British Journal of Pharmacology

July 1993

Volume 109 Proceedings Supplement 1P–142P

1P INHIBITION BY PROSTANOID EP RECEPTOR AGONISTS OF FORMYL-METHIONYL-LEUCYL-PHENYLALANINE (FMLP)-STIMULATED NEUTROPHIL SUPEROXIDE PRODUCTION AND RELATIONSHIP TO INCREASES IN cAMP

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Prostaglandins of the E series suppress a variety of neutrophil functions, such as superoxide production by a mechanism that involves increases in cAMP formation (Hecker et al, 1990; Ney & Schror, 1991). However, the coupling between cAMP and neutrophil modulation has not been analysed pharmacologically, either to verify whether the two functional responses are mediated by the same receptor, or to determine the quantitative relationship between them. In this study we have used PGE_2 and some other EP receptor agonists to define this relationship.

Human neutrophils were isolated from venous blood by centrifugation with Polymorphprep (Nycomed). Neutrophils (10^6 cells ml^{-1}) were preincubated (37°C) with cytochalasin B (5µg ml^{-1}) and prostanoids or vehicle (≤ 0.25% ethanol) for 5 min in phosphate buffered saline (containing 0.9mM $CaCl_2$, 0.5mM $MgCl_2$ and 11mM glucose) prior to stimulation with FMLP (submaximal concentration, 10^{-7}M) for 5 min at 37°C. Superoxide production was measured as the reduction of cytochrome C (0.08mM, A550nm). The cAMP generation was determined from $2x10^7$ cells ml^{-1} pretreated for 5 min with isobutylmethyl xanthine (500µM) and stimulated for 5 min with the EP receptor agonists or vehicle (≤ 0.25% ethanol). After addition of perchloric acid (5 % final concentration, 4°C) and centrifugation (30s at 10,000g) cAMP was extracted using tri-n-octylamine/Freon, and assayed by ^{3}H-RIA (Amersham).

All the agonists tested behaved as full agonists against FMLP (10^{-7} M) stimulated superoxide production (9.9 ± 0.5nmol 10^6 $cells^{-1}$) whereas two of the agonists, PGA_1 and AH 13205, behaved as partial agonists of cAMP formation. α and pEC_{50} values obtained by fitting a logistic equation to concentration-effect data are shown in the Table 1.

Table 1

pEC_{50} (and α values for cAMP) for PGE_2, 11-deoxy PGE_1, misoprostol, PGA_1 and AH 13205 in superoxide and cAMP assays

Agonist	PGE_2	11-deoxy PGE_1	Misoprostol	PGA_1	AH 13205
Superoxide	7.2±0.1 (n=30)	6.1±0.2 (n=7)	6.3±0.4 (n=3)	6.3±0.2 (n=3)	5.5±0.2 (n=7)
cAMP	6.8±0.1 (α =1, n=21)	5.6±0.2 (α =1, n=4)	5.2 (α =1, n=4)	6.3±0.2 (α =0.62, n=3)	5.8±0.1 (α =0.34, n=3)

Analysis of the two sets of data using operational model-fitting (Leff et al, 1990) showed that they differ only in terms of the receptor reserve operating in the two assays. It is concluded, therefore, that the receptors subserving cAMP elevation and superoxide inhibition are the same. Additionally, affinity and efficacy values could be estimated for PGA_1 and AH 13205 demonstrating that cAMP measurement provides a more quantitative analysis of agonist effects than inhibition of superoxide production.

AH 13205 and misoprostol were gifts from Glaxo and Searle respectively.

Hecker, G., Ney, P. & Schror, K. (1990) *Naunyn - Schmiedeberg's Arch Pharmacol* 341, 308 - 315.
Leff, P., Prentice, D.J., Giles, H., Martin, G.R. and Wood, J. (1990) *Journal of Pharmacol Methods* 23, 225 - 237.
Ney, P. & Schror, K.(1991) *Eicosanoids* 4, 21 - 28

2P ASPIRIN IS A MORE POTENT INHIBITOR OF PROSTACYCLIN BIOSYNTHESIS BY ENDOTHELIAL CELLS THAN BY LPS-TREATED J774 MACROPHAGES

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In endothelial and other cells, a constitutive cyclo-oxygenase (COX-1) metabolizes arachidonic acid (AA) to the prostaglandin (PG) endoperoxides PGG2 and PGH2. Prostacyclin (PGI2) synthase then converts PGH2 to PGI2. Lipopolysacharide (LPS) induces human monocytes to express a different isoform of cyclo-oxygenase (COX-2). This induction of COX-2 by LPS requires protein biosynthesis and is prevented by dexamethasone (Fu et al., 1990). Its induction is likely to account for PG release in inflammation. The inhibition of cyclo-oxygenase by aspirin and other non-steroid anti-inflammatory drugs (NSAID) is the basis for their therapeutic activity and shared side effects (Vane, 1971). Here we investigate the relative potencies of aspirin, ibuprofen and sodium salicylate as inhibitors of the conversion of AA to PGI2 in bovine aortic endothelial cells (BAE; COX-1 activity) and LPS-stimulated J774 macrophages (COX-2 activity). J774 cells were cultured to confluence in 96-well plates and incubated with LPS (1 mg/ml) for 10-12 h. Subsequently, the culture medium was removed and fresh medium containing aspirin, ibuprofen or sodium salicylate was added. After 30 min, AA (30 mM) was added and conversion to PGI2 (assayed as 6-keto-PGF1a by radioimmunoassay) was measured 10 min later as an indicator of COX-2 activity. BAE were cultured (de Nucci et al., 1988) to confluence in 96-well plates. Immediately prior to the experiment the culture medium was replaced with fresh medium containing aspirin, sodium salicylate or ibuprofen and the conversion of AA to 6-keto-PGF1a was measured as above an indicator of COX-1 activity.

Different plates of BAE form 2-10 ng of 6-keto PGF1a/ml/10 min in response to AA. Unstimulated J774 macrophages made 0.2-0.5 ng of 6-keto-PGF1a/ml/10 min, whereas J774 macrophages treated with LPS for 12 h released between 1.5-10 ng of 6-keto-PGF1a. Only plates of J774 cells which produced COX-2 activity representing a >4 fold increase were used in this study. Aspirin (IC_{50} of 0.3 mg/ml for BAE; 50 mg/ml for J774 cells) or ibuprofen (IC_{50} of approximately 4 mg/ml for both BAE and J774 cells) dose-dependantly inhibited 6-keto-PGF1a formation by both cell types (n=6-15). Sodium salicylate also inhibited the release of 6-keto-PGF1a equally in BAE and J774 cells but the maximum inhibition at 100 mg/ml was 55±7% in BAE and 50±5 in J774 cells (n=7-11). Interestingly, the concentration of sodium salicylate which produced half maximum effect was 6 mg/ml for BAE and 0.6 mg/ml for J774 cells. Thus, the greater potency of aspirin against COX-1 correlates with its gastric ulcerogenic activity when given in anti-inflammatory (anti-COX-2) doses. The weaker ulcerogenic activities of ibuprofen and salicylate in anti-inflammatory doses reflect their weaker actions on COX-1. The identification of selective inhibitors of different isoforms of COX will lead to clinical advances in the therapy of inflammation.

Fu, J.Y., Masferrer, J.L., Seibert, K., Raz, A. and needlemann, P. (1990). J.Biol. Chem., 265, 16737-16740.
Vane (1971). Nature, 231, 232-235.
de Nucci., G., Grygewski, R., Warner, T.D. and Vane, J.R. (1988). Proc. Natl. Acad. Sci., 85, 9797-9800

3P THE EFFECTS OF CHRONIC FLURBIPROFEN AND L-N^G-NITRO-ARGININE TREATMENT ON NERVE FUNCTION IN NON-DIABETIC AND DIABETIC RATS

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Reduced peripheral nerve conduction velocity in streptozotocin-diabetic rats depends on diminished endoneurial blood flow (Cameron *et al.*, 1991a). Sciatic nerve epi/perineurial vessel prostacyclin synthesis is impaired by reduced substrate availability in diabetes, and there is a deficit in nitric oxide mediated endothelium-dependent relaxation. Prostacyclin production and nerve function may be restored by ω-6 essential fatty acid treatment (Cameron *et al.*, 1991b). Aldose reductase inhibitors also improve nerve function and endothelium-dependent relaxation (Cameron & Cotter, 1992). Thus, it is plausible that endothelial abnormalities contribute to the aetiology of diabetic neuropathy because of diminished perfusion due to deficits in prostacyclin and NO synthesis.

To test whether this hypothesis could result in conduction deficits in the absence of diabetes, groups of mature male Sprague-Dawley rats were treated with flurbiprofen (5 mg kg^{-1} day^{-1} p.o.), L-N^G-nitro-arginine (NOARG) (5 or 25 mg kg^{-1} day^{-1} p.o.), or were given combined treatment for 2 months. *In vivo* measurements (1-1.5 g kg^{-1} urethane anaesthesia i.p.) revealed the following sciatic motor conduction velocity deficits compared to age-matched rats (n=10): 5.5±1.3% (mean ± s.e. mean) for flurbiprofen (n=10) (Analysis of variance and Bonferroni-corrected Student's t-test, $P < 0.01$), 6.0±1.1% ($P < 0.001$) for low-dose NOARG (n=10), 16.9±0.4% ($P < 0.001$) for high-dose NOARG (n=10), 17.2±0.6% ($P < 0.001$) for flurbiprofen + low-dose NOARG (n=14), and 21.9±1.5% for flurbiprofen + high-dose NOARG (n=14) treatments. Saphenous nerve sensory conduction velocity was only significantly reduced by high-dose NOARG (9.8±0.5%, $P < 0.001$), flurbiprofen + low-dose NOARG (9.3±0.9%, $P < 0.001$) and flurbiprofen + high-dose NOARG (11.9±0.5%, $P < 0.001$). The effects of flurbiprofen or low-dose NOARG on nerve conduction were also examined in 2-month streptozotocin-induced (40 mg kg^{-1} i.p.) diabetic rats. For untreated diabetes (n=12), there were 18.6±1.5% ($P < 0.001$) motor and 10.0±2.0% ($P < 0.001$) sensory conduction velocity deficits compared to onset control rats. Flurbiprofen (n=20) or low-dose NOARG (n=12) treatments caused further 13.9±1.3% ($P < 0.001$) or 7.8±1.2% ($P < 0.01$) reductions in sciatic motor conduction but did not significantly affect sensory fibres.

Thus, when the endothelial dysfunction found in diabetes was partly mimicked by pharmacological blockade, similar nerve conduction abnormalities developed in non-diabetic rats. It is possible, therefore, that such microvascular phenomena could contribute to the aetiology of diabetic neuropathy. Nerve function was further compromised by endothelial inhibition in diabetic rats.

We thank the British Diabetic Association, ICI and Scotia for financial support.

Cameron N.E., Cotter M.A. & Low P.A. (1991a) Am. J. Physiol. 261, E1-E8.
Cameron N.E., Cotter M.A. & Robertson S. (1991b) Diabetes 40, 532-539.
Cameron N.E. & Cotter M.A. (1992) Diabetologia 35, 1011-1019.

