250 u ml^{-1}; maximal ACh-induced relaxation 91.2 ± 2.1 and 92.9 ± 1.7%, respectively). In DETCA (0.3 mM)-treated tissues, the ability of HX/XO and LY-83583 to inhibit ACh-induced relaxation was powerfully potentiated (maximal relaxation 42.4 ± 9.0 and 27.2 ± 4.7 %, respectively). This enhanced blockade induced by HX/XO and by LY-83583 in DETCA-treated tissues was prevented following treatment with SOD (250 u ml^{-1}, maximum ACh-induced relaxation 74.0 ± 1.0 and 84.0 ± 7.3 %, respectively).

The inability of exogenous SOD, which cannot penetrate cells, to reverse the blockade of ACh-induced relaxation produced by DETCA either reflects a non-selective action by DETCA or impairment of intracellular Cu-Zn SOD. The ability of DETCA to augment the blockade of ACh-induced relaxation produced by HX/XO and LY-83583, and the reversal of this action by exogenous SOD, suggests that endogenous extracellular Cu-Zn SOD protects endothelium-derived NO from destruction by O_2^- in rabbit aorta.

Mian, K.B. & Martin, W. (1995). Br. *J. Pharmacol., 115, 933-1000.*

160P EFFECTS OF SODIUM NITROPRUSSIDE, LEVCROMAKALIM AND NICORANDIL IN ISOLATED PIGLET PULMONARY AND MESENTERIC ARTERIES

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We have evaluated the relaxant effects of the potassium channel opener (PCO) levcromakalim, the nitric oxide (NO) donor sodium nitroprusside (SNP) and the mixed compound (PCO and NO donor) nicorandil in isolated pulmonary and mesenteric arteries of two week old piglets.

Endothelium-denuded isolated arterial rings (2-3 mm in length, 0.5-2 mm in diameter) were mounted for isometric tension recording in Krebs solution (Pérez-Vizcaíno et al., 1996). Rings were contracted with the thromboxane A_2 analogue U46619 and noradrenaline (NA) and cumulative concentration-response curves were carried out for the three vasodilator drugs.

U46619 (10^{-7}M) and NA (10^{-5}M) induced contractile responses of a similar magnitude (897 ± 116 mg, n = 18, and 750 ± 23 mg, n = 23, respectively, P > 0.05) in pulmonary arteries. U46619-induced contractions were partially resistant to the vasodilator responses to SNP and levcromakalim whereas nicorandil fully relaxed these contractions (Figure 1). In arteries contracted by U46619 (10^{-6}M) and then relaxed by 3 x 10^{-7}M levcromakalim (final tone 744 ± 144, n = 6, P > 0.05 vs that induced by 10^{-7}M U46619), SNP induced a complete relaxation. When the contractile agent was noradrenaline (NA), levcromakalim again induced partial relaxation, whereas SNP and nicorandil fully relaxed the arteries. In arteries contracted by NA, the K^+ ATP-dependent channel inhibitor glibenclamide (10^{-5}M) inhibited the relaxant responses to levcromakalim but not those to SNP or nicorandil. In contrast, the guanylate cyclase inhibitor ODQ (10^{-6}M) inhibited the relaxation induced by SNP and nicorandil but not that induced by levcromakalim. Mesenteric arteries were contracted by equieffective concentrations of NA (10^{-6}M) and U46619 (10^{-6}M) (1815 ± 220 mg, n = 17, and 2244 ± 324 mg, n = 18, respectively). Levcromakalim, SNP and nicorandil produced a full relaxation in these arteries independently of the vasoconstrictor employed. Furthermore, in U46619-contracted vessels all vasodilators were 3-5 fold more potent to relax mesenteric than pulmonary arteries, whereas in NA-contracted arteries this was true only for nicorandil and levcromakalim.

In conclusion, the combination of PCO activity and NO release abolishes the component of contraction of pulmonary arteries which is resistant to dilate by any of the mechanisms alone. Nicorandil vasodilates pulmonary arteries mainly by an activation of the soluble guanylate cyclase but its PCO activity may also participate in its relaxant effects.

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Pérez-Vizcaíno, F., Villamor, E., Moro, M. & Tamargo, J. (1996) *J. Vasc. Res.* 33,249-257.

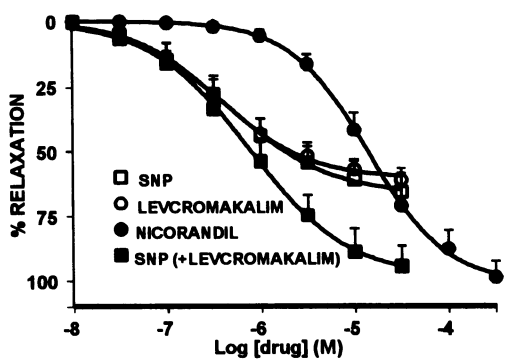

Figure 1 Effects of SNP (in the absence/presence of 3 x 10^{-7}M levcromakalim), levcromakalim and nicorandil in U46619-contracted pulmonary arteries.

161P ARTERIAL BLOOD PRESSURE AND NITRIC OXIDE IN RATS FED ON CALCIUM-DEFICIENT DIETS

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It has been suggested that the quantity of calcium in the diet affects the arterial blood pressure (ABP), because calcium administration causes, among other changes, variations in endothelial nitric oxide (NO) production (Porsti et al., 1990; López-Jaramillo et al., 1991). In this study we evaluate the ABP and plasma NO levels in normotensive Sprague-Dawley (SDR) and spontaneously hypertensive (SHR) rats fed from weaning with three possible diets: control with a normal-calcium content (Ca 1%), low-calcium (Ca 0.1%), and calcium-free (Ca 0.01%). The systolic (SBP) and the diastolic (DBP) arterial blood pressure were measured in 9-week-old conscious SDR and weekly in conscious SHR from the 6 to the 20 week of life by the tail cuff method. Plasma NO levels were estimated by the Griess reaction at the moments when maximal differences in the ABP were observed between the animals fed on the normal-calcium diet and those fed on the other two diets. The results are expressed as mean values ± s.e.mean for a minimum of 8 rats, and Student's t-test was used for comparison of mean values.

The calcium-free diet did not change the ABP of the SDR, but the low-calcium diet did cause an increase in their SBP and DBP. The SHR fed on a normal-calcium diet showed a gradual increase in SBP and DBP which reached maximum values between weeks 16-20. The calcium-free diet clearly lessened the development of hypertension in this rat strain. On the contrary, hypertension developed most rapidly in SHR fed with the low-calcium diet. In this strain the maximum differences in the ABP between animals fed on the calcium-free diet and those fed on the normal-calcium diet were obtained in 20-week-old rats, and the maximum differences between animals fed on the low-calcium diet and those fed on the normal-calcium diet were obtained in 8-week-old rats. Plasma nitrite and nitrate (NO_x^-) were always lower in those animals with higher ABP values (see table 1). These results suggest that the ABP changes induced by dietary calcium deficiency may well be associated with alterations in endothelial NO production.

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López-Jaramillo, P. & De Felix, M. (1991) Bull. Pan. Am. Health Organ 25, 109-117.
Porsti, I., Arvola, P., Wuorela, H., Ilkka, M., Saynavalammi, P., Huhtala, H., Metsa-Ketela, T. & Vapaatalo, H. (1990). J. Hypertens. 8, 835-841.

TABLE 1. Baseline SBP and DBP (mm Hg), and plasma NO_x^- (μmol/l) levels in SDR and SHR fed on three diets with a different calcium content.

	% Dietary Ca		
	1	0.1	0.01
9-week-old SDR			
SBP	144 ± 2	168 ± 3***	149 ± 1
DBP	111 ± 2	125 ± 2***	111 ± 2
NO_x^-	16 ± 2	13 ± 1	17 ± 2
8-week-old SHR			
SBP	191 ± 5	241 ± 4***	-
DBP	140 ± 5	189 ± 7***	-
NO_x^-	10 ± 1	5 ± 1***	-
20-week-old SHR			
SBP	225 ± 5	-	200 ± 2***
DBP	175 ± 13	-	133 ± 9
NO_x^-	6 ± 1	-	4 ± 1

The asterisks show significant differences compared with animals fed on the Ca 1% diet (*p<0.05; **p<0.01; ***p<0.001).

162P CONTRACTILE RESPONSES OF RABBIT AORTA RINGS TO OUABAIN

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Ouabain (OUA), a well-known inhibitor of the membrane Na^+-K^+ ATPase, contracts a variety of arterial smooth muscles in different ways. A biphasic increase in tension has been described when this drug was administered in the isolated rabbit aorta. It has been suggested that the contractile effect of OUA in this artery is due to the inhibition of the Na^+-K^+ pump in the sarcolemmal membrane of the vascular smooth muscle cells, and to the release of catecholamines caused by the inhibition of the Na^+-K^+ pump in the nerve terminal (Stewart et al., 1992; Motley et al., 1993). Both mechanisms may contribute in a different degree to the time-related and dose-dependent OUA response in this tissue. We have studied the 24-hour course and magnitude of the dose-dependent response to OUA of aorta rings from untreated and reserpine-treated (1.5 mg/kg/day i.p. for 3 days) rabbits. Male New-Zealand rabbits (2-2.5 kg) were used in this study. OUA was used in concentrations ranging from 10^{-7} M to 10^{-4} M, and individual rings were exposed to only a single concentration. We measured isometrically the magnitude of the contractile response elicited at 37° C every 5 min for the first hour following the drug administration, and then every 15 min for the next four hours and every hour after that. The results are expressed as mean values ± s.e.mean for a minimum of 8 preparations, and Student's t-test was used for comparison of mean values.

Control rings without OUA, and those exposed to 10^{-7} M OUA showed no change in tone. The initiation of three different increases in tone were observed when 10^{-4} M OUA was administered to the aorta rings from untreated rabbits. Therefore in these preparations we characterized three contractile responses. In the preparations exposed to 10^{-5} M and 10^{-6} M OUA we saw two contractile responses which, according to the time when they appeared, might correspond to the second and the third response caused by the highest OUA dose. On the other hand, OUA always caused only one contractile response in the preparations obtained from reserpine-treated animals. This response probably corresponds to that characterized as the second one, which would be the one mainly caused by the inhibition of the Na^+-K^+ ATPase of the arterial smooth muscle cells (see table 1).

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Motley, E.D., Paul, R.J. & Matlib, M.A (1993) Am. J. Physiol. 264:H1028-H1040.
Stewart, L., Hamilton C., Ingwall, J., Naomi, S., Graves, S., Canessa, M., Williams, G. & Hollenberg, N. (1993). Circ. Res. 73:1113-1120.

TABLE 1: Time-to-peak tension (min), and maximum tension (g) corresponding to the contractile responses of aorta rings from untreated (U) and reserpine-treated (R) rabbits exposed to three different concentrations of OUA

	Responses					
mol/l	1st		2nd		3rd	
	min	g	min	g	min	g
10^{-4}						
U	30	5.3±0.7	135	6.2±0.9	1050	3.4±0.7
R	--	-------	135	4.0±1.3	----	-------
10^{-5}						
U	--	-------	195	5.9±0.8	1170	5.1±0.9
R	--	-------	75	4.0±0.7	----	-------
10^{-6}						
U	--	-------	285	2.9±0.7	660	3.2±0.1
R	--	-------	75	3.3±0.4	---	-------

163P α-ADRENOCEPTOR-INDUCED CONTRACTIONS OF RAT AORTA AFTER THE INHIBITION OF NITRIC OXIDE SYNTHESIS

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Previous studies carried out in our laboratory showed that the administration of the inhibitor of nitric oxide (NO) synthase N$^{\omega}$-nitro-L-arginine methylester (L-NAME) decreased the α-pressor responses in pithed rats when the animals had received a chronic treatment with this drug. Nevertheless, the acute treatment with L-NAME did not alter the responses to α-adrenoceptor agonists in pithed rats (Aleixandre et al., 1996).

We have studied the rat aorta ring dose-dependent contractions induced by α-adrenoceptor agonists (Methoxamine and Phenylephrine) in the following preparations: controls, preparations from acutely (100 mg/kg i.p.) L-NAME-treated animals, and preparations from chronically (100 mg/kg/day in drinking water for 7 or 21 days) L-NAME-treated animals. Male Sprague-Dawley rats (250-300 g) were used in this study. The aorta contractile responses elicited by the agonists at 37° C were measured isometrically, and we obtained only one Methoxamine (10^{-7} M - 10^{-4} M) or Phenylephrine (10^{-9} M - 10^{-6} M) dose-response curve for each aorta ring. The preparations were first contracted by 80 mM KCl. Then, they were washed with physiological saline solution until the basal tension was reestablished, and the α-adrenoceptor agonist was administered in increasing cumulative doses. The contraction produced by 80 mM KCl was taken as 100% response and served to evaluate subsequent α-adrenoceptor agonist responses. Results are expressed as mean values ± s.e.mean for 8-12 experiments and Student's t-test was used for comparison of mean values.

In the control preparations, the maximum responses were obtained when 10^{-4} M Methoxamine or 10^{-6} M Phenylephrine were administered. Table 1 shows the responses obtained with both agonists in the different preparations and demonstrates that the contratile responses were similar in the controls and in the preparations from acutely L-NAME treated rats. In addition, it can be seen that the chronic treatment with L-NAME only caused a decrease in the responses when the agonist used was Phenylephrine and when the treatment lasted longer.

We had previously described a compensatory decrease in α-vasoconstrictory mechanisms after the chronic inhibition of NO synthesis. The present results show that the *in vivo* chronic inhibition of NO synthesis could also cause a decrease in the α-contractile responses of the isolated rat aorta.

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Aleixandre, M.A., López-Miranda, V., Puerro, M. & Ortega, A. (1996) Br. J. Pharmacol. (Bath meeting, abstract in press).

TABLE 1. Responses to 10^{-4} M Methoxamine (MTX) and 10^{-6} M Phenylephrine (PHE) in rat aorta

		From L-NAME-treated rats		
	Control	Acutely	7 days	21 days
MTX	126 ± 3	123 ± 2	128 ± 4	129 ± 8
PHE	100 ± 4	111 ± 5	110 ± 4	52 ± 4***

The asterisks show significant differences compared with controls (***p<0.001).

164P INHIBITION OF ACETYLCHOLINE-EVOKED DILATATION OF THE RAT ISOLATED MESENTERIC BED BY L-CITRULLINE

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Endothelium-derived nitric oxide (NO) is synthesised by the enzyme nitric oxide synthase (NOS) which catalyses the conversion of L-arginine to L-citrulline and NO (Palmer & Moncada, 1989). In the rat mesenteric bed, endothelium-dependant relaxation evoked by acetylcholine (ACh) has been shown to be largely resistant to the actions of NOS inhibitors, and thus, it has been suggested that both NO-dependant and -independent mechanisms may mediate endothelium dependant relaxation in this bed (Parsons *et al.*, 1994). However, a recent study has indicated that a combination of NOS inhibitors may be required to completely inhibit NO production (Plane *et al.*, 1996). In this study we have investigated the effect of combining two NOS inhibitors on ACh-evoked relaxation of rat mesenteric arteries. In addition, the effect of the co-product of NO synthesis, L-citrulline, and its analogue L-thiocitrulline, on endothelium-dependent responses was also examined.

Male Wistar rats (~250g) were killed by cervical dislocation. Isolated mesenteric preparations were perfused with oxygenated Krebs buffer (37°C; 5 ml min^{-1}) via a catheter inserted in the superior mesenteric artery. Bolus doses of ACh (0.02-2000 nmol) evoked dose-dependent dilation of preparations pre-constricted with phenylephrine (PE; 30 μM). All data are expressed as mean ± se mean % relaxation of PE-induced constriction. Differences between mean values was calculated using the Student's t-test or ANOVA.

ACh (2000 nmol) produced a maximum relaxation of 81.4 ± 3.5 % (n=21). In the presence of of L-N^{G}-nitroarginine methyl ester (L-NAME; 100 μM; 30 mins), this relaxation was attenuated and the maximum response reduced from 69.5 ± 6.8 % to 60.2 % ± 3.4 % (n=6; P>0.05). Exposure to both L-NAME and N^{G}-nitro-L-arginine (L-NOARG; both 100 μM) together caused a further significant inhibition of ACh-evoked dilatation reducing the maximum relaxation to 43.0% ± 5.4% (n=6; P<0.05).

Perfusion of preparations with L-citrulline (10 mM) or L-thiocitrulline (10 mM) for 1 hr reduced ACh-evoked dilatation to a similar extent as the combination of L-NAME and L-NOARG (P>0.05). In the presence of L-citrulline and L-thiocitrulline the maximum response to ACh was reduced by around 60 % to 36.8 ± 7.5 % (n=10; P<0.05) and 38.5 ± 9.6 % (n=6; P<0.05), respectively.

L-citrulline and L-thiocitrulline did not significantly inhibit relaxation to the endothelium-independent relaxant sodium nitroprusside (n=4; P>0.05) and did not alter contraction to PE (n=4; P>0.05). In addition, D-citrulline (10 μM) did not significantly inhibit ACh-evoked responses (n=5; P>0.05).

These data indicate that in the rat mesenteric bed, exposure to either L-NAME or L-NOARG alone may not be sufficient to completely inhibit the production of endothelium-derived NO and that a combination of inhibitors may be required. Furthermore, it appears that, in this preparation, both L-citrulline and L-thiocitrulline are potent inhibitors of NO-meditated responses.

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Palmer, R.M.J. & Moncada, S. (1989) *Biochem. Biophys. Res. Commun.*, **158**, 348-352.
Parsons, S.J.W., Hill, A., Waldron, G.J. *et al.*, (1994) *Br. J. Pharmacol.*, **113**,1275-1280.
Plane, F., Najibi, S., Cohen, R.A. & Garland, C.J. (1996) *Br. J. Pharmacol.*, **117**, 77P.

165P THE ROLE OF NITRIC OXIDE AND ENDOGENOUS PROSTAGLANDINS IN THE RESPONSES OF THE OVINE DIGITAL ARTERY TO PHENYLEPHRINE AND BRADYKININ

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Altered blood flow to the foot contributes to the syndrome of laminitis (Hood *et al.*, 1993), an important cause of lameness in domestic ungulates. The nature of the vascular changes has yet to be established. The aim of this study was to investigate the vascular responses of the normal sheep digital artery, specifically the role of endothelium-derived nitric oxide (NO) and prostaglandins such as prostacyclin, which have a potentially key role in regulating local blood flow (Vane *et al.*, 1990).

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 arterial rings, 2mm long, were mounted between stainless steel wires in 10ml tissue baths containing warmed (30°C), oxygenated Krebs. Vessels were maintained at a baseline tension of 2g, measured using an isometric force transducer, for a minimum of 2 hours before the addition of drugs. Cumulative concentration-response curves to phenylephrine (PHE; 10^{-9}M - $3x10^{-4}$M) and non-cumulative concentration response curves to bradykinin (BK; 10^{-10}M - $3x10^{-6}$M) were constructed in intact (unrubbed) and endothelium-denuded (rubbed) digital arterial rings. To study relaxation the rings were precontracted with a standard dose of PHE ($6x10^{-7}$M). To investigate the role of endogenous NO and prostaglandins, the dose response curves were repeated in intact digital arterial rings after incubation for 20 minutes with inhibitors of cyclooxygenase (COX; 10μM indomethacin, INDO) and/or nitric oxide synthase (NOS; 300μM N^G-nitro-L-arginine methyl ester, L-NAME).

PHE responses were expressed as tension in g/g tissue wet weight, BK responses as percentage change in precontracted tension. Values for log EC_{50}(M) and E_{max} were calculated from a sigmoid curve fitting procedure (Microcal Origin) and were compared by one-way analysis of variance. $P < 0.05$ was taken to indicate statistical significance and where differences occurred the results were further analysed by Tukey's pairwise comparisons. A summary of the results is presented in Table 1.

TABLE 1: Effect of endothelium-removal or drug treatment on dose-response curve parameters to PHE and BK in ovine digital arteries

	PHENYLEPHRINE			BRADYKININ		
	n	EC50 ± SEM log[PHE]	Emax ± SEM g/g	n	EC50 ± SEM log[BK]	Emax ± SEM %
UNRUBBED	12	-6.13 ± 0.07	2107 ± 76	8	-8.68 ± 0.13	-61.8 ± 7.4
RUBBED	12	-6.28 ± 0.05	2188 ± 129	8	-7.91 ± 0.27*	+76.0 ± 12.1*
CONTROL	8	-6.22 ± 0.08	2479 ± 213	6	-8.89 ± 0.15	-61.7 ± 5.6
INDO	7	-6.55 ± 0.07*	2603 ± 349	7	-8.92 ± 0.16	-50.9 ± 5.5
L-NAME	7	-6.37 ± 0.12	2732 ± 251	7	-8.56 ± 0.20	-34.0 ± 2.1*
INDO + L-NAME	6	-6.68 ± 0.05*	2731 ± 149	7	-8.18 ± 0.22	-9.6 ± 2.8 **

* indicates $p < 0.05$ when compared to unrubbed or control group
** indicates $p < 0.05$ when compared to L-NAME group

NOS inhibition produced no significant effect on PHE responses under the conditions of the study, however COX inhibition decreased the log EC_{50} indicating that endogenous prostaglandins modulate the PHE response in ovine digital arteries. BK-induced relaxation occurred only in intact digital arteries and was significantly reduced by NOS inhibition. COX inhibition alone had no effect on BK-induced relaxation however it enhanced the inhibition produced by L-NAME. This implies a role for both NO and prostaglandins in the BK response and may demonstrate a reciprocally inhibitory interaction between the two mediators as demonstrated in human saphenous veins (Barker *et al.*, 1996).

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Barker, J.E. *et al.* (1996) *Br. J. Pharmacol.* **118**, 643-648.
Hood, D.M. *et al.* (1993) *J. Vet. Int. Med.* 7, 228-234.
Vane, J.R. Änggard, E.E. & Botting, R.M. (1990) *N. Engl. J. Med.* **323**, 27-36.

166P THE ISOPROSTANE 8-ISO PROSTAGLANDIN $F_{2\alpha}$ VASODILATES RAT PULMONARY ARTERY VIA THE RELEASE OF NITRIC OXIDE

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8-iso prostaglandin (PG) $F_{2\alpha}$ is an isomer of a newly-described group of PG-like compounds (isoprostanes), the majority of which are formed independently of the enzyme cyclo-oxygenase (COX) during conditions of oxidative stress (Morrow and Roberts, 1996). 8-iso $PGF_{2\alpha}$ contracts various smooth muscle preparations at least in part via the activation of thromboxane (TP) receptors. We have shown previously that 8-iso $PGF_{2\alpha}$ contracts rat pulmonary artery, an effect that is increased by the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), (Jourdan et. al. 1996). These results suggested that 8-iso $PGF_{2\alpha}$ could release nitric oxide. Here we have therefore investigated the possible vasodilator properties of 8-iso $PGF_{2\alpha}$.

Wistar rats (300g) were killed by cervical dislocation, and the PAs removed. Tissues were mounted in 2 ml organ baths filled with Krebs' buffer as previously described (Curzen et al., 1995). Rings were incubated with L-NAME, D-NAME (both at 100 μM), the TP receptor antagonist ICI 192605 (100 μM) or Krebs' alone (control) for 30 minutes. In the absence of sympathetic tone the PAs were contracted with U46619 (1μM) or phenylephrine (PE) (10μM) before the start of the relaxation experiments.

8-iso $PGF_{2\alpha}$ caused a concentration-dependent dilation of the rat PA and this was significantly ($P<0.05$) reduced by the addition of L-NAME, but not by it's inactive enantiomer D-NAME (figure1,A). This suggests that the dilator function of 8-iso $PGF_{2\alpha}$ is mediated by nitric oxide. When the PA was precontracted with PE instead of the thromboxane mimetic U46619, the TP receptor antagonist ICI 192605 increased the dilator potential of 8-iso $PGF_{2\alpha}$ presumably by inhibiting it's contraction via TP receptors.

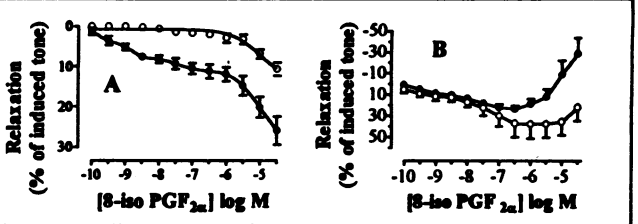

Figure 1. Effect of 8-iso $PGF_{2\alpha}$ on rat PA precontracted with either U46619 (A) or PE (B); A: Closed circle, control; open circle, plus L-NAME. B: Closed circle, control; open circle, plus ICI 192605. (Data is the mean ± SEM of n=5-6).

These results demonstrate for the first time a dilator action for 8-iso $PGF_{2\alpha}$ that is mediated by NO. Moreover 8-iso $PGF_{2\alpha}$ is approximately ten fold more potent as a vasodilator than contractile agent (Jourdan et. al. 1996). Thus, in the pulmonary vasculature where oxidant stress is a feature of diseases such as sepsis, the production of 8-iso $PGF_{2\alpha}$ may modulate vascular tone.

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Morrow and Roberts, 1996, Biochem. Pharmacol. 51;1-9
Jourdan, Curzen, Evans and Mitchell,1996, Br. J. Pharmacol.118,86P.
Curzen, Griffiths and Evans, 1995, Am. J. Physiol. 268:H2260-H2266.

167P EFFECTS OF CHRONIC INHIBITION OF NITRIC OXIDE SYNTHASE ON MEAN ARTERIAL PRESSSURE AND *IN VITRO* NORADRENERGIC VASOREACTIVITY IN THE RAT

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Altered endothelium-dependent vasodilatation, mediated by changes in metabolism of nitric oxide (NO), may be involved in the pathogenesis of diabetic microangiopathy. Several *in vivo* studies in humans and animal models suggest that NO is reduced in diabetes, due to reduced synthesis by the constitutive isoform of NO synthase (reviewed by Poston & Taylor, 1995). However, other evidence such as the beneficial effect of aminoguanidine (AG), a specific inhibitor of inducible NO synthase, on diabetic vascular dysfunction in streptozotocin-induced diabetic rats suggests that diabetes is associated with increased NO production (Corbett et al, 1992).

We are studying the role of NO in diabetic vascular dysfunction in the spontaneously diabetic, insulin-dependent BB rat, the best animal model of insulin-dependent diabetes currently available (Baird, 1989). As a preliminary to a larger investigation, we report the effects of chronic *in vivo* administration of either Lω-nitro-L-arginine methyl ester (L-NAME, a non-specific inhibitor of NO synthase) or AG on mean arterial pressure (MAP) and vasoreactivity to noradrenaline *in vitro* in perfused mesenteric arterial bed preparations from non-diabetic rats from the Edinburgh (BB/E) colony of spontaneously diabetic BB rats.

Age-matched (mean±SEM=30±1d) and weight-matched (56±3g) groups of non-diabetic diabetes-prone BB/E rats were either untreated (n=10), L-NAME treated ($27\pm1 mgkg^{-1}d^{-1}$ p.o.; n=9) or AG treated ($481\pm38 mgkg^{-1}d^{-1}$ p.o.; n=14) for four months. At the end of this period, MAP was measured directly via an indwelling carotid arterial cannula inserted two days previously under halothane anaesthesia. Mesenteric artery preparations were then isolated under anaesthesia (sodium pentobarbitone) and intralumenally perfused as described by McGregor (1965) and modified by Douglas and Hiley (1990). Non-cumulative dose-response curves to noradrenaline (0.2-2μmol) were performed and the maximal response above basal perfusion pressure (MAX) and pEC_{50}, the negative logarithm of the noradrenaline concentration required to give half-maximal response, were calculated using a commercial curve-fitting program (Polyfit). MAP was not significantly different between the untreated (116±3mmHg) and AG treated (120±2mmHg) groups. L-NAME treatment significantly ($p<0.001$) increased MAP (165±6mmHg). There was no significant difference in basal perfusion pressure between control, L-NAME treated and AG treated rats (27.0±2.6mmHg, 25.9±4.8mmHg and 25.4±3.2mmHg respectively). L-NAME treatment significantly increased the response to noradrenaline across the concentration range studied compared to untreated (ANOVA F value=7.3, $P<0.001$) and AG treated ($F=3.9$, $P<0.001$) rats. There was no significant difference in the dose-response curve to this agonist between the untreated and AG-treated groups ($F=1.7$). MAX was significantly increased in L-NAME treated rats (224±8mmHg) compared to untreated (166±7mmHg, $p<0.001$) and AG treated (190±10mmHg, $p<0.02$) rats. Although the mean MAX value for AG treated rats was higher than that for the control group, this difference was not significant. There were no significant differences in pEC_{50} between the three groups (5.74±0.05, 5.71±0.05 and 5.75±0.05 for untreated, L-NAME treated and AG treated rats respectively).

These results indicate a parallel alteration in MAP *in vivo* and microvascular vasoreactivity to noradrenaline *in vitro* in non-diabetic rats treated chronically with the non-selective NO synthase inhibitor L-NAME *in vivo*. These differences were not observed in rats treated with AG, a compound which selectively inhibits inducible NO synthase. We suggest that the use of these two compounds in studies with diabetic BB/E rats may assist in the clarification of the controversial role of NO in diabetic vascular dysfunction.

Baird, J.D. (1989) in *Recent Advances in Endocrinology and Metabolism* Vol. 3. Eds Edwards C.R.W. & Lincoln D.W. pp253-280. Edinburgh: Churchill Livingstone
Corbett, J.A. Tilton, R.G., Chang, K., et al (1992) *Diabetes* **41**, 552-556
Douglas, S.A. & Hiley, R. (1990) *Br.J.Pharmacol.* **101**, 81-88.
McGregor, D.D. (1965) *J.Pharmacol. Exp. Ther.* **253**, 1057-1062.
Poston, L. & Taylor, P.D. (1995) *Clin. Sci.* **88**, 245-255.

168P DIFFERENTIAL EFFECTS OF ACUTE HYPEROSMOTIC GLUCOSE AND MANNITOL ON MICROVASCULAR STRUCTURE AND PERMEABILITY TO SMALL AND LARGE MOLECULAR WEIGHT SOLUTES

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Hyperglycaemia *per se* is a determinant factor in the induction of microangiopathy associated with diabetes (King *et al.*, 1996). The deleterious effect of high glucose concentrations on microvascular ultrastructure and function, however, is thought to occur as a result of long term exposure (Tooke, 1995; Tucker, 1990). The aim of the present study was to examine the very early effect of high glucose concentrations on the permeability and ultrastructure of microvascular endothelium in the isolated perfused rat heart and to compare these effects with those obtained using high concentrations of mannitol.

Hearts obtained from non-diabetic rats were retrogradely perfused at constant flow via the aorta with well-oxygenated Krebs solution containing 5 mM glucose for the first 15 min (equilibration period) and the perfusate was switched to Krebs solution containing 25 mM glucose or 5 mM glucose+20 mM mannitol for a further 60 min. The permeability-surface area (*PS*) products (ml min^{-1} g^{-1}) for the diffusible solutes were determined every 15 min throughout the perfusion period by rapid infusion (50 μl) of a mixture containing ^{131}I-γ-globulin (as an intravascular reference)+^{125}I-albumin+^{57}Co-cyanocobalamin (as diffusible tracers). The *PS* products for albumin and cyanocobalamin were computed from the initial fractional extractions obtained from the dilution profile curves for these tracers (Renkin, 1959). At the end of the perfusion study the heart was fixed by perfusion with 2.5% glutaraldehyde containing 1% lanthanum nitrate and ultrathin sections from these hearts were examined in an electron microscope.

The *PS* products for albumin and cyanocobalamin obtained from hearts perfused with normal Krebs solution were 0.2±0.04 and 3.4±0.8 (n=8). These values remained stable throughout the entire perfusion period. The *PS* products for these solutes were 0.75±0.14 ($P<0.05$) and 2.3±0.22 ($P>0.05$; n=6) 15 min after switching the perfusate to Krebs solution containing 25 mM glucose which remained at the same levels throughout the perfusion with high glucose concentrations. These values were 0.26±0.04 ($P>0.05$) and 11.10±0.87 ($P<0.05$; n=6) 15 min after perfusion with Krebs solution containing 5 mM glucose + 20 mM mannitol which remained stable throughout the experimental procedure. The effect of mannitol was associated with a 31±2% reduction in aortic perfusion pressure. Electron microscopic examination of biopsies obtained from these hearts showed an absence of lanthanum binding sites on the glycocalyx and endothelial gap formation (~1 μm) in hearts perfused with a high glucose concentration but not in those perfused with normal or high mannitol-containing Krebs solution.

We conclude that in absence of influence from blood born factors, perfusion with a high glucose concentration produced a fast and sustained increase in coronary microvascular albumin permeation and that the likely route of this effect is the endothelial gaps (Al-Haboubi, *et al.* 1995). These effects of glucose cannot be explained by hyperosmolarity of the solution.

Al-Haboubi, H.A. *et al.*, (1995) J.Physiol. **482**,157-166.
King, G.L. *et al.*, (1996) Diabetes **45**, S105-S108.
Renkin, E.M. (1959) Am.J.Physiol. **197**, 1205-1210.
Tooke, J.E. (1995) Diabetes **44**, 721-726.
Tucker, B.J. (1990) Diabetes **39**, 919-923.

169P DETERMINATION OF THE COMPONENTS INVOLVED IN THE FLOW-INDUCED DILATION OF A RAT PERFUSED CORONARY ARTERY

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The flow-induced dilation, initially demonstrated by Schretzenmayr (1933) in whole animal, has also been observed in various *in vitro* preparations like rabbit femoral artery (Hecker *et al.*, 1993) and rat coronary artery (Pourageaud & Freslon, 1995). In rat gracilis muscle arteriole (Koller *et al.*, 1994), this phenomenon has been demonstrated to be partly dependent on the release of both NO and PGI_2. Thus, the aim of the study was first to define the role of the endothelium in the flow-induced dilation of a perfused rat coronary artery. We also tried to determine the involvement of the various endothelial factors (like NO and PGI_2) in this phenomenon. For this purpose, segments of the right interventricular coronary were taken from 10-15 week-old male Wistar rats (250-300 g). Arteries were mounted in an arteriograph where internal diameter was continously 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. Arteries were preconstricted with serotonin (10 µM), and the dilation induced by flow (0.1-0.8 ml/min) was quantified. This dilator effect was tested in control conditions, after incubation with L-NAME (100 µM) or indomethacin (100 µM), and after mechanical destruction of the endothelium (-E).

Dilations were expressed as percent of the serotonin-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 at maximal value of flow (0.8 ml/min) with n=10 preparations are given in the Table 1.

In control conditions, the increase in flow led to a progressive increase in shear stress. From 0.4 to 0.8 ml/min, the rate of rise of shear stress as a function of flow showed a tendancy to plateau. The flow-induced dilation of the vessels appeared to counterbalance and regulate the rise in shear stress.

With L-NAME, this regulation was greatly altered, with the loss of the plateau. Therefore, this flow-induced dilation appears to be mainly dependent on the synthesis and release of NO. In contrast, with indomethacin, our results did not differ from those obtained in control conditions and they do not support a significant involvement of a prostanoid vasodilating substance in this phenomenon. When endothelium was removed, the flow-induced-dilation was similar to that obtained with L-NAME, with a large reduction of the vasodilation. The mechanism(s) involved in this endothelium-independent component remain(s) to be determined.

P.V. is a MESR Student.

Hecker M., Mülsch A., Bassenge E. & Busse R.(1993) *Am. J. Physiol.* 265,H828-H833

Koller A., Sun D., Huang A., & Kaley G.(1994) *Am. J. Physiol.* ; 267,H326-H332

Pourageaud F. & Freslon J.-L. (1995) *J. Vasc. Res.* 32,190-199

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

	Maximal dilation (% of induced tone)	Maximal shear stress
Control	63 ± 4	76 ± 4
+ L-NAME	39 ± 3 ***	173 ± 14 ***
+ Indomethacin	69 ± 4	81 ± 6
(-E)	40 ± 2 ***	150 ± 13 ***

Values are mean ± SEM; *** : $p < 0.001$ vs control.

170P PHARMACOLOGY OF RS 25560-197, A NOVEL AND SELECTIVE INHIBITOR OF DOPAMINE-β-HYDROXYLASE

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Inhibition of the sympathetic nervous system (SNS) in congestive heart failure (CHF) may favorably alter the natural course of the disease (Packer, 1992). RS 25560-197 (S-5-aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-1,3-dihydroimidazole-2thione hydrochloride) is a novel compound which modulates the SNS by inhibition of dopamine-β-hydroxylase (DBH), the enzyme responsible for conversion of dopamine (DA) to norepinephrine (NE) in sympathetic nerves.

In vitro experiments: DBH activity was assayed by measuring the conversion of tyramine to octopamine under appropriate conditions (pH 5.2, 32°C) (Feilchenfeld et al., 1982). RS 25560-197 was a potent inhibitor of bovine (IC_{50}(± s.e.mean))= 8.5 ± 0.8 nM) and human (IC_{50}= 9.0 ± 0.8 nM) DBH. RS 25560-197 had negligible activity (IC_{50} > 10 µM) at a range of other enzymes including tyrosine hydroxylase, NO synthase, phosphodiesterase III, phospholipase A_2, neutral endopeptidase, Ca^{2+}/calmodulin protein kinase II, acetyl CoA synthetase, acyl CoA-cholesterol acyl transferase, HMG-CoA reductase and cyclooxygenase-I. RS 25560-197 also had negligible affinity (Ki > 10 µM) for α_{1A}, α_{1B}, α_{2A}, α_{2B}, β_1, β_2, M_1, D_1, D_2, µ, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors.

Effects on tissue catecholamines in spontaneously hypertensive rats (SHR) and normal dogs: Male SHR's (15-16 weeks old) were dosed with either vehicle or RS 25560-197 (three consecutive doses of either 3, 10, 30 or 100 mg.kg^{-1}; po; 12 h apart) and tissues were harvested 6 h after the third dose. Male beagle dogs were dosed with either an empty capsule or RS 25560-197 (0.05, 0.5, 1.5 or 5 mg.kg^{-1}; po; b.i.d, for 5 days) and tissues were harvested 6 h after the first dose on Day 5. DA and NE content in tissues were assayed by HPLC using electrochemical detection. Tissues studied were left ventricle (LV), mesenteric artery (MA), renal artery (RA) and cerebral cortex (CC).

Table 1: Effects on tissue DA/NE ratio (mean ± s.e.mean) in SHR's.

Dose	MA	LV	CC
0	0.03 ± 0.00	0.02 ± 0.00	0.19 ± 0.01
3	0.07 ± 0.01*	0.05 ± 0.01*	0.19 ± 0.00
10	0.13 ± 0.01*	0.09 ± 0.01*	0.21 ± 0.01
30	0.25 ± 0.01*	0.15 ± 0.01*	0.29 ± 0.01*
100	0.43 ± 0.08*	0.22 ± 0.01*	0.6 ± 0.03*

Doses are in mg.kg^{-1},po; n = 9; * $p < 0.01$ vs control (0) group.

At the doses tested, RS 25560-197 produced maximum decreases in tissue NE of 47%, 35% and 42% and maximum increases in tissue DA of 820%, 800% and 86% in MA, LV and CC, respectively.

Table 2: Effects on tissue DA/NE ratio (mean ± s.e.mean) in dogs.

Dose	RA	LV	CC
0	0.02 ± 0.00	0.03 ± 0.01	0.12 ± 0.01
0.05	0.05 ± 0.01*	0.06 ± 0.01*	0.16 ± 0.01*
0.5	0.19 ± 0.02*	0.3 ± 0.05*	0.35 ± 0.03*
1.5	0.52 ± 0.07*	0.85 ± 0.11*	1.41 ± 0.26*
5	1.91 ± 0.58*	4.54 ± 1.25*	9.5 ± 1.39*

Doses are in mg.kg^{-1},po; n = 8; * $p < 0.01$ vs control (0) group.

At the doses tested,RS 25560-197 produced maximum decreases in tissue NE of 88%, 91% and 96% and maximum increases in tissue DA of 627%, 700% and 166% in RA, LV and CC, respectively.

Conclusions: RS 25560-197 is a potent and selective inhibitor of DBH *in vitro*. Oral administration of the drug to SHR's and normal dogs produces dose-dependent modulation of tissue catecholamine levels (decrease in NE, increase in DA and DA/NE ratio), consistent with inhibition of DBH *in vivo*. These findings suggest that RS 25560-197 may be of value in the treatment of CHF.

Feilchenfeld, N. B., Richter, H. & Waddell, W. H.(1982) *Anal. Biochem.* 122, 124-128.

Packer, M. (1992) *J. Am. Coll. Cardiol.* 20, 248-254.

171P IMPLANTED PREGANGLIONIC TRUNK FUNCTIONALLY REINNERVATES CAROTID ARTERY IN CAT

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The superior cervical ganglion in cat is reinnervated by original preganglionic fibres after chronic homologous cross-anastomosis (Taniguchi et al, 1983) and also by vagal afferent fibres after chronic heterologous cross-anastomosis in cat (Fujiwara et al,1978). We report herein on functional reinnervation of carotid artery by implanted preganglionic trunk of superior cervical ganglion in cat.

The preganglionic trunk of left side in cats of either sex, weighing 4.0 to 6.0 kg, was cut at 10 mm below superior cervical ganglion. The caudal cut end of the trunk was implanted into common carotid artery and the animals were kept for 6 months. Then, the animals were examined for functional reinnervation of preganglionic fibres to carotid artery. The common carotid artery with implanted preganglionic trunk at left side was cut into ring preparation with the nerve. The preparations were set in organ bath containing Krebs-Henseleit solution. The implanted nerve was stimulated by bipolar platinum electrodes (1 msec in duration, 10 Hz in frequency, maximal V in intensity for 20 sec).

The electrical stimulation of the implanted nerve caused a definite contraction of carotid artery and the presence of $PGF_{2\alpha}$ (5×10^{-7} M) augmented the contraction. The contractions in the presence of $PGF_{2\alpha}$ were as follows: 22 ± 22 mg (n=4) in the arteries within 1.0 month after the implantation; 81 ± 26 mg (n=7) in the arteries, 1.1 to 2.0 months; 106 ± 44 mg (n=9) in the arteries, 2.1 to 3.0 months; 199 ± 43 mg (n=15) in the arteries, 3.1 to 4.0 months; 186 ± 71 mg (n=10) in the arteries, 4.1 to 5.0 months; 56 ± 17 mg (n=15) in the arteries, 5.1 ± 6.0 months. The contraction was higher at the implanted period, 3.1 to 4 months, than at the rest of periods. The presence of prazosin (10^{-6} M) nearly abolished the contraction. In the presence of prazosin (10^{-6} M) (without washout) and physostigmine (10^{-6} M), the stimulation of the implanted trunk caused a relaxation. The relaxation (396 ± 61 mg, n=3) was higher at the period within 1 month after the operation than at the other operation periods. The relaxations were decreased post-operative period dependently. Atrpoine (10^{-6} M) abolished the relaxation and reversed to a contraction (274 ± 106 mg, n=3). Hexamethonium (10^{-6} M) did not affect the relaxation. In non-operated right side carotid artery, the electrical transmural stimulation (0.3 msec induration, 30 Hz in frequency, maximal V in intensity for 20 sec) caused a contraction and the contraction was nearly abolished by prazosin (10^{-6} M) but not affected by hexamethonium (10^{-6} M) and atropine (10^{-6} M).

The present experiments clearly provide pharmacological evidence that in addition to adrenergic fibres, the preganglionic cholinergic fibres are able to reinnervate the carotid artery after chronic implantation of preganglionic trunk in cat, suggesting that the transmissions are mediated by both α-adrenoceptor and muscarinic cholinoceptor. The determinant of the involvement of muscarinic receptor may be due to postsynaptic events, (smooth muscle cells), since original cholinergic transmission in superior cervical ganglion is mediated by nicotinic receptor.

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Taniguchi, T., Kurahashi, K. & Fujiwara, M. (1983) J. Pharmacol. Exp. Ther. 224, 674-678.
Fujiwara, M., Kurahashi, K., Mizuno, N. & Nakamura, Y. (1978) J. Pharmacol. Exp. Ther. 205, 77-90.

172P EFFECT OF α_2-ADRENOCEPTOR AGONISTS ON BRADYKININ-INDUCED PLASMA PROTEIN EXTRAVASATION IN GUINEA-PIG SKIN

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Local administration of α_1-adrenoceptor agonists inhibits bradykinin (BK)-induced plasma protein extravasation (PPE) in guinea pig skin (Beets & Paul, 1980). Systemic administration of the α_2-adrenoceptor agonist, clonidine (Clon), has been reported to inhibit acute paw oedema in rats (Kulkarni *et al.*, 1986) whereas it has no significant effect on BK-induced airway permeability in guinea pigs (Biyah & Advenier, 1995). Here, we report studies of the effects of intradermal (i.d.) administration of α_2-adrenoceptor agonists on BK-induced PPE in guinea pigs.

Intradermal injections (0.1 ml site^{-1}) were made in duplicate in shaved flank skin of conscious, male Dunkin Hartley guinea pigs (490-570 g) according to a balanced block design. PPE was measured as local accumulation of intravenously injected ^{125}I-albumin over a 40 min period, calculated as µl plasma (^{125}I counts in skin site/^{125}I counts in 1µl plasma) and corrected by subtracting leakage in diluent (phosphate buffered saline) or drug alone control sites. Mean ± s.e. mean values (n = 5 or 6) were calculated and have been expressed as % of the BK (0.5 µg site^{-1}) alone control. The significance of differences between means has been assessed on the original data by analysis of variance followed by Tukey's test.

Co-injection of the α_2-adrenoceptor agonists (1 ng -10µg site^{-1}) Clon, naphazoline (Naph), oxymetazoline (Oxy) and tramazoline (Tram) produced reductions in BK-induced PPE which were significant ($P<0.01$) at doses ≥ 10 ng site^{-1}. In the same dose range, xylometazoline had no significant effect. The four effective agonists were unable to produce complete inhibition of the BK response with maximal reductions to 33 ± 3% (Tram), 36 ± 5% (Naph), 40 ± 9% (Oxy) and 49 ± 5% (Clon) of the BK control. In contrast, noradrenaline (Nor; 1 µg site^{-1}) reduced the BK response to 2 ± 3% of control. Nor (100 ng site^{-1}), Clon (3 µg site^{-1}) and phenylephrine (Phe; 150 ng site^{-1}) reduced the BK response to 22 ± 5% ($P<0.01$), 60 ± 4% ($P<0.01$) and 54 ± 5% ($P<0.01$), respectively. The response in BK sites injected with Nor + Phe was 22 ± 2% whereas that in BK sites injected with Nor + Clon was 54 ± 6%. The latter was significantly ($P<0.01$) different from the response in BK sites injected with Nor alone.

These results show that locally administered α_2-, like α_1-, adrenoceptor agonists can reduce BK-induced PPE in guinea pig skin. However, α_2-, unlike α_1-, adrenoceptor agonists are unable to completely suppress the response. Moreover, Clon appears to be acting as a partial agonist since it significantly reduced the inhibitory effect of Nor on BK-induced PPE.

Beets, J.L. & Paul, W. (1980) *Br. J. Pharmacol.* 70, 461-467.
Biyah, K. & Advenier, C. (1995) *Neuropeptides* 28,197-207.
Kulkarni, S.K., Mehta, A.K. & Kunchandy, J. (1986) *Arch. Int. Pharmacodyn. Ther.* 279,324-334.

173P ON THE USE OF ASYMMETRICAL SIGMOIDAL MODELS FOR THE ANALYSIS OF α_1-ADRENOCEPTOR AGONIST CONCENTRATION-EFFECT CURVES IN RAT AORTA

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Recently, analysis of competitive antagonism suggested the presence of a heterogeneous α_1-adrenoceptor population in rat aorta (Van der Graaf *et al.*, 1996). In that study, the first indication of heterogeneity was given by small, but systematic, deviations of the curve fit obtained with the Hill equation from the noradrenaline (NA) and phenylephrine (PE) concentration-effect (E/[A]) curves. Accordingly, we have investigated the utility of asymmetrical sigmoidal models for the analysis of α_1-adrenoceptor agonist E/[A] curves in rat aorta.

Cumulative E/[A] curves (n=6-10) to NA, PE, methoxamine (METHOX), oxymetazoline (OXYMET), cirazoline (CIRAZ), Sgd 101/75 (SGD), ST 587 (ST) and SK&F 89748-A (SK&F) were obtained on ring segments of rat (Wistar) aorta as described before (original data presented previously to the British Pharmacological Society, Van der Graaf *et al.*, 1995). By use of the software package NONMEM (see Schoemaker & Cohen, 1996), individual E/[A] data were fitted to the four-parameter Richards (1959) model (see insert), which is an extension of the three-parameter logistic model, to provide estimates (Table 1) of the upper asymptote (α, expressed as % of a PE calibration response), the slope at the point of inflection (p), the point of inflection (EC_i) and the asymmetry factor (δ). When δ=1, the Richards model is identical to the logistic model (which is equivalent to the Hill equation). In the case of OXYMET and ST, δ was found to be not significantly different from unity and when the data were refitted with δ constrained to unity, the goodness-of-fit did not decrease significantly. In contrast, however, the δ estimates associated with the E/[A] curves for the other agonists were significantly less than unity and the asymmetrical Richards model fitted significantly better than the symmetrical model with δ constrained to unity. In the case of PE, SGD and SKF, the δ parameter was not significantly different from 0 and the data could be fitted equally well by the three parameter asymmetrical Gompertz model, which is a special case of the Richards model when $\delta \rightarrow 0$ (see Richards, 1959).

$$E = \frac{\alpha}{\left(1 + \delta \cdot e^{-2.3026 \cdot p \cdot (\log[A] - \log EC_i)}\right)^{1/\delta}}$$

Overall, this analysis shows that the Richards model can discriminate between α_1-adrenoceptor agonists which produce symmetrical (OXYMET and ST) and asymmetrical (NA, METHOX, CIRAZ, PE, SGD and SKF) E/[A] curves in rat aorta. This heterogeneous behaviour is not consistent with expectations for the involvement of a simple one-receptor-one-transducer system. The curve fitting method used in this study is objective, sensitive, provides a quantitative measure of asymmetry and allows for discrimination between agonists on the basis of their E/[A] curve shape, a discrimination which could not have been made by simple inspection of the experimental data points. Like the routinely used Hill equation, the Richards model (either unconstrained or reduced to the less flexible Gompertz model) may prove to be a useful tool for the analysis of pharmacological data.

Richards FJ (1959) *J Exp Bot* **10**, 290-300.
Schoemaker RC & Cohen AF (1996) *Br J Clin Pharmacol* **42**, 283-290
Van der Graaf PH *et al.* (1995) *Br J Pharmacol* **115**, 125P.
Van der Graaf PH *et al.* (1996) *Br J Pharmacol* **118**, 299-310.

Table 1 **Richards model parameter estimates (mean ± s.e.)**

agonist	pEC_i	p	α(%)	δ
NA	8.06±0.08	0.51±0.04	145±7	-0.20±0.06
METHOX	5.73±0.09	1.07±0.18	125±8	0.43±0.20
CIRAZ	7.41±0.10	0.86±0.05	126±7	0.34±0.14
PE	7.69±0.15	0.66±0.06	128±4	0.13±0.17*
SGD	5.89±0.04	0.74±0.05	83±2	-0.06±0.10*
SKF	6.81±0.07	0.70±0.04	93±3	-0.14±0.11*
OXYMET	6.31±0.37	1.61±0.44	103±2	1.63±0.75#
ST	6.07±0.04	1.59±0.40	54±5	1.63±0.52#

*** and #: not significantly different from 0 and 1, respectively**

174P A VOLTAGE-DEPENDENT NORADRENALINE-SENSITIVE INTRACELLULAR Ca^{2+} STORE IN GUINEA-PIG AORTIC SMOOTH MUSCLE

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In vascular smooth muscle, noradrenaline (NA) and caffeine (CAF) release Ca^{2+} from a common intracellular Ca^{2+} store, referred to as the NA/CAF-sensitive store, of the sarcoplasmic reticulum (SR)(Leijten *et al.*, 1984). Itoh *et al.* (1992) using rabbit mesenteric artery, showed that in Ca^{2+} free medium, contractions induced by NA, but not CAF, increased with $[KCl]_o$. They found that with NA stimulation, $[Ca]_i$, but not the IP_3 production, also increased with $[KCl]_o$, and suggested that membrane depolarization might enhance the sensitivity of the IP_3-induced Ca^{2+} release mechanism to IP_3. Our study shows that the high K^+-induced enhancement of the contractile response to NA is related to Ca^{2+} mobilization from a voltage-dependent NA-sensitive intracellular store (VD-NAS store).

Endothelium-denuded rings (3-4mm width) of Albino guinea pig thoracic aorta were suspended in 20ml organ baths under 2g tension and equilibrated in normal Krebs solution (KS) for 90min. Contractions were recorded using a Grass Force Transducer (FT03) on a Grass Polygraph (7PIA). Regular Ca^{2+}-free KS was prepared by omitting $CaCl_2$, and high-KCl Ca^{2+}-free KS by substituting NaCl with equimolar KCl. The effects of $[KCl]_o$ on contractions induced by NA (1μM) and CAF (10mM) in the absence of extracellular Ca^{2+} with or without EGTA, were studied. The ratio of the NA response in high K^+ solution to its preceding NA response in regular Ca^{2+}-free KS was calculated and expressed as means±s.e. mean, unless stated otherwise.

In regular Ca^{2+}-free KS, the first application of either 1μM NA or 10mM CAF elicited a contraction. The second CAF stimulation failed to induce a contraction, but repetitive NA stimulations induced progressively declining responses, such that the response to the 5th NA stimulation was 20% of the first (n=6). The progressive decline in the response to repeated NA-, but not CAF-, stimulations was not only reversed, but also enhanced by bathing tissue in high KCl Ca^{2+}-free KS. Responses to repetitive NA stimulations under this condition did not decline and remained about 133% of the first response in regular Ca^{2+}-free KS (n=6). The enhancement of response to NA was still evident when 2mM EGTA was present in this high K^+ solution (ratio: 0.4±0.04 vs 0.1±0.04; n=3, $P<0.05$, paired *t* test), and was dose dependently related to the $[KCl]_o$. The contractile ratio was 1.6±0.1 at 25mM KCl; 2.4±0.1 at 65mM KCl; 3.3±0.4 at 85mM KCl; 4.0±0.4 at 105mM KCl, respectively (n=6, $P<0.05$, one way ANOVA with Fisher's method for multiple comparison). These results suggest that, under this condition, Ca^{2+} was mobilized from a voltage-dependent store, instead of the NA/CAF-sensitive one. Treatment with cyclopiazonic acid (CPA, 10μM), a putative SR Ca-ATPase inhibitor, did not affect the first NA induced contraction in 105mM KCl Ca^{2+}-free KS. However, the second NA stimulation was significantly inhibited in this high K^+ solution (about 38% of the control, ratio: 0.28±0.13 vs 0.75±0.05, n=3, $P<0.05$, unpaired *t* test), suggesting inhibition of the Ca^{2+} recycling function by CPA. Our results are consistent with the finding of Itoh *et al.* that membrane depolarization enhances NA-induced contraction. However, based on our findings we propose that, in guinea pig aortic smooth muscle, there are two intracellular Ca^{2+} stores: one is the well-known NA/CAF-sensitive store, and the other is a VD-NAS store with efficient recycling capability, which is highly susceptible to inhibition by CPA.

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Itoh, T., Seki, N., Suzuki, S. *et al.* (1992) *J.Physiol.*, 451:307-328
Leijten, P.A.A. and Van Breemen, C.(1984) *J.Physiol.*, 357:327-339

175P INHIBITION OF PLATELET ACCUMULATION IN THE CEREBRAL VASCULATURE OF THE RABBIT BY DOPAMINE DOES NOT OCCUR VIA β-ADRENERGIC RECEPTORS

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We have previously shown (Emerson et al, 1996a) that dopamine can inhibit thrombin induced platelet accumulation in the cerebral vasculature of the rabbit. Dopamine exerts its effects via α- and β-adrenergic receptors as well as specific dopamine receptors; all of these classes of receptors are found on platelet membranes. It has been suggested (De Keyser et al, 1988) that inhibition of *in vitro* platelet aggregation by dopamine occurs via D1-receptors. However, *in vitro* platelet aggregation is poorly predictive of platelet function *in vivo* and selective D1-agonists failed to inhibit *in vivo* platelet accumulation (Emerson et al, 1996b). It is possible therefore that dopamine exerts its inhibitory effects on platelets via β-adrenergic receptors.

In the experiments presented here we have examined the effects of the β-adrenergic antagonist, propranolol, *in vivo* in a model of thromboembolism using continuous monitoring of ^{111}In-labelled platelets in the cerebral and pulmonary vasculature of male NZW rabbits (May et al, 1990). Animals were anaesthetised with diazepam (4mg/kg i.p.) followed 10 min later by Hypnorm (0.4ml/kg i.m.). Administration of dopamine and propranolol began 40 min prior to injection of thrombin (80U/kg i.c.) and infusions continued for the duration of the recording period.

Platelet accumulation is expressed as mean ± s.e. mean (n=at least 4) of the maximum % increase in counts above baseline values. Control and experimental values were compared using an unpaired t-test.