4P REGULATION OF PROSTACYCLIN SYNTHESIS BY THE RELEASE OF ENDOGENOUS NITRIC OXIDE IN RESPONSE TO BACTERIAL LIPOPOLYSACCHARIDE

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Nitric oxide (NO) and prostacyclin (PGI_2) are released from endothelial cells when intracellular calcium is elevated, for instance, by receptor activation. NO and PGI_2 are formed respectively by constitutive isoforms of NO synthase (NOS1) and cyclooxygenase (COX1). In addition cytokines and/or lipopolysaccharide (LPS) induce the synthesis of a different isoform of NO synthase (NOS2) and cyclooxygenase (COX2). Cells which contain NOS2 release greater amounts of NO than constantly stimulated endothelial cells. Here we have studied the time-course of induction of NOS2 and COX2 in a macrophage cell line and investigated whether NO released endogenously influences the release of PGI_2 or vice versa.

J774 macrophages were cultured in 96-well plates to 80-90% confluence. All drugs were dissolved in distilled water and sterilized by filtration through a 0.2 μ filter. To induce NOS2 and COX2, cells were incubated with LPS (1 μg/ml) for 1-24 h. The release of NO was measured by the accumulation of nitrite in the culture medium, using the Griess reaction adapted for a 96-well plate reader (Gross et al., 1991). The release of PGI_2 was measured by radioimmunoassay for 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$).

LPS caused a time-dependent increase in the accumulation of both nitrite and 6-keto $PGF_{1\alpha}$ in the culture medium. The release of nitrite over 24 h from untreated cells was 1.4±0.3 μM which was increased to 43±4 μM after 24 h incubation with LPS (n=12). Similarly there was a >10 fold increase in the release of 6-keto $PGF_{1\alpha}$ (control, 0.2±0.05 ng/ml; + LPS, 2.5±0.3 ng/ml). Co-incubation with cyclohexamide (1 μM) and LPS reduced the stimulated release of both nitrite and 6-keto $PGF_{1\alpha}$ by 90% (n=3). In separate experiments, J774 macrophages were incubated with LPS for 1-24 h and then fresh culture medium containing arachidonic acid (30 μM) was added and the cells incubated for 10 min at 37°C. COX2 activity was maximum after 12 h of incubation with LPS (2.8±0.1 ng/ml). At 24 h COX2 activity had declined to near control levels (1.25±0.15 ng/ml; n=6; control activity 0.7±0.08 ng/ml). However, in the presence of N^G-monomethyl-L-arginine (MeArg; 10 mM) COX2 activity was still near maximum after 24 h incubation with LPS treatment (2.3±0.25 ng/ml; n=3). MeArg (1 μM-10 mM) or N^G-nitro-L-arginine methyl ester (1 μM-10 mM) caused a concentration-dependent inhibition of nitrite release and a concentration-dependent elevation in 6-keto $PGF_{1\alpha}$ in the medium of J774 macrophages treated with LPS for 24 h (n=9). The formation of 6-keto $PGF_{1\alpha}$ by J774 macrophages treated with LPS was inhibited in a concentration-dependent manner by sodium nitroprusside (SNP; 0.1-1000 μM; n=3) with an IC_{50} value of 1 μM. Inhibition of COX2 by indomethacin (10 μM) had no significant effect on nitrite accumulation in J774 macrophages (n=12).

Thus, LPS stimulates the release of both NO and PGI_2 from cells which normally release very low levels of these two autocoids and this release is dependent upon protein synthesis. The formation of 6-keto $PGF_{1\alpha}$ in response to LPS was potentiated by inhibition of NOS2. Furthermore, increased COX2 (and/or PGI_2 synthetase) activity persisted up to 24 h only in the absence of NO production. These findings suggest that under conditions where NOS2 and COX2 are induced, NO represents an endogenous inhibitor of PGI_2 release. Whether NO inhibits COX2 and/or prostacyclin synthetase at a pre- or post translation level remains to be investigated.

Gross et al. (1991). Biochem. Biophys. Res. Commun., 187, 823-829.

This work was supported by a grant from Glaxo Research Group Ltd. and T.S. is a recipient of a British Heart Foundation grant.

5P DUAL ACTION OF PROSTAGLANDINS (PG's) IN THE MOUSE SKIN OEDEMA TEST

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PGE_1, PGE_2 and PGI_2 have been shown to possess pro-inflammatory effects in the skin (Williams, 1979). This action is believed to be due to the vasodilatory capacity of these substances. Alone, the PG's produce little oedema but when given in combination with agents which increase venular permeability, they have been shown to have a strong synergistic effect. We now report that this effect is stimulus-dependent.

Male Swiss Albino mice (28-32g) were anaesthetised and the backs shaved. Following this 0.05μCi of ^{125}I-labelled human serum albumin in 100μl of 0.5% Evan's blue solution was injected i.v. into each mouse. Platelet activating factor (PAF) and zymosan activated serum (ZAS) were given intradermally either alone or in combination with the natural prostaglandin PGE_1 or either of the two selective EP_3-receptor agonists sulprostone or misoprostol. In the case of PAF blood was collected by intracardiac puncture after 30 min and in the case of ZAS, 2 h. The mice were then killed the skin removed and 6mm sites punched out. Both the skin sites and plasma samples prepared from the collected blood were assessed for radioactivity using a gamma counter. Oedema was expressed as a measure of ^{125}I-labelled albumin extravasation (Williams, 1979).

Both PAF (0.01-5.0 nmol per site) and ZAS (5.0-50.0% per site) caused dose-related plasma extravasation with comparable effects at 1.0 nmol PAF (11.0 ± 0.84 μl of oedema, n=21) and 50% ZAS (10.4 ± 0.57 μl, n=25). PGE_1 (0.3 nmol) potentiated the magnitude of the response to 1.0 nmol PAF (19.1 ± 2.57, n=11, $p<0.01$) but lost this potentiatory effect at a dose of 3.0 nmol (12.0 ± 1.90 μl, n=9). The latter dose of PGE_1 inhibited the response to ZAS 50% by 55% (n=9, $P<0.01$). The EP_3-receptor agonist sulprostone caused a dose-related (0.003 - 3.0 nmol) attenuation of the oedema produced in response to both PAF and ZAS with maximal inhibition of 60% (n=9, $P<0.01$) and 83% (n=7, $P<0.01$) respectively. Misoprostol, the selective EP_2/EP_3-receptor agonist inhibited the PAF response at the lowest dose of 0.03 nmol only (7.45 ± 0.66 μl, n=4, $P<0.01$), while it inhibited the oedema response to ZAS at the 3.0 nmol dose (5.98 ± 1.40, n=8, $P<0.01$). Pretreatment of animals with the thromboxane-receptor antagonist GR32191B (0.1 mg kg^{-1} i.v., -15 min) had no effect upon the inhibitory activity of either PGE_1 (3.0 nmol) or sulprostone (3.0 nmol) upon 50% ZAS-induced oedema.

These results show that PGE_1, in the mouse skin, may potentiate or inhibit oedema formation dependent upon the type of inflammatory stimuli used. Secondly it is clear that PGE-analogues with selective activity for the EP_3-receptor show potent inhibitory activity upon the oedema produced in response to both direct-acting and neutrophil-dependent stimuli.

This work was funded by Ono Pharmaceutical Co. (Osaka, Japan). RJF is funded by an endowment from Lilly, UK.

Williams, T.J. (1979). *Br.J.Pharmacol.*, **65**, 517-524.

6P ROLE OF ENDOGENOUS NITRIC OXIDE IN LEUKOCYTE ACCUMULATION AND OEDEMA FORMATION IN GUINEA-PIG SKIN

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Nitric oxide (NO) is an important mediator in the control of microvascular tone (Moncada et *al*, 1991, Paul et *al*, 1992). Oedema formation, a feature of inflammation, is dependent on the synergism between mediators which alter blood flow and mediators which increase vascular permeability (Williams and Peck, 1977). Leukocyte accumulation is also modulated by changes in blood flow (Issekutz, 1981). In this study, we have investigated the role of endogenous NO on oedema formation and leukocyte accumulation induced by different inflammatory mediators in guinea pig skin.

Accumulation of ^{111}In-labelled leukocytes (eosinophils and neutrophils) and ^{125}I-human serum albumin leakage in response to intradermal (i.d.) injection of inflammatory agents were measured over 2 hours in guinea pig skin as previously described (Teixeira et *al*, 1992). Cell accumulation was expressed as ^{111}In-labelled leukocytes per skin site and oedema formation as μl of plasma per skin site. Blood flow was analysed by a laser doppler flow meter system (Perimed, Sweden) before and 10 minutes after the i.d. injections. Blood flow results were expressed as % increase (+) or decrease (-) from baseline.

Oedema formation induced by bradykinin (Bk, 10^{-10}moles/site) was inhibited in a dose-dependent manner by the NO synthesis inhibitor N_W-Nitro-L-Arginine-Methyl-Ester (L-NAME). For example, a dose of 10^{-8} moles/site of L-NAME inhibited Bk-induced oedema formation by 56.1 ± 3.1% (n=4), whereas a dose of 10^{-6} moles/site gave an inhibition of 89.3 ± 6.0% (n=4). L-arginine (L-arg, dose 100 times that of L-NAME) only partially reversed this effect, but both prostaglandin E_1 (PGE_1, 3 x 10^{-10}moles/site) and sodium nitroprusside (SNP, 10^{-7}moles/site) totally reversed the inhibition caused by 10^{-6} moles/site of L-NAME (Bk, 30.9 ± 1.8 μl; Bk+L-NAME, 17.2 ± 0.6; Bk+L-NAME+PGE_1, 30.6 ± 3.8; Bk+L-NAME+SNP, 33.2± 2.6; n= 7). L-NAME also dose-dependently inhibited oedema formation (up to 73.2%), eosinophil accumulation (up to 46.8%) and neutrophil accumulation (up to 34.0%) induced by zymozan-activated plasma (ZAP 30% in saline). PGE_1 and NPS also reversed the inhibition of ZAP-induced oedema formation or neutrophil accumulation by L-NAME (10^{-6} moles/site). However, only NPS was able to reverse the inhibition of ZAP-induced eosinophil accumulation (ZAP+saline, 12813 ± 741 ^{111}In-labelled eosinophils/site; ZAP+L-NAME, 6814 ± 574; ZAP+L-NAME+SNP, 15051 ± 1308; ZAP+L-NAME+PGE_1, 9539 ± 539; n=4). Blood flow experiments showed that L-NAME inhibited basal blood flow by 47.34 ± 6.74% (n=6). PGE_1 or NPS also reversed this effect (PGE_1+L-NAME, +0.68 ± 14.50% basal flow; SNP+L-NAME, -2.86 ± 9.53%). Bk did not cause an increase of blood flow above baseline.

These results demonstrate the flow dependence of cell accumulation in guinea pig skin and suggest an important role for basal NO production in the control of microvascular tone in this species.

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

Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1991) *Pharmacol. Rev.* 43:109-142.
Paul, W., Lawrence, L., Douglas, G.J., Schachter, M. and Page, C. (1992) *Br.J.Pharmacol.* 107: 287p.
Williams, TJ and Peck, MJ (1977) *Nature*, 270:530-532.
Issekutz, A.C. (1981) *Lab. Invest.* 45(3): 234-240.
Teixeira, M.M., Williams, T.J. and Hellewell, P.G. (1993) *BPS Cambridge* C12.