Dopamine (2mg/kg/min i.c.) significantly ($P<0.05$) reduced platelet accumulation (to 63.8±9.1) compared to saline controls (111.3±11.1). Pre-treatment with propranolol alone (0.5 mg/kg) had no significant effect ($P>0.05$) on subsequent platelet accumulation and this dose of propranolol did not significantly affect accumulation inhibited by dopamine (74.9±4.1). A higher dose of propranolol (0.5mg/kg + 20μg/kg/min i.c.) also failed to affect inhibition of platelet accumulation by dopamine (55.2±8.0).

These results demonstrate that inhibition of platelet accumulation *in vivo* by dopamine does not occur *via* β-adrenergic receptors. It is possible therefore that this effect of dopamine may occur *via* selective dopamine D1 receptors. However if this is so it is not clear why dopamine agonists failed to inhibit platelet accumulation *in vivo*.

De Keyser, J., Dewaela, M., Convents, A. et al. (1988) Life Sci. 42, 1797-1806.
Emerson, M., Page, C.P. & Paul, W. (1996a) Br. J. Pharmacol. 117 (Suppl.), 231P.
Emerson, M., Page, C.P. & Paul, W. (1996b) Br. J. Pharmacol. 119 (Suppl.), 149P.
May, G.R., Herd, C.M., Page, C.P. et al (1990) J. Pharmacol. Meth. 24, 19-35.

176P FUNCTIONAL RESPONSES TO VARIOUS DRUGS IN RAT CAROTID ARTERIES WITH INTIMAL HYPERPLASIA

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An important problem associated with restenosis is the occurrence of intimal hyperplasia, due to injury of the arterial wall after percutaneous transluminal coronary angioplasty. Vascular injury caused by balloon catheter de-endothelization is a frequently and well established model to study this problem (Clowes *et al.*, 1989). Most pharmacological studies have examined the effects of balloon denudation on vascular contractile and dilator functions, but only after one fixed time period. This study examines the time dependency of the pharmacological responses of these tissues.

After inducing balloon injury in male Wistar rat (250-300g) left carotid artery, the animals were sacrificed after 0,1,3,7,14,28,56,84 and 112 days, respectively. The contractile responses of the injured and control carotid arteries to potassium chloride (K^+) and phenylephrine (PhE) were evaluated, together with the endothelium-dependent and -independent relaxation induced by methacholine (MCh) and sodium-nitroprusside (SNP) in an isometric wire myograph (PSS, 37°C, pH7.4, gassed with 95%O_2/5%CO_2).

Morphological studies have shown that the experimental procedure causes significant intimal hyperplasia in the left carotid artery after 14 days. From this moment onwards, the neointimal surface increases with time and reaches equilibrium after 84 days (data not shown). The maximal contractile responses (E_{max}) to K^+-depolarisation of the injured carotid artery did not differ significantly, when compared to the control preparation, for all time points measured (immediately after the vascular injury: E_{max}=3.3±0.4 vs. E_{max}=3.6±0.2; injured and control artery respectively, n=8). The E_{max} to PhE appeared not to be significantly influenced immediately after the vascular injury (E_{max}=2.9±0.3 vs. E_{max}=2.8±0.1; injured and control artery respectively, n=8), but became significantly decreased at 7 days (E_{max}=1.6±0.2 vs. E_{max}=2.5±0.1; injured and control artery respectively, n=8). After 84 days, the maximal response to PhE appeared fully restored (E_{max}=2.9±0.2 vs. E_{max}=3.2±0.1; injured and control artery respectively, n=6; at 112 days, E_{max}=3.0±0.1 vs. E_{max}=2.7±0.2; injured and control artery respectively, n=5). The sensitivity to PhE (pD_2) was significantly increased immediately after damaging the artery (pD_2=7.6±0.1 vs. pD_2=7.1±0.1, injured and control artery respectively, n=8; $P<0.05$). This increase in sensitivity was not observed at later time points. The endothelium-dependent vasodilator responses of the injured artery to MCh were completely abolished for all time points shorter than 84 days. At 84 days, this response of the injured artery to MCh recovered, but it still proved significantly lower than in the control artery (E_{max}=47.8%±8.6 vs. E_{max}=77.8%±3.4; injured and control artery respectively, n=6; $P<0.05$). However, the sensitivity of the preparation to MCh was equal to that of the control vessel (pD_2=6.5±0.3 vs. pD_2=6.9±0.1; injured and control artery respectively, n=6). After 112 days, both E_{max} and pD_2 for MCh of the injured artery, when compared to the control preparation, were fully restored (E_{max}=69.6%±2.6 vs. E_{max}=73.4%±5.7; pD_2=7.1±0.1 vs. pD_2=7.1±0.1; injured and control artery respectively, n=5). No significant changes were observed for the vasodilator responses to sodium-nitroprusside (E_{max} and pD_2).

In conclusion, the contractile responses to potassium of rat carotid arteries appear not be affected after vascular injury by balloon denudation. This also holds true for the endothelium-independent vasodilation after sodium-nitroprusside. The E_{max} for α_1-adrenoceptor stimulation as well as the endothelium-dependent vasodilator responses of the rat carotid artery become severely impaired after balloon denudation. The increased sensitivity to phenylephrine seen immediately after the vascular injury might be due to the removal of the protective endothelial cell layer. Apparently, after 84 to 112 days both endothelium-dependent vasodilator and α_1-adrenergic contractile functions return to normal values, indicating that both the smooth muscle cells and the endothelial layer have fully recovered.

Clowes, A.W., Clowes, M.M., Fingerle, J. *et al.* (1989) *J Cardiovasc Pharmacol* 14(6), S12.

177P INFLUENCE OF OXIDATIVE STRESS ON β-ADRENOCEPTOR-MEDIATED INOTROPIC EFFECTS

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In the present investigation we have studied the influence of oxygen derived free radicals (OFR) on the effects of β-adrenoceptor stimulation in isolated rat left atria. OFR's were generated by means of electrolysis of the medium. Free radical generation via this method was first described by Jackson et al. (1986). For this purpose we have developed a special stimulation electrode with two additional platinum wire electrodes (each 0.7 cm) circular at the bottom of the organ baths (Ø 1.5 cm). Terephthalic acid (TPA) was used in control experiments as a hydroxyl radical specific fluorescent probe. TPA (non-fluorescent) can be hydroxylated by hydroxyl radicals to yield fluorescent monohydroxy terephthalate (MHT). These experiments showed that the production of hydroxyl radicals by electrolysis is current-dependent (relative fluorescense after 75 s electrolysis: 5mA = 1.8 ± 0.1; 15 mA = 6.0 ± 0.4 and 30 mA = 21.3 ± 1.87). The hydroxyl radical scavengers DMSO (100 mM) and mannitol (100 mM) were both able to reduce the formation of MHT after electrolysis (30 mA, 75 s) by 79% and 45%, respectively ($p<0.05$). Exposure of isolated rat left atria to the medium which had been subjected to electrolysis caused a current dependent decrease in contractile force due to field-stimulation (Table 1). DMSO (10 mM), catalase (150 U/ml) and a combination of catalase (150 U/ml) and superoxide dismutase (SOD) (100 U/ml) were able to counteract the reduction in contractile force induced by electrolysis of the bath fluid (30 mA, 75s). However, SOD (100 U/ml) alone proved ineffective in this respect (Table 1). Electrolysis of the medium induced significant and substantial rightward shifts of the concentration response curves (30 min. after electrolysis (30 mA, 75 s)) for the inotropic responses to isoprenaline (pD_2: 7.56 ± 0.10 to 6.77 ± 0.11, $p< 0.05$) and forskolin (pD_2: 6.17 ± 0.12 to lower than 4.5). The increase in contractile force caused by $1 \cdot 10^{-5}$ M dibutyryl cAMP amounted to 2.15 ± 0.01 mN under control conditions and to 1.21 ± 0.10 mN after electrolysis of the medium ($p<0.05$). Measurement of adenylyl cyclase activity in membrane fractions indicated that the basal adenylyl cyclase activity in atria subjected to oxidative stress was decreased by 11.1% when compared to control organs ($p<0.05$). In addition, the amount of cAMP formed in response to 10^{-6} M forskolin proved significantly decreased in atria subjected to oxidative stress: 155.0 ± 5.1 versus 48.0 ± 1.8 pmol cAMP/mg protein/min. for control and electrolysis groups, respectively. In functional experiments, DMSO (10 mM) proved able to counteract the dimished response to isoprenaline. Without DMSO in the medium the rise to 10^{-5} M isoprenaline was 2.6 ± 0.3 (control) versus 1.7 ± 0.2 mN after electrolysis (30 mA, 75s) of the medium ($p<0.05$). In a DMSO (10 mM) containing medium the rise in contractile force to 10^{-5} M isoprenaline was not significant different between both groups (2.4 ± 0.4 mN for control, versus 2.4 ± 0.3 mN for electrolysis group).

Table 1. Influence of electrolysis and radical scavengers on contractile force

75 s electrolysis		$F_{contr.}$ (%) t=30 min.	$F_{contr.}$ (%) t=60 min.
Control	(0 mA)	96.4 ± 1.4	82.8 ± 3.5
	(5 mA)	88.2 ± 2.9	61.3 ± 6.5*
	(15 mA)	59.5 ± 4.9*	38.7 ± 6.7*
	(30 mA)	36.3 ± 3.3*	11.8 ± 4.3*
DMSO (10 mM)	(30 mA)	69.8 ± 6.5**	36.1 ± 6.5**
catalase (150 U/ml)	(30 mA)	53.7 ± 2.4	45.5 ± 5.6**
SOD (100 U/ml)	(30 mA)	28.9 ± 4.3	16.2 ± 5.0
SOD + catalase	(30 mA)	61.4 ± 8.1**	47.5 ± 9.1**

* $p<0.05$ compared to control ** $p<0.05$ compared to electrolysis (30 mA)

From these experiments we may conclude that electrolysis of a physiological salt solution is a suitable method to study the influence of OFR's on atrial tissue and that free radical generation is current-dependent. Accordingly, the exposure of atrial tissue to OFR's results in a current-dependent, gradual decrease in contractile force and biochemical alterations. The impaired response to β-adrenoceptor stimulation may be due to a decreased adenylyl cyclase activity or caused by an injured contractile apparatus.

Jackson, C.V., Mickelson, J.K., Stringer, K. *et al.* (1986) *J. Pharmacol. Methods* 15:305-320

178P ADENOSINE RECEPTORS MEDIATING VASORELAXATION IN THE SMALL MESENTERIC ARTERY OF THE RAT

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Adenosine mediates relaxation in isolated mesenteric arterial rings of the rat (Vuorinen *et al.*, 1992), but the receptors involved have not been characterised. This study was intended to identify the receptors involved in the third generation of the mesenteric artery by use of selective agonists and antagonists. Since this vessel contributes to the resistance of the mesenteric bed, the study may therefore show the receptors involved in the regulation of mesenteric blood flow by adenosine.

Segments (1.5-2.0 mm long) of third generation mesenteric artery (normalised internal diameter 200-400 μm) from male Wistar rats (250-500g) were mounted on 40 μm wire in a myograph (JP Trading, Aarhus, Denmark) for isometric tension recording. Vessels were bathed in physiological 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 5) equilibrated with 95% O_2/5% CO_2 at 37°C and equilibrated for 45 min before normalisation (Mulvany & Halpern, 1977). After a further 30 min, they were precontracted with a submaximal concentration of methoxamine (10 μM). Presence of endothelium was assessed by determining the relaxation to 10 μM carbachol; any artery giving <50% relaxation was discarded. Vessels were left for a further 30 min before obtaining concentration/response curves to adenosine agonists. Responses were measured as changes in tension and expressed as percentage relaxation of methoxamine-induced tone. Concentration/effect curves were analysed by fitting to a logistic function (Randall et al., 1989)

NECA (5'-N-ethyl-carboxamidoadenosine; Sigma Chemical Co., Poole), R-PIA (N^6-R-phenylisopropyladenosine; RBI, Natick, MA, USA) and APNEA (aminophenyl N'-ethyladenosine; RBI) gave responses with the following order of potency NECA > R-PIA ≥ APNEA which is consistent with activation of A_2 receptors. All the cocnentration/response curves were monophasic and the maximal response (E_{max}) to each agonist was near complete relaxation of the methoxamine-induced tone (Table 1).

Table 1 Relaxation of mesenteric artery to adenosine agonists

Agonist	E_{max} (% induced tone)	EC_{50} (μM)	*n*
NECA	83.1 ± 3.3	0.25 ± 0.01	8
R-PIA	96.3 ± 1.2	1.44 ± 0.01	8
APNEA	108.0 ± 6.0	2.64 ± 0.64	8

Addition of 10nM DPCPX (8-cyclopentyl-1-3-dipropylxanthine; RBI) an A_1-selective antagonist did not affect the responses to any of the three agonists ($n = 4$ for all). Addition of 8-SPT (150 μM), an A_1 and A_2 adenosine receptor antagonist, antagonised the responses to NECA, shifting the curve 88 fold ($n = 4$) suggesting the presence of A_2 receptors. Responses to R-PIA were unaffected by 150 μM 8-SPT ($n = 4$) but the antagonist did cause an approximate 10 fold rightwards shift of the curve for APNEA, a selective A_3 receptor agonist ($n = 2$). CGS 21680 (2-[p-(carboxyethyl)phenylethylamine]- 5 - N-ethylcarboxamido-adenosine; RBI), a selective A_{2a} agonist did not relax this tissue.

In conclusion, these results suggest the presence of A_{2b} receptors and another as yet unidentified site which is resistant to 8-SPT in the small mesenteric artery. NECA and APNEA appear to activate both sites but only the unidentified site is activated by R-PIA. A similar site has been noted by Prentice et al. (1996) in the first generation mesenteric artery. It seems unlikely that the A_1 or A_{2a} receptors play an important role in the vasodilator responses to adenosine in the small mesenteric artery.

SPC is an Medical Research Council Research Student.

Mulvany, M.J. & Halpern, W. (1977). *Circ. Res.*, 41, 19-26.
Prentice, D.J., Payne, S.L. & Hourani, S.M.O. (1996). *Drug Dev. Res.*, 162.
Randall, M.D., Douglas, S.A. & Hiley, C.R. (1989). *Br. J. Pharmacol.*, 98, 685-699.
Vuorinen, P., Pörsti, I., Metsä-Ketelä, T. *et al.* (1993). *Br. J. Pharmacol.*, 105, 279-284.

179P HYPOXIA-INDUCED STUNNING IN ISOLATED CARDIAC TISSUES: THE EFFECT OF AN A_3 RECEPTOR AGONIST

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Studies of the effect of hypoxia and ischaemia on cardiac function have usually been performed in Langendorff or working hearts (Schultz *et al.* 1995). This study was therefore undertaken to characterise the effect of hypoxia on isolated guinea-pig left atria and papillary muscles. The effects of the A_3 adenosine receptor agonist IB-MECA (N^6-(3-iodobenzyl)adenosine-5'-N-methylcarboxamide) on the recovery of contractility after hypoxia was then investigated.

Left atria and a left ventricular papillary muscle from Male Dunkin-Hartley guinea pigs (250-300g) were set up in 50ml organ baths, containing Krebs bicarbonate solution (composition mM : NaCl 118.4, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4.7H_2O$, $NaHCO_3$ 24.9, $KH_2PO_4.2H_2O$ 1.2, glucose 11.6) maintained at 37± 0.5°C and gassed with 5% CO_2 in oxygen. Tissues were paced at 2Hz with threshold voltage + 50%. Resting tensions of 0.5-1.0g were applied and isometric tension recorded. All figures shown are the means of at least four experiments ± standard error of the mean. The results were taken to be significantly different if unpaired Student t-test returned values of $p<0.05$.

After 15 min equilibration, hypoxia was induced by switching the 5% CO_2 in oxygen gassing the bathing solution to 5% CO_2 in nitrogen, followed by several changes of the bathing solution. Hypoxia was maintained for 30 min before reoxygenation by returning to gassing with 5% CO_2 in oxygen. Tissues were paced throughout. The effects of IB-MECA were investigated by the addition of a single bolus concentration ($3x10^{-7}$M) either 10 min into hypoxia or at the point of reoxygenation.

In control left atria and papillary muscles the induction of hypoxia caused a fall in developed tension to 16.9±3.4% and 20.3±1.6% of the pre-hypoxic level respectively. During hypoxia diastolic tension increased. This contracture is an index of Ca^{2+} overload. The onset of contracture occurred at 14±0.7 and 14.3±1.0 min into the hypoxic period and reached a peak of 30.5±13% and 21.9±7.5% above the pre-hypoxia resting diastolic tension in left atria and papillary muscles, respectively. On reoxygenation, the developed tension returned to 75.4±3.9% and 78.5±5.5% of the pre-hypoxic value in left atria and papillary muscles, respectively, at 5min after reoxygenation. These levels were significantly ($p<0.05$) below the pre-hypoxia resting level and indicate myocardial stunning.

IB-MECA added 10 min into hypoxia did not affect the fall in developed tension during hypoxia compared to controls, the values being at the end of hypoxia 17.6±1.3% and 13.8±3.1% of the pre-hypoxic level in left atria and papillary muscles, respectively. The onset and extent of the hypoxic contracture were also not significantly ($p>0.05$) affected by the addition of IB-MECA in both preparations. On reoxygenation no significant ($p>0.05$) improvement in developed tension over control values was observed, the values being 86.5±8.2% and 74.7±6.3% at 5 min and 74.0±5.8% and 94.4±9.5% at 15 min for left atria and papillary muscles, respectively. Addition of IB-MECA at reoxygenation, however, caused a significant ($p<0.05$) improvement in developed tension at recovery from the hypoxic episode in both left atria and papillary muscles. The values were 96.7±6.5% and 106.6±7.8% of the pre-hypoxic value at 5min post reoxygenation in left atria and papillary muscle respectively. This improvement was maintained after 15 min (98.5±5.1% and 125.9±3.5%, respectively).

Hence the A_3 receptor agonist IB-MECA added at reoxygenation facilitates an improved recovery from an hypoxic episode in both left atria and papillary muscle and thus attenuates the myocardial stunning.

Schultz,R., Ehring, T & Heusch, G. (1995). Basic Res. Cardiol., 90, 294-296.

180P CARDIOPROTECTIVE EFFECTS OF GR79236, AN ADENOSINE A1 AGONIST, ARE INDEPENDENT OF ITS BRADYCARDIC AND HYPOTENSIVE ACTIVITY

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Adenosine A_1-receptor agonists have been shown to reduce infarct size (Martin, *et al.*, 1993) and ischaemia-induced dysrhythmias (Wainwright *et al.*, 1993) in pigs. GR79236, (N-[(1S, trans)-2-hydroxycyclopentyl]adenosine) is a potent and selective agonist at adenosine A_1-receptors (Gurden, *et al.*, 1994) and we therefore investigated its cardioprotective actions in a model of myocardial ischaemia/reperfusion in anaesthetised pigs.

Myocardial infarction was induced in pentobarbitone anaesthetised Large White pigs (either sex, 20-35 kgs) by occluding a branch of the left anterior descending coronary artery for 50min followed by 180min reperfusion. Arterial blood pressure (MBP) and heart rate (HR) were measured and from these data the pressure-rate product (PRP) was calculated and taken as an index of myocardial work (Nelson *et al.*, 1974). In one group of animals electrodes were attached to the right atrium to allow HR to be fixed by electrical pacing. Infarct (I) was determined by negative tetrazolium staining and expressed as a percentage of the area at risk (AAR) of infarction which was delineated by fluorescent beads. All areas were measured using computerised planimetry. Incidence, and time to onset, of ventricular fibrillation were noted.

Either GR79236 ($3.5\mu g.kg^{-1}$, iv) or saline (vehicle control) was administered 10 min prior to the occlusion. Initial MBP and HR were similar in all of the treatment groups. GR79236 reduced MBP from 58±2 to 50±3mmHg and HR from 128±9 to 100±7 bpm, however both changes could be reversed by electrical pacing. GR79236 significantly reduced infarct size in both paced and unpaced groups relative to vehicle treated controls (Table 1). There was no difference in the incidence of VF in any of the groups; however, in GR79236 treated animals the time to onset of VF was prolonged. The delay in VF was reversed in paced, GR79236-treated animals. Furthermore, both the haemodynamic and cardioprotective effects of GR79236 were blocked by prior administration of the adenosine A_1-receptor antagonist DPCPX ($1mg.kg^{-1}$ iv)

Table 1 (All data are mean ± s.e.m., n=5-6. *$p<0.05$ vs control)

	Control	GR79236 (unpaced)	GR79236 (paced)
I/AAR (%)	45.8±5.2	14.0±2.3*	19.4±4.8*
AAR/LV (%)	11.9±1.6	16.9±3.4	15.8±3.0
PRP($mmHg.min^{-1}$)	7630±630	4372±467*	8192±532

We conclude that the cardioprotective effects of GR79236 are independent of its bradycardic activity and are mediated by adenosine A_1-receptors. However, delay in onset of VF in unpaced animals treated with GR79236 is a result of reduced myocardial work.

Gurden, M.F., Coates,J., Ellis,F.,*et al.* (1994) *Br. J. Pharmacol.*; **109**, 693-698.
Martin, B.J., McClanahan, T.B., Hamilton, H.W. *et al.*,(1993) *Circulation*; **88(4)**:I430
Nelson, R.R., Gobel F.L., Jorgensen, C.R. *et al.*,(1974) *Circulation*; **50**:1179-1189
Wainwright, C.L. & Parratt, J.,(1993) *Cardiovasc. Res.*; **27**:84-89

181P 8-EPI-PROSTAGLANDIN $F_{2\alpha}$ IS A VASOCONSTRICTOR IN THE RAT ISOLATED HEART AFTER PERFUSION WITH XANTHINE AND XANTHINE OXIDASE

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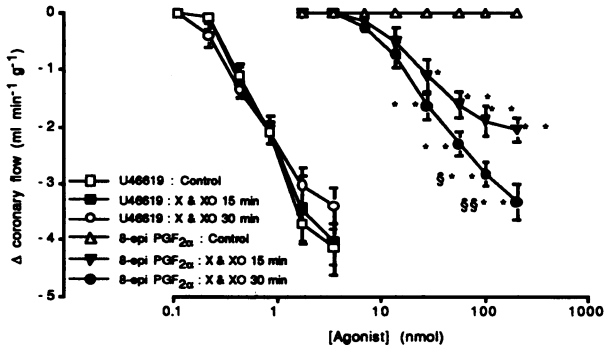
Previously we reported that 8-epi prostaglandin $F_{2\alpha}$ (8-epi $PGF_{2\alpha}$), an F_2-isoprostane, has a vasoconstrictor effect on rat coronary circulation following low flow ischaemia (Kromer & Tippins, 1996). To assess further the role of this compound in oxidant stress, the effects of a thromboxane mimetic (U46619) and 8-epi $PGF_{2\alpha}$ were studied on rat isolated Langendorff heart preparation, before and after perfusion with xanthine (X) and xanthine oxidase (XO).

Sprague-Dawley rats (200g) were anaesthetised with hypnorm/ hypnovel (2.7ml/kg; 2:5 v/v). Hearts were perfused at constant pressure (80mm Hg) and coronary flow (11.8±1.26 ml min^{-1} g^{-1} tissue wet weight in control hearts, mean ± s.e.mean, n=14) recorded as an indication of coronary resistance. A latex balloon in the left ventricle measured ventricular pressure from which rate was calculated. Bolus doses (10µl) of U46619 or 8-epi $PGF_{2\alpha}$ were given into the coronary perfusion cannula. The tissue was left for at least 5 minutes between doses or until a stable baseline had been reached.

U46619 caused a dose-dependent decrease in flow, indicative of coronary constriction, with an ED_{50} of 0.77±0.13 nmol (n=5). 8-epi $PGF_{2\alpha}$ had no effect on normal rat coronary vasculature. After perfusion with Krebs containing 0.23mM xanthine and 0.5mU ml^{-1} xanthine oxidase for either 15 or 30 minutes, the dose responses were repeated. The U46619 dose response curve shifted slightly to give an ED_{50} values of 0.77±0.12 nmol (n=4) and 0.57±0.09 nmol (n=5) for 15 and 30 minute perfusions respectively. This shift was not statistically significant. However, X and XO perfusion caused 8-epi $PGF_{2\alpha}$ to give significant coronary constriction (Unpaired *t* test; n=5, Figure 1) in a time-dependent fashion (* $P<0.05$; ** $P<0.001$: X & XO vs control; § $P<0.05$; §§ $P<0.001$: 30 min vs. 15 min X & XO perfusion).

Figure 1. Effect of U46619 and 8-epi prostaglandin $F_{2\alpha}$ on coronary flow in the isolated heart.



Thus dose-dependent response of rat coronary vasculature to 8-epi $PGF_{2\alpha}$ occurs after perfusion with X and XO with no response in control hearts. This is of interest, since isoprostanes are formed in physiological conditions of oxidant injury including stable angina and myocardial infarction (Catella *et al.*, 1995), and may therefore play an important pathophysiological role in such conditions.

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Catella, F., Reilly, M.P., Delanty, N. *et al.* (1995). *Adv. in Prostaglandin Thromboxane and Leukotriene Research*, 23, 233-236.

Kromer, B. & Tippins, J.R. (1996). *Br. J. Pharmacol.*, 118, 86P.

182P BRADYKININ B_1 RECEPTOR AGONIST STUDIES IN PORCINE ENDOTOXIN SHOCK

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There are two main types of bradykinin (BK) receptor, namely B_1 and B_2 (Regoli & Barabe, 1980). The B_2 receptor is normally constitutive whereas the B_1 receptor appears to be upregulated in response to a variety of noxious stimuli including experimental endotoxic shock in rabbits (Regoli & Barabe, 1980; Nwator & Whalley, 1989) pigs (Siebeck *et al.*, 1989) and rats (Tokumasu *et al.*, 1995).

Bradykinin B_2 receptor antagonists have been shown to increase survival in endotoxic shock in rabbits, rats (Whalley *et al.*,1992) and pigs (Siebeck *et al.*, 1996). Recent studies have also demonstrated that the protective effect of a B_2 antagonist, with respect to survival and other parameters in porcine endotoxic shock, is reversed when a B_1 antagonist is given simultaneously (Siebeck *et al.*, 1996). These latter data would suggest that activation of the B_2 receptor in this model has a detrimental effect whereas the upregulated B_1 receptor may be protective.

The present study describes preliminary experiments to assess the effect of continuous intravenous infusions of the B_1 agonist, Lys0-des-Arg9-BK (LDABK), on hypotensive respon-ses to bolus doses of the same agonist. Endotoxic shock was induced in healthy anesthetized pigs using a constant intravenous infusion of LPS from *S. abortus equi*, 2 ug/kg/min for 8h and blood pressure recorded as described previously (Siebeck *et al.*, 1996). In this model, upregulation of the B_1 receptor, as reflected by increased hypotensive response to bolus doses of LDABK, was found to occur within 3-4 h. After 4 h, animals received repeated intra-arterial bolus injections of LDABK (10 ug) before and during continuous intravenous infusions of LDABK at 3, 10, 30 and 100 ng/kg/min for 15 min.

Infusion of LDABK at all doses had no effect on resting b.p. Responses (mean ± s. e. mean diastolic arterial b.p. in mmHg) to LDABK (before, during, n=4-6) were found to be -35.8 ± 2.8 (before); -35.5 ± 4.0 (saline, n.s.); -35.3 ± 2.6 (3ng/kg/min, n.s.); -16.1 ± 2.7 (10ng/kg/min, $p<0.05$); -23.3 ± 2.2 (30 ng/kg/min, $p=0.2$); -11.4 ± 4.7 (100ng/kg/min, $p<0.005$).

It can be concluded that tachyphylaxis was seen with bolus doses (10ug) of LDABK when administered during a continuous intravenous infusion of LDABK at doses of 10 and 100 ng/kg/min but not at 3 ng/kg/min. Such low dose infusions of a B_1 receptor agonist may be of benefit in such conditions as endotoxic shock in which this receptor appears to have an apparent protective effect. Such agonist studies are in progress.

Nwator I.A.A. & Whalley E.T. (1989) Eur. J. Pharmacol., 160, 125-132

Tokumasu T., Ueno A. & Oh-ishi S. (1996) Eur. J. Pharmacol., 274, 225-228.

Regoli D., & Barabe J. (1980) Pharmacol. Rev., 32, 1-46.

Siebeck M., Whalley E.T., Hoffmann H. *et al.* (1989) Adv. Exp. Med. Biol., 247B, 389-393.

Siebeck M., Spannagl E., Schorr M. *et al.* (1996) Immuno-pharmacology, 33, 81-84.

Whalley E.T., Solomon J., Modafferi D. *et al.* (1992) Agents Actions, 38, 202-206.

183P INTERACTION OF NEUROMUSCULAR BLOCKING DRUGS WITH RECOMBINANT HUMAN m1-m3 MUSCARINIC RECEPTORS

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There have been reports of neuromuscular blocking drugs (NMBD) producing adverse cardiovascular effects (see Hunter, 1995). This could result from an interaction with cardiac m2 muscarinic receptors. In this study we have investigated if NMBDs interact with recombinant human m1, m2 and m3 muscarinic receptors in CHO cells (Buckley et al., 1989).

CHO cells were cultured in minimal essential medium at 37°C in 5% carbon dioxide/air mixture. The cells were passaged weekly and fed twice weekly. Muscarinic receptor binding studies were performed in 1ml volumes of HEPES 20mmol litre^{-1} / Mg^{2+} 1mmol litre^{-1} (pH 7.4) buffer containing approximately 120 µg of membranes. In saturation experiments to determine receptor density (B_{max}) and equilibrium dissociation constant (K_d), membranes were incubated with increasing concentrations (0.007-3.23 nmol litre^{-1}) of a radiolabelled muscarinic antagonist *n*-methyl-scopolamine ([^{3}H-]NMS). The affinity of a range of NMBDs and muscarinic receptor subtype selective antagonists were estimated by displacement of a fixed concentration of NMS (~ 0.3 nmol litre^{-1}) and increasing concentrations of displacer. Tissue was incubated for 1h at 37°C and non-specific binding was determined in the presence of atropine 10µmol litre^{-1}. Bound and free radioactivity were separated by rapid vacuum filtration onto Whatman GF/B filters. B_{max} and K_d were calculated according to Scatchard (1949). The concentration of drug producing 50% displacement of specific binding was corrected for the competing mass of NMS according to Cheng and Prussoff (1973) to yield the affinity constant K_i.

The binding of NMS was dose-dependent and saturable in CHOm1, m2 and m3 membranes with B_{max} and K_d of 2242±75 fmol mg^{-1} protein and 0.11±0.02 nmol litre^{-1} (*n*=5), 165±13 fmol mg^{-1} protein and 0.15±0.01 nmol litre^{-1} (*n*=5) and 1877±33 fmol mg^{-1} protein and 0.12±0.01 nmol litre^{-1} (*n*=5) respectively. The binding of [^{3}H]-NMS was displaced dose-dependently (pK_i) by, pirenzepine in CHOm1 cells (7.97±0.04, *n*=5), methoctramine in CHOm2 cells (8.55±0.1, *n*=5) and by 4-diphenylacetoxy-N-methyl piperidine methiodide (4-DAMP) in CHOm3 cells (9.38±0.03, *n*=5). All NMBDs displaced NMS in a dose dependent manner with pK_i values shown in table 1.

Table 1. pK_i values of NMBD's for m1-m3 muscarinic receptors ($n \geq 5$)

	CHOm1	CHOm2	CHOm3
Pancuronium	6.43±0.12	7.68±0.02[a]	6.53±0.06
Vecuronium	6.14±0.04	6.90±0.05[a]	6.17±0.04
Pipecuronium	6.34±0.11	6.58±0.03[b]	5.94±0.01
Rocuronium	5.42±0.01	5.40±0.02[b]	4.34±0.02
Gallamine	6.83±0.05	7.67±0.04[a]	6.06±0.06

[a] $p<0.05$ higher affinity than m1 and m3. [b] $p<0.05$ higher affinity than m3 only.

These results show an interaction of NMDB's with m2 muscarinic receptors which may account for the adverse effects of these agents on the cardiovascular system. Investigations to determine possible agonist or antagonist effect are currently underway.

We thank Dr N.J. Buckley (University College, London) for providing CHO transfects and Organon Teknika for providing vecuronium, pipecuronium and rocuronium. TMC holds a University of Leicester studentship.

Buckley, N.J., Bonner, T.I., Buckley, C.M., *et al.* (1989) *Mol. Pharmacol.* **35**, 469-476.
Cheng, Y.C., Prusoff, W.M. (1973) *Biochem. Pharmacol.* **22**, 3099-3108.
Hunter, J.M. (1995) *N. Engl. J. Med.* **25**, 1691-1699.
Scatchard, G. (1949) *Ann. N. Y. Acad. Sci. USA.* **52**, 660-672.

184P MODIFICATION OF TETANIC CONTRACTIONS IN RAT PHRENIC NERVE-DIAPHRAGM PREPARATION BY DIAZEPAM AND PK 11195

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Myorelaxation produced by benzodiazepines is a well-known side effect in humans. Using animal preparations, biphasic effects of diazepam have been described by Driessen et al. (1984); other derivatives produce exclusive inhibition of muscle contractions. The site of origin of these actions is unknown. Using the antagonist PK 11195 [1-(2-chlorophenyl-N-methyl-N-(1-methylpropyl)3-isoquinolinecarboxamide], this study is a preliminary attempt to establish possible involvement of peripheral binding sites.

Phrenic nerve-diaphragm preparations from female rats, killed by neck blow, were fixed in a vessel containing Krebs-Ringer solution (35° C), gassed with 5% CO_2 in O_2, and equilibrated during one hour. Preparations were stimulated indirectly with supramaximal rectangular pulses (0.2 msec width). Stimulation frequences were 0.1 Hz during 5 min and 10 to 50 Hz during 3 sec applied with intervals of 20 sec. This cycle was repeated 6 times. Diazepam (10^{-4} M) was added to the bath 2.5 min before the start of the first cycle and PK 11195 (10^{-5} M) one hour before. Each experiment was preceded by 3 control cycles without drugs. Each compound or combination was investigated in 6 experiments. Contractions were recorded isometrically. Effects were expressed as percentage ± SEM of the area under the curve of the third control recording. $P < 0.05$ was considered the level of significance.

PK 11195 when added alone did not interfere with tetanic contractions. Diazepam at all frequencies, except at 50 Hz during cycle 5 and 6, enhanced the tetanic contraction force. The enhancement proceeded in two steps and was time- and frequency-dependent. Initially an abrupt increase of the contractions was observed, which was subject to gradual reduction in subsequent cycles and at higher frequencies. At each point in time the contraction force was yet significantly larger as compared to control contraction force. The maximum increase of the contraction amounted to 233% ± 36.2 in the second cycle at 40 Hz. In subsequent cycles this maximum gradually decreased to 126% ± 17.5 in the 6th cycle at 30 Hz. Only in the 5th and the 6th cycle at 50 Hz contractions were inhibited: to 80% ± 16.0 and 66% ± 11.4 respectively, as compared to control. In the presence of PK 11195 the response to diazepam became biphasic in shape: initial enhancement of tetanic contractions was followed by inhibition after the second cycle. At 40 Hz reversal was observed after the first cycle, whereas at 50 Hz only relaxation was recorded.

In conclusion, diazepam evoked enforcement of neuromuscular transmission; this exclusive potentiation proceeded in two steps. In the presence of PK 11195 the response was modified to a biphasic one; i.e. stimulation followed by inhibition of muscle contraction. It is suggested that benzodiazepine receptors may be involved in the effects of diazepam.

Driessen, J.J., van Egmond, J.J., van der Pol, F. et al. (1984), J. Pharm. Pharmacol. 36, 244-247.

185P PK 11195 AND FLUMAZENIL MODIFY RESPONSES OF CORONARY FLOW RATE AND INOTROPY OF THE PERFUSED RAT HEART TO Ro 05-4864

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The benzodiazepine analogue Ro 05-4864 increases coronary flow rate (Grupp et al., 1987) and contraction force (Zeegers et al., 1996) of the rat heart. Responses of the isolated rat heart to Ro 05-4864 [chlorodiazepam] in the absence and in the presence of PK 11195 [1-(2-chlorophenyl-N-methyl-N-(1-methylpropyl)-3-isoquinoline-carboxamide] or flumazenil are described.

Hearts of female rats (160-180 g), anaesthetized with pentobarbitone, heparinized and cannulated aortae were excised and perfused retrograde with conventional Tyrode's solution at 37 °C (pH 7.0), gassed with 5% CO_2 in O_2. Coronary flow rate was measured by catching the perfusate leaving the heart into a tumbling vessel (5 ml); the time expiring for the vessel to tumble was measured and the flow rate expressed as ml/min perfusate caught. Contraction force (mm Hg) was recorded isometrically, using a latex balloon filled with water and inserted into the left ventricle. Each heart was exposed to Ro 05-4864, in successively increasing concentrations between $2x10^{-5}$ and $4x10^{-4}$ M with (n=6) or without (n=12) an antagonist continuously in the perfusate. For each concentration of an antagonist a separate group of heart preparations was used. Changes in coronary flow rate or contraction force during exposure were expressed as percentage ± SEM of control rate or force, immediately before exposure. $P < 0.05$ was considered the level of significance.

Both coronary flow rate and contraction force were increased concentration - dependently by Ro 05-4864 between $2x10^{-5}$ to $1x10^{-4}$ M. PK 11195 in concentrations of 10^{-7} to $5x10^{-5}$ M significantly antagonized the increase of both coronary flow rate and contraction force; $5x10^{-5}$ M PK 11195 even abolished the positive inotropic effect. Both responses were displayed in a concentration-dependent manner. Emax values (according to Lineweaver-Burke plots) of Ro 05-4864 at 10^{-7}, 10^{-6}, 10^{-5} and $5x10^{-5}$ M PK 11195 were 76% ± 6.2, 52% ± 4.1, 18% ± 2.5 and 5.0% ± 2.1 (coronary flow rate) and 59% ± 6.4, 46% ± 2.8 and 19% ± 1.9 (inotropy), and pD'2 values 5.74 ± 0.11 and 5.64 ± 0.15 respectively. In the presence of flumazenil, 10^{-7} to 10^{-5} M, both the increase of coronary flow rate and of contraction force produced by Ro 05-4864 were significantly ($p < 0.05$) reduced as well, depending on concentration of flumazenil. Emax values of Ro 05-4864 on coronary flow rate were 85% ± 3.7 (10^{-7} M flumazenil), 65% ± 2.2 (10^{-6} M) and 46% ± 3.0 (10^{-5} M) and on contraction force 49% ± 2.3, 46% ± 1.8 and 31% ± 1.7.

In conclusion, the results indicate that peripheral-type of benzodiazepine receptors may be involved in cardiac responses to benzodiazepines; the finding that flumazenil reduced the actions of Ro 05-4864 as well, indicates that other mechanisms may be involved.

Grupp, I.L., French, J.F. and Matlib, M.A. (1987), Eur. J.Pharmacol., 143, 143-147.
Zeegers, A., Leeuwin, R.S. and van Wilgenburg, H. (1996), Br.J. Pharmacol., 117, 266P.

186P BLOCK OF EVOKED ACTION POTENTIALS OF THE RAT SCIATIC NERVE *IN VITRO* BY BENZODIAZEPINES

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Depression of vagal nerve activity by diazepam has been reported by Wesselman et al. (1991). This presentation describes effects of diazepam and midazolam on evoked action potentials in the rat sciatic nerve *in vitro*.

Female rats (appr. 180g) were killed by neck blow. Sciatic nerves were dissected from the spinal cord downwards, including its branches in the tibial muscle and fixed in a chamber, constructed especially for the purpose of stimulation and recording of nervous activity, which was perfused with Krebs-Ringer solution (12 ml/hr) gassed with 5% CO_2 in O_2 at 25°C. Suction electrodes were used for both stimulation and recording. The frequency of stimulation was 0.25 Hz, duration 0.05 msec, with a constant current of 1 mA. The recording electrode was connected to an amplifier, enabling recording on a tape recorder. The nerve was pre-incubated for 1 hr. The experiment started with recording action potentials at 10, 3 and 1 min, each lasting 1 min, before administration of a benzodiazepine. Perfusion was subsequently continued with Krebs-Ringer solution containing the desired concentration of a benzodiazepine ($3x10^{-5}$ to $6x10^{-4}$ M). Subsequent recordings were performed under continuous stimulation of the preparation between 1 and 60 min. After washing additional recordings were made at 15 and 60 min. N=6. Effects were expressed as percentage of control values ± SEM. Statistics were performed by means of the independent sample Student's t-test, using the SPSS programme. $P< 0.05$ was considered the level of significance.

Inhibition of evoked action potentials by diazepam was significant and concentration-dependent (from $6x10^{-5}$ to $6x10^{-4}$ M); the response was also time-dependent (from 10 to 40 min of exposure). The response to midazolam was more complex: initially, 3 to 30 min, at $3x10^{-5}$ M the action potentials were inhibited; at $6x10^{-5}$ M, this inhibitory response was first reversed; and subsequently, from $1x10^{-4}$ M onwards, further inhibition of the action potentials was observed, which was statistically significant. At the higher concentrations of midazolam reduction of action potentials was time-dependent. EC_{50} values of diazepam were $2.2x10^{-4}$ M (30 min), $1.9x10^{-4}$ M (40 min), $1.7x10^{-4}$ M (50 min) and $1.4x10^{-4}$ M (60 min); and of midazolam $4.2x10^{-4}$ M (30 min), $3.2x10^{-4}$ M (40 min), $2.6x10^{-4}$ M (50 min) and $1.8x10^{-4}$ M (60 min). The relation between EC_{50} and time (t in min) was 3.0-0.03xt for diazepam and 6.6-0.08xt for midazolam respectively. The difference was significant. Recovery after washing was always complete.

In conclusion, benzodiazepines reduce evoked action potentials in isolated nerve preparations. Diazepam appears to be more potent in blocking the response than midazolam. The mechanism underlying the action is unknown.

Wesselman, J., van Wilgenburg, H. and Long, S. (1991), Neurosc. Letters, 128, 261-264.

187P ACTIVATION OF NICOTINIC RECEPTORS ON SYMPATHETIC NERVE TERMINALS INCREASES ACTION POTENTIAL-EVOKED ATP RELEASE IN THE GUINEA-PIG ISOLATED VAS DEFERENS

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Nicotinic cholinergic receptors occur peripherally at the neuromuscular junction as well as in the CNS. It is less well known that prejunctional facilitatory nicotinic receptors are present on some postganglionic nerve terminals. Here we have studied the action of nicotine on transmitter release from pre- and postganglionic sympathetic nerves in the guinea-pig isolated hypogastric ganglion and vas deferens.

Conventional intracellular recording techniques were used to record EPSPs as a measure of ACh release from preganglionic sympathetic nerves and EJPs as a measure of ATP release from postganglionic nerves. The prostatic third of the vas deferens was removed, when appropriate, to ensure ganglion free preparations. Trains of stimuli were delivered through Ag/AgCl electrodes positioned around the prostatic end of the vas deferens. In electrophysiological experiments, prazosin (0.3 μM) and nifedipine (1 μM) were present.
When ganglia were present, nicotine infusions (100 μM) induced immediate and sustained contractions (> 30s). Contraction was associated with asynchronous discharges of EPSPs and action potentials in the hypogastric ganglion. Both of these phenomena were abolished by hexamethonium (100 μM). EJPs evoked by stimulation of postganglionic nerves in aganglionic preparations were potentiated by nicotine, an effect abolished by hexamethonium (Fig. 1). The amplitude of the first EJP in a train (0.5 or 1 Hz) in control cells was 6.4 ± 1.0 mV and 21.7 ± 3.9 mV (mean ± s.e.mean, n = 6 in 3 preparations) 4 minutes after nicotine (100 μM), an increase of some 288% ($P < 0.01$). Subsequent EJPs in a train had similar amplitudes and facilitation did not occur (Fig. 1). The potentiating effect of nicotine on EJPs declined during the infusion but were repeatable 1 hour after wash.

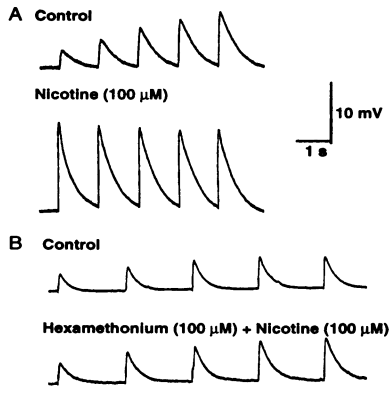

Fig. 1. Effects of nicotine and hexamethonium on EJPs

In conclusion, nicotinic receptors located on postganglionic sympathetic nerve terminals can powerfully increase the action potential-evoked release of ATP. Similar conclusions were reached by von Kügelgen & Starke (1991) from overflow studies of both ATP and noradrenaline. It is fair to say that the role of nicotinic receptors on nerve terminals remains poorly understood. The vas deferens should prove a useful model system in which to study electrophysiologically the mechanisms by which prejunctional nicotinic receptor activation enhances neurotransmitter release on an impulse to impulse basis.

von Kügelgen, I. & Starke, K. (1991) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 344, 419-429

188P EFFECT OF PLATELET-ACTIVATING FACTOR (PAF) ON FLUID SECRETION IN HUMAN COLON *IN VITRO*

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Platelet-activating factor (PAF) has been implicated as a putative mediator of the diarrhoea associated with inflammatory bowel disease, endotoxic shock and cholera (Guerrant *et al.*, 1994; Nassif *et al.*, 1996). In rat colon, application of PAF has been shown to induce fluid secretion, the response was independent of specific PAF receptors or prostaglandin synthesis (Buckley & Hoult, 1989). The present study aims to investigate the effects of PAF on fluid secretion in human colon, and to determine it's mechanism of action.

Sections of macroscopically-normal human sigmoid colon were obtained from specimens removed at operations for carcinoma. After removal of muscle layers, intact sheets of mucosa were mounted in Ussing chambers (window area 1.43cm^2), bathed either side by gassed (95% O_2 :5% CO_2) Krebs solution at 37°C, and changes in short-circuit current (I_{SC}; an indicator of electrogenic fluid secretion) were monitored. After 60 min equilibration, either a PAF receptor antagonist (WEB 2170; MacNaughton & Gall, 1991), indomethacin or control vehicle were applied to both sides of the tissue, and left to equilibrate for a further 30 min. After this time, a single concentration of PAF (10^{-9} to 10^{-5} M) was applied to the serosal surface of each preparation, and the resulting change in I_{SC} was monitored. After a maximum response had been attained, carbachol (100μM) was applied to the serosal side of all preparations. Data are given as mean±s.e.mean, except EC_{50} values which are given as geometric mean with 95% confidence limits (95% C.L.), n indicates the number of specimens used. Data analysis used the Mann-Whitney U-test, with $p<0.05$ being taken to indicate statistical significance.

After 60 min equilibration, basal I_{SC} across the mucosa of human sigmoid colon was 59.7±12.5 μAcm^{-2} (n=11). Serosal application of PAF was shown to cause a significant, concentration-dependent increase in I_{SC}, with a maximum response of 74.3±9.5 μAcm^{-2} at 10^{-5}M and an EC_{50} of 18.2nM (95% C.L. 5.5 - 61.4nM, n=6). Prior treatment with indomethacin (1μM, n=3) or WEB 2170 (10μM, n=4) significantly reduced the secretory response to 1μM PAF (increase in I_{SC} of 4.4±1.0 μAcm^{-2} and 6.1±3.1 μAcm^{-2} respectively, compared to 62.4±14.2 μAcm^{-2} in control tissues, n=6, $p<0.05$). Mucosal application of PAF (10^{-6}M) had no effect on I_{SC} (n=6). Application of carbachol caused a significant increase in I_{SC} in all preparations (210.8±11.7 μAcm^{-2}, n=11), proving tissue viability.

In addition, in the 30 min following their application, both indomethacin and WEB 2170 were shown to significantly reduce basal I_{SC} of human colonic mucosa, by 35.1±6.7 μAcm^{-2} and 23.2±3.3 μAcm^{-2} respectively (compared to 9.9±5.7 μAcm^{-2} in control tissues, $p<0.05$). This may indicate that both PAF and prostaglandins play a role in mediating the basal secretory tone of human colonic mucosa.

In conclusion, application of PAF induced significant fluid secretion across human colonic mucosa, an effect mediated via a specific PAF receptor and dependent on a product of cyclo-oxygenase enzymes. In addition, this study may have uncovered a role for cyclo-oxygenase products and PAF in the maintenance of basal fluid secretion in human colon.

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Buckley, T.L. & Hoult, J.R.S. (1989) *Eur. J. Pharmacol.*, 163, 275-283.
Guerrant, R.L., Fang, G.D., Thielman, N.M. *et al.* (1994) *Proc. Natl. Acad. Sci. USA*, 91, 9655-9658.
MacNaughton, W.K. & Gall, D.G (1991) *Eur. J. Pharmacol.*, 200, 17-23.
Nassif, A., Longo, W., Mazuski, J. *et al.* (1996) *Dis. Colon Rectum*, 39, 217-223.

189P BTS 67 582 ACTS AS A GLUCOSE SENSITISING AGENT IN RAT PERFUSED PANCREATIC ISLETS

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We have previously shown that BTS 67 582 (1,1-dimethyl-2-(2-morpholinophenyl)]guanidine fumarate) lowers blood glucose concentrations in normal rats with a concomitant increase in plasma insulin concentration, but is unable to bind to the sulphonylurea receptor (Kaul *et al.* 1995). In this study we have characterised the effects of BTS 67 582 on first and second phase insulin release from perifused pancreatic islets isolated from normal rats.

Pancreatic islets were isolated from fed male Charles River Wistar rats (175-225g) following the injection of collagenase into the pancreatic duct (10 ml of 9 mg ml^{-1} Sigma Type XI in buffer). Oxygenated (95% O_2/5% CO_2) Gey & Gey buffer was used throughout with the following composition (in mM): NaCl (111), $NaHCO_3$ (27), KCl (4.96), $MgCl_2.6H_2O$ (0.98), $MgSO_4.7H_2O$ (0.28), Na_2HPO_4 (0.63), KH_2PO_4 (0.22), $CaCl_2$ (2), 1 mg ml^{-1} bovine serum albumin (Sigma, Fraction V, RIA grade) and 4 mM glucose (unless otherwise indicated). Batches of 20 islets (pooled from 3 rats per islet preparation) were placed into disposable syringe filters (Gelman Acrodisc 0.8 μm) and perifused at 37°C with buffer at 1 ml min^{-1} and samples collected at 2 minute intervals for insulin assay (Linco, rat insulin RIA kits RI-13K). BTS 67 582 (1 μM to 1 mM) was dissolved in buffer and perifused for 60 minutes following a 30 minute drug free preequilibration period. Data from 3-4 separate experiments were pooled for each treatment and presented as the mean ± s. e. mean.

The perifused islets were responsive to glucose between 4 mM and 20 mM with an approximate EC_{50} value of 11.8 ± 0.7mM. BTS 67 582 dose dependently increased both first and second phase insulin release. In islets preequilibrated with 8 mM glucose, the EC_{50} value for the effect of BTS 67 582 on the peak phase 2 response was 9.1 ± 1.5 μM and maximal insulin secretion was increased from the control value of 23 ± 7 up to 97 ± 6 pg insulin min^{-1} $islet^{-1}$. The effect of BTS 67 582 on the first phase insulin release at 8 mM glucose was only seen at high concentrations (100 μM and 1 mM) and maximal insulin secretion was raised from a control value of 76 ± 17 up to 124 ± 15 and 164 ± 50 pg insulin min^{-1} $islet^{-1}$ respectively. In contrast, at the substimulatory glucose concentration of 4 mM there were only slight effects of 100 μM and 1 mM BTS 67 582 on insulin release (peak phase 2 release increased from 2 ± 1 to 9 ± 4 and 11 ± 3 pg insulin min^{-1} $islet^{-1}$, respectively). There were no effects of BTS 67 582 at concentrations up to 1 mM on insulin release when islets were perifused with 15 mM glucose.

These data demonstrates that BTS 67 582 in perifused islets acts to potentiate the action of glucose on insulin release within the normal physiological range (5-8 mM). BTS 67 582 has little effect on insulin release at either sub-stimulatory (4 mM) or maximally stimulatory glucose concentrations (15 mM).

Kaul, C.L., Marita, A.R., Dickinson, K. *et al.* (1995) *Br. J. Pharmacol.* **114**, 256P.

190P EVIDENCE FOR TYROSINE KINASE INVOLVEMENT IN NORADRENALINE-INDUCED VASOCONSTRICTION OF THE RABBIT PERFUSED OVARIAN VASCULAR BED

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We have recently characterised the adrenoceptor sub-type mediating vasoconstrictor responses of the perfused rabbit ovarian bed as being α_{1A} (Yousif et al., 1996).

The present experiments were carried out to determine the mechanism by which activation of such receptors by noradrenaline (NA) led to vasoconstriction.

Sexually-mature female New Zealand white rabbits (3-4 Kg) were anaesthetised with sodium pentobarbitone 50 mg/Kg i.v. and exsanguinated. The ovarian artery was cannulated and the ovarian bed *in vitro* was perfused with Krebs' solution at 6 mls/min at 37°C. Basal perfusion pressure was 41.7±3.3 mm Hg (mean±s.e.mean, n=20). Changes in perfusion pressure are expressed as a % of the maximum pressor response elicited by 1 μM NA, 198±11.2 mm Hg (n=8).

Perfusion of the ovarian vascular bed with Krebs' solution (calcium-omitted) reduced the NA response (1 μM) to 16.7±7.8% of control values ($p<0.01$, n=4). Perfusion (30 min) with verapamil (10^{-7} M) or nifedipine (10^{-7} M) caused partial but significant reductions in the responses to NA (1μM) to 60.4±9.4% and 74.6±6.6% of controls respectively ($p<0.01$, n=4). However responses to 200 μM KCl were completely abolished by these drugs.

Genistein, an inhibitor of tyrosine kinase was found to produce a significant reduction in the responses to NA (0.3μM) which was dose-related, 69.1±5.8% relative to control at 10^{-6} M and 34.5±12.1% at 10^{-5} M respectively (both n=4, $p<0.01$). However, the protein kinase C (PKC) inhibitor polymyxin B (10^{-4} M) had no significant effect on NA-induced (1μM) vasoconstrictor responses (86.6±11.7% relative to control, n=5). These results suggest that in the perfused rabbit ovarian artery NA produces vasoconstriction via the influx of extra-cellular calcium. The major portion of this influx was sensitive to calcium channel blockade and was therefore presumably via voltage-activated channels (VOC), as reported previously (Nelson et al., 1988). As blockade of VOC was only partially-effective in inhibiting NA-induced vasoconstriction, activation of receptor-operated calcium channels may also play a role. If this is the case then Ca influx via ROC activation in the ovarian artery appears to be linked to tyrosine kinase activation, not PKC stimulation as has been reported in other tissues (Henrion & Laher, 1993).

Henrion, D. & Laher, I. (1993) Can. J. Physiol. Pharmacol. 71, 521.

Nelson, M. T., Standen, N. B., Brayden, J. E. & Worley, J. F. (1988) Nature, 336, 382.

Yousif, M. H., Williams, K. I. & Oriowo, M. A. (1996) FASEB J. 10, A424.

191P ELECTROPHYSIOLOGICAL STUDY ON THE ACTIVITY OF THE TACHYKININ NK_2 RECEPTOR ANTAGONIST MEN 11,420 IN THE GUINEA-PIG CIRCULAR COLON

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We have investigated the activity of the tachykinin NK_2 receptor antagonist MEN 11,420, or [Asn(2-AcNH-β-D-Glc)-Asp-Trp-Phe-Dap-Leu]c(2β-5β), in the circular muscle of the guinea-pig proximal colon by means of sucrose-gap technique (Hoyle, 1987) which enables the simultaneous recording of changes in membrane potential and contractile activity of smooth muscle.

In resting conditions, the circular muscle of guinea-pig proximal colon generated spontaneous slow waves which were accompanied by low amplitude contractions. MEN 11,420 (0.1-3μM) did not affect the spontaneous motor activity neither the resting membrane potential, nor the size of the hyperpolarizing electrotonic potentials (change in conductance 5.3±2.6%, n=4, n.s.).

The NK_1 receptor selective agonist, [Sar^9]SP sulfone (0.3μM for 10s) induced depolarization of the membrane (11.7±0.9mV, n=4) accompanied by action potentials and contraction (8.6±0.8mN, n=4). MEN 11,420 (3μM) failed to antagonize the electrical (13.5±0.7mV, n=4, n.s.) and contractile activity (9.2±1.4mN, n=4, n.s.) induced by [Sar^9]SP sulfone.

The NK_2 receptor selective agonist, [βAla^8]NKA(4-10) (0.3μM for 10s) induced depolarization of the membrane (12.4±1.3mV, n=18) accompanied by action potentials and contraction (7.2±0.7mN, n=18). MEN 11,420 (0.1-3μM) concentration-dependently inhibited both depolarization and contraction produced by [βAla^8]NKA(4-10), the IC_{50} being 0.34μM(0.22-0.43μM) and 0.32μM(0.31-0.33μM) respectively.