7P INSENSITIVITY OF MESENTERIC SMALL VEINS TO NITROVASODILATORS AND ENDOTHELIUM-DERIVED RELAXING FACTOR

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Nitrovasodilators (the organic nitrates, nitrites and nitroprusside) are regarded as arterial- and venodilators, whose actions in smooth muscle (the liberation of nitric oxide, activation of guanylate cyclase and accumulation of cyclic GMP) are identical to those of endothelium-derived relaxing factor (EDRF). 60-80% of blood volume resides in the venous system: 25-50% of this in veins of < 1mm diameter. Pooling in these veins might therefore contribute substantially to the haemodynamic effects, including orthostatic hypotension, seen clinically with nitrate administration. Our aim was to study the dilator effects of glyceryl trinitrate (GTN), sodium nitrite (SNI), sodium nitroprusside (SNP) and the endothelium-dependent relaxants, acetyl choline (ACh), pilocarpine (PC), bradykinin (BK) and the calcium ionophore, A23187 in isolated small veins.

Male Sprague-Dawley rats (270-450g) were anaesthetized by ketamine and xylazine. 1-2mm long segments were excised from jejunal mesenteric veins accompanying the 3rd and 4th order arteries, placed in oxygenated physiological salt solution (PSS) at 37°C, pH 7.4, containing indomethacin 49μm, and cannulated in a small vessel arteriograph (Living Systems Instrumentation, Burlington, VT, USA). Intraluminal pressure was maintained, without flow, at 3-4mmHg by a pressure servo unit. Lumen diameter was measured by a Video Dimension Analyzer coupled to a MacLab data acquisition system (AD Instruments, Castle Hill, NSW, Australia) and MacIntosh PC. Drugs were added cumulatively to the circulating extraluminal perfusate. Veins (diameters 110-430μm, mean 275μm) were submaximally precontracted by 30mM potassium PSS (K30: by a mean of 68.2%, sd 5.5, n=11) or by phenylephrine (PE) 7×10^{-7}M (mean 41.2%, sd 12.6, n=44). 2-3 experiments were carried out per vessel in 22 animals. Dilators, in random order, were tested at 10^{-8} to 10^{-5}M, except BK (10^{-10} to 5×10^{-6}M).

K30 and PE contractions were sustained and reproducible over 60-160 min. No dilator responses were seen with GTN (n=20), SNI (n=4), ACh (n=16), PC (n=2), A23187 (n=1) or BK (n=1). In 7 of 11 experiments with SNP the lowest dose was followed by a gradual (20-30 min) loss of tone. No stepwise concentration-response was seen.

Mesenteric small veins *in vitro* show significant active myogenic responses to potassium depolarization and to α-adrenergic activation, but are insensitive to the nitrovasodilators and to endothelium-derived relaxing factor. Relaxation in these vessels may be accomplished by the withdrawal of active tone.

8P CHAPS ATTENUATES BOTH ENDOTHELIUM-DEPENDENT DILATATION AND RESPONSE TO VASOCONSTRICTORS IN THE PERFUSED SUPERIOR MESENTERIC ARTERIAL BED IN THE RAT

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The zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate (CHAPS) removes vascular endothelium, as shown histologically by silver staining (Poole *et al.*, 1958), and attenuates the endothelium-dependent dilatation induced by acetylcholine (ACh) (Hiley *et al.*, 1987). However, it is not known whether CHAPS also damages the underlying vascular smooth muscle, thus affecting responses to vasoconstrictors. The present *in vitro* experiments therefore, examined whether different durations of exposure to CHAPS affected responses to the vasoconstrictors noradrenaline (NA), methoxamine (MTX), arginine vasopressin (AVP) and potassium chloride (KCl), as well as to the vasodilator ACh, in the rat mesenteric arterial bed.

Male Wistar-Han rats (200-400g) were anaesthetised (sodium pentobarbitone; 60mg/kg) and heparinised (1000u/kg) with a combined intraperitoneal injection. The mesenteric arterial bed was perfused according to the protocol of McGregor (1965), at a constant rate of 2 ml/min, with oxygenated Krebs-Henseleit solution (95% O_2:5% CO_2) maintained between 36.5 and 37.5°C. Pressure was measured using a transducer inserted into the perfusion circuit close to the cannula. Following 30 min equilibration, 3 bolus doses of NA (30nmol) were given to assess viability. Responses were then recorded to bolus (100µl) injections of NA, MTX, AVP and a constant infusion of KCl, followed by bolus injections of ACh in the presence of a continuous infusion of 0.1mM MTX, before and after perfusion with Krebs (control), or 0.3% w/v CHAPS for 15, 30 & 45s. Doses of drugs and results are shown in Table 1.

Table 1. Effects of perfusion with CHAPS, shown as mean increase (+) or decrease (-) in pressure (mmHg) ± s.e.mean, n=6 or 4(†)

DRUG	DOSE	CONTROL	15s CHAPS	30s CHAPS	45s CHAPS	
NA	0.1µmol	+62±6	+76±11	+54±9	+35±4**	*p<0.05,
MTX (bolus)	0.1µmol	+60±4	+71±8†	+51±15†	+18±2**†	**p<0.01,
AVP	5.0nmol	+121±9	+131±10	+93±9	+68±11**	(unpaired t-test
KCl	120mmol	+121±10	+112±11	+95±4*	+67±19**	compared to control)
MTX (infusion)	0.1mmol	+60±6	+73±12	+44±7	+50±7 .	
ACh	3nmol	-23±4	-17±2	-14±2*	-6±1**	

Basal perfusion pressures,11±1, 13±2, 14±2 & 11±3 mmHg, were not affected by treatment with CHAPS for 0, 15, 30 & 45s respectively. Responses to the vasoconstrictors were unaltered after exposure to 15s CHAPS. The response to KCl was attenuated by 23%, but other vasoconstrictors were unaffected, by 30s CHAPS. Responses to NA, MTX, AVP & KCl were attenuated by 41, 70, 44 & 44% respectively with 45s CHAPS. The responses to MTX infusion were not affected even after 45s exposure to CHAPS. Perfusion with CHAPS for 15, 30 & 45s attenuated the ACh responses by 26, 40 & 76% respectively. Histological studies confirmed that CHAPS duration-dependently removed the endothelium. Exposure to 45s CHAPS was sufficient to completely removed the endothelium.

The degree to which 0.3% w/v CHAPS attenuates the vasodilation to ACh depends on the duration of exposure, probably reflecting removal of the endothelium. However, administration of CHAPS for more than 30s can substantially attenuate the responses to a wide range of vasoconstrictors and may reflect damage to the underlying vascular smooth muscle. These findings emphasise that the duration of exposure to CHAPS must be precisely monitored when using this agent to de-endothelialise the rat mesenteric arterial bed, particularly when vasoactive agents are administered as bolus injections.

Hiley, R. *et al.*, (1987). *Br. J. Pharmacol.* 21, 378P.
McGregor, D.D. (1965. *J. Physiol.* (Lond.) 177, 21-30.
Poole, J.C.F. *et al.*, (1958). *J. Pathol. Bacteriol.* 75, 133-143.

IDR is the recipient of an MRC studentship.

9P AMIDES AND ESTERS OF N$^{\omega}$-NITRO-L-ARGININE (e.g. L-NAME) MUST BE HYDROLYZED TO BECOME ACTIVE NO SYNTHASE INHIBITORS

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Esters and amides of N$^{\omega}$-nitro-L-arginine (NO_2-ARG), such as the methyl ester (NAME), have been used as specific inhibitors of nitric oxide synthase (NOS). Furthermore, Babbedge et al. (1992) have suggested that the NOS isoform selectivity of the p-nitroanilide derivative (NAPNA) differs from that of the parent molecule. Nevertheless, there have been no reports defining the ability of NO_2-ARG amides or esters to inhibit NOS directly. Since amides and esters are susceptible to enzymic hydrolysis, yielding free NO_2-ARG - a potent NOS inhibitor by itself - such studies require highly purified NOS. For this purpose, NOS from cytokine-activated smooth muscle cells (SMC) of the rat (Gross and Levi, 1992), was purified to >95% by ion exchange chromatography on DEAE-dextran followed by affinity chromatography with 2',5'-ADP-sepharose. Purified NOS was incubated with test agents and cofactors (0.5 mM L-arginine, 0.5 mM NADPH and 10 µM tetrahydrobiopterin in 80 mM TRIS; pH 7.6) and NOS activity was quantified spectrophotometrically using a kinetic assay based on the oxidation of Fe^{2+}-myoglobin by NO (Gross and Levi, 1992). The hydrolysis of NAPNA to NO_2-ARG was measured spectrophotometrically, based on the absorption of released p-nitroaniline at 405 nm.

	INHIBITION OF NOS (% of control; mean ± SEM, n=3)							
ANALOG (µM)	PURIFIED NOS				PURIFIED NOS + CYTOSOL			
	10	30	100	300	10	30	100	300
NO_2-ARG	12.5±1.8	29.9±0.7	53.3±4.6	77.5±0.4	11.7±2.7	28.2±1.3	54.0±0.7	77.3±0.5
NAME	2.9±1.7	8.0±8.0	6.3±2.5	0.9±0.3	10.2±1.5	21.7±2.0	42.7±2.2	64.4±1.9
NAPNA	5.6±2.4	1.0±0.9	2.6±1.0	1.8±1.6	0.7±1.3	21.1±7.9	44.5±0.5	66.0±0.2

The table above compares the efficacy of NO_2-ARG, NAME and NAPNA as inhibitors of purified NOS, before and after a 30 min exposure to NOS-free cytosol (380 µg protein, prepared as the 100,000 x g supernatant of untreated SMC). Whereas NO_2-ARG inhibits purified NOS equally well, before and after exposure to cytosol (EC50 = 90 µM), NAME and NAPNA inhibit NOS only after exposure to cytosol. Inhibition of NOS by NAPNA was found to increase with the duration of pre-exposure to cytosolic protein; EC50 values approached, but did not exceed, that found with NO_2-ARG. Hydrolysis of NAPNA to free NO_2-ARG, undetectable during a 90 min incubation with purified NOS, proceeded linearly in the presence of cytosol at a rate proportional to the concentrations of cytosolic protein and NAPNA (650 pmol/min/mg protein for 1 mM NAPNA). A plot of percent inhibition of NOS vs. the concentration of NO_2-ARG produced from NAPNA (n = 48) was indistiguishable from that for inhibition of NOS by authentic NO_2-ARG. Thus, the degree of inhibition by NAPNA at these concentrations could be fully accounted for by hydrolysis to NO_2-ARG. Nevertheless, inhibition of purified NOS by NAME and NAPNA could be detected with much higher concentrations of these agents (EC50 ≈ 3 mM and 10 mM, respectively). The present studies demonstrate that NAME and NAPNA are potent NOS inhibitors because they are prodrugs for NO_2-ARG. As the potency of NAPNA for inhibiting NOS in brain, relative to endothelium, can be attributed to enhanced NAPNA hydrolysis (Bishop-Bailey et al., this meeting), perhaps such prodrugs can be designed to target NOS inhibitors to specific tissues based on differences in the distribution of hydrolyzing enzymes.

Babbedge, R.C. et al., (1992). Br. J. Pharmacol., **107**, 233.
Gross S.S. and Levi, R, (1992) J. Biol. Chem., **267**, 25722-25729.