Cholinergic excitatory junction potentials (e.j.p.) (15.2±3.9mV, n=3) followed by action potentials and contractions (10.5±2.6mN, n=3) were evoked by low pulse width electrical field stimulation (EFS, 40V, 0.02-0.03ms). These EFS-induced responses were abolished by atropine (1μM). MEN 11,420 (3μM) had no effect either on electrical (13.8±2.8mV, n=3, n.s.) or contractile activity (11.2±2.9mN, n=3, n.s.).

Endogenous tachykininergic responses mediated by NK_1 and NK_2 receptor activation were obtained by prolonged period of EFS (3Hz for 3min) in the presence of atropine (1μM), guanethidine (3μM), indomethacin (10μM), Nω-nitro-L-arginine (0.1mM) and apamin (0.1μM). In these conditions EFS produced a long lasting depolarization (LLD) with superimposed action potentials (APs) and a concomitant contraction showing a distinct first phasic and late tonic component. Nifedipine (1μM) blocked APs and the phasic component of contraction while LLD was only partially affected (21% inhibition, $p<0.01$) and the tonic component of contraction was unaffected. Application of MEN 11,420 (1-3μM) almost completely abolished the nifedipine-resistant tonic contraction (92% inhibition, $p<0.01$) and partially affected the LLD (19% inhibition, $p<0.01$).

In the presence of atropine (1μM) and indomethacin (10μM) single pulse EFS (20-30V, 0.2ms) evoked inhibitory junction potentials (i.j.p.) (11.4±2.8mV, n=3) which were abolished by the combined administration of Nω-nitro-L-arginine (0.1mM) and apamin (0.1μM). MEN 11,420 (3μM) did not affect the amplitude or duration of non-cholinergic non-adrenergic i.j.p. (11.1±2.6mV, n=3).

The present results demonstrate that MEN 11,420 is a potent antagonist of tachykinin NK_2 receptors in the circular muscle of the guinea-pig colon. The activity of MEN 11,420 is highly selective: it does not affect the mechanical or the electrical activities induced by activation of tachykinin NK_1 receptors, nor the cholinergic and NANC inhibitory transmission nor the general ionic permeability of the membrane.

Hoyle, C.H.V. (1987) *J. Pharmac. Meth.* 18, 219-226.

192P SB 207266 IS A POTENT 5-HT_4 RECEPTOR ANTAGONIST IN HUMAN ISOLATED GASTROINTESTINAL TISSUE

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5-HT_4 receptor-mediated responses have been identified in human isolated colon (Hillier *et al.*, 1994). SB 207266 has recently been identified as a highly potent, highly selective 5-HT_4 receptor antagonist with good oral bioavailability (Wardle *et al.*, 1996). In the present study the effects of SB 207266 were investigated in human isolated colon and ileum.

Specimens of terminal ileum and sigmoid colon were obtained at surgery for benign or malignant disease. Circular muscle strips (2 - 4 mm wide and 20 - 30 mm long) were suspended under 1 g load in Krebs solution containing methiothepin (0.1 μM) and granisetron (1 μM) at 37°C and bubbled with 5% CO_2 in O_2. Concentration-effect curves to 5-HT were constructed cumulatively by adding increasing concentrations at 3 min intervals. Antagonists were incubated for 40 min prior to construction of a second 5-HT curve.

Traces were analysed by integrating the area under the spontaneous activity during the 3 min challenge period with each concentration of 5-HT. Results were expressed as a % of the maximum 5-HT-evoked response in the control curve and were expressed as mean ± s.e.mean of a number (n) of observations in different patients.

In human isolated colon, 5-HT (0.1 nM - 1 μM) evoked a concentration-dependent inhibition in the amplitude of spontaneous activity with a pEC_{50} value of 7.8 ± 0.3 (n = 13 from 6 patients). Following washout, spontaneous activity returned to 89 ± 3% of the control level. A second concentration-effect curve to 5-HT was not significantly ($P > 0.05$; Student's t-test on pEC_{50} and E_{max}) different from the first curve. SDZ 205 557 (0.3 μM, n = 2) evoked a rightward and surmountable displacement of the 5-HT curve, yielding individual pK_B values of 7.8 and 6.9. SB 207266 (1 nM, n = 1 and 100 nM, n = 2) caused a rightward surmountable displacement and an abolition respectively of the 5-HT curve. The pK_B for 1 nM SB 207266 was 10.7.

Human isolated ileum circular muscle displayed minimal spontaneous activity. 5-HT (0.1 nM - 10 μM) evoked a concentration-dependent increase in phasic and tonic activity with a pEC_{50} value of 6.7 ± 0.1 (n = 20 from 5 patients). Following washout, spontaneous activity returned to control levels. A second concentration-effect curve to 5-HT was not significantly ($P > 0.05$) different from the first curve. SDZ 205 557 (0.3 μM, n = 5) evoked a rightward surmountable displacement of the 5-HT curve, yielding a mean pK_B value of 7.4 ± 0.3. SB 207266 (1 nM, n = 5) caused a rightward surmountable displacement of the 5-HT curve. The mean estimated pK_B was 9.8 ± 0.1.

These results suggest that SB 207266 is a highly potent antagonist at the 5-HT_4 receptor in human isolated colon and ileum and as such is an important tool in understanding the patho-physiology of this receptor in man.

Hillier, K., Tam, F.S.-F., Bunce, K.T. and Grossman, C. (1994). *Br. J. Pharmacol.*, 112, Proc Suppl, 102P.

Wardle, K.A., Bingham, S., Ellis, E.S. *et al.* (1996). *Br. J. Pharmacol.*, 118, 665 - 670.

193P THE INFLUENCE OF 5-HT RECEPTOR ANTAGONISTS ON THE CONTRACTILE RESPONSE TO 5-HT IN THE ISOLATED INTESTINE OF *SUNCUS MURINUS*

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In previous studies (Javid *et al.*, 1996) we have demonstrated that the contractile response to 5-HT in proximal intestine of *Suncus murinus* were antagonised by methysergide, a 5-HT_1 and 5-HT_2 antagonist, and ritanserin, a 5-HT_2 antagonist. This suggested the involvement of 5-HT_2 receptors in mediating the contractile response to 5-HT. The aim of the present studies is to investigate the influence of antagonists for these and other 5-HT receptors on the contractile response to 5-HT in the *Suncus murinus* intestine, to characterise further the receptors that mediate the response.

Segments (1-1.5 cm long) were taken from the intestine (2-3 cm distal to the pyloric sphincter) of adult Japanese House Musk shrew, *Suncus murinus* (38-88 g) of either sex. These were mounted in 10 ml organ baths containing Krebs' solution (37°C, 95% O_2, 5% CO_2). The tissues were allowed to equilibrate for 60 min and washed every 20 min. The resting tension was maintained at 0.5 g and recorded isometrically. Non-cumulative concentration-response curves to 5-HT were established with a 1 min contact time and 22 min intervals between doses. The procedure was repeated in the constant presence of ondansetron (ond), a 5-HT_3 antagonist, (1μM), SB204070 (SB), a selective 5-HT_4 antagonist, (1nM) (Wardle *et al.*, 1994), a combination of methysergide (methy) (1μM) and ond (1μM), a combination of methy (1μM) and SB (1nM), a combination of ond (1μM) and SB(1nM) or a combination of methy (1μM), ond (1μM) and SB (1nM). Tissues were allowed to equilibrate for 1 h in the presence of antagonist before the application of 5-HT. Tension changes were expressed as the mean±s.e.mean of n=4-10 and analysed using one-way ANOVA followed by Bonferroni/Dunnett's t-test.

Ond (1μM) or SB (1nM), or a combination of ond (1μM) and SB (1nM) did not affect the contractile response to 5-HT, Figs. 1.a-b. Methy (1μM) in the presence of ond (1μM) or SB (1nM) significantly ($p<0.05$) shifted the lower part (10nM-0.3μM) of the concentration-response curve to the right without affecting the maximum response, Fig. 1-b. A combination of methy (1μM), ond (1μM) and SB (1nM) significantly ($p<0.05$) shifted the lower part of the concentration-response curve to the right without affecting the maximum response, Fig. 1-c.

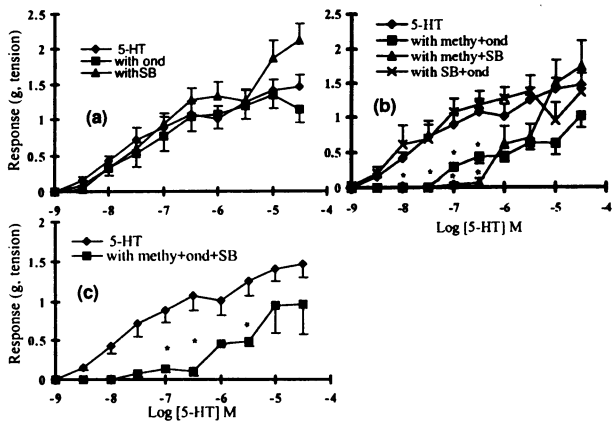

Figure 1. The effect of 5-HT receptor antagonists on the contractile response to 5-HT in the *Suncus murinus* intestine.

The data suggest that the involvement of 5-HT_3 and 5-HT_4 receptors in mediating the contractile response to 5-HT is unlikely. They also support the involvement of 5-HT_2 receptors in mediating the contractile response to 5-HT in the segments distal to the pyloric sphincter.

Javid, F. A., Naylor, R. J. & Tuladhar, B. R. (1996) *Br. J. Pharmacol.* 119, 197P.

Wardle, K. A., Ellis, E. S., Baxter, G. S. *et al.* (1994) *Br. J. Pharmacol.* 112, 789-794.

194P PROTECTIVE EFFECT OF NIRAVOLINE, A KAPPA-OPIOID AGONIST, ON ENDOTOXIN-INDUCED ACUTE GASTRO-INTESTINAL LESIONS IN THE RAT

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It has previously been shown that vasopressin played a significant aggressive role in acute endotoxin shock-induced gastro-intestinal injury (Laszlo et al. 1994). Indeed, a pretreatment with a V1 antagonist was able to partially protect the gastro-intestinal mucosa and, in addition, Brattleboro homozygous rats were less prone to damage following the endotoxin injection. Kappa-opioid agonists have been shown to induce an inhibition of vasopressin secretion in various species (Hamon and Jouquey, 1990). The aim of the present study was to assess the effects of such an inhibition of vasopressin secretion on the gastro-intestinal injury induced by LPS injection in the rat.

Fasted female Sprague-Dawley rats (body weight 200-230 g) were injected intravenously with either E. coli endotoxin (lipopolysaccharide, LPS serotype 0111:B4, 50 mg/kg in saline) (n = 8 rats per group) or sterile saline (1 ml/kg) (n = 5) and then sacrificed 15 min later. Saline or niravoline (0.1, 0.3 or 1 mg/kg), a novel kappa agonist (Hamon et al. 1995) were injected intravenously 10 min before LPS. The animals were sacrificed by decapitation, trunk blood was collected on EDTA and centrifuged at 4°C for 20 min. The plasma was then stored at -80°C until vasopressin concentration measurement, using a specific radioimmunoassay. The stomach and duodenum were then removed and cut open in order to evaluate the gross macroscopic mucosal damage induced by the LPS treatment. Following a visual examination, the lesions were scored from 0 to 3 according to their severity. Statistical analysis of the mean lesion scores was performed using Dunnett's test.

LPS induced clear lesions in both the stomach and the duodenum (respective lesion scores 1.1 ± 0.4 and 2.9 ± 0.1) the severity of which was dose-dependently decreased by pretreatment with niravoline as follows: for stomach lesions, -51% (ns) and -95% ($p < 0.05$) at 0.3 and 1 mg/kg respectively ; for duodenum lesions, -17% ($p < 0.05$), - 24% ($p < 0.05$) and -48% ($p < 0.01$) at 0.1, 0.3 and 1 mg/kg respectively.

LPS treatment induced a very important increase in vasopressin plasma level (from 0.51 ± 0.01 pg/ml in the saline control group to 375.68 ± 69.70 pg/ml) which was dose-dependently inhibited by niravoline : -21% (ns), -64% ($p < 0.01$) and -82% ($p < 0.01$) at 0.1, 0.3 and 1 mg/kg respectively.

The results of this study indicate that a pretreatment with a kappa opioid agonist is able to protect the gastro-intestinal mucosa against LPS-induced damage. This effect parallels the decrease of vasopressin release in the blood stream. This data lends support to an involvement of vasopressin release in LPS-induced acute gastro-intestinal injury.

Laszlo, F., Kavacsony, G., Pavo, J., Varga, Cs., Rojik, I. and Laszlo, F.A. (1994), Eur. J. Pharmacol. **258**, 15-22.

Hamon, G., Clémence, F., Fortin, M., Le Martret, O., Jouquey, S., Vincent, J.C., Petit, F. and Bichet, D.G. (1995), Br. J. Pharmacol. **114**, suppl. 310P.

Hamon, G. and Jouquey, S. (1990), Horm. Res. **34**, 129-132.

195P INHIBITION OF POTASSIUM (K_{ATP}) CHANNELS REDUCES SHORT-CIRCUIT CURRENT RESPONSE OF RAT COLONIC MUCOSA TO ACETYLCHOLINE

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Intestinal secretion depends upon electrogenic chloride transport into the gut lumen. Such transport requires the maintenance of an electrically negative cell membrane voltage, which is achieved through basolateral outward movement of potassium ions (Dawson and Richards, 1990). The aim of the investigation was to determine whether secretory responses of rat colonic mucosa to acetylcholine were sensitive to inhibition of potassium channels and whether such inhibition could indicate the nature of channel involved. Short-circuit current (I_{sc}) was used as a functional index of acetylcholine-induced secretion. Muscle-stripped preparations of rat (male, Wistar, 200-300 g) large intestine (excluding striated portion) were mounted in Ussing chambers containing Krebs solution at 37°C and gassed with 5% CO_2 in O_2. Mucosal sheets were voltage clamped at zero potential and transmucosal I_{sc} continuously recorded. Drugs were added to the basolateral side of preparations. Statistical analysis used the Mann-Whitney U test for unpaired data, with $P<0.05$ being taken to represent a significant difference. Values quoted are mean ± s.e.mean. After 60 min equilibration, basal I_{sc} was 15 ± 3 $\mu A.cm^{-2}$ (n=27). Serosal application of acetylcholine (10^{-6} - 10^{-3}M) gave a concentration-dependent increase in I_{sc} with an EC_{50} value of 14 ± 1 μM and a maximal response of 188 ± 16 $\mu A.cm^{-2}$ (n=4). Using a 30 min contact time, potassium channel inhibitors were tested against I_{sc} responses to acetylcholine (14 μM). Barium (1 - 20 mM, n=4) produced a concentration dependent inhibition of 38 ± 13 to 90 ± 5%, the values for 4-aminopyridine (1-5 mM, n = 5) and glibenclamide (dissolved in DMSO, 100 - 500 μM, n=5) were 9 ± 5 to 66 ± 5% and 29 ± 6 to 95 ± 1% inhibition respectively. A combination of charybdotoxin (0.3 μM) plus apamin (0.3 μM) did not reduce responses to ACh (n=8, $P>0.05$). Responses to acetylcholine were also reduced by the K_{ATP} channel inhibitors 5-hydroxydecanoate (10 mM, 26 ± 6%, n=6, $P<0.05$) and phentolamine (100 μM, 92 ± 2%, n=5, $P<0.05$). Parallel time-matched control experiments showed no significant change in responses to acetylcholine either for aqueous or DMSO vehicles ($P>0.05$).

These preliminary experiments indicate that a basolateral outward movement of potassium ions is required for the I_{sc} response of rat colonic mucosa to acetylcholine. The particular potassium channel involved appears to be ATP-dependent and calcium insensitive. ATP-regulated channels are also known to be active in the basolateral membrane of human colonic epithelial cells. (Cuffe *et al.*, 1995).

N.D. Palmerley gratefully acknowledges financial support from The Nuffield Foundation.

Cuffe, J., McNamara, B., O'Sullivan, G. *et al.* (1995) J. Physiol. 489, 122-123P
Dawson, D.C. & Richards, N.W. (1990) Am. J. Physiol. 259, C181-C195.

196P RADIOLIGAND BINDING PROFILE OF MUSCARINIC RECEPTORS IN HUMAN COLON AND ILEUM

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Radioligand binding studies of intestinal smooth muscle from animal species (eg Giraldo *et al.*, 1988) and from human colon (Gomez *et al.*, 1992) have demonstrated a heterogeneous population of muscarinic receptors. The present study utilises a wider range of antagonists than previously reported (Gomez *et al.*, 1992) to investigate the binding profile of human colon and ileum smooth muscle muscarinic receptors.

Human colon and ileum specimens were obtained at autopsy (white Caucasian and Hispanic) and prepared separately. Adhering fat and connective tissue, mucosa and submucosa were removed by blunt dissection before microsomal preparation of samples. Experiments were carried out in Tris buffer (pH 7.4) at room temperature. The binding affinities of known muscarinic antagonists for human muscarinic receptors was determined by displacement of [^{3}H]-N-methylscopolamine (NMS), at a concentration close to the derived equilibration dissociation constant (K_D), for each microsomal preparation using 12 concentrations of antagonist. Specific binding was determined using atropine (1 μM). Equilibrium binding parameters (K_D, B_{max} (fmol/mg protein)) for colon smooth muscle were 4.88 ± 0.95 nM and 1821 ± 195 (n=3) and for ileum smooth muscle were 4.70 ± 1.68 nM and 1370 ± 324 (n=4).

The pK_i values of all antagonists in colon and ileum preparations are shown in Table 1 (Graphpad Prism software). All unlabelled drugs displaced [^{3}H]NMS with Hill coefficients not significantly different from unity, suggesting a homogenious population of receptors in colon and ileum. The affinity of atropine suggests the displacement of [^{3}H]NMS from muscarinic receptors in both colon and ileum preparations. Affinities for 4-DAMP and p-F-HHSiD suggest that they are not of the M_3 subtype. pK_i values for AF-DX-116 and methoctramine, and pirenzepine are not consistent with the presence of M_2 subtype or M_1 subtype, respectively.

In conclusion, these binding affinities appear not to be consistent with the presence of M_1, M_2 nor M_3 -like muscarinic subtypes in the smooth muscle from human colon and ileum.

Giraldo, E.,Vigano, M.A., Hammer, R. *et al.* (1988) *Mol. Pharmacol.* **33**, 617-625.
Gomez, A., Martos, F., Bellido, I. *et al.* (1992) *Biochem. Pharmacol.* **43**, 2413-2419.

Table 1 pKi values for muscarinic antagonists for human colon and human ileum smooth muscle (mean ± sem; n=3-4 for each antagonist)

	Atropine	4-DAMP (M_3)	p-F-HHSiD (M_3)	AF-DX 116 (M_2)	Methoctramine (M_2)	Pirenzepine (M_1)
Colon	8.36± 0.07	7.60±0.10	5.67±0.20	5.53±0.07	6.40±0.20	5.80±0.06
Ileum	8.52±0.15	7.58±0.08	5.72±0.18	5.55±0.05	6.50±0.06	5.82±0.16

197P IDENTIFICATION OF A SPECIFIC BINDING SITE FOR MENTHOL IN GUINEA-PIG AIRWAY

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L-menthol inhibits both neurokinin A- and capsaicin-induced bronchoconstriction in the guinea pig and relaxes pre-constricted isolated guinea pig bronchi (Wright *et al* 1996). Structure-activity relationships have been defined for the action of L-menthol on cold receptors suggesting a drug receptor interaction at the site of the peripheral cold receptors (Watson 1978). We have determined a specific binding (SB) site for [^{3}H] L-menthol in guinea pig lung using ligand binding studies.

18 male Dunkin Hartley guinea pigs (355-700g) were killed by dislocation of the neck. Lung tissue was dissected out and membrane pellets prepared, with protein concentration adjusted to 2 mg/ml and stored at -80 °C. For association studies 50 µl [^{3}H] L-menthol (20 nM) was incubated with 400µl lung membranes and 50µl incubation buffer, for times ranging 1-120 min at 37 °C (total bound). Non specific binding (NSB) was defined by incubating adjacent membranes with 50 µl, 100 µM unlabelled L-menthol. For dissociation studies, at full association, an excess of L-menthol 100 µM was added over a range of time points (0.5-30 min) and (SB) was quantified.

Displacement of specifically bound [^{3}H] L-menthol was studied with, L-menthol, D-menthol, camphor, cineole, capsaicin, capsezepine, 5-hydroxytryptamine, adrenaline, spiperone, SCH23390, bepridil, nicardipine and flunarazine. Results are expressed as mean±s.e.mean. SB was quantified from total bound-NSB. Binding parameters were determined using SIGFIT program.

[^{3}H] L-menthol became fully associated to its site within 45 min, $t_{1/2}$ = 5 min. L-menthol was fully dissociated from its binding site within 8 min, $t_{1/2}$ = 2 min. Positive results from the inhibition studies are in Table 1. Noradrenaline, 5-hydroxytryptamine, spiperone, flunarazine, bepridil and nicardipine did not effect [^{3}H] L-menthol binding.

We have identified a putative menthol receptor with binding characteristics similar to the known rank order of potency of menthol agonists. The association and dissociation kinetics indicate an abundant low affinity binding site.

Wright, C.E., Laude, E.A., Grattan, T.J. *et al.* (1996) Br. J. Pharmacol (submitted)

Watson, H.R., Hems, R., Rowsell, D.G., *et al.* (1978) J. Soc. Cosmet. Chem. 29,185-200

Table 1 Inhibition of [^{3}H] L-menthol binding

	L-menthol	D-menthol	Camphor	Cineole	Capsaicin	Capsezepine	SCH23390
n	8	6	6	6	6	6	6
mean slope	-1.21±0.1	-1.0±0.3	-1.1±0.7	-0.9±0.7	-1.2±0.3	-1.0±0.02	-0.8±0.4
mean IC_{50}	-5.55±0.1	-4.74±0.1	-4.3±0.1	-3.59±0.1	-4.26±0.1	-5.02±0.1	-5.3±0.2

198P CAPSAICIN- AND NEUROKININ A-INDUCED BRONCHOCONSTRICTION IN THE ANAESTHETISED GUINEA-PIG: EVIDENCE FOR A BRONCHODILATOR EFFECT OF L-MENTHOL

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In the guinea pig (GP) the irritants capsaicin and citric acid produce both cough and a release of neurokinins to initiate bronchoconstriction, possibly via stimulation of sensory afferents. L-menthol has been reported to reduce cough in both man and guinea-pig (Morice *et al.*, 1994; Laude *et al.*, 1994) but its mechanism of action has not been identified. We have studied the effects of L-menthol on capsaicin- and exogenous neurokinin A (NKA)- induced bronchoconstriction *in vivo* and studied the effect of L-menthol upon isolated GP bronchi *in vitro*.

Male Dunkin Hartley GP 420-645g (n=13) were anaesthetised with urethane (1.5g.kg^{-1}, *i.p.*) and received either a bolus injection of capsaicin (7.5 µg.kg^{-1}, *i.v.*) or an infusion of NKA (1µg.min^{-1}, *i.v.*) while freely breathing air (0.8 l.min^{-1}) or air impregnated with L-menthol vapour (6mg. l^{-1}.min^{-1}) from a tracheal cannula. Airways resistance (Raw) was determined via the pleural pressure/tracheal airflow relationship and was measured continuously together with ventilation. Results are expressed as mean±s.e.mean. Statistical analysis was two tailed paired t test.

Isolated bronchi (n=24) of mean internal diameter (1029±73.6 µm) were mounted in a myograph and bathed in physiological saline solution aerated with 95%O_2 & 5%CO_2. Bronchi were maximally pre-contracted to 80mM potassium chloride (KCl) or 2mM acetylcholine (ACh) and the effect of a cumulative dose response to L-menthol (1-3000 µM) on bronchoconstriction was investigated. Results are expressed as mean±s.e.mean. Statistical analysis was ANOVA and two tailed paired t test.

In vivo, capsaicin, with air pretreatment caused an increase in Raw of 24.3±3.8 mmHg l min^{-1} from basal. L-menthol pretreatment significantly (P<0.01) reduced the capsaicin-induced increase in Raw to 10.9±2.8 mmHg.l.min^{-1}. Air inhalation had no effect upon the NKA-induced increase in Raw. However, L-menthol inhalation significantly (P<0.01) reduced NKA-induced increase in Raw from 12.6±3.22 to 7.38±2.34 mmHg l min^{-1}. *In vitro*, L-menthol caused a highly significant (P<0.001) dose dependent relaxation of KCl and ACh contractions with an IC_{50} of 40 µM and 110 µM respectively.

L-menthol in the Guinea pig attenuates both capsaicin- and NKA-induced bronchoconstriction *in vivo* and relaxes pre-constricted bronchi *in vitro*. It is possible that menthol may have a direct action on bronchial smooth muscle.

Laude, E. A., Morice, A.H., Grattan, T.J. (1994) Pulm. Pharmacol.7, 179-184.
Morice, A.H., Marshall, A.E., Higgins, K.S., *et al.* . (1994) Thorax. 49, 1024-1026

199P THE ANTITUSSIVE ACTION OF ORAL MENTHOL ON CITRIC ACID-INDUCED COUGH IN GUINEA-PIGS

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Menthol vapour has been shown to be effective against induced cough in both guinea-pig (Laude *et al*, 1995) and man (Marshall *et al.*, 1995) but its effective duration is relatively short (less than 30 min in guinea-pigs). By increasing the systemic reserve of menthol by oral administration it may be possible to prolong the therapeutic effects. This investigation was designed as two crossover studies balanced for carryover effects to establish an effective dose of oral menthol and to assess whether menthol may have an additive or synergistic anti-tussive action with oral dextromethorphan (DM).

Cough challenges were performed on conscious male Dunkin Hartley guinea-pigs (512±12 g) by exposure to ultrasonically nebulised solutions of either 0.9% saline or citric acid (300 or 500 mM) for 2-4 min in a perspex chamber (800ml) with a constant airflow (800 ml/min). Airflow was continuously monitored via a pneumotachograph. Cough was determined from changes in the airflow and observation of the animal. A minimum of 48 h was allowed between challenges. Animals were screened to establish the duration of exposure and concentration of tussive agent required to produce >10 coughs /10min for each animal. This dose was given 30 min post-treatment in Study 1 and 60 min in Study 2. Oral treatments in Study 1 (n=10) were menthol 30, 100, 300 mg/kg, 0.9% saline and vehicle (5% methyl cellulose). In Study 2 (n=10) treatments were menthol (300mg/kg), DM (40mg/kg), menthol (300mg/kg) + DM (40mg/kg) and vehicle (5% methyl cellulose). Results are presented as mean coughs/10 min ± s.e.mean. Statistical analysis were ANOVA two by two factorial and two tailed paired t test where applicable.

In Study 1, no significant difference was found in cough frequency between pretreatment with methyl cellulose, saline and 30 mg/kg menthol (15.2 ± 1.3, 13.8 ± 1.8, 14.5 ± 2.4 respectively). The reduction with 100 mg/kg (12.6±.7 *cf* 15.2 ± 1.3) was not significant but 300 mg/kg menthol produced a significant ($p<0.02$) 46% reduction ((8.2±2.2 *cf* 15.2±1.3). In Study 2, the 16% and 24% reductions in cough frequency 60 min post-treatment with 300 mg/kg menthol and 40 mg/kg DM were not significant (17.5±1.4, 15.8±1.9 *cf* 20.8±2.2 methyl cellulose). However, DM (40 mg/kg) + menthol (300 mg/kg) significantly ($p<0.002$) reduced cough frequency to 13.4±1.8, a 36% reduction.

In the guinea-pig, oral menthol was found to have an anti-tussive action of greater duration than that reported for vapour administration. When given in combination with oral DM, a more marked anti-tussive action was evident than with either agent alone although analysis was unable to demonstrate synergy.

Laude E.A., Grattan T.J., Morice A.H (1994) Pulm Pharmacol. 7,179-184.
Morice A.H., Marshall A.E., Higgins K.S. *et al* (1995) Thorax. 49,1024-1026

200P VENTILATORY ACTION OF THE PULMONARY VASODILATOR ANP (ATRIAL NATRIURETIC PEPTIDE)

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ANP, released from cardiac myocytes in response to hypervolaemia, is an effective vasodilator in certain vascular beds including the pulmonary. It improves pulmonary hypertension in chronic obstructive lung disease and maintains oxygenation by increasing ventilation (VE) (Rogers et al 1994; Andrivet et al 1994). An increased VE on *iv* infusion of ANP in both normoxic and chronically hypoxic rats was abolished by section of the carotid sinus nerve thus localising its action to the peripheral chemoreceptor the carotid body (Bee 1995). We have attempted to characterise the means whereby ANP acts on the carotid body by use of the specific ANP-A receptor antagonist HS 142-1 (Kyowa Hakko Kogyo Co Ltd, Japan) and the phosphodiesterase inhibitors milrinone (PDE III) and zaprinast (PDE V) to prevent the breakdown of the second messengers cAMP and cGMP respectively.

Seventeen normoxic male rats (BW 281g±4 g) were anaesthetised with thiobarbiturate (Inactin, BYK, 100mg/Kg, *ip*). The trachea and a femoral vein and artery were cannulated. VE was measured using body plethysmography (@ 30°C ambient temperature) and blood gases and blood pressure were monitored throughout. 4-5μg iv ANP was given as a 1 min. infusion before and after 5 min *iv* blockade. Data are presented as mean±s.e.mean and analysed by paired or unpaired non parametric tests (Mann-Whitney-U) where appropriate.

As reported previously ANP increased VE significantly from 112.5±8.1 ml/min/100g body weight to 121.7±7.8 ($p<0.05$; a rise of 8.6±1.9%). After receptor blockade with HS 142-1 (8mg/Kg) the increase in VE caused by ANP was significantly attenuated (in 6 rats VE changed from 105.9±6.6 to 108.4±7.1 ml/min/100g; a rise of 2.4±2.2 %; $P<0.05$). This is likely to be underestimated as repeated doses of ANP potentiate the VE effect. At the concentrations used milrinone (50μg/Kg) and zaprinast (4μg/Kg) gave variable results and did not significantly affect the ANP response.

The increase in ventilation caused by ANP was blocked by the specific receptor antagonist HS-142-1 and so is apparently mediated by an ANP-A receptor similar to those found in the kidney and vasculature.

Andrivet, S., Chabrier, P., Defouilley, C. *et al*, (1994) Chest, 106:118-124
Bee, D. (1995) European Respiratory Journal, 8(19s):61s
Rogers, T., Sheedy, W., Waterhouse, J. *et al*, (1994) Thorax, 49:233-239;

201P THE EFFECT OF THE SELECTIVE PHOSPHODIESTERASE 4 INHIBITOR, Ro 20-1724, ON ANTIGEN-INDUCED IL-5 RELEASE IN GUINEA-PIG AIRWAYS

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We have previously demonstrated an airway eosinophilia and hyperreactivity to inhaled U46619 24h post antigen challenge in sensitized guinea-pigs (Danahay & Broadley, 1996), which were both attenuated by the selective inhibitor of phosphodiesterase (PDE) type 4, Ro 20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) (Beavo & Reifsnyder, 1990). Airway hyperreactivity and eosinophilia in guinea-pigs may also be induced by interleukin (IL)-5 (Van Oosterhout *et al*, 1993). Inhibition of the synthesis or release of IL-5 may be a possible anti-inflammatory mechanism of PDE 4 inhibitors. The aim of this study was to examine any effect of Ro 20-1724 on IL-5 levels in the airways of OvA challenged guinea-pigs.

Male Dunkin-Hartley guinea-pigs (200-250g) were sensitized by an i.p. injection containing ovalbumin (OvA, 10μg) and aluminium hydroxide (100mg), and were used 14-21 days later. Animals were exposed to aerosols of saline or OvA (0.5%) for 10 min. 30 min. after mepyramine (30mgkg^{-1}, i.p.) and either Ro 20-1724 (1 & 3mgkg^{-1}, i.p.) or DMSO (50% in saline, i.p.). A second identical dose of Ro 20-1724 or vehicle was given at 6h post challenge. At regular intervals post challenge (0,1,3,6,12,24h) animals were overdosed with pentobarbitone (100mgkg^{-1} ip.) and the lungs were lavaged. Total and differential cell counts were performed and the lavage supernatant was assayed for IL-5. A scintillation proximity assay (SPA) was used to measure IL-5, using an SPA bead coated with TRFK-5, the rat antibody directed against murine IL-5.

Challenge	Treatment	Eosinophils (10^5ml^{-1}) 24h	$[IL5]_{BALF}$ (pM) 3h	24h
Saline	vehicle	1.2 ±0.2	107 ±20	124 ±50
OvA	vehicle	13.0 ±2.4[a]	291 ±44[a]	468 ±94[a]
OvA	Ro20-1724 (1mgml^{-1})	7.8 ±2.8	124 ±60[b]	74 ±23[b]
OvA	Ro20-1724 (3mgml^{-1})	6.7 ±1.5[a,b]	140 ±20[b]	57 ±27[b]

Table 1. Eosinophil and IL-5 levels in the bronchoalveolar lavage fluid of aerosol challenged guinea-pigs ([a] indicates significant difference from saline group, [b] indicates significant difference from OvA group, using ANOVA with *Neuman Keuls* ad hoc t-test, $P<0.05$, n=3-9)

24h post OvA challenge, there was a marked airway eosinophilia which was significantly attenuated by Ro 20-1724 (Table 1). IL-5 levels initially peaked at 3h returning towards baseline levels by 6h. There was then a second rise in IL-5 levels which was significantly elevated above baseline levels by 24h. (Fig. 1). Both the 3h and 24h peak increases were significantly attenuated by Ro 20-1724 (Table 1).

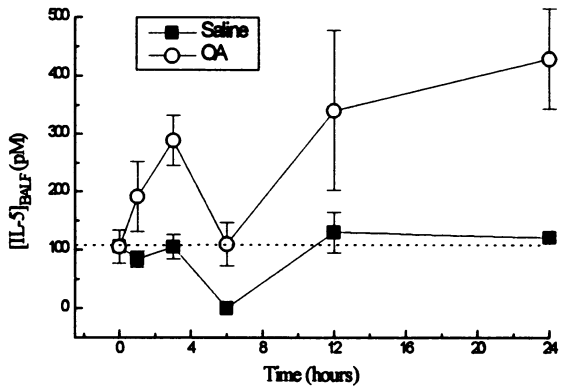

Figure 1. Time course for the appearance of IL-5 in bronchoalveolar fluid of saline and OvA challenged guinea-pigs

These results suggest that Ro 20-1724, attenuates an airway eosinophilia possibly by inhibiting IL-5 synthesis and/or release.

Beavo & Reifsnyder (1990) *TiPS* 11, 150-155
Danahay & Broadley (1996) *Br. J. Pharmacol.* (in press).
Van Oosterhout et al. (1993) *Am. Rev. Resp. Dis.* 147, 548-552

202P MEN 11149, A POTENT ANTAGONIST OF THE TACHYKININ NK_1 RECEPTOR

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The potential involvement of substance P (SP) in a number of physiological and pathological processes has fostered the research for tachykinin NK_1 receptor antagonists. We report here the pharmacological profile of a novel potent and selective antagonist of tachykinin NK_1 receptor, MEN 11149 (2-(2-naphthyl)-1-N-{(1R,2S)-2-N-[1(H)indol-3-yl-carbonyl]aminocyclohexanecarbonyl}-1-[N'-methyl-N'-(4-methylphenylacetyl)]diaminoethane).

MEN 11149 inhibited the binding of [^{3}H]SP (0.3nM) to IM9 cells (NK_1 sites) with an inhibition constant (K_i) of 2.8±0.1 nM (n=6). Mathematical analysis provided by the program LIGAND (Munson & Rodbard, 1980) of four families of homologous competition curves for SP in the presence or in the absence of increasing concentrations of MEN 11149 (1-30 nM), indicated an insurmountable interaction. The antagonist, in fact, affected both the dissociation constant (K_d) and the number of binding sites (B_{max}) of the ligand.

MEN 11149 was selective for the NK_1 receptors, since it did not compete for [^{3}H][βAla^8]neurokinin A(4-10) binding to hamster urinary bladder membranes (NK_2 sites) or for [^{3}H]senktide binding to guinea-pig cerebral cortex (NK_3 sites) (K_i=1.6±0.2 and >10 μM, respectively) (n=3). In addition, it did not compete (up to 1 μM) for the binding of several ligands (n=28) to other receptors or ion channels.

In the isolated guinea-pig ileum, MEN 11149 (0.1-100 nM) produced non-parallel shifts of the cumulative concentration-response curves induced by SP-methyl ester (SPOMe), with a progressive inhibition of maximal contraction (n=14). Mathematical analysis obtained with program ALLFIT (DeLean et al., 1978) of these curves futher indicated an insurmountable behaviour, since MEN 11149 affected both EC_{50} and E_{max} of SPOMe ($P<0.001$). An apparent pK_B estimate of 9.55±0.1 was calculated according to Kenakin (1984).

MEN 11149 did not significantly interact with L-type Ca^{++} channel, since the compound: a) did not affect the binding of [^{3}H]desmethoxyverapamil to rat cerebral cortex (K_i=3.1±0.3 μM; n=3); b) did not induce smooth muscle relaxation, up to 10 μM, in rat portal vein preparation (n=4) and c) did not induce, up to 3 μmol/Kg i.v., any hypotensive effect in anaesthetized rat (n=3).

The in vivo activity of MEN 11149 was investigated in the anaesthetized male Dunkin-Hartley guinea-pigs (300-400 g) in two models. MEN 11149, administered by intravenous route, dose-dependently antagonized [Sar^9,Met(O$_2$)^{11}]SP (1 nmol/kg)-induced bronchoconstriction (ED_{50}=83±31 nmol/kg, n=20). The duration of the effect exceeded 3 hours. In addition, the compound dose-dependently inhibited the [Sar^9,Met(O$_2$)^{11}]SP-induced plasma protein extravasation in guinea-pig bronchi either when administered intravenously (ED_{50}=0.2±0.02 μmol/kg, n=25) or orally (ED_{50}=0.97±0.2 μmol/Kg, n=14).

These results demonstrate that MEN 11149 is a potent, highly selective and orally effective insurmountable antagonist of tachykinin NK_1 receptors.

DeLean, A., Munson, P.J. & Rodbard, D. (1978) Am. J. Physiol. 235, E97.
Kenakin, T.P. (1984) Pharmacol. Rev. 36, 165.
Munson, P.J. & Rodbard, D. (1980) J. Pharmacol. Meth. 14, 213.

203P EOSINOPHIL PEROXIDASE: A RELIABLE MARKER OF ANTIGEN-INDUCED PULMONARY EOSINOPHILIA IN THE GUINEA-PIG

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Previous studies have demonstrated the importance of pulmonary eosinophil infiltration in the aetiology of human bronchial asthma (Walker et al., 1991). Contributing to this knowledge has been information obtained from animal models of pulmonary eosinophilia (Gulbenkian et al., 1992). The aim of this study was to investigate whether measurement of eosinophil peroxidase (EPO) activity could be used as an alternative to cell counting for the quantification of ovalbumin (OA) - induced pulmonary eosinophil infiltration in conscious sensitized guinea-pigs.

Male guinea-pigs (250-300g) were sensitized by injection to 50mg ovalbumin s.c. and i.p. and used in the challenge procedure from day 25 onwards. Animals were pretreated with pyrilamine maleate (10mg.kg^{-1} i.p.) 55 minutes (min) prior to a 5 min challenge with an aerosol of either saline vehicle (0.9%) or OA (0.025, 0.05, 0.1, 0.25 or 2.0% (w/v)). Animals were killed 24 hours post-challenge, BAL performed and the lungs removed. EPO activity was determined in BAL cell lysate and lung tissue homogenate samples (Yeadon et al., 1993) at pH 6.8 by a colorimetric method at 340nm (Bos et al., 1981). Myeloperoxidase (MPO) was extracted and assayed at pH 4.6 using the same colorimetric method. The effect of 3-amino 1,2,4-triazole (3-AT), a selective inhibitor of EPO (Cramer et al., 1984), was also investigated. 3-AT was used to verify that the peroxidase activity at pH 6.8 found in lung tissue homogenate samples from animals exposed to 2% OA was EPO, and not MPO (Cramer et al., 1984).

A significant BAL eosinophilia was seen after 0.1, 0.25, and 2.0% OA inhalation, with a significant increase in BAL EPO activity ([dA/min]) after 0.05, 0.1, 0.25 and 2% OA challenge. There was also a significant BAL neutrophilia after 0.1 and 2.0% OA inhalation (Table 1). Correlation analysis between total BAL eosinophil number and BAL EPO activity resulted in a significant correlation after saline and 0.025, 0.05, and 0.1% OA inhalation only (r=0.94, 0.92, 0.97, 0.94 respectively ; Pearson correlation coefficient ; $p \leq 0.001$; n=7-10). The addition of 3-AT(100μM) to the assay of lung tissue homogenate EPO samples at pH 6.8 obtained from animals exposed to 2% OA, resulted in a significant inhibition of activity per milligram wet weight tissue ([dA/min]/wt wght tissue (mg)) (97% inhibition ; $p \leq 0.001$) (Table 1). Addition of 3-AT to the pH modified (pH 4.6) assay mixture from lung tissue homogenate samples taken from the same animals resulted in no significant change in MPO activity. The inhibition of enzymatic activity at pH 6.8 by 3-AT confirms the peroxidase activity to be that of EPO. Since 3-AT was unable to inhibit the enzyme activity extracted and assayed at pH 4.6, this peroxidase activity may represent MPO, indicating the presence of tissue neutrophilia. In conclusion, EPO can be used as a sensitive and reliable marker of eosinophilia in this model when a correlation exists between BAL EPO and BAL eosinophil number.

Table 1:
The effect of OA exposure on pulmonary cell numbers and peroxidase activity.

Aerosol Exposure	Total Neutrophils x10^5	Total Eosinophils x10^5	BAL EPO [dA/min]	Tissue EPO [dA/min] / wt wgt tissue(mg) pH 6.8	Tissue MPO [dA/min] / wt wgt tissue(mg) pH 4.6
VEHICLE	0.4±0.3	5.5±0.3	101.6±28.8	0.05±0.07	nt
OA(0.025%)	0.4 ±0.1	13.4±7.5	198.7±138.7	0.02±0.6	nt
OA(0.05%)	0.5 ±0.4	15.6±13.8	270.7±145.3*	0.9 ±0.6*	nt
OA(0.1%)	0.8 ±0.6*	24.9±10.3**	284.7±138.0**	1.0±0.7***	nt
OA(0.25%)	1.1±0.9	24.9±12.9***	469.3±26.7***	1.2±0.2***	nt
VEHICLE	0.8 ±0.6	6.5 ±4.1	159.8±51.3	0.08±0.01 (0.03±0.01)	0.15±0.03 (0.15±0.04)
OA(2%)	17.2±12.0**	59.4±32.9***	1263.5±478.5***	1.2±0.2*** (0.04±0.02)###	1.1±0.2*** (1.0±0.3)

Data represents median ± semi-interquartile range, n=7-10, *$p \leq 0.05$, **$p \leq 0.01$,***$p \leq 0.001$, significance between vehicle and OA groups. Values in brackets represent peroxidase activities in the presence of 100μM 3-AT ; ### $p \leq 0.001$ significance between OA 2% without 3-AT and OA 2% with 3-AT ; Mann-Whitney Statistics. nt = not tested.

Bos, J. et al. (1981) Infec.Immunol. 32, 427-431.
Cramer, R. et al. (1984) J.Immunol.Methods. 70, 119-125.
Gulbenkian, A.R. et al. (1990) Am. Rev. Respir. Dis. 142,680-685.
Walker, C. et al. (1991) J. Allergy. Clin. Immunol. 88, 935-942.
Yeadon, M. et al. (1993) Agents Actions 38, 8-18.

204P CHARACTERISATION OF FUNCTIONAL MUSCARINIC RECEPTORS IN HUMAN SUBMANDIBULAR SALIVARY GLAND

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The parasympathetic nervous system plays a major role in the control of salivary secretion and dry mouth is a common clinically-observed side effect with muscarinic antagonists (Ekstrom, 1989). Five human muscarinic receptors have been cloned and four physiological receptor subtypes have been identified in functional studies (Caulfield, 1993). Currently, little is known regarding the nature of the muscarinic receptor subtypes which regulate salivary secretion in man. However, studies in rat and guinea-pig sub-mandibular salivary glands show that responses to cholinergic agonists in these tissues are mediated via M_3 receptors (Laniyonu *et al.*, 1989; Newgreen *et al.*, 1995). The aim of the present study was to characterise the functional muscarinic receptors in human sub-mandibular salivary gland.

Segments of human sub-mandibular salivary gland were obtained from patients undergoing surgical removal of the glands (Kent and Canterbury Hospital). All tissue used appeared normal to the eye and was transferred immediately to Krebs-Henseleit solution on removal. Within 1 h., the tissue was finely minced and incubated in Krebs-Henseleit solution (37°C, gassed with 95%O_2/5%CO_2) containing ^{86}RbCl (10 μCiml^{-1}) for 90 min. The tissue was then aliquoted into chambers of a Brandel superfusion apparatus. Following a 10 min. wash, muscarinic antagonists or vehicle were superfused in Krebs-Henseleit solution (37°C, gassed with 95%O_2/5%CO_2) for 20 min. prior to a carbachol (10^{-5}M) challenge. The potencies of muscarinic antagonists to inhibit the ensuing carbachol-induced increase in the ^{86}Rb efflux rate constant (Durbin & Jenkinson, 1961) were expressed as the log of the concentration required to produce a 50% inhibition (pIC_{50}). The data are shown in Table 1.

Table 1: pIC_{50} values for muscarinic antagonists on human submandibular salivary gland (mean ± s.e. mean, n = 4).

Compound (selectivity)	Antagonist potency - pIC_{50}
Atropine (none)	8.0 ± 0.25
Pirenzepine (M_1/M_4)	5.83 ± 0.27
Methoctramine (M_2/M_4)	5.2 ± 0.14
4-DAMP (M_3/M_1)	7.79 ± 0.14
Himbacine (M_4/M_2)	5.63 ± 0.16

The pIC_{50} values of the standard muscarinic antagonists used in this study and their rank order of potency relative to atropine (Caulfield, 1993), is consistent with functional muscarinic responses in human submandibular salivary gland being mediated via the M_3 muscarinic receptor subtype.

Caulfield, M. P. (1993). *Pharmacol. Ther.*, 58, 319-379
Durbin, R. P. & Jenkinson, D. H. (1961). *J. Physiol.*, 157, 74-89
Ekstrom, J. (1989). *Proc. Finn. Dent. Soc.*, 85, 323-331
Laniyonu, A., Sliwinski-Lis, E., & Fleming, N. (1990). *Eur. J. Pharmacol.* 188, 171-174
Newgreen, D.T., Leahy, D.J. & Naylor, A.M. (1995). *Br. J. Pharmacol.*, 116, 146P

205P THE EFFECTS OF DARIFENACIN AND OXYBUTYNIN ON BLADDER FUNCTION AND SALIVATION IN THE CONSCIOUS RAT

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Darifenacin is a novel and selective muscarinic M_3 antagonist (Wallis *et al.* 1995), which shows some selectivity for bladder versus salivary gland *in vitro* (Newgreen & Naylor, 1996). Darifenacin is currently undergoing clinical evaluation for the treatment of urinary urge incontinence (UUI). In this study, the relative selectivities of darifenacin and oxybutynin (a current antimuscarinic therapy for UUI, Thüroff *et al.*, 1991) were compared in conscious rat cystometry and salivation models.

For cystometry studies, male rats (SD, CD,Charles River, 250-350 g) were anaesthetised (fentanyl/medetomidine *ip*, which was reversed post-surgery using atipamezole/nalbuphine *ip*) and the bladder exposed *via* a mid-line incision and cannulated. A jugular vein was also cannulated. The cannulae were exteriorised *via* a sub-cutaneous tunnel to the retroscapular area and attached to a sealable injection port. Rats were left to recover for at least 72h prior to cystometrical investigation. For the study each rat was placed in a Bollman's cage and the bladder infused with 0.9% saline, at room temperature, *via* the bladder cannula, at a rate of 200 µl.minute^{-1}. Pressure recordings were made *via* a transducer in series with the infusion line. At micturition, the time to micturition (micturition interval, MI), micturition volume (MV) and peak micturition pressure (MP) were recorded. When a series of three consistent micturitions had been recorded, the study compound or vehicle was injected *via* the jugular vein cannula. MV, MI and MP were measured for 1h post dose. For each parameter the maximum effect was expressed as a percentage of the mean pre-dose value.

For salivation studies male rats (SD, CD,Charles River, 250-350 g) were dosed *via* the tail vein with test compound or vehicle. Five min post dose, methacholine (3 mg.kg^{-1}) was administered sub-cutaneously. After a further five min the methacholine-evoked salivation was scored subjectively by an operator blinded to the dose received by each rat.

Darifenacin and oxybutynin (0.1-3.0 mg.kg^{-1} *iv*) caused dose related decreases in MI, MV, MP and salivation consistent with the actions of a muscarinic antagonsist. ED_{50}s are given in Table 1.

Table 1. Potencies on micturition parameters (MI, MV & MP) and salivation (ED_{50} ± s.e. mean, mg.kg^{-1}, *iv*, n=3-6) in conscious rats for darifenacin and oxybutynin.

	MI	MV	MP	Salivation
Darifenacin	0.37(±0.12)	0.23(±0.07)	0.089(±0.06)	0.48(±0.05)
Oxybutynin	1.37(±0.27)	0.85(±0.15)	0.83(±0.22)	0.51(±0.08)

Darifenacin is significantly ($p<0.05$, Student's t-test) more potent than oxybutynin on all micturition parameters and equipotent on salivation. Darifenacin has up to an 8-fold selectivity advantage over oxybutynin for bladder over salivation. The profile of darifenacin in this study is indicative of symptomatic benefit in the treatment of UUI with a lower incidence of dry-mouth compared to oxybutynin at therapeutic doses.

Newgreen, D.T. & Naylor, A.M., (1996), *Br. J. Pharmacol*, 117, 107P

Thüroff, J.W., Bunke, B., Ebner, A. *et al.*, (1991), *J. Urol.*, 145, 813-817

Wallis, R.M., Burges, R.A., Cross, P.E. *et al.*, (1995), *Pharmacol. Res.*, 315, 54

206P EFFECTS OF 5-HT AND THE TETRAPEPTIDE FMRFamide UPON THE CONTRACTION OF THE PHARYNGEAL MUSCLE OF *HELIX ASPERSA*

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The pharyngeal muscle of *H. aspersa* runs from the columella part of the shell to the buccal mass and is innervated by a nerve from the suboesophageal ganglia (Ramsay 1940, Lloyd 1980).

We examined muscle sections immuno-cytochemically to investigate the localization of 5-HT and FMRFamide. The effects of 5-HT and FMRFamide on the contractile properties of the muscle were studied using an electrically stimulated pharangeal muscle preparation of *H. aspersa*. Stimulus 70V, 5ms duration, at 3min intervals).

Immuno-cytochemistry revealed a dense network of varicose fibres of extrinsic origin for both 5-HT-like and FMRFamide-like substances. However the pattern of distribution was found to be different for 5-HT and FMRFa-immunoreactive elements, suggesting different localization of the two signal molecules.

Application of low concentrations of 5-HT had no significant effect upon the amplitude of the twitch but significantly increased the rate of muscle relaxation in a dose dependent way, measured by the time to half relaxation from twitch peak (mean 0.65 sec, ± 0.04 s.e.mean, n=9). Threshold for the effect of 5-HT on the half relaxation time was less than 10^{-9}M, with maximal change of 36.8 ± 3.7% s.e.mean, n=9, $P<0.01$ (Students t-test) occurring at 10^{-7}M. At concentrations above 10^{-7}M, 5-HT often showed a biphasic effect on the amplitude of the twitch response. An initial reduction in twitch amplitude was accompanied by a reduced time to half relaxation. After three minutes the twitch amplitude was enhanced above control but the half relaxation time remained elevated, this effect lasted for over nine minutes.

At a concentration of 10^{-4}M, FMRFamide significantly reduced the time to half relaxation by 32.3 ± 5.1% s.e.mean, n=9, $P < 0.01$. The effect of FMFRamide on the half relaxation was not sustained and failed to produce a biphasic change in the twitch tension at concentrations as high 10^{-4}M.

These results indicate both 5-HT and FMRFamide may have a role in controlling *H. aspersa* pharyngeal muscle contractions.

References:

J. A. Ramsay (1940) *J. Exp. Biol.* **17:** 96-115

P.E. Lloyd (1980) *J. Comp. Physiol.* **139**: 341-347

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207P EVIDENCE FOR A FUNCTIONAL β_3-ADRENOCEPTOR IN HUMAN ISOLATED TAENIA COLI

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β_3-adrenoceptors have been shown to be present in a number of isolated gastrointestinal preparations in animals (see Arch & Kaumann, 1993) and man (de Ponti et al, 1996). The present study demonstrates the functional presence of similar β_3-adrenoceptors in the smooth muscle of the human isolated taenia coli.

Macroscopically normal segments of human sigmoid colon were obtained from patients undergoing colonic resection. Strips of taenia coli smooth muscle were suspended under a resting tension of 2 g in organ baths containing Krebs' medium at 37°C, aerated with 95 %/ 5% O_2/CO_2. The Krebs medium also contained cocaine, normetanephrine, EDTA, l-ascorbic acid (all at 30μM) and phentolamine (0.1μM). Tissues were equilibrated for 2 hours and allowed to either develop spontaneous tone, or if no tone developed, pre-contracted by the addition of KCl (20 mM) to the organ baths.

Isoprenaline (ISO), noradrenaline (NA) and fenoterol (FEN) all relaxed the pre-contracted taenia coli with pD_2's of 7.2 ± 0.11 (n=21), 6.89 ± 0.15 (n=9), 6.21 ± 0.10 (n=10) respectively. This order of potency suggests a β_1-adrenoceptor-like response. The β_3-adrenoceptor agonist BRL 37344 (0.01-100 μM, n=4) failed to relax the human isolated taenia coli. A subsequent addition of ISO (10 μM, n=4) after the highest concentration of BRL 37344 produced its characteristic relaxatory response. In contrast, CGP 12177A (CGP), a β_1/β_2-adrenoceptor antagonist with partial agonist properties at β_3-adrenoceptors in the rat (Mohell & Dicker, 1989) and human adipocytes (Lonnqvist et al. , 1993), induced concentration-dependent relaxations of this tissue with a pD_2 value of 6.19 ± 0.20 (n=5), and was a partial agonist with an intrinsic activity of 0.41 ± 0.07 (n=5). Further additions of ISO (10 & 100 μM, n=5) after the highest concentration of CGP (100 μM) failed to induce any additional relaxations. ISO-induced relaxations were antagonised by the selective β_1-adrenoceptor antagonist CGP 20712A (30nM; estimated pA_2 of 8.9, n=5), while the selective β_2-adrenoceptor antagonist ICI 118,551 (30nM, n=5) produced only a small shift of the ISO concentration-response curve. The non-selective β - adrenoceptor antagonist nadolol (1, 10 & 100 μM) antagonised ISO-induced relaxations in a competitive manner and a resultant pA_2 = 6.74 (n=20) with a Schild gradient of 0.95 ($p < 0.05$) but failed to antagonise CGP-induced relaxations (n=5).

The present data suggests that the smooth muscle of the human isolated taenia coli contains a population of both inhibitory β_1- and β_3-adrenoceptors. The results obtained with the antagonist nadolol suggest that ISO and CGP may bind in different ways or to different conformations of the human β_3-adrenoceptor.

Arch, J.R.S. and Kaumann, A.J. (1993), *Med. Res. Rev.* **13** 663-729
de Ponti, F., Gibelli, G., Croci, T. et al., *Br. J. Pharmacol.* (1996) 117(7) 1374-1376
Mohell, N. and Dicker, A. (1989), *Biochem J.* **261** 401-405
Lonnqvist, F., Krief, S., Strosberg, D., et al., (1993), *Br. J. Pharmacol.* **110** 929-936

208P THE MECHANISM OF COOLING-INDUCED CONTRACTION OF OVINE TRACHEALIS MUSCLE

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Cooling ovine tracheal and bronchiolar preparations rapidly induces contractile responses inversely proportional to temperature, which are not dependent on the presence of epithelium. These responses do not appear to involve release of mediators or a neurogenic process, but extracellular calcium appears to be necessary (Mustafa et al., 1996). This study aimed to determine more precisely the roles of extra- and intracellular Ca^{2+} in cooling-induced contraction (CIC) of ovine trachealis muscle.

Tracheal strips were prepared from Merino sheep as previously described (Thulesius & Mustafa, 1994). They were suspended in Krebs' solution at 37°C, pre-tensioned at 2g, gassed (95%O_2, 5%CO_2) and isometric tension recorded. Responses were examined at 5°C intervals over the range 30°C-5°C under different conditions of Ca^{2+} availability and expressed as mg/mg tissue weight (mean±s.e.mean)

In previous experiments prolonged incubation (45-90 min) in EGTA-containing Krebs' solution significantly suppressed CIC and it was concluded that extracellular Ca^{2+} is necessary. However, in the present experiments exposure of tracheal strips to this medium for only 5 min had no effect on CIC but reduced K^+-induced contractions by 75±4% (n=5; P<0.05).