10P THE INHIBITORY EFFECTS OF HYDROQUINONE ON NITRIC OXIDE-INDUCED RELAXATION OF THE MOUSE ANOCOCCYGEUS ARE PREVENTED BY NATIVE THIOLS

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While there is little doubt that nitric oxide (NO) is involved in non-adrenergic, non-cholinergic (NANC) relaxations in the mouse anococcygeus, certain experimental observations suggest that the NO may be 'protected' in some way within the synaptic gap; for example, the free radical scavenger hydroquinone reduces responses to NO but has no effect on NANC relaxations (Hobbs et al., 1991). Chemical incorporation of NO into several S-nitrosothiol compounds affords protection from hydroquinone (Gibson et al., 1992). However, in this study we have investigated whether native thiols, by themselves, might also act to protect NO.

Anococcygeus muscles from male mice (LACA; 25-35g) were set up for the recording of isometric tension responses to drugs and NANC stimulation (5Hz; 50V; 10s), and NO solutions prepared, as described previously (Gibson et al., 1992). Muscle tone was raised with carbachol (50µM). Under these conditions, NO (15µM) reduced tone by 56±3% (n=27) and NANC stimulation reduced tone by 57±4% (n=30). The thiols studied were glutathione (GSH), cysteine (CYSH) and coenzyme A (CoASH). Results were expressed as mean ± s.e.; statistical evaluation was by Student's t test.

As shown previously (Hobbs et al., 1991), hydroquinone (100µM) reduced responses to NO (15µM) by 73±4% (n=55), but had no effect on NANC relaxations. All 3 thiols produced a concentration-related (1-10µM) protection of NO against hydroquinone, such that, in the presence of 10µM GSH, CYSH and CoASH, hydroquinone reduced NO-induced relaxations by only 11±4% (n=14), 2±1% (n=8) and 6±2% (n=6) respectively. By themselves, GSH (100µM), CYSH (100µM) and CoASH (10µM) had no effect on relaxations to either NO or NANC stimulation. The concentration of CoASH used was restricted to 10µM, since above this the thiol tended to reduce carbachol-tone.

Oxidised glutathione (GSSG; 100µM) did not protect NO from hydroquinone (58±11% inhibition of NO in the absence, and 68±11% in the presence of GSSG; n=10 in both cases; P>0.05). Similarly, the protective effects of GSH, CYSH and CoASH were lost in the presence of the thiol-oxidising agent diamide (100µM).

Thus, GSH, CYSH and CoASH protected NO from inhibition by hydroquinone; the similar potencies of the three compounds and the lack of protection afforded by oxidised species (GSSG, or the thiols in the presence of diamide) indicates that the thiol group is responsible for the protective effect. Whether the thiols interact with the NO to protect it from hydroquinone, or act as 'guardians' by preferentially attacking the hydroquinone, is yet to be determined. However, if thiols are present in sufficient concentrations in the synapse this might explain the lack of effect of hydroquinone on NANC relaxations.

The support of the MRC is appreciated.

Gibson, A., Babbedge, R., Brave, S.R., Hart, S.L., Hobbs, A.J., Tucker, J.F., et al., (1992) *Br. J. Pharmacol.*, 107, 715-721.
Hobbs, A.J.,. Tucker, J.F. & Gibson, A. (1991) *Br. J. Pharmacol.*, 104, 645-650.

11P AN ANTAGONIST OF PLATELET-ACTIVATING FACTOR (WEB 2086) INHIBITS THE INDUCTION OF NITRIC OXIDE SYNTHASE BY BACTERIAL LIPOPOLYSACCHARIDE

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Antagonists of platelet-activating factor (PAF) including the hetrazepine WEB 2086 (Casals-Stenzel, 1987) have protective effects in circulatory shock induced by endotoxin (bacterial lipopolysaccharide, LPS). This is partially related to their ability to inhibit the release and action of various cytokines triggered by LPS. The circulatory failure in endotoxin shock is associated with an induction of nitric oxide synthase (NOS) resulting in an enhanced formation of nitric oxide (NO) in the vasculature. Here we have investigated whether WEB 2086 influences the induction of NOS by LPS in vitro and in vivo.

J 774.2 macrophages were cultured in 96-well plates to 80-90% confluence. Cells were incubated with LPS (1 μg/ml for 24 h). Nitrite accumulation was measured by the Griess reaction using a 96-well plate reader (Gross et al, 1991). Cell respiration was measured using the MTT colorimetric assay (Gross et al, 1991). Mean arterial blood pressure (MAP) and heart rate were measured via a carotid arterial cannula in rats anaesthetized with sodium thiopental (120 mg/kg i.p.). LPS (10 mg/kg; i.v. bolus) was administered in vehicle-treated control rats (n=8) and in rats pretreated with WEB 2086 (5 mg/kg i.v., 20 min prior to LPS). At 60 and 180 min after LPS, pressor responses to noradrenaline (NA, 1 μg/kg i.v.) were obtained to measure hyporeactivity. Calcium-dependent (constitutive) and calcium-independent (induced) NOS activity was measured by the conversion of ^{3}H-arginine to ^{3}H-citrulline in lung homogenates ex vivo (Mitchell et al, 1991).

Pretreatment of J774.2 macrophages with WEB 2086 (10^{-12} - 10^{-8} M) for 30 min inhibited nitrite accumulation stimulated by LPS, without affecting cell respiration. Maximal inhibition (35±5%) was observed at 10^{-9} M (n=6, $p<0.01$). When added 120 min after LPS, WEB 2086 did not affect nitrite accumulation.

LPS caused a fall in MAP (from 127±5 to 71±5, 77±10 and 61±7 mmHg, at 5, 120 and 180 min, respectively, $p<0.01$; n=8). Pressor responses to NA were significantly reduced (from 33±3 to 18±2 and 17±2 mmHg at 60 and 180 min; n=8). Heart rate increased from 434±19 (control) to 506±10 beats/min at 180 min ($p<0.01$, n=8). At 180 min there was a significant elevation of the induced NOS activity in the lung (154±7 pmol citrulline/mg/20 min, n=5). Animals pretreated with WEB 2086 maintained higher MAP (the corresponding values at 5, 60 and 180 min after LPS were 106±5 to 102±2, and 98±6 mmHg; $p<0.05$ when compared to LPS-control, n=9), and exhibited more pronounced pressor responses to NA at 180, but not at 60 min (n=9). LPS, however, caused similar increase in the heart rate in the WEB 2086 treated rats as in LPS-controls (from 414±6 to 512±25 beats/min at 180 min; n=9). WEB 2086 treatment reduced the activity of the induced NOS in the lung by 23±7% n=5; $p<0.05$). When added in vitro to the lung homogenates, WEB 2086 (10^{-12} - 10^{-6} M) did not directly affect the activity of the induced NOS (n=3).

Thus, WEB 2086 attenuates NOS induction in vitro and in vivo, suggesting that PAF is an endogenous mediator of NOS induction by LPS. (This work was supported by a grant of Glaxo Group Research Ltd.)

Casals-Stenzel J. (1987) Eur. J. Pharmacol. 135, 117
Gross, S.S., Jaffe E.A., Levi R. & Kilbourn R.G. (1991) Biochem. Biophys. Res. Comm. 187, 823
Mitchell J.A., Sheng H., Forstermann U. & Murad F. (1991) Br. J. Pharmacol. 104, 289

12P INHIBITORS OF GTP CYCLOHYDROLASE CAUSE SUPERINDUCTION OF NITRIC OXIDE SYNTHASE BY IMMUNOSTIMULANTS IN AORTIC SMOOTH MUSCLE CELLS

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Tetrahydrobiopterin (BH4) is a cofactor of all known isoforms of nitric oxide synthase (NOS). The *de novo* synthesis of BH4 is via an enzymic pathway whose first and rate-limiting step is catalysed by GTP cyclohydrolase (GTPCH) and selectively inhibited by 2,4-diamino-6-hydroxypyrimidine (DAHP). Additionally, BH4 can be produced from pre-existing dihydropterins by a salvage pathway; the final step of this pathway is catalysed by dihydrofolate reductase (DHFR) and blocked by methotrexate (MTX). Induction by LPS of NOS in aortic smooth muscle cells (SMC) of the rat is concomitant with induction of BH4 synthesis; each event is required for NOS expression (Gross and Levi, 1992). While BH4 synthesis inhibitors are known to attenuate LPS-induced NO synthesis by SMC, we have now investigated the effect of these agents on induction of NOS enzyme, *per se*.

NOS was induced in SMC in culture by a combination of LPS (30 μg/ml) and interferon-γ (IFN; 50 ng/ml), alone, and in the presence of drugs which alter intracellular BH4 levels. Cytosolic NOS activity was quantified by the increase in A_{405} resulting from the oxidation of Fe^{2+}-myoglobin by NO (Gross et al., 1991). Optimal concentrations of L-arginine and NADPH were present (0.5 mM each) to ensure that NOS activity would be proportional to NOS protein concentration (Gross et al., 1991). Nitrite was assayed as a marker for NO production by intact SMC. As shown below, the production of NO by intact SMC was markedly reduced by DAHP and MTX (values are means ± SEM after 24 hr treatment, n = 4) . Predictably, cytosol prepared from these BH4-depleted cells contained insignificant NOS activity when assayed without added BH4. Nonetheless, remarkably high levels of NOS were detected after addition of exogenous BH4 (> 400% of that found in cells treated with LPS/IFN alone).

	Nitrite production by SMC (% of LPS/IFN stimulated)		Cytosolic NOS activity (nmoles/min/mg protein)	
TREATMENT	- BH4	+ BH4	-BH4	+BH4
LPS/IFN	100	115.0 ± 2.4	1.06 ±0.13	1.35 ±0.10
LPS/IFN + DAHP/MTX	28.9 ±2.2	20.7 ± 0.8	0.23 ± 0.8	5.89 ± 0.

BH4 = 10 μM. LPS/IFN = 30 μg/ml and 50 ng/ml, respectively. DAHP/MTX = 3 mM and 10 μM, respectively.

Studies of the timecourse of NOS induction by LPS/IFN, revealed "superinduction" with DAHP/MTX at all timepoints (6 - 24 hr post-LPS/IFN), with a peak effect at ≈12 hr. Superinduction was also seen with DAHP alone, but not with MTX, suggesting that inhibition of GTPCH, but not DHFR, is responsible. Superinduction was not a consequence of BH4 depletion, since it did not occur with N-acetylserotonin, an agent which inhibits BH4 synthesis and NO production by blocking the final step of *de novo* BH4 synthesis. Moreover, superinduction of NOS *was* observed with another GTPCH blocker, sepiapterin (SEP), which increases both intracellular BH4 and production of NO by intact SMC. Although SEP is a potent GTPCH inhibitor, it is also a precursor of BH4 via the pterin salvage pathway, thereby eliciting a large increase in intracellular BH4 (Gross and Levi, 1992). Since increased gene transcription is the only mechanism known to upregulate inducible NOS activity, our findings suggest that GTPCH inhibitors potentiate cytokine-induced NOS transcription in SMC. (This work was supported by the GLAXO Group Reasearch Ltd. and NIH grant HL46403).

Gross S.S. and Levi, R.L., (1992) J. Biol. Chem., **267**, 25722-25729.
Gross et al., (1991) Biochem. Biophys. Res. Commun., **178**, 823-829.
Stuehr et al., (1991) Procs. Natl. Acad. Sci. (U.S.A)., **88**, 7773-7777.