Cooling to 20°C also inhibited the contractile responses to KCl (10-160mM), causing a decrease in the E_{max} from 393.4±27.0 to 192.5±35.0 mg/mg (n=5; P<0.05) and an insignificant shift in EC_{50} from 2.85±0.5 to 2.25±0.12 $x10^{-2}$ M Incubation with the calcium channel antagonists, nifedipine (0.1μM) or verapamil (1,10μM) or with $MnCl_2$ (100μM) raised the basal tone to 9.66±2.7, 22.19±12.0, 85.46±43, 10.8±6.0 mg/mg respectively (n=5; P<0.05). These drugs also potentiated the effects of CIC; e.g. at 20°C control and post-drug tensions were 24.2±7.4 and 137.0±34.0; 6.0±2.8 and 86.0±37.0; 6.0±2.8 and 324.0±86.0; 15.5±11.6 and 90.5±40.0 mg/mg respectively (n=5; P<0.05).

These results suggest that cooling suppressed Ca^{2+} influx and may explain why calcium channel antagonists and Mn^{2+} failed to decrease CIC. It is possible that the prevention of extracellular Ca^{2+} influx caused a compensatory rise in the concentration of free intracellular Ca^{2+} (Ohta *et al*, 1995) which would explain the potentiation of CIC seen with these agents.

Addition of caffeine (100 μM) after 60 min incubation in calcium-free Krebs solution plus EGTA (1mM) induced a contraction of the trachea but a second addition failed to elicit an effect. Responses to cooling in calcium-free medium were virtually abolished after caffeine pre-treatment. Furthermore, cooling significantly augmented contractions of the trachea induced by carbachol with E_{max} of 290±8.5 mg/mg at 37°C increasing to 462±73 at 20°C (n=5; P<0.05).

These results suggest that extracellular Ca^{2+} entry via voltage-operated channels is not involved in CIC. The responses to cooling appear to involve intracellular calcium release from a caffeine-sensitive pool. The potentiation of CCh responses suggests that cooling may also inhibit the re-uptake of calcium into the intracellular storage sites.

Mustafa, S., Pilcher, C.W.T. & Williams, K.I. (1996) Br. J. Pharmacol. 118, 147P.
Ohta, T., Kawai, K., Ito, S. & Nakazato, Y. (1995) Br. J. Pharmacol., 114, 1165-1170.
Thulesius, O. & Mustafa, S. (1994) Clinical Physiol. 14, 135-143.

Supported by Kuwait University grant no.MR 029

209P CONFORMATIONAL RESTRAINTS TO FIND BIOLOGICALLY RELEVANT STRUCTURES OF $CGRP_{8-37}$ IN RAT PROSTATIC VAS DEFERENS, PULMONARY ARTERY AND INTERNAL ANAL SPHINCTER

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The antagonist $CGRP_{8-37}$ has been used to characterize responses to CGRP but its important structural features have not been fully identified. Previous work demonstrated that substitution of proline at position 16 abolished antagonism, presumably by disrupting the biologically active conformation (Wisskirchen *et al.*, 1994). However, this substitution was accepted in the putative bend region at position 19. Present experiments have used bend inducing residues and a bicyclic template (BTD; Nagai & Sato, 1985), to confirm two putative bend regions and their exact position within $CGRP_{8-37}$ by relating the effect of substitution to a functional assay. In addition, the importance of charge and side chain in the N-terminal region of $CGRP_{8-37}$ has been investigated.

Tissues were removed from male Sprague-Dawley rats (325-450g) and set up in Krebs solution at 37°C (95/5% O_2/CO_2). The prostatic half of the vas was field stimulated (60v, 1ms, 0.2Hz) to contract, pulmonary artery rings were contracted using phenylephrine $3x10^{-8}$M while the internal anal sphincter increased in tone spontaneously. The isometric tone in these preparations was reduced by the cumulative addition of hαCGRP. Potential antagonists were equilibrated with tissues for at least 20 minutes before repeating the agonist curve. $CGRP_{8-37}$ and its derivatives were synthesised on an ABI 430 peptide synthesiser utilising FastMoc chemistry, cleaved and de-protected by conventional protocols, purified to homogeneity by RP-HPLC and fully characterized by high field NMR and mass spectrometry.

Human αCGRP had a pEC_{50} of 8.0±0.1, 8.5±0.1 and 7.5±0.1 in the vas, pulmonary artery and internal anal sphincter respectively. The pA_2 for $CGRP_{8-37}$ was 6.0 (Schild plot slope 0.9±0.1) while the pK_Bs for $CGRP_{8-37}BTD^{19,20}$ and $CGRP_{8-37}BTD^{33,34}$ were 6.0±0.2 and 6.1±0.1 respectively in the vas deferens. As found previously in the vas, in the pulmonary artery $CGRP_{8-37}Pro^{19}$, and $CGRP_{8-37}Ala^{16}$ were antagonists while $CGRP_{8-37}Pro^{16}$ was inactive at 10^{-5}M. In addition $CGRP_{8-37}BTD^{19,20}$ was an antagonist in the pulmonary artery (pK_B 6.1±0.1).

Substitution of valine at the N-terminus by either proline, glycine, *des*-NH_2Val or N-acetyl valine gave peptides with no antagonist activity at 10^{-5}M in the vas deferens. However, $CGRP_{8-37}Pro^{8}$, $CGRP_{8-37}Gly^{8}$ and $CGRP_{8-37}$ *des*-NH_2Val^{8} were all antagonists in the internal anal sphincter (pK_B 5.5±0.1, 5.8±0.1 and 5.8±0.1 respectively compared with $CGRP_{8-37}$ 5.7±0.3).

For the first time a correlation has been made between inducible structure and function for $CGRP_{8-37}$. The results show that an enforced β-bend in positions 19,20 and 33,34 is consistent with the biologically active conformation of $CGRP_{8-37}$. The difference in activity of the 8-substituted compounds between the vas and internal anal sphincter is consistent with the hypothesis that at least two subtypes of CGRP receptor exist in the rat that are not distinguished by $CGRP_{8-37}$.

Nagai, U. & Sato, K. (1985), *Tetrahedron Lett.*, **26**, 647-650.
Wisskirchen *et al.*, (1994), *Br. J. Pharmacol.*,**112**, 239P.

210P CHARACTERIZATION OF THE α_1-MEDIATED RESPONSES OF ISOLATED RABBIT CORPUS CAVERNOSUM

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Previous studies have established that α_1 adrenoceptors play an important role in regulating the tone of corpus cavernosal smooth muscle (Andersson, 1993). In this series of experiments we have determined the pharmacological properties of functional α_1 mediated responses of isolated rabbit corpus cavernosum.

Strips of corpus cavernosum (1 x 2 x 5 mm) from New Zealand White rabbits (2.5-3.5 kg) were mounted in 15 ml organ baths under a resting tension of 1 g in gassed modified Krebs Ringer bicarbonate at 37 °C containing propranolol (1 μM) and yohimbine (0.5 μM). Cocaine (10 μM) and corticosterone (10 μM) were also added for experiments using noradrenaline (NA).

The α_{1A} selective agonists A-61603 and PNO-49B (Knepper et al., 1995; Muramatsu et al., 1995) caused concentration-dependant contractions of rabbit corpus cavernosum. Mean pD_2 values (n=3) for agonists tested were in the following rank order: A-61603 (8.11) > oxymetazoline (7.08) > PNO-49B (5.93) > methoxamine (5.38) > phenylephrine. (5.25). All were full agonists, except oxymetazoline which behaved as a partial agonist (intrinsic activity, 0.73).

Concentration response curves to NA (control EC_{50} = 2.28 ± 0.04 μM) were relatively unaffected by treatment with chloroethylclonidine (CEC; 100μM for 30 mins, 60 min washout; EC_{50} = 4.22 ± 0.01μM, n=4). (Data are mean ± s.e.m.).

Prazosin caused dextral, surmountable shifts in NA concentration response curves, resulting in a pA_2 of 8.04 (Schild slope = 0.99) suggesting competitive antagonism. WB-4101 and 5-methyl-urapidil were also potent antagonists on this tissue but displayed low schild slopes. The potent and selective α_{1A} antagonist, RS-17053 (Ford et al., 1996), was a weak antagonist of NA mediated responses (Table 1).

Table 1. Antagonist affinity estimates (pA_2) against noradrenaline mediated contractions of rabbit corpus cavernosum (n=4).

COMPOUND	pA_2	(slope, 95% CL)
Prazosin	8.04	(0.99, 0.8-1.2)
WB-4101	8.47	(0.72, 0.6-0.9)
5-methyl-urapidil	8.35	(0.66, 0.4-0.9)
RS-17053*	6.01	
BMY-7378	5.89	(0.77, 0.6-1.0)

*Estimated from a single concentration of RS-17053 (3μM) due to insurmountable antagonism at >3μM.

The lack of effect of CEC on responses to NA, together with the rank order of agonist potency, suggests that α_{1A} adrenoceptors mediate contractions of rabbit corpus cavernosum and contrasts the proposal that α_{1B} adrenoceptors predominate (Chess-Williams *et al.*, 1995). However, in comparison to their profile at α_{1A} adrenoceptors (Kenny et al., 1995), the relatively low functional potency for antagonists exhibited in functional studies suggest that contractions of rabbit corpus are mediated by the putative α_{1L} adrenoceptor (Ford *et al.*, 1996). Data from the present study suggests that this may not be a homogeneous population.

Andersson, K.-E. (1993). *Pharmacol. Rev.*, 45, 253-308.
Chess-Williams, R. et al. (1995). *Br. J. Pharmacol.*, 114, 421p.
Ford, A.P.D.W. et al. (1996). *Mol. Pharmacol.*, 49, 209-215.
Kenny, B.A. et al. (1995). *Br. J. Pharmacol.*, 115, 981-986.
Knepper, S.M., et al. (1995). *J. Pharmacol. Exp. Ther.*, 274, 97-103.
Muramatsu, I. et al. (1995). *Naunyn-Schmied. Arch. Pharmacol.*, 351, 2-9.

211P PHARMACOLOGICAL ANALYSIS OF 5-HT-RECEPTOR-MEDIATED VASOCONSTRICTION OF HUMAN MIDDLE MENINGEAL ARTERIES: DETERMINING THE CONTRIBUTION OF 5-HT_{1B} AND 5-HT_{1F} RECEPTOR ACTIVATION

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It has been suggested that in human cerebral arteries sumatriptan-evoked contractions are 5-HT_{1B}-receptor (i.e. 5-HT_{1D_β}) mediated (Hamel et al., 1993). However, sumatriptan also has affinity for 5-HT_{1F}-receptors (Adham et al., 1993) and activation of this receptor may underlie the vasoconstrictor response. The aim of the present study was to determine whether the vasoconstrictor response in human isolated blood vessels is 5-HT_{1B}- or 5-HT_{1F}-receptor mediated.

Middle meningeal (MMA) and temporal arteries (TA), obtained from neurosurgical patients, were cut into ring segments (2-3mm) and mounted for isometric tension recordings in organ baths containing physiological salt solution (37°C, aerated with 95%O_2/ 5%CO_2). Cumulative concentration effect curves to 5-HT-receptor agonists (1nM-100μM) were performed (see Table 1; n ≥ 4). Responses to sumatriptan were determined following incubation (30min) with GR127,935 and GR125,743 (Clitherow et al, 1994; selective 5-HT_{1D}-receptor antagonists, n≥3). Polyclonal antibodies specific for human 5-HT_{1D_α}-, 5-HT_{1B}- and 5-HT_{1F}-receptors were used to detect the presence of receptor-like protein in sections of vascular tissue (formalin fixation, paraffin embedded). [^{3}H]-5HT (2nM) displacement binding studies were carried out on membranes prepared from stable CHO cell lines expressing human 5-HT_{1D_α}, 5-HT_{1B}- or 5-HT_{1F}-receptors.

There was a significant correlation (determined using linear regression analysis) between vasoconstrictor potency (EC_{50} values in MMA) and affinity (IC_{50} values) for cloned 5-HT_{1B}-receptors ($r = 0.93$; $P = 0.019$) but not for 5-HT_{1D_α}- or 5-HT_{1F}-receptors ($r < 0.53$; $P > 0.3$). In MMA, sumatriptan evoked contractions were non-competitively antagonised by GR127,935 (with 10nM reducing the maximum contraction by 75%). GR125,743, acted as a competitive antagonist (apparent $pA_2 = 9.1$). 5-HT_{1B}-receptor-like immunoreactivity (ir) (but not 5-HT_{1D_α}-receptor-like ir) was detected in the smooth muscle from MMA. In TA, sumatriptan was a poor vasoconstrictor and 5-HT_{1B}-receptor like ir was not detected.

Table 1: Measurements of affinity for cloned 5-HT-receptors (IC_{50}, nM) and vasoconstrictor potency (EC_{50}, nM) in MMA

	5-HT_{1D_α}	5-HT_{1B}	5-HT_{1F}	MMA (EC_{50})
5-HT	6.6	6.1	7.6	32.0
5-CT	1.9	2.3	954.9	2.2
Sumatriptan	7.3	9.3	17.8	70.0
CP122,288	6.1	8.1	2884.0	142.9
Zolmitriptan	1.3	6.2	46.8	42.5

In MMA, vasoconstrictor potency correlated with 5-HT_{1B}-receptor affinity but not with 5-HT_{1F}-receptor affinity and sumatriptan evoked contractions were antagonised by selective 5-HT_{1D}-receptor antagonists. These data suggest that contraction is mediated via 5-HT_{1B}-receptors. Immunohistochemical studies confirmed the presence of 5-HT_{1B}-receptor protein like in MMA but not in TA.

Hamel E., et al. (1993) Mol. Pharmacol., 44, 242-246.
Adham N., et al. (1993) Proc. Natl. Acad. Sci. USA, 90, 408-412.
Clitherow JW et al (1994) J. Med Chem 37, 2253

We are grateful to the neurosurgeons at Addenbrooke's Hospital for providing tissue samples.

212P INHIBITION OF NICOTINE-EVOKED RELAXATION OF THE GUINEA-PIG ISOLATED BASILAR ARTERY BY SUMATRIPTAN, 5-HYDROXYTRYPTAMINE (5-HT) AND 5-CARBOXAMIDOTRYPTAMINE (5-CT)

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An agonist action at inhibitory 5-HT_{1D} receptors on trigeminal nerve terminals may be a mechanism of action of sumatriptan (sumat) in migraine (Moskowitz, 1992). Sumat blocked neurogenic plasma extravasation from blood vessels in the dura mater of rat and guinea-pig *in vivo* (Buzzi & Moskowitz, 1990). 5-HT was not active but 5-CT was potent in this model. O'Shaughnessy & Connor (1994) described an *in vitro* method for stimulating trigeminal terminals with nicotine in the guinea-pig basilar artery (GPBA). A nicotine-evoked relaxation was substance P-mediated, endothelium-dependent and blocked by sumat. We have used the GPBA to compare the effect of 5-HT, 5-CT and sumat in this *in vitro* model of trigeminal activation.

Male, Hartley strain guinea-pigs (250-350g) were killed by stunning and exsanguination. Ring segments (1.5-2.5mm in length) of the GPBA were mounted on tungsten wires (0.1mm diameter) in 10 ml tissue baths under a tension (isometric recording) of 0.4g. Tissues were maintained in Krebs' solution (1.25mM Ca^{++}) at 37°C, gassed with 5% CO_2 in O_2. After the tissues had stabilized for 90 min a cumulative concentration-response curve to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10nM-10μM) was obtained. Substance P (3 or 10nM) was added to confirm an intact endothelium. Tissues were then washed and equilibrated with guanethidine (3μM) and atropine (3μM). After 1h tissues were re-contracted with $PGF_{2\alpha}$ (1μM) and the nicotine (0.1mM)-evoked relaxation was determined. Tissues were washed, allowed 70 min to recover and re-contraction with $PGF_{2\alpha}$ (1μM). 5-HT (30nM-1μM), 5-CT (1nM-1μM), sumat (0.3μM-30μM) or vehicle (Krebs') was added to the bath. A response to nicotine was evoked 4 min after addition of the 5-HT agonist and was expressed as a percentage of the first response to nicotine in the same tissue. One concentration of agonist was tested in a tissue, n=3-6 at each concentration. Concentration-response curves were analysed using Allfit.

The maximum increase in tension with $PGF_{2\alpha}$ in control tissues (n=17) was 0.54±0.040g (mean±s.e.mean). Only 50% of tissues responded to nicotine. The relaxation evoked by the first exposure to nicotine in these tissues was 0.16±0.031g. These values were not significantly different (ANOVAR) to the initial responses in groups treated with 5-HT agonists. Concentration-related inhibition of the response to nicotine was observed with sumat (pIC_{50} 5.5), 5-HT (6.9) and 5-CT (8.2). The calculated maxima of concentration-response curves were 96, 92 and 61% inhibition for sumat, 5-HT and 5-CT respectively. All agonists evoked small contractions of the basilar artery in the presence of $PGF_{2\alpha}$. The calculated maximum contractions for sumat, 5-HT, 5-CT and vehicle respectively were 8.7, 11.0, 7.2 and -0.3 % of the initial maximum response to $PGF_{2\alpha}$ and agonist pED_{50} values were 5.5, 7.0 and 7.5 respectively.

The potency of agonists in inhibiting nicotine-evoked relaxation of the GPBA is consistent with a 5-HT_{1D} receptor-mediated response. The reported lack of activity of 5-HT in *in vivo* models of trigeminal nerve stimulation may reflect multiple 5-HT receptor activation. This method may be a suitable *in vitro* assay of activity of compounds at trigeminal terminals.

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Buzzi, M.G. & Moskowitz M.A. (1990) *Br. J. Pharmacol.* 99, 202-206.
Moskowitz, M.A. (1992) *Trends Pharmacol.Sci.* 13, 307-311.
O'Shaughnessy, C.T. & Connor, H.E. (1994) *Eur. J. Pharmacol.* 259, 37-42.

213P RIZATRIPTAN INHIBITS TRIGEMINAL NOCICEPTIVE RESPONSES IN AN ELECTROPHYSIOLOGICAL ASSAY IN THE ANAESTHETIZED RAT

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The caudal portion of the trigeminal nucleus is a convergent relay site for nociceptive information from pericranial and intracranial tissues. Prejunctional $5HT_{1B/1D}$ receptors have been proposed to modulate the release of neuropeptides from sensory afferent nerve fibres in the meninges (Buzzi et al. 1995) and recent evidence suggests that similar mechanisms may occur at the central terminals of the same fibres (Kaube et al. 1993; Shepheard et al. 1995). We have used an in vivo electrophysiological technique in the anaesthetized rat to test whether the $5\text{-}HT_{1B/1D}$ receptor agonist rizatriptan (MK-462; L-705,126; Street et al. 1995) can activate central $5\text{-}HT_{1B/1D}$ receptors after systemic dosing and thereby reduce central trigeminal nociceptive neurotransmission.

Eleven male Sprague-Dawley rats (320-430 g) were anaesthetised with halothane (2% in O_2) and surgically prepared for single unit recording from the trigeminal nucleus caudalis; the carotid artery, jugular veins and trachea were cannulated for artificial ventilation, monitoring blood pressure and for drug/anaesthetic administration. The brainstem was exposed and the skull was thinned, but not opened, over the temporal lobe to visualize the meningeal blood vessels. Anaesthesia was maintained with a constant infusion of sodium pentobarbitone (20-30 mg $kg^{-1}h^{-1}$). The animal was paralysed with pancuronium (1 mg $kg^{-1}h^{-1}$); adequate anaesthesia was ensured by monitoring cardiovascular responses to noxious stimuli. Body temperature was maintained between 36-37.5°C. Extracellular action potentials were recorded from single caudal trigeminal neurones in response to electrical stimulation of the dura mater via an electrode placed on the thinned skull in the vicinity of the middle meningeal artery. The action potentials evoked by this stimulation regime were counted using a window discriminator and stored on computer. The dura was stimulated at 1 Hz for 20 s; this was repeated every 200 s. Once a series of stable responses were obtained rizatriptan was administered i.v. in a cumulative dose regime (0.3, 1 and 3 mg kg^{-1} base weight; every 10 min) and compared with repeat administration of vehicle in separate experiments. All data are mean ± s.e.mean.

Rizatriptan dose dependently attenuated the responses of single neurons to dural stimulation by -7 ± 6 %, -24 ± 11 % and -52 ± 9 % at 0.3, 1 and 3 mg kg^{-1} respectively (n = 6). A maximum inhibition of -73 ± 9 % was attained 19 ± 4 min after the 3 mg kg^{-1} dose suggesting that the magnitude of the inhibitory effects seen after the lower doses may not have been maximal. Sequential administration of 3 doses of vehicle using the same protocol had no effect on durally evoked responses (+6 ± 3 %, -4 ± 3 % and -1 ± 5 % respectively; n = 5). Statistical analysis using a 2-way ANOVA with repeated measures showed the inhibition by rizatriptan to be highly significant ($p<0.0005$) when compared to the vehicle effects.

Rizatriptan dose-dependently inhibited the responses of single trigeminal nucleus caudalis neurones to noxious stimulation of the dura mater in the vicinity of the middle meningeal artery. These data indicate that rizatriptan penetrates the blood-brain barrier and has central trigeminal antinociceptive activity that could contribute to its clinical effectiveness against migraine headache pain.

Buzzi M. G., Bonamini M. & Moskowitz M. A. (1995) *Cephalalgia* 15, 277-280.
Kaube, H., Hoskin, K. L. & Goadsby, P. J. (1993) *Br. J. Pharmacol.* 109, 788-792.
Shepheard S. L., Williamson D. J., Williams J. *et al* (1995) *Neuropharmacol.* 34, 255-261.
Street, L. J., Baker R., Davey A. R. *et al.* (1995) *J. Med. Chem.* 38, 1799-1810.

214P 5-HT, OR THE $5\text{-}HT_{2B}$ RECEPTOR AGONIST, BW 723C86, DO NOT ALTER MARKER EXTRAVASATION IN RAT DURA MATER

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Activation of $5\text{-}HT_{2B}$ / $5\text{-}HT_{2C}$ receptors have been proposed as an initiating event in migraine (Fozard 1990). This study investigates the effects of a localised administration of 5-HT and BW 723C86 (Martin G.R *et al.*, 1993) , a selective $5\text{-}HT_{2B}$ receptor agonist in the extravasation of sodium fluorescein (NaFlu) from cerebral vessels in the meninges and cortex.

Male Sprague-Dawley rats (250-320g) were anaesthetised with sodium pentobarbitone (60mgkg^{-1}). The left linguofacial artery was cannulated for local administration of drugs into the left external carotid artery. Left and right femoral veins were cannulated for administration of NaFlu (50mgkg^{-1}) and anaesthetic infusion (sodium pentobarbitone, 18mgkg^{-1}hr^{-1}), respectively. Blood pressure was recorded via a right femoral artery cannula. Drug doses were selected such that effects on mean arterial blood pressure were subthreshold. Following equilibration of NaFlu for 10 min, saline (control), histamine (20µgkg^{-1}), 5-HT (20µgkg^{-1} and 2µgkg^{-1}) or BW 723C86 (20µgkg^{-1}), delivered at 45µlmin^{-1} over 15 min, were infused into the left external carotid artery. The animal was then euthanased and transcardiac perfused (50 U ml^{-1} heparinised saline, 100mmHg). The lip, conjunctiva, dura and cortex were then analysed for ng of marker / g wet weight of tissue. Data represent means ± s.e.mean and were analysed for significance from saline controls using one way ANOVA followed by post hoc Dunnetts T-test. A significance level of $p<0.05$ was adopted.

All doses of drug and vehicle had no significant effect on mean arterial blood pressure.

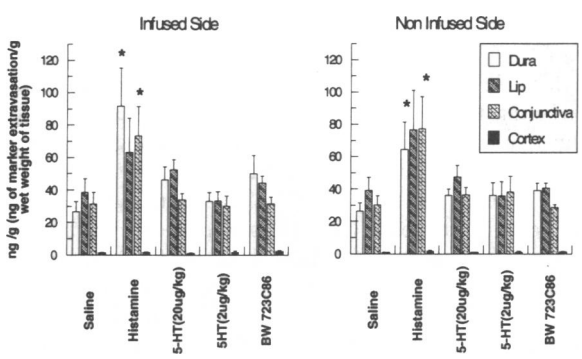

Figure 1. Infused and non infused side NaFlu extravasation per wet weight of tissue (ng/g) following saline, histamine, 5-HT or BW 723C86 treatment. Data represent mean ± s.e. mean. *P<0.05 significant extravasation of marker vs saline, ANOVA followed by post hoc Dunnetts T-test. n=5-7.

Our results indicate that histamine, but not 5-HT or BW 723C86, produces extravsation of NaFlu in the dura and conjuctiva of the rat at doses without cardiovascular liability. Additionally, it was found that histamine induced contralateral extravasation of marker, indicating a spread of dose to both hemispheres.

Fozard J.R.(1990) In: Sandler M, Collins G.M. Migraine:a spectrum of ideas. pp 128 - 146. Oxford University Press, Oxford.
Martin G.R., Browning C., Giles H. (1993), Br. J. Pharmacol 110, 137P.

215P NON-ADRENERGIC, NON-CHOLINERGIC (NANC) RELAXATION OF THE RAT ANOCOCCYGEUS MUSCLE *IN VITRO* DUE TO ACTIVATION OF CAPSAICIN-SENSITIVE NEURONES

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The rat anococcygeus muscle contains neurones which stain positively for NO synthase (NOS) that may be responsible for the NANC relaxation produced by electrical field stimulation (EFS) of the muscle *in vitro* (Gibson et al., 1995). Many sensory neurones in dorsal root ganglia stain positively for NOS (Farkas-Szallasi et al., 1995). It is possible that some of the NOS positive fibres in the muscle are sensory. Hence the relaxant NANC response to EFS could be due to NO released from sensory fibres. We have tested this hypothesis by using capsaicin (caps) to excite sensory fibres and compared responses of the muscle to caps and to EFS.

Anococcygeus muscles from adult Wistar rats were suspended in organ baths filled with modified Krebs' solution containing 0.5 mM $CaCl_2$ and either 5 μM prazosin or 20 μM phentolamine. Tension in the muscles was measured using an isometric force transducer. Muscle tone was raised by adding carbachol (10 μM) and relaxant responses to 6 s EFS (70V, 5 ms pulse width, 10-30 Hz) were obtained using a pair of platinum strips placed one on either side of the muscle. Data are reported as mean ± s.e.m. and statistical significance was tested by unpaired, 2-tailed Student's t-test.

Caps caused a transient, dose-dependent relaxation with an EC_{50} of 0.61 ± 0.18 μM and a maximum effect, at 10 μM, of 41.9 ± 4.8% (n=38) reduction in tone. The muscle desensitized completely to caps after a single application. The response to 10 μM caps was reduced to 10.5 ± 3.7 % (n=4, P=0.016) by tetrodotoxin (TTX, 1.0 μM) and to 6.4 ± 2.5 % (n=5, P=0.011) by ruthenium red (RR, 10 μM), consistent with an effect on sensory nerves. N-monomethyl-L-arginine (L-NMMA, 100 μM) reduced the response to 10 μM caps to 18.1 ± 3.7 % (n=15, P<0.001) and to EFS (10 Hz) from 55.4 ± 4.6% (n=35) to 23.8 ± 4.6 % (n=16, P=0.004). This was prevented when L-arginine (100 μM) was applied with L-NMMA. Haemoglobin (Hb, 25 μM) reduced responses to 10 μM caps to 8.5 ± 3.6 % (n=10, P<0.001) and EFS to 22.6 ± 4.7 % (n=10, P<0.001). Relaxant responses to sodium nitroprusside (SNP, 0.2 μM SNP) were reduced by Hb from 62.5 ± 4.1 % (n=33) to 28.3 ± 3.6 % (n=7, P<0.001). SNP responses were not significantly reduced by TTX, RR, or L-NMMA. Relaxant responses to EFS and caps-evoked relaxations were less than additive.

Caps-evoked relaxation and EFS induced NANC responses are not additive but are TTX-sensitive and NOS-dependent so it is likely that both responses are due to excitation of the same intrinsic nerve population. We suggest therefore that the NANC response of the anococcygeus to EFS, like the caps response, may be due to stimulation of intrinsic sensory nerves.

Supported by the Wellcome Trust

Gibson, A., Brave, S.R., McFadzean, I. *et al.* (1995). *Arch. int. Pharmacodyn.* 329, 39-51.
Farkas-Szallasi, T., Lundberg, J.M., Wiesenfeld-Hallin, Z. *et al.* (1995). *Neuroreport* 6. 2230-2234.