13P *IN VIVO* INCREASE OF PROSTAGLANDIN H_2 SYNTHASE (CYCLO-OXYGENASE) AND NITRIC OXIDE SYNTHASE ACTIVITY BY BACTERIAL LIPOPOLYSACCHARIDE

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Nitric oxide (NO) and prostacyclin (PGI_2) are synthesised in endothelial cells (EC) by the constitutive calcium-dependent NO synthase (NOS) and prostaglandin H_2/prostacyclin synthases, respectively. They exert potent vasodilator and anti-thrombotic activities. Many cells, when stimulated with cytokines or endotoxins, express a distinct calcium-independent form of NOS (Stuehr et al., 1991). Recently, a novel, inducible isoform of prostaglandin H_2 synthase (cyclo-oxygenase, COX2) was described in cultured macrophages (Fu et al., 1990). Here we have investigated the possibility that bacterial lipopolysaccharide (LPS) induces COX2 *in vivo*.

Male Wistar Rats were injected intraperitoneally (i.p.) with LPS (5 mg/kg) to induce endotoxaemia. At 0, 6 and 24 h, 4 rats were killed and the spleens and hearts removed, frozen in liquid nitrogen and stored at -80°C until measurements of NOS and COX activity were performed. NOS activity was measured by the ability of organ homogenates to convert ^{3}H-L-arginine (L-Arg) to ^{3}H-L-citrulline (L-Cit: Mitchell et al., 1991). COX activity was measured by the ability of organ homogenates to metabolise arachidonic acid (AA) to PGI_2 (measured as 6-keto-prostaglandin $F_{1\alpha}$: 6-keto $PGF_{1\alpha}$), thromboxane B_2 (TXB_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), as determined by radioimmunoassay.

NOS and COX activity increased in a time-dependent manner after LPS administration, peaked at 6 h and returned to near basal levels at 24 h (see table 1; results as mean ± s.e.m.; n=3).

TABLE 1.	SPLEEN			HEART		
Time (h)	control (0)	6	24	control (0)	6	24
6-keto $PGF_{1\alpha}$ (ng $30min^{-1}g^{-1}$)	630±60	2330±210	835±140	610±10	1430±115	685±55
TXB_2 (ng $30min^{-1}g^{-1}$)	2093±50	6235±155	2884±213	37±20	71±26	81±59
$PGF_{2\alpha}$ (ng $30min^{-1}g^{-1}$)	263±14	730±32	500±21	39±5	64±0.1	41±0.6
L-Cit (pmoles)	805±39	10060±373	2515±426	707±97	1738±200	1258±70

Similar results were obtained using homogenates from 2-3 other animals. The COX activity in the spleen and the heart was abolished when samples of organ homogenates were pretreated with indomethacin (10 μM) or boiled before adding AA (30 μM). Similarly, the NOS activity in the spleen and the heart was abolished when samples were pretreated with N^G-monomethyl-L-arginine (1 mM) or boiled prior to the assay. The metabolism of AA was time-dependent with an optimal time of incubation of 30 min.

Thus the activity of both COX2 and NOS2 increases in parallel following the administration of LPS *in vivo*.

Fu et al. (1990). J. Biol. Chem., 265, 16737-16740.
Mitchell et al. (1991). Br. J. Pharmacol., 104, 289-291.
Stuehr et al. (1991). J. Biol. Chem., 266, 6259-6263.

This work was supported by a grant from GLAXO and Dr Tomcsz Swierkosz is a recipient of a grant from the British Heart Foundation.

14P EFFECT OF THE PROSTANOID EP_2-RECEPTOR AGONIST, AH13205, ON AIRWAY RAPIDLY-ADAPTING STRETCH RECEPTORS IN THE CAT

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Inhalation of AH13205, a selective prostanoid EP_2-receptor agonist (Nials et al, 1991) which was developed as a potential bronchodilator, has been shown to evoke airway irritancy in normal volunteers and cough in mild asthmatics (Nials et al, 1993). The irritant properties of AH13205 could be the result of stimulation of airway sensory nerves. In the present study, we have examined the effects of inhaled AH13205 on airway rapidly adapting stretch receptors (RARs) in cats.

Cats (n=6) were anaesthetised (chloralose, 60-80mg.kg^{-1}, i.v.), paralysed with dimethyl tubocurarine and artificially ventilated. Impulse discharges from isolated vagal fibres arising from airway RARs were recorded using conventional electrophysiological techniques. Aerosols were administered to the lower airways via the respiration pump (6 breaths) using an ultrasonic nebulizer (Devilbis Pulmosonic). Histamine, administered intravenously (10mg.kg^{-1}) and by aerosol (1mg.ml^{-1} solution) stimulated RARs in all six animals (D increase in impulses).s^{-1} from baseline levels, 2.5; 0.99imp.s^{-1} and 2.09; 0.73imp.s^{-1} respectively).

Administration of AH13205 (1mg.ml^{-1} solution) had no effect on the spontaneous discharge of 3 RARs (D increase in imp.s^{-1}, 0.52; 0.27) compared with vehicle (0.60 ; 0.27 imp.s^{-1}). In contrast, AH13205 at 3mg ml^{-1} stimulated 2 out of the 3 RARs examined (D increase in imp.s^{-1}, 1.1; 0.69). In the same fibres (n=3), sequential inhalation of PGE_2 (0.01 and 0.1mg ml^{-1}) increased the spontaneous discharge of the RARs in a dose-dependent manner (D increase in imp.s^{-1} from baseline levels being, 1.5; 0.61 and 2.7; 0.92 respectively). Inhalation of PGE_2 (1mg ml^{-1} solution) also increased the spontaneous discharge of all six fibres (D increase in imp.s^{-1} being 3.15; 0.72).

In these studies therefore, inhaled PGE_2 caused dose-related stimulation of RARs in cats, and in 2/3 animals, AH13205 had similar activity at doses in the same range as those causing airways irritancy in man. Furthermore, AH13205 appears to be at least 30 times less potent than PGE_2, which is similar to its observed potency as an EP_2-receptor agonist (Nials et al, 1991). In conclusion, therefore, EP_2-receptors appear to be involved in the prostanoid-induced stimulation of airway RARs in the cat, a mechanism which could account for the irritant effects of inhaled prostanoids in man.

Nials, A.T. Coleman, R.A., Hartley, D. & Sheldrick, R.L.G. (1991). Br. J. Pharmacol. Proc. Suppl., 102, 24P.
Nials, A.T., Vardey, C.J., Denyer, L.H., Thomas, M., Sparrow, S.J. Shepherd, G.D. & Coleman, R.A. (1993). Cardiovasc. Drug Rev. (in press).

15P EVIDENCE THAT INHIBITION OF HISTAMINE-INDUCED PLASMA PROTEIN EXTRAVASATION BY SALMETEROL IN GUINEA-PIG LUNG IS NOT SUBJECT TO TACHYPHYLAXIS

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In man, repeated administration of salbutamol or salmeterol by inhalation leads to desensitization to the cardiovascular side-effects of these drugs, but not to their bronchodilator actions (Lipworth *et al.*, 1989; Minton, *et al.*, 1991). More recently, however, it has been suggested that some of the potentially beneficial, non-bronchodilator effects of β-adrenoceptor agonists may also be subject to desensitization (O'Connor *et al.*, 1992). We have previously shown that inhaled salmeterol or salbutamol inhibits vascular permeability in guinea-pig lung (Whelan and Johnson, 1992), and in the present study, we have investigated whether there is evidence of tachyphylaxis to this effect of salmeterol in guinea-pig lung.

Male guinea-pigs (300-350g) were exposed to salmeterol (nebuliser conc. 100μg.ml^{-1}) twice daily for 3 min for 4 days. On day 5, the inhibitory effects of inhaled salmeterol (10 and 100μg.ml^{-1}) on histamine (Hist)-induced plasma protein extravasation (PPE) were determined as described previously (Whelan and Johnson, 1992), and compared with those in vehicle-treated animals. When vehicle-treated guinea-pigs were challenged with Hist, the plasma content of the recovered bronchoalveolar lavage fluid (BALF) increased from 0.66 (95% C.L. 0.50-0.87, n=11) to 6.74 (3.58-12.67, n=11) μl plasma.ml^{-1}. Pretreatment of vehicle-treated guinea-pigs with a single exposure of salmeterol (10 and 100μg.ml^{-1}) for 3 min significantly ($P<0.05$) reduced Hist-induced PPE to 1.82 (1.00-3.32, n=6) and 1.07 (0.80-1.43, n=8) μl plasma.ml^{-1} BALF respectively. Similarly, in animals which had been treated with salmeterol for 4 days, salmeterol (10 and 100μg.ml^{-1}) significantly ($P<0.05$) reduced Hist-induced PPE to 0.93 (0.45-1.91, n=8) and 1.08 (0.78-1.50, n=8) μl plasma.ml^{-1} BALF, respectively. There was no significant difference ($P>0.05$) in the inhibitory potency of salmeterol between groups which had been treated with the β_2-adrenoceptor agonist for 4 days and vehicle-treated animals. Furthermore, in animals which had been treated with salmeterol (100μg.ml^{-1} b.d.) for 4 days, but received no salmeterol on day 5, Hist-induced PPE was not significantly different from that seen in control animals ($P>0.05$).

These data show that repeated administration of even high doses of inhaled salmeterol, does not result in tachyphylaxis to the inhibition of vascular permeability in guinea-pig airways.

Lipworth, B.J. *et al.*, (1989). Am. Rev. Respir, Dis., 140, 586-592.
Minton, N.A. *et al.*, (1991). Br. J. Clin. Pharmacol., 31, 241-242P.
O'Connor, B.J. *et al.*, (1992). New Eng. J. Med., 327, 1204-1208.
Whelan, C.J. and Johnson, M. (1992). Br. J. Pharmacol., 105, 831-838.

16P DIFFERENT EFFECTS OF TWO ANTI-CD18 ANTIBODIES ON ANTIGEN-INDUCED AIRWAY HYPERRESPONSIVENESS AND CELL ACCUMULATION IN GUINEA-PIGS

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Asthma is characterised by airway hyperresponsiveness to spasmogens correlated with increased numbers of eosinophils in bronchoalveolar lavage fluid (BALF) (Wardlaw et al. 1988). In the airway, leukocyte integrins of the CD18 family are involved in the adhesion of leukocytes to both endothelium and epithelium via intercellular adhesion molecule-1 (ICAM-1). In primates, antibodies to ICAM-1 reduced both bronchial hyperresponsiveness and eosinophil infiltration of the lung (Wegner et al. 1990). In other experimental models of asthma, involvement of eosinophils in the generation of bronchial hyperresponsiveness is less clear. We have used two anti-CD18 antibodies, R15.7 and 6.5E, to study the importance of eosinophils in mediating bronchial hyperresponsiveness in a guinea-pig model of antigen-induced airway inflammation.
Guinea-pigs were sensitised to ovalbumin over two weeks and challenged with an aerosol of ovalbumin on day 15. 24hrs later, the animals were anaesthetised and airway resistance (R) in response to acetylcholine (Ach) was measured. Lungs were lavaged (40ml buffer) and a total and differential count made of cells in BALF. Animals treated in this way were hyperresponsive to Ach (3 fold change in EC_{50}), and had increased total cells (3 fold) and eosinophils (16 fold) in BALF. The anti-CD18 antibodies R15.7 or 6.5E, or a control antibody MOPC21, were given i.v. 1h pre challenge and again 4h post challenge, at either 1 mg/kg or 3 mg/kg.

Treatment Group		Increase in R with 10ug/kg Ach ($cmH_2O/l/s$)	Number of cells in BALF ($X10^6$/ml)	
1 mg/kg	n		Total	Eosinophils
R15.7	4	933±164	28.0±2.3	11.5±1.8
6.5E	4	900±254	30.0±10.8	8.7±4.6
MOPC21	4	610±130	34.7±5.8	9.2±3.0
3 mg/kg				
R15.7	7	264±30*	28.1±5.8	5.4±1.6*
6.5E	5	868±98	21.8±2.8	3.3±0.8*
MOPC21	5	661±128	41.2±9.3	16.1±4.8

Values shown are mean±s.e.mean. *$p<0.05$, significantly different from MOPC21.

MOPC21 had no effect on bronchial hyperresponsiveness or numbers of cells in BALF compared to untreated animals. Both CD18 antibodies reduced eosinophil numbers equally at the higher dose (3 mg/kg), but whereas R15.7 also reduced hyperresponsiveness, 6.5E was ineffective. These results suggest that increased numbers of eosinophils are not an essential correlate of bronchial hyperresponsiveness in this model.

Wardlaw A.J. et al. Am Rev Respir Dis, 137:62-69, 1988.
Wegner C.D. et al. Science, 247:456-459, 1990.

17P BINDING CHARACTERISTICS OF THE DELTA ANTAGONIST (1,5'-^{3}H)NALTRINDOLE IN RAT BRAIN MEMBRANE

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Naltrindole (NTI), a delta opioid selective antagonist was originally synthesized by Portoghese, *et.al* (1988), and it was used in radiolabelled form by Yamamura, *et.al.* (1989). In the present paper we describe the binding properties of [1,5'-^{3}H]naltrindole. In this case the radioactive form was prepared from 1,5'dibromonaltrindole and tritium gas in the presence of PdO, catalyst. With the introduction of two tritium atoms the specific radioactivity was greatly enhanced and reached 46.1 Ci/mmol. In our saturation experiments with the NTI ligand an equilibrium constant (K_d) of 0.63 ± 0.2 nM and the maximal number of binding sites (B_{max}) of 244 ± 6.0 fmol/mg was found. NTI exhibited the greatest affinity for the delta opioid sites in rat brain membranes (PVG/C strain). The rank order of potencies of different ligands can be seen in Table 1.

Table 1. K_i values for the inhibition of [^{3}H] Naltrindole by various ligands

LIGANDS Specificity	Naltrindole delta	DSLET delta	DELTORPHIN-II delta	DPDPE delta	DAMGE mu	U50,488H kappa
K_i(nM)	0.6±0.1	5.6±0.1	7.4±1.0	33.0±2.0	465.0±1.6	10,325.0±1675.0

A significant proportion of the binding becomes wash resistant after 2 hrs of incubation. Therefore, washing experiments were carried out to determine the irreversibility of the NTI binding. Our results show, that more than 90% of the ligand remains irreversibly bound after several washing steps. Further studies lead us to believe, that the NTI binding is not of the covalent type but tends to have a pseudo-irreversible nature as reported with other ligands by Garzon-Aburbeh, *et.al.*(1989) and Wild, *et.al.* (1993). From these results it can be concluded, that the tritiated naltrindole has a very high affinity and selectivity for delta opioid binding sites. This potential can be used as an appropriate tool for the further characterisation of the delta opioid receptor, especially for the identification of the antagonist binding site.

Garzon-Aburbeh, A., Lipowski, A.W., Larson, D.L., *et.al.* (1989) *Neurochemistry International*, 15, 207-214
Portoghese, P., Sultana, M. & Takemori, A. (1988) *European Journal of Pharmacology*, 146, 185-186
Wild, K.D., Horan, P., Misicka, A., Lipkowski, A., Haaseth, R., *et.al.*(1993) *European Journal of Pharmacology*, (In Press).
Yamamura, M.S., Horvath, R., Toth, G., Otvos, F., Malatynska, E., *et.al.* (1991) *Life Sciences*, 50, PL119-124

18P *IN VITRO* AND *IN VIVO* ACTIVITY OF A PERIPHERALLY ACTING μ OPIOID RECEPTOR AGONIST/BK_2 RECEPTOR ANTAGONIST HETERODIMER

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The kallikrein-kinin system and neurogenic processes appear to be involved in a variety of inflammatory disorders including asthma, pain,oedema and migraine (Barnes et al., 1992; Moskowitz, 1992, Morton and Chahl,1980). We have described a series of peptide-peptide heterodimers with effects at BK_2/BK_1, BK_2/NK_1, BK_2/NK_2 and BK_2/opioid receptors (Whalley et al., 1992; Whalley et al., 1993). We have now developed a series of combined BK_2 antagonists (CP-0126 and CP-0347) coupled to the non-peptide opioid agonist oxymorphone (OXY).

In vitro studies were performed on the isolated rat uterus (RU) for BK_2 antagonism against BK and the electrically stimulated guinea-pig ileum (ESGPI) for opioid agonist activity. The results are shown in Table 1. Monomer BK_2 antagonists were active on RU but not the ESGPI, whereas OXY inhibited the ESGPI and was inactive on the RU. The heterodimers CP-0494 ([CP-0126]-[OXY]) and CP-0499 ([CP-0347]-[OXY]) were active on both preparations. In the mouse formalin test (50ul of 5% formalin subplantar), OXY produced a dose dependent (0.3 - 0.9 umoles/kg s.c. 15 min before formalin) inhibition of the 1st (0-5 min) and 2nd (15-30 min) phase responses and at the highest dose (0.9 umoles/kg s.c.) used caused marked behavioural obtundation. At equi-analgesic doses CP-0494 (0.1 umoles/kg s.c.; 69% and 86% inhibition of the 1st and 2nd phases respectively) was approximately 10 times more potent than OXY (0.9 umoles/kg s.c.; 72% and 96% inhibition of 1st and 2nd phases respectively) and, more importantly, had no observable CNS effects. The homodimer BK_2 antagonist CP-0127 at 12.6 umoles/kg s.c. inhibited both the 1st (55%) and 2nd (58%) phases. In the rat blood pressure assay CP-0494 but not OXY produced total blockade of BK-induced (5,10 and 20 nmole) hypotension.

Compounds such as CP-0494 and CP-0499 may be useful for the treatment of disorders which involve both a bradykinin and a neurogenic component and, where peripheral selectivity is desired.

Table 1.

	COMPOUND	RAT UTERUS (pA_2)	ESGP (IC_{50})[a]
CP-0126	Hyp[3]-Cys[6]-DPhe[7]-Leu[8]-DArg[BK]	7.1±0.2	inactive
CP-0127	Hyp[3]-Cys[6]-DPhe[7]-Leu[8]-DArg[BK] Hyp[3]-Cys[6]-DPhe[7]-Leu[8]-DArg[BK]	8.5±0.2	inactive
CP-0347	Hyp[3]-Thi[5]-Cys[6]-DTic[7]-Oic[8]-DArg[BK]	9.5±0.2	inactive
	oxymorphone	inactive	21.7
CP-0494	[CP-0126]-[oxymorphone]	8.4±0.2	24
CP-0499	[CP-0347]-[oxymorphone]	8.8±0.2	17

Table 1. Values shown are means ± s.e.m. g n=3-7. a=nmolar.

Barnes,P.J.,Belvisi,M.G. and Rodgers,D.F. (1990) Trends Pharmacol Sci. 11,185-189.
Morton ,C.R. and Chahl,L.A.(1980),N-S Arch. Pharmacol.,314,271-276
Moskowitz, M. (1992),Trends Pharmacol Sci., 13, 307-311
Whalley,E.T.,Loy,S.D.,Modaferri,D.,Blodgett,J.K. and Cheronis,J.C.(1992), Br.J.Pharmacol. 107,257P.
Whalley,E.T.,Loy,S.D.,Cheronis,J.C.,Blodgett,J.K. and Allen,L.G. (this meeting)

19P NOVEL PEPTIDE HETERODIMERS WITH ACTIONS AT BK_2 AND EITHER μ OPIOID, NK_1 OR NK_2 RECEPTORS: *IN VITRO* STUDIES

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Recently we described the pharmacological activity of a novel class of potent peptide homo- and heterodimers with antagonist activity against BK_2 receptors (Cheronis et al,1992) and both BK_2 and BK_1 receptors (Whalley et al,1992) respectively. Both BK and various neuropeptides are involved in a variety of neurogenically mediated processes including asthma (Barnes et al,1992), migraine (Moskowitz,1992) and oedema (Morton and Chahl, 1980). We describe here a novel class of peptide-peptide heterodimer compound with antagonist activity at the bradykinin BK_2 receptor and either antagonist activity at NK_1, NK_2 or agonist actions at mu opioid receptors in vitro.

Compounds were assayed on the isolated rat uterus (RU)(for BK_2 antagonist activity against BK), guinea-pig ileum (GPI) (for NK_1 antagonist activity against SP), rabbit pulmonary artery (RbPA) (for NK_2 antagonist activity against NKA) and the electrically stimulated guinea-pig ileum (ESGPI) (for opioid receptor agonist activity).

The results are seen in Table 1. CP-0088, CP-0544 and CP-0558 were antagonists only at BK_2-,NK_1- and NK_2- receptors respectivley, whereas CP-0577 was an agonist at opioid receptors. In contrast, the heterodimes, CP-0433 (BK_2/opioid), CP-0394 (BK_2/NK_1) and CP-0411 (BK_2/NK_2) posessed activity at both their respective receptors.

	COMPOUND	RU[a]	ESGPI[b]	GPI[a]	RbPA[a]
(CP-0088)	Hyp³-DPhe⁷-Leu⁸-DArg[BK]	7.2±0.2	-	-	-
(CP-0577)	Tyr-DAla-Gly(p-Nitro-Phe)Pro-Cys-NH_2*	-	6.8±0.1	-	-
(CP-0433)	Tyr-DAla-Gly(p-Nitro-Phe)Pro-Cys-NH_2 Hyp³-Cys⁶-DPhe⁷-Leu⁸-DArg[BK]	8.0±0.1	6.6±0.4	-	-
(CP-0544)	DArg-DPro-Lys-Pro-Gln-Asn-DPhe-Phe-DTrp-Leu-Nle	-	-	5.6±0.2	-
(CP-0394)	DArg-DPro-Lys-Pro-Gln-Asn-DPhe-Phe-DTrp-Leu-Nle Hyp³-Cys⁶-DPhe⁷-Leu⁸-DArg[BK]	7.9±0.1	-	5.8±0.3	-
(CP-0558)	Asp-Tyr-DTrp-Val-DTrp-DTrp-Arg	-	-	-	6.9±0.2
(CP-0411)	Cys-Tyr-DTrp-Val-DTrp-DTrp-Arg Hyp³-Cys⁶-DPhe⁷-Leu⁸-DArg[BK]	7.5±0.1	-	-	5.5±0.3

Table 1. Values (a=pA_2; b=-log IC_{50}) are means ± s.e.m. of n=3-10. CP-0433 and CP-0411 were dimerized using a Cys-Cys *bis*succinimidohexane linkage and CP-0394 by an (N-succinimido)-ε-aminocaproyl linkage. *Cys analogue of BW443C81 (Buchan and Adcock, 1992).