216P NITRIC OXIDE (NO) MODULATES RABBIT CHOLINERGIC AMACRINE CELLS BY REDUCING GLYCINERGIC NEGATIVE FEEDBACK

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Acetylcholine (ACh) release from the rabbit retina in response to flickering light is regulated by a negative feedback circuit. Cholinergic amacrine cells synapse with glycinergic amacrines which feed back either directly onto the cholinergic neurones or onto the bipolar cell terminals [Neal and Cunningham, 1995]. As nitric oxide synthase (NOS) has also been localized in a population of amacrine cells [Koistinah et al, 1993], eye-cup preparations and isolated retinae were used to study the interaction of cholinergic, nitrergic and glycinergic amacrine cells. Here we report on a novel role for NO within this circuit, modulating glycine release and indirectly ACh release.

Adult New Zealand white rabbits were anaesthetised with urethane (1.5g/kg I.P.) and an eye-cup was prepared. Eye-cups were filled with Krebs bicarbonate containing [^{3}H] choline (Ch) (0.5μM) for 30 min, followed by irrigation for 60 min with fresh medium containing eserine (30μM). Medium (0.5ml) was then replaced at 5 min intervals and the resulting samples were analysed for radioactivity. Isolated rabbit retinae were incubated in Krebs bicarbonate medium containing [^{3}H] Ch (50nM)and then placed in a small (1ml) chamber. Medium containing eserine was replaced every 10 min and the samples assayed for radioactivity (ACh) and analysed by HPLC (glycine). The retinae were exposed to two separate periods of K^+ (50mM) depolarisation (S1, S2) with NO donors/inhibitors included during the second K^+ stimulation period (S2).

In the rabbit eye-cup, the NOS inhibitor N^G-monomethyl-L-arginine.monoacetate (LNMMA) (1mM) reduced light evoked release of ACh to 41±4.1% of control values (p<0.005, n=7). This was blocked by strychnine (10μM, n=5) and L-arginine (200μM, n=4) neither of which had an effect alone. N^G-nitro-L-arginine (LNA) (5mM) also decreased light evoked ACh release to 58±7.3% of control values (p<0.0005, n=7). In contrast to the inhibitory effects of NOS inhibitors, the NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) (1mM) increased the light evoked release of ACh to 215±33% (P<0.001, n=11). This effect was blocked by haemoglobin (25μM, n=4), which had no effect alone. Sodium nitroprusside (SNP) (1mM) similarly increased light evoked ACh release to 176±13% (P<0.001, n=10). Experiments using isolated retinae confirmed these findings. Both SNP (1mM) and SNAP (500μM) increased K^+ evoked ACh release, the S2/S1 ratio increased from 0.6±0.08 to 0.88±0.07 (p<0.05, n=4) and 0.99±0.09 (p<0.01, n=5) respectively. In the absence of any drug, K^+ evoked glycine release, (S2/S1 ratio) was 1.26±0.19 (n=6). Both SNP (1mM) and SNAP (500μM) decreased this value to 0.51±0.05 (p<0.03, n=3) and 0.68±0.15 (p<0.03, n=4) respectively.

These results suggest that NO modulates retinal ACh release by reducing glycinergic feedback inhibition.

Neal MJ & Cunningham JR (1995) *J Physiol* **482,** 363-372
Koistinah J, Swanson RA, de Veure J & Shegar SM (1993) *Neuroscience* **57 (3),** 587-597

217P THIOGLYCOLLATE-INDUCED PERITONITIS IN THE CD1 MOUSE IS DEPENDENT ON L- AND P-SELECTIN

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L-, P- and E-selectin have been shown to mediate leukocyte rolling and cell recruitment in several experimental animal models of acute inflammation (Bevilacqua & Nelson 1993; Bosse & Vestweber, 1994; Mulligan *et al*, 1992; 1994). However, the contribution of each selectin appears to depend on the model. The thioglycollate induced peritonitis model in the mouse is associated with a marked neutrophil recruitment into the peritoneal cavity. We have used monoclonal antibodies to assess the contribution of the selectins to cell recruitment in this inflammatory model.

Peritonitis was induced in male CD1 mice (30 -38g) by intra-peritoneal injection of thioglycollate (1ml, 6%, Difco batch T1055). Four hours later invading cells were collected by peritoneal lavage (4ml). Total cell numbers in the lavage fluid were estimated by haemocytometer counting and differential counts obtained from cytospin preparations stained with May-Grunwald/Giemsa.

Thioglycollate increased neutrophil (PMN) numbers within the peritoneal cavity (see table). Antibodies to L-selectin (MEL14, 20μg) and P-selectin (RB40, 20μg), given into the tail vein (200μl) immediately before thioglycollate, reduced PMN infiltration. Given together inhibition produced by these two antibodies was additive. An anti-E-selectin antibody (10E9, 40μg, given just before and 2 hours after thioglycollate), previously shown to inhibit neutrophil infiltration by 65% in a similar model of thioglycollate induced peritonitis in the BALB/c mouse (Bosse & Vestweber, 1994), had little effect on PMN infiltration in our hands and did not increase the inhibition seen with anti-L and anti-P antibodies. By contrast, control antibodies had no important effects on PMN infiltration in this model.

Treatment Groups	antibody	neutrophils in peritoneal cavity ($x\ 10^{-5}$)	n
saline control		5 ± 1	30
thioglycollate control		30 ± 2	46
anti-L selectin	MEL14	17 ± 2*	21
anti-P selectin	RB40	24 ± 2*	22
anti-E selectin	10E9	31 ± 2	24
anti- L + P		7 ± 1*	16
anti- L + E		13 ± 2*	15
anti- P + E		25 ± 2*	14
anti- L + P + E		8 ± 1*	8

*Significantly different ($P < 0.05$) from thioglycollate control using Analysis of Variance and Students t-test.

We conclude that the PMN infiltration into the peritoneal cavity of the CD1 mouse induced by thioglycollate is dependent on L- and P-selectin mediated events. These results contrast with those previously reported by Bosse & Vestweber (1994) using the same antibodies in a similar experimental model.

Bevilacqua MP & Nelson RM, J. Clin. Invest. 1993;91:379-387.
Bosse R. & Vestweber D., Eur. J. Immunol. 1994;24:3019-3024.
Mulligan MS, *et al.*, J. Clin. Invest. 1992;90:1600-1607.
Mulligan MS, *et al.*, J. Immunol. 1994;152:832-840.

218P SENSITISATION OF CUTANEOUS AFFERENT NEUROTRANSMISSION TO INNOCUOUS AND NOXIOUS MECHANICAL STIMULI USING TOPICAL PROSTAGLANDIN E_2 (PGE_2)

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Extracellular recording techniques in the rat spinal cord *in vivo* can be used to selectively study second order dorsal horn neurones and their role in nociceptive neurotransmission. Previous studies have used von Frey hairs (VFHs) to stimulate mechanoreceptive primary afferent neurones and have also sensitised these afferent neurones with intraplantar injection of PGE2 (Martin *et al.*, 1987). The aim of this study was to further investigate innocuous and noxious cutaneous neurotransmission, using VFHs to stimulate mechanoreceptors on the rat hindpaw and in addition, to develop a model of cutaneous hypersensitivity, using a non-invasive method, by topical administration of PGE2.

Male Wistar rats (250-350g) were anaesthetised with sodium pentobarbitone (60 $mgkg^{-1}$ ip; 3-5$mgkg^{-1}h^{-1}$ iv) and prepared for single unit extracellular recording from L4-L5 regions of the spinal cord. The mechanical threshold and receptive fields for dorsal horn neurones responding to both touch and noxious pinch, were established using VFHs (0.22-46.5g). All results (mean ± s.e.m.) were calculated as a mean of three, 5s, VFH applications (minus basal firing) and were expressed as either a percentage of the maximum of the first pressure response curve to VFHs or of the response to 46.5g, which ever was greater. Repeated Measures ANOVA and the Mauchly test (treatment by time interaction) were used to determine statistical significance between treatment groups ($p \leq 0.05$ is significant).

It was possible to construct 3 reproducible pressure response curves in 5 cells tested (123.8% ±28 & 103.3% ±32.5 for 2nd & 3rd curves with respect to first). Prior to PGE2 application, the mechanical thresholds (range 0.45-4.19g) were estimated on 19 cells and receptive fields mapped and found to be located primarily on the toes and pads of the hind paw. Following topical PGE2 application (3μg in 20μl acetone), responses to selected VFH increased in 8 out of 19 cells tested. The response to the 46.5g VFH was the only hair to show a significant increase after PGE2 application (Repeated Measures ANOVA, $p<0.05$) compared to control responses, this was not time dependent up to 120min (Mauchly, $p=0.87$). The maximum increase was seen at 70min (197.4%±29). PGE2 application also reduced the mechanical threshold in 6 cells (from 0.45-4.19g to 0.22-0.75g) and increased receptive field size in 4 cells.

In conclusion, at least 3 reproducible pressure-response curves can be constructed in dorsal horn neurones, when applying graded VFHs to the rat hind paw. Topically PGE2 applied to the rat hindpaw induces a mechanical cutaneous hypersensitivity but only in a proportion of neurones and only significantly at higher mechanical pressures.

Martin, H.A., Basbaun, A.I., Kwiat, G.C. *et al.* (1987) *Neuroscience*, 22, 651-659.

219P VALIDATION OF THE DUAL CHANNEL WEIGHT AVERAGER AS AN INSTRUMENT OF THE MEASUREMENT OF CLINICALLY RELEVANT PAIN

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Unilateral hindpaw injection of carrageenan elicits an inflammation of the injected paw which is associated with a mechanical hyperalgesia, as measured by a reduction in the paw withdrawal threshold (Randall and Selitto, 1957). This reaches a maximum after 3 hours and is resolved by 9 hours (Vinegar *et al.*,1968). A reduction in mechanical paw withdrawal threshold is not a measure of "spontaneous" or "incident" pain. Furthermore, it provides no indication of allodynia ie perceived pain response to something which is normally innocuous. We have therefore investigated a dual channel weight averager (Bioengineering, GlaxoWellcome/Linton Instruments) which measures the distribution of weight of the two hindpaws of a rat. Normal or saline injected rats distribute their body weight equally between the two hind paws, but when the left hind paw is inflamed and/or painful, the rats re-distribute their body weight so that less weight is placed on the inflamed paw. We have compared the efficacy of four commonly used analgesics or anti-inflammatory agents, dexamethasone, indomethacin, ibuprofen and morphine in inhibiting the reduction in weight bearing with mechanical paw withdrawal threshold. Male Random Hooded rats (200-250g) were fasted overnight and were dosed orally (subcutaneous for morphine) with drug or vehicle, 30 minutes prior to an intraplantar injection (100µl, left hind paw) of 2% carrageenan to all animals. Three hours later mechanical paw withdrawal threshold and weight bearing (after 8 seconds) on the left paw was determined for the vehicle treated group and the drug treated group. Morphine, dexamethasone, ibuprofen and indomethacin all produced a dose related inhibition of the decrease in mechanical paw withdrawal threshold and reversed the decrease in weight bearing (Table 1). There was no significant difference between the effect on paw withdrawal threshold compared to that on weight bearing for each of the drugs ($p>0.05$; paired t test). In conclusion, the dual channel weight averager provides an assessment of analgesic potency, which compares favourably with that determined using the Randall and Selitto method. However, the former is certainly less subjective, less variable and possibly offers a better instrument for the measurement of clinically relevant pain.

Randall, L.O. & Selitto, J.J. (1957) Arch. Int. Pharmacodyn., 61 409-419.
Vinegar, RW. & Hugo, R. (1968). Fed Proc. 46 (1), 118-126.

Table 1. Morphine, dexamethasone, ibuprofen and indomethacin produced a dose-related inhibition of the carrageenan-induced decrease in paw withdraw threshold and decrease in weight bearing.

	ED_{50} mgkg $^{-1}$ with 95% confidence limits n=6-7 per group	
Drugs	Mechanical paw withdrawal threshold	Weight bearing
Morphine	0.85 (0.4-1.70)	0.33 (0.13-1.26)
Dexamethasone	0.15(0.01-0.47)	0.02(0.004-0.07)
Ibuprofen	26.50(18.60-35.40)	21.50(0.99-49.60)
Indomethacin	0.64(0.29-1.34)	0.46(0.30-0.60)

220P THE USE OF LOCOMOTOR ACTIVITY EQUIPMENT TO ASSESS ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

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Acute inflammation produced by uniplantar injection of carrageenan is associated with marked oedema and hyperalgesia, that reaches a maximum after 3 hours, and starts to resolve after 7 hours (Vinegar *et al.*, 1968). In this study we have investigated the Benwick locomotor activity equipment (Iwamoto, 1984) as a readout of analgesia and anti-inflammatory activity, with the more conventional readouts of mechanical paw withdrawal (MPW; Randall & Selitto, 1957) and paw volume. Four commonly used analgesics and/or anti-inflammatory compounds were tested in the model: dexamethasone, indomethacin, paracetamol and morphine. Male Random hooded rats (200-250g) were dosed with drug or vehicle, 30 minutes prior to an intraplantar injection (100µl; left hindpaw) of 2% carrageenan. Three hours later, MPW latency and paw volume were determined. Locomotor activity was measured by placing the animals in mobility recording boxes and slow rearing activity recorded on line, over a 15 minute period. To indicate statistical significance, a Dunnett's multiple comparison test was used ($p<0.05$ considered significant). Dexamethasone (0.01-1mgkg^{-1}p.o.) and indomethacin (0.1-3mgkg^{-1}p.o.) produced a dose-related reversal of the decrease in slow rearing activity, MPW latency and the increase in paw volume, following intraplantar carrageenan (see Table 1 for ED_{50s}). Morphine (0.4-4mgkg^{-1}s.c.) also produced dose related effects in all three tests, but an ED_{50} could only be calculated for the reversal in MPW latency. Maximum effects for the other tests are shown in Table 1. Paracetamol (50-150mgkg^{-1}p.o.) showed only weak analgesic activity in the MPW test and had no significant effect (NSE) on locomotor activity or paw oedema. The possibility of sedative side effects was eliminated by only testing doses of the compounds, which did not cause an impairment in locomotor activity in the absence of carrageenan. In conclusion the locomotor activity equipment can be used to assess drugs with both analgesic and anti-inflammatory properties, but it does not provide an effective pain readout for drugs that are mainly analgesic.

Iwamoto T.O. (1984). *Psychopharmacology*, 84, 374-382.
Randall L.O. & Selitto J.J. (1957). *Arch. Int. Pharmacodyn.*, 61, 409-419.
Vinegar, R., Schreiber, W. & Hugo, R. (1968). *Fed. Proc.*, 46(1), 118-126.

Table 1. Effects of compounds on carrageenan induced decrease in locomotor activity, mechanical paw withdrawal and increase in paw volume (n = 5-7 for all groups).

		ED_{50} mgkg^{-1} (95% C.L.)	
Compound	Locomotor activity	Mechanical paw withdrawal latency	Paw volume
Dexamethasone	0.34 (0.22-0.45)	0.41 (0.13-5.75)	0.94 (0.45-3.18)
Indomethacin	1.34 (0.75-3.13)	1.95 (0.84-13.85)	2.38 (1.05-15.95)
Morphine	33% at 4mgkg^{-1}	1.12 (0.76-1.64)	43% at 4mgkg^{-1}
Paracetamol	23% at 150mgkg^{-1}	37% at 150mgkg^{-1}	NSE

221P THE DIFFERENTIAL EFFECTS OF FENTANYL ON DORSAL HORN NEURONES RESPONSIVE TO COLORECTAL DISTENSION WITHIN THE LUMBOSACRAL CORD OF THE ANAESTHETISED RAT

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A proportion of neurones within the lumbosacral dorsal horn have been shown to respond to colorectal distension (CRD), the magnitude of the response increasing with intraluminal pressure. Four neuronal subtypes have been classified according to their response to CRD: SL-A (Short-latency abrupt, no firing after cessation of CRD), SL-S (Short-latency sustained, firing continues after CRD), L-L (Long-latency, firing continues after CRD) and Inhib (inhibition of ongoing spontaneous activity by CRD)(Ness & Gebhart, 1987). In decerebrate, spinalized rats, morphine has been shown to inhibit responses to CRD in both SL-A and SL-S neurones (Ness & Gebhart, 1989).We have investigated the effect of another μ-opioid receptor agonist, fentanyl, on similar neuronal responses in the intact, pentobarbitone-anaesthetised rat.

Male Wistar rats (220-320g) were anaesthetised with sodium pentobarbitone (60mgkg^{-1} ip; 3-5mgkg^{-1}h^{-1} iv) and prepared for single unit extracellular recording from the lumbosacral spinal cord (L5-S1). A latex balloon was inserted into the colorectum; a noxious search stimulus (80mmHg for 30s, 5min intervals) was used to locate neurones responsive to CRD. Data are given as mean ± s.e.mean. The ED_{50} for fentanyl is given with 95% confidence limits. A paired students t-test was used to assess statistical significance ($p<0.05$ considered significant).

Fifty seven neurones located 0.1-1.1mm from the cord dorsum responded to CRD and were classified as SL-A (58%), SL-S (25%), L-L (9%) and Inhib (8%). All neurones showed a graded response to CRD (20-100mmHg, 30s) except 4 SL-A neurones which responded maximally at 10mmHg and may therefore reflect low threshold mechanosensitive afferent input. Cutaneous receptive fields (CRFs) were mapped for 45 neurones; responses were classified as low threshold (5), wide dynamic range (21) and high threshold (10), according to the response to innocuous touch and noxious pinch . CRFs were located on the back, thigh, scrotal and tail regions.

In SL-S neurones, fentanyl (1-8μgkg^{-1} iv) inhibited the response to CRD in a dose related manner, with an ED_{50} of 2.5 μgkg^{-1} (1.7-3.7). At 4μgkg^{-1} the response was 44.4±14.6% of control values ($p<0.05$, n=6). Fentanyl also significantly reduced the duration of the response in these neurones (31.4±7.8% of control at 4μgkg^{-1}, $p<0.05$). Both effects were blocked by naloxone (3mgkg^{-1} iv); at 4μgkg^{-1} fentanyl, responses were 101.1±9.5% & 109.9±14% of control respectively. In contrast, SL-A neurones were not inhibited by fentanyl (81.9±22.7% of control at 8μgkg^{-1}, n=6). In conclusion, fentanyl has differential inhibitory effects on subgroups of dorsal horn neurones responsive to CRD in intact, anaesthetised rats.

Ness, T.J.& Gebhart, G.F.(1987) *J.Neurophysiol.* 57(6),1867-1892.
Ness, T.J.& Gebhart, G.F.(1989) *J.Neurophysiol.* 61(1),220-230

222P ACTIVATION OF THE δ-OPIOID RECEPTOR IN CHO CELLS INCREASES INTRACELLULAR CALCIUM

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It is now well established that opioids produce both stimulatory and inhibitory effects on cell signaling, including activation of the Ins(1,4,5)P_3-$[Ca^{2+}]_i$ system (Smart & Lambert, 1996a). We have previously reported a stimulation of Ins(1,4,5)P_3 formation by [D-Pen^{2,5}]enkephalin (DPDPE) in CHO cells expressing recombinant δ-opioid receptors with a pEC_{50} of 7.26±0.13 (Smart et al., 1996). In this study we examine the $[Ca^{2+}]_i$ response to DPDPE stimulation using CHO cells expressing the recombinant δ-opioid receptor.

CHO cells expressing around 350fmol/mg protein of δ-opioid receptors were maintained in supplemented Hams F12 medium. $[Ca^{2+}]_i$ was measured fluorimetrically in Fura-2 loaded suspensions of whole cells in Krebs-HEPES buffer, pH7.4 at 37°C as described previously (Smart & Lambert 1996b). All data presented are mean±s.e.mean (n≥4)

In the presence of extracellular Ca^{2+} DPDPE produced a dose-dependent (Figure 1) and naloxone reversable biphasic increase in $[Ca^{2+}]_i$ with an pEC_{50} of 8.53±0.09 (2.94nmol litre^{-1}). The κ agonist spiradoline (1μmol litre^{-1}) and the μ agonist [D-ala^2,MePhe^4,gly(ol)^5]enkephalin (1μmol litre^{-1}) were ineffective. In Ca^{2+} free buffer (+0.1mmol litre^{-1} EGTA) the increase in $[Ca^{2+}]_i$ was monophasic. When $[Ca^{2+}]_o$ (2.5mmol litre^{-1}) was replaced $[Ca^{2+}]_i$ increased above the normal plateau phase. In addition, if naloxone (Nal, 10μmol litre^{-1}) is added 90s after DPDPE challenge an increase is still observed. (Table 1)

These data show that recombinant δ-opioid receptors increase $[Ca^{2+}]_i$ utilising both intracellular and extracellular Ca^{2+}. The EC_{50} for peak phase $[Ca^{2+}]_i$ lies to the left of that for the increase in Ins(1,4,5)P_3 (55nmol litre^{-1}) suggesting that DPDPE releases intracellular stored Ca^{2+} and that an amplification process may occur. The observation that $[Ca^{2+}]_i$ still inreases following Ca^{2+} readdition after naloxone implicates receptor independent Ca^{2+} influx. Further studies are required to address this question.

Figure 1. Dose-dependent increase in $[Ca^{2+}]_i$ with DPDPE

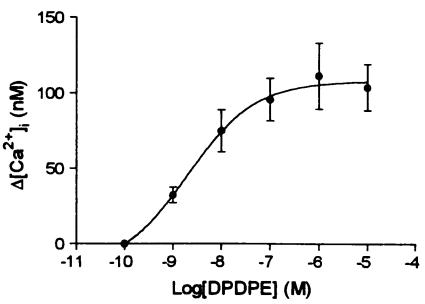

Table 1. Effects of Ca^{2+} replacement on DPDPE stimulated increase in $[Ca^{2+}]i$. (Ca^{2+} was replaced 190s after DPDPE)

	Δ$[Ca^{2+}]_i$ (nmol litre^{-1})		
	Peak	Plateau (160s)	After Ca^{2+}
+$[Ca^{2+}]_o$	150+24	106+28	-
-$[Ca^{2+}]_o$	128+12	10+ 3	209+24
-$[Ca^{2+}]_o$+Nal	109+ 5	22+ 2	272+29

Smart D., Devi. L.A. & Lambert, D.G. (1996) *Br. J. Pharmacol.* **118,** 28P.
Smart, D. & Lambert, D.G. (1996a) *Trends Pharmacol. Sci.* **17**, 264-269.
Smart, D. & Lambert, D.G. (1996b) *J. Neurochem.* **66**, 1462-1467.

223P COMPARISON OF THE REGIONAL DISTRIBUTION OF ORL_1- AND μ-, δ- AND κ-OPIOID BINDING SITES IN HOMOGENATES OF RABBIT, GUINEA-PIG AND RAT BRAIN

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The novel basic neuropeptide nociceptin or orphaninFQ is the endogenous ligand for the ORL_1 receptor but has little activity at opioid receptors (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). We have compared the regional distribution of the binding of [^{3}H]-nociceptin at the ORL_1-sites with that found with ligands selective for each of the μ, δ and κ-opioid binding sites in the rabbit, guinea-pig and rat brain.

Brains from male New Zealand White rabbits, Dunkin-Hartley guinea-pigs, and Hooded Lister rats were dissected into seven regions as described by Glowinski and Iversen (1966). Membranes, prepared in 50mM Tris.HCl (pH 7.4), were incubated with tritiated ligands for 60 min at 25°C (Paterson & McKnight, 1996). Samples were filtered over GF/B filters pre-soaked in 1% polyethyleneimine. Non-specific binding was determined with 100nM nociceptin or 1μM diprenorphine for opioid ligands. [^{3}H]-nociceptin (1.5nM) and [^{3}H]-bremazocine (3.5nM) were used to determine the total number of ORL_1 and opioid sites. [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (1nM), [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin (1.5nM) and [^{3}H]-CI-977 (0.25nM) were used to label the μ, δ and κ-opioid binding sites .

In homogenates of rabbit, guinea-pig and rat brain [^{3}H]-nociceptin labels a single population of binding sites with high affinity (Table 1). In the rabbit, the highest level of [^{3}H]-nociceptin binding was found in the cortex and the lowest in the medulla. there was no significant differences between the binding in the other five regions. In the hippocampus, there were equal numbers of ORL_1 and opioid sites, whereas in the other regions there were more opioid sites than ORL_1 sites. However, the distribution of the ORL_1 sites did not correspond with that of the μ, δ or κ-opioid sites. Similarly, in the guinea-pig and rat brain, the distribution of the ORL_1 sites did not match that of any of the three opioid receptors. These results confirm that the ORL_1 receptor is not a sub-type of opioid receptor.

Table 1. Characteristics of the binding of [^{3}H]-nociceptin in homogenates of rabbit, guinea-pig and rat brain at 25°C.

Species	Kd (nM)	B_{max} (fmol.mg^{-1} protein)
Rabbit	0.049± 0.011	143±24 (4)
Guinea-pig	0.089± 0.029	166±23 (4)
Rat	0.124± 0.041	254±16 (4)

The values are the mean ±s.e. mean.

Glowinski , J. & Iversen, L.L. (1966). *J. Neurochem.*13, 655-669.
Meunier, J.-C., Mollereau, C., Toll, L. *et al.* (1995). *Nature* 377, 532-535.
Paterson, S.J. & McKnight, A.T. (1996). *Br. J. Pharmacol.* 119, 35P
Reinscheid, R.K., Nothacker, H.-P., Bourson, A. *et al.* (1995). *Science* 270, 792-794.

224P EFFECTS OF NOCICEPTIN ON cAMP AND INS(1,4,5)P_3-[Ca^{2+}]I SIGNALLING IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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The heptadecapeptide nociceptin/orphanin FQ is the endogenous ligand for the orphan receptor, ORL-1/LC132 that inhibits cAMP formation in cells expressing recombinant ORL-1/LC132 (Meunier et al., 1995; Reinscheid et al., 1995). In addition, nociceptin increases inwardly rectifying K^+ currents in dorsal raphe nucleus neurones (Vaughan & Christie, 1996) and inhibits N-type voltage sensitive Ca^{2+} channels in SH-SY5Y cells (Connor et al., 1996). In a comparitive study using SH-SY5Y cells, we have examined the effects of nociceptin and [D-ala^2,MePhe^4,gly(ol)] enkephalin (DAMGO) on 3',5'-cyclic adenosine monophosphate (cAMP) formation. The effects of nociceptin on Ins(1,4,5)P_3-[Ca^{2+}]$_i$ signaling have also been examined.

SH-SY5Y cells were maintained in supplemented minimal essential medium. cAMP, Ins(1,4,5)P_3 and [Ca^{2+}]$_i$ were measured in suspensions of whole cells in Krebs/HEPES buffer containing 0.1% bovine serum albumin pH7.4 at 37°C as described previously (Hirst et al., 1995; Smart & Lambert, 1996). When nociceptin was used bestatin, amastatin, captopril and phosphoramidon were included at 30μM to prevent its breakdown. All data are presented as mean±s.e.mean (n≥3).

Nociceptin produced a dose-dependent inhibition of cAMP formation in SH-SY5Y cells with a pIC_{50} and I_{max} of 7.14±0.14 and 47.4±5.8% respectively. In addition, activation of μ-opioid receptors on SH-SY5Y cells also produced a dose-dependent inhibition of cAMP formation with pIC_{50} and I_{max} of 6.69±0.21 and 52.9±3.6% respectively. The inhibition produced by DAMGO but not nociceptin was reversed by naloxone, table 1. Nociceptin (300nM) did not increase Ins(1,4,5)P_3 formation (basal 11.0±1.2, 15s 9.2±3.7, 1min 12.9±1.9, 5min 11.0±2.8pmol.mg protein^{-1}) or enhance the sustained carbachol (10μM) stimulated increase in [Ca^{2+}]$_i$ when added acutely.

Table 1. Effects of nociceptin and DAMGO on forskolin (1μM) stimulated (6.8±0.9 fold basal) cAMP formation in SH-SY5Y cells.

	Inhibition cAMP formation (%)
Nociceptin (1μM)	38.4±2.9
Nociceptin+Naloxone (10μM)	30.5±3.4
DAMGO (1μM)	43.2±4.2
DAMGO+Naloxone	0.0±0.0

These data confirm the expression of orphan receptors on SH-SY5Y cells and their functional coupling to adenylyl cyclase. In our hands nociceptin does not activate phospholipase C in these cells.

Meunier, J-M., Mollereau, C., Toll L. et al. (1995) *Nature* 377, 532-535.
Reinscheid, R.K., Nothacker, H-P., Bourson A. et al. (1995) *Science* 270, 792-794.
Vaughan, C.W. & Christie, M.J. (1996) *Br. J. Pharmacol.* 117, 1609-1611.
Connor, M., Yeo, A. & Henderson, G. (1996) *Br. J. Pharmacol.* 118, 205-207.
Hirst, R.A. & Lambert, D.G. (1995) *Biochem. Pharmacol.* 49, 1633-1640.
Smart, D. & Lambert, D.G. (1996) *J. Neurochem.* 66, 1462-1467.