Such heterodimers as described above with polypharmacological actions may form the basis for the development of compounds for use in disorders where both a bradykinin and a neurogenic component are involved.

Barnes,P., Belvisi, M.G., Rodgers,D.F. (1990) Trends Pharmacol Sci. **11**, 185-189.
Buchan, P.J., and Adcock, J.J. (1992) Br. J. Pharm., 105, 448-452.
Cheronis, J.C., et. al., (1992)J.Med.Chem.35,1563-1572.
Morton,C.R., and Chahl,L.A. (1980) N-S Arch. Pharmacol., **314**, 271-276.
Moskowitz, M. (1992) Trends Pharmacol Sci., **13**, 307-311.
Whalley,E.T., Loy,S.D., Modaferri,D., Blodgett,J.K., and Cheronis,J.C. (1992), Br.J. Pharmacol. **107**, 257P.

20P SELECTIVE DOWN-REGULATION OF HEPATIC CYTOCHROME P450 ISOZYMES BY MORPHINE - A NEUROENDOCRINE MECHANISM?

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The cytochrome P450 (CYP) enzyme superfamily consists of a large number of hemoproteins that catalyze the oxidation of numerous drugs and endogenous substrates. Many of the families are influenced by drugs and other chemicals with induction as the most common consequence. We now describe an unusual down-regulation of some of the isozymes by morphine.

Groups of male Sprague-Dawley rats (n = 5-6, b.w. 170 g) were given single daily i.p. injections of saline, or escalating daily doses of morphine HCl for 4 or 9 days. For comparison, other groups of rats were treated for 4, 9, or 14 days with escalating single daily i.p. doses of pethidine. The initial and final doses of morphine and pethidine were 20-30 (or 20-80) and 11.5-17.3 (or 11.5-25.9 or 11.5-44) mg x kg^{-1} b.w., respectively. Other groups of rats were treated for 4 days with morphine, clonidine, or yohimbine, or combinations thereof, via osmotic mini-pumps (Alzet) (morphine, yohimbine) or via the drinking water (clonidine). *Enzyme activities* were studied with androstenedione (A), dehydroepiandrosterone (DHA), or ethylmorphine (EM) as substrates. *Expression* of the P450 isozymes was studied in Western blots.

The male rat specific 16α-hydroxylation of A (CYP2C11) and DHA decreased to 47 and 42 % of control, respectively, after 4 days' treatment with *morphine* (control values 0.60 and 2.02 nmol/mg^{-1} x min^{-1}, respectively). The N-demethylation of EM was also decreased to about 40 % after 4 days' treatment (control value 15.21 nmol/mg^{-1} x min^{-1}). In contrast, the female rat specific A 5α-reductase increased 4-fold (from 1.53 nmol/mg^{-1} x min^{-1}) after 9 days' treatment. These changes signify a feminization of the male rat liver metabolism. Immunoblotting experiments showed a marked down-regulation of CYP2C6, CYP2C11, CYP3A2 and CYP4A1, whereas CYP1A2, CYP2B1, and CYP2E1 were increased. No clear change was observed for CYP2C7.

None of the P450 isozymes was down-regulated by *pethidine*. In contrast, pethidine gave a marked increase of CYP2B1 and CYP2C6. A moderate increase of CYP1A2, CYP2C7, CYP3A2, and CYP4A1 was observed after longer treatment periods. When combined with yohimbine or clonidine, the down-regulating effect of morphine was augmented or unaffected, respectively, indicating an involvement of an α_2-adrenergic mechanism.

We have previously demonstrated similar effects on the P450 isozymes by growth hormone (GH) [Blanck *et al.*, 1990]. The morphine effects were shown to be dependent on an intact pituitary. Thus, previous and present results suggest an alteration of the GH secretion as the common denominator for the observed biochemical effects of morphine on the hepatic P450 isozyme pattern.

Blanck, A., Hansson, T., Assefaw-Redda, Y., Rane, A. (1990) *Biochem. Pharmacol.* 40, 2177-2180

21P GLUCOCORTICOIDS MODULATE BASAL AND INFLAMMATION-INDUCED OPIOID GENE EXPRESSION IN RAT SPINAL CORD

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Peripheral inflammation induces upregulation of proenkephalin (PRO-ENK) and prodynorpin (PRO-DYN) gene expression in rat spinal neurons. The present study investigates whether corticosteroid treatment of normal and inflamed rats influences spinal opioid gene expression and immunoreactivity.

Four groups of rats were used: group 1 was untreated; group 2 was subjected to collagen II-induced arthritis (Persson et al., 1992); group 3 was subjected to collagen II arthritis and treated with the corticosteroid budesonide; group 4 received budesonide only. The spinal cords of the different groups were investigated by semiquantitative in situ hybridisation (Schafer et. al., 1993) with ^{35}S-UTP labeled riboprobes for PRO-ENK and PRO-DYN and by immunocytochemistry for the PRO-ENK derivative met-enkephalyl-arg-gly-leu (MERGL) and the PRO-DYN-derivative dynorphin A_{1-17} (DYN A) (Weihe et al., 1989). In the arthritic group, PRO-DYN gene expression and immunoreactivity in the dorsal horn was upregulated (ca. 10 fold) as compared to the low basal expression typical for group 1. Budesonide treatment of arthritic rats caused a reduction of inflammation-induced upregulation of PRO-DYN gene expression and immunoreactivity. Control rats treated with budesonide responded with a slight upregulation of PRO-DYN gene expression and DYN immunoreactivity. Changes in PRO-ENK gene expression and MERGL immunoreactivity in the dorsal horn were essentially similar, but started from a much higher basal level. Motoneurons exhibited basal expression of Pro-ENK mRNA and MERGL immunoreactivity, but Pro-DYN expression was absent. There were no obvious changes in Pro-ENK gene expression in motoneurons in response to inflammation.

In conclusion, corticosteroids moderately upregulate basal and markedly downregulate inflammation-induced opioid gene expression in superficial and deep dorsal horn neurons relevant in nociceptive processing. This may suggest differential molecular mechanism mediating the glucocorticoid effect on basal and evoked spinal opioid expression which depends on the level of cellular activity.

Persson, S., Post, C., Holmdahl, R. & Nyberg, F. (1992) *Brain Res.* 581, 273-282.
Schafer, M.K., Herman, J.P. & Watson, S.J. (1993) in *Imaging Drug Action in the Brain* ed. London, E.D. pp 337-378. Boca Raton: CRC Press.
Weihe, E., Millan, M.J., Höllt, V., Nohr, D., Herz, A. (1989) *Neuroscience* 31, 77-95.

Supported by the German Research Foundation.

22P UPREGULATION OF NEUROKININ 1 RECEPTOR GENE EXPRESSION IN DORSAL HORN NEURONS EVOKED BY UNILATERAL PERIPHERAL INFLAMMATION IN THE RAT

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Spinal tachykinin receptors are involved in the processing of noxious impulses in peripheral inflammation. While radioligand binding studies have revealed the presence of tachykinin receptors in the spinal cord, the spinal sites of synthesis of tachykinin receptors are not clear. The aim of this study was 1) to determine the distribution of neurokinin 1 receptor synthesis in rat spinal cord at the cellular level using in situ hybridization with a ^{35}S labelled cRNA probe (Schafer et. al, 1993) and 2) to investigate changes of neurokinin 1 receptor gene expression in unilateral Freund adjuvant-induced inflammation by semi-quantitative image analysis.

Neurokinin 1 receptor mRNA was detected in neurons throughout the cervical, thoracic and lumbosacral grey matter, but not in white matter. Neurons of the superficial dorsal horn, motoneurons and preganglionic sympathetic neurons exhibited the highest concentration of neurokinin 1 receptor mRNA. Unilateral inflammation of the right hind paw (n = 4; 6 days) caused a selective increase in neurokinin 1 receptor mRNA levels in a distinct subpopulation of neurons in the ipsilateral superficial and deep dorsal horn as compared to the contralateral side and to control animals. Image analysis of spinal cord autoradiograms indicated a two fold increase in the ipsilateral dorsal horn of L4 to L5 segments as compared to the contralateral side. The distribution of neurons showing upregulation of neurokinin 1 receptor gene expression coincided with that of neurons exhibiting increased levels of prodynorphin mRNA.

In conclusion, we have revealed multiple cellular sites of synthesis of the neurokinin 1 receptor in rat spinal cord supporting the view that the neurokinin 1 receptor is involved in spinal sensory, motor and autonomic functions. The synchronous and spatially coinciding plasticity of the spinal prodynorphin gene and of the neurokinin 1 receptor gene in peripheral inflammation lends further support to the crucial and interactive role of tachykinin and opioid systems in spinal nociception and processing of inflammatory signals from the periphery.

Schafer, M.K., Herman, J.P., Watson, S.J. (1993) in *Imaging Drug Action in the Brain* ed. London, E.D. pp 337-378. Boca Raton: CRC Press.

Supported by the German Research Foundation (E.W.) and NIH grant NS21937 (J.E.K).

23P MULTIPLE α_2-ADRENOCEPTOR SUBTYPES: EVIDENCE FOR A ROLE OF α_{2D}-ADRENOCEPTORS IN THE CONTROL OF NOCICEPTION AND MOTOR BEHAVIOUR IN RODENTS

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Alphaα_2-adrenoceptors play a major role in the control of motor behaviour and nociception and the discovery of multiple α_2-adrenoceptor subtypes, currently classified as α_{2A}, α_{2B}, α_{2C} and α_{2D} (the rat homologue of the human α_{2A}-adrenoceptor), raises the question as to their individual roles. We have addressed this question in rodents.

The binding of the α_2-adrenoceptor antagonist, [^{3}H]-RX 821002 (methoxyidazoxan), was evaluated in homogenates of rat cerebral cortex, human platelets (α_{2A}), neonatal rat lung (α_{2B}) and rat submaxillary gland (α_{2D}). Antinociception was evaluated in mice using the hot-plate (55°C) test (latency to hind-paw lick) and the acetic acid induced writhing test. Motor effects were determined in mice using the rotarod test (latency to fall) and in rats using the loss of righting reflex. The ability of antagonists to displace [^{3}H]-RX 821002 binding (K_i) and to inhibit the functional actions of the agonists, UK 14,304 or xylazine (Inhibitory $Dose_{50}$), was determined. A correlation coefficient (Pearson Product-Moment) matrix was computed amongst all binding sites (together with the data of Blaxell *et al.* (1991) for α_{2C}-adrenoceptors in opposum kidney) and all functional paradigms. The antagonists were: atipamezole, L 657,743, efaroxan, idazoxan, fluparoxan, SKF 86466, 1-pyrimidinylpiperazine, corynanthine, RX 821002, yohimbine, rauwolscine, idazoxan, WB 4101, BRL 44408, ARC 239 and prazosin.

The single population of binding sites identified by [^{3}H]-RX 821002 in cerebral cortex were indistinguishable from the α_{2D}-sites of rat submaxillary gland ($r = +0.99$). Amongst all 4 functional assays, coefficients were very high (range = 0.85-0.96/median = 0.91). For each procedure, correlations were excellent to the α_2-sites in cerebral cortex (0.76-0.89/0.84) and to the α_{2D}-sites in the submaxillary glands (0.78-0.92/0.84), moderate to α_{2A}-sites in platelets (0.68-0.79/0.74) and to α_{2C}-sites in oppossum kidney (0.61-0.77/0.74) and poor to α_{2B} sites in lung (0.55-0.73/0.60). Further, in every test, ARC 239 and prazosin, preferential antagonists at α_{2B}- (and α_{2C})-adrenoceptors were completely ineffective while BRL 44408, which has low affinity for α_{2B}- (and α_{2C}-) adrenoceptors, was fully active.

These data suggest that the binding site recognized by [^{3}H]-RX 821002 in the cortex may an α_{2D}-adrenoceptor (as suggested by Mackinnon *et al.* (1992) but do *not* exclude the presence of further type(s). They suggest that a common α_2-adrenoceptor type may mediate the antinociceptive and motor effects of α_2-adrenoceptor agonists in rodents and indicate that this is likewise an α_{2D}-adrenoceptor. The data do *not*, however, exclude a role of other adrenoceptor types. The development of improved, subtype-selective agents will be necessary for the further definition of the putative functional roles of α_{2D}- and other α_2-adrenoceptor subtypes.

Blaxell, H.S., Murphy, T.J. Baker, J.C., Ray, C. & Bylund, D.B. (1991) *J. Pharmacol. Exp. Ther.* 259, 323-329.
Mackinnon, A.C., Kilpatrick, A.T., Kenny, B.A, Spedding, M. & Brown, C.M. (1992) *Br. J. Pharmacol.* 106, 1011-1018.

24P NEW 2-IODO SUBSTITUTED DERIVATIVES OF IDAZOXAN WITH HIGH AFFINITY FOR MAMMALIAN α_2-ADRENOCEPTORS

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[^{3}H]-Idazoxan has been extensively used for the identification of α_2-adrenoceptors, but its affinity for non-adrenoceptor sites complicates interpretation of radioligand binding data (Michel and Insel., 1989). More recently, the tritiated form of RX821002 (2-methoxy-idazoxan) has proved superior to [^{3}H]-idazoxan, having higher affinity and selectivity for α_2-adrenoceptors and low affinity for non-adrenoceptor sites (Mallard *et al.*, 1992). These characteristics of [^{3}H]-RX821002 have allowed its use for the autoradiographic mapping of α_2-adrenoceptors in rat brain (Hudson *et al.*, 1992). An ^{125}I labelled form of RX821002 may provide a more rapid tool for the identification of these receptors. We have therefore investigated the affinities of three analogues of RX821002 containing iodine atoms in the alkoxy substituent, namely, BR347 (2-(3-iodobenzyloxy)-idazoxan), BR351 (2-(4-iodobenzyloxy)-idazoxan) and BR352 (2-(3-iodopropoxy)-idazoxan) for rat and rabbit central α_2-adrenoceptors.

Crude whole brain membranes were prepared from male Wistar rats and ex-breeding New Zealand white rabbits (200-300 g and 2.5-4 kg respectively). Washed Membrane aliquots (300-450 µg protein) were incubated with 2nM [^{3}H]-RX821002 and test drugs over the concentration range 0.1 - 10,000 nM, in triplicate, for 30 min at 22°C in a final buffer volume of 1 ml (50mM Tris-HCl, 1mM Mg^{2+}, pH 7.4). Bound and free radioligand were separated by rapid filtration and estimated by liquid scintillation counting. Non-specific binding was determined in the presence of 10 µM rauwolscine.

Specific [^{3}H]-RX821002 binding accounted for >89% and >95% of total bound for rabbit and rat respectively. Test compounds displaced all specific binding at 10,000 nM. Inhibition curves for competing drugs were steep with Hill coefficients close to unity. Affinity constants (Ki) for the displacing agents are shown in Table 1. Each value represents the mean ± s.e.mean of observations from 3 - 4 separate experiments.

Table 1	Ki (nM) α_2-adrenoceptors	
	Rat	Rabbit
Idazoxan	11.2 ± 0.2	55.4 ± 4.9
RX821002	1.2 ± 0.2	5.1 ± 1.2
BR347	5.9 ± 3.0	3.8 ± 1.4
BR351	38.9 ± 7.6	77.9 ± 10.2
BR352	3.3 ± 0.9	6.7 ± 2.9

BR347 and BR352 have similar affinity to RX821002 and higher affinity than idazoxan for rat and rabbit central α_2-adrenoceptors. These preliminary results indicate BR347 and BR352 maybe suitable precursors for radiolabelling with ^{125}I, which would facilitate rapid autoradiography of α_2-adrenoceptors. Moreover addition of ^{123}I may produce a SPECT (single photon emission computerised tomography) ligand for these receptors.

Hudson, A.L., Mallard, N.J., Tyacke, R. & Nutt, D.J. (1992). *Mol. Neuropharmacol.*, 1, 219-229.
Mallard, N.J., Hudson, A.L. & Nutt, D.J. (1992). *Br.J. Pharmacol.*, 106, 1019-1027.
Michel, M.C. & Insel, P.A. (1989). *Trends in Pharmacol. Sci.*, 10, 342-344.

25P EFFECTS OF SYSTEMIC MEMANTINE ON THE RESPONSES OF RAT SPINAL NEURONES TO EXCITATORY AMINO ACIDS AND PERIPHERAL NOXIOUS STIMULI

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Memantine (1-amino-3,5-dimethyladamantane) is used for its antispastic and antiparkinsonism actions (see Bormann, 1989). The mode of action of this drug is not clear. Whilst memantine can block NMDA channels, acting probably at the PCP site (Bormann, 1989), it has not yet been shown whether this action occurs under *in vivo* conditions, nor whether it is a selective effect, nor how effective memantine may be at affecting NMDA receptor-mediated synaptic events. We have now addressed these aspects in an electrophysiological study performed *in vivo*.

Experiments were performed on 10 chloralose-anaesthetized rats. Following a lumbar laminectomy and low thoracic spinalization, multibarrel pipettes were used for microelectrophoretic ejection of excitatory amino acid analogues and for recording the activity of single spinal dorsal and ventral horn neurones. Systemic memantine (1-32mg.kg^{-1}) was tested on responses elicited by cycles of *N*-methyl-D-aspartate (NMDA), (RS)-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate and peripheral noxious heat or pinch stimuli (for details of methods see Headley *et al.*, 1987). In all 10 experiments memantine selectively and dose-dependently reduced responses to NMDA, with a calculated mean ID_{50} of 26mg.kg^{-1}; the reduction was significant at doses of 8mg.kg^{-1} and above. Spontaneous activity was reduced at $\geq$16mg.kg^{-1}. Responses to AMPA, kainate and peripheral noxious stimuli were not affected over this dose range. Mean arterial blood pressure was unaffected at doses up to 16mg.kg^{-1} but was transiently reduced, by 25mm Hg, at the 32mg.kg^{-1} dose. Respiration remained normal as judged by visual observation, except in two animals in which it became fast and shallow at 32mg.kg^{-1}. Ketamine was tested on 9 of these cells. The calculated mean ID_{50} for NMDA responses was 3.5mg.kg^{-1}. Nociceptive responses were weakly reduced, to 88% control, at the highest dose tested of 4 to 8mg.kg^{-1}.

These results indicate that under *in vivo* conditions memantine can selectively reduce responses to applied NMDA, suggesting that this activity may contribute to its therapeutic actions. It is most likely that memantine binds to the PCP site in the NMDA channel (Kornhuber *et al.*, 1989). It is however some 7 times weaker than ketamine, and over 50 times weaker than MK-801 (see Salt *et al.*, 1988) at blocking responses to NMDA *in vivo*.

Bormann, J. (1989) *Eur. J. Pharmacol.* 166, 591-592.
Headley, P.M., Parsons, C.G. & West, D.C. (1987) *J. Physiol.*, 385, 169-188.
Kornhuber, J., Bormann, J., Retz, W., Huberg, M. & Rieder, P. (1989) *Eur. J. Pharmacol.* 166, 589-590.
Salt, T.E., Wilson, D.G. & Prasad, S.K. (1988) *Br. J. Pharmacol.* 94, 443-448.

26P CARBETAPENTANE ANALOGUES: ANTICONVULSANT AGENTS ACTING VIA DEXTROMETHORPHAN RECEPTORS?

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It is unclear whether the anticonvulsant activity of the antitussive agent dextromethorphan (DM) is mediated via DM binding sites in rat brain or via the NMDA receptor-associated phencyclidine (PCP) site, to which DM also binds (Newman *et al.*, 1992). Carbapentane (2-[2-(diethylamino)-ethoxy] ethyl-1-phenyl-1-cyclopentylcarboxylate, CBP) which is another antitussive and anticonvulsant is also a ligand for the DM site (Tortella & Mussachio, 1986). In an attempt to determine the involvement of DM, as compared to PCP, sites in the action of CBP a series of analogues were synthesised (Calderon *et al.*, 1991) and their affinities for the DM and PCP sites determined. Within the CBP molecule, two sites were modified (Table): the cyclopentyl ring was expanded (n) and/or the ester function was replaced (X). The activity of these compounds as anticonvulsants was assessed using the rat supramaximal electroshock (MES) test and compared with binding activities at the DM and PCP binding sites in rat brain, using [^{3}H]DM and [^{3}H]thienylcyclohexyl-piperidine ([^{3}H]TCP) respectively (Newman *et al.*, 1992).

Protection against MES-induced seizures was observed with CBP and three of the analogues (Table), with one of these (2) being more potent than the parent compound. All of the CBP analogues inhibited [^{3}H]DM binding in rat brain. Although there appears to be only a weak correlation between anticonvulsant activity and affinity for the DM site, there may still be a functional relationship between binding and *in vivo* activity. The compounds appear to bind to more than one site (low slope factors), and the significance of the high and low affinity sites in the anticonvulsant effect of these compounds is not known. Importantly, however, none of the compounds (up to 10μM) showed any activity at the PCP site in rat brain and thus it seems unlikely that the anticonvulsant effect is mediated *via* the NMDA receptor. Neither CBP nor compound 2 produced any behavioural toxicity at 100 mgkg^{-1}. In addition, compound 2 had no pro-convulsant activity at doses up to 7 times its MES ED_{50}. This protective index exceeds those of currently available anticonvulsant agents, and could have exciting implications for future anticonvulsant therapy.

Compound	n	X	[^{3}H]DM K_i (μM ± s.e.m.)	slope	MES ED_{50} (μmolkg^{-1} s.c., 95% CL)
1(CBP)	1	$-CO_2$	2.8±0.92	-0.37	48 (31-72)
2	1	$-CH_2O-$	3.2±0.9	-0.33	16 (9-31)
3	2	$-CH_2O-$	0.6±0.2	-0.25	86 (50-151)
4	1	-CONH-	>10	---	>50mgkg^{-1}
5	2	$-CO_2-$	0.5±0.2	-0.29	173 (80-375)
6	1	$-CH_2NH-$	4.2±1.2	-0.42	>50mgkg^{-1}
7	1	$-CH_2N(CH_3)-$	5.0±1.8	-0.31	>50mgkg^{-1}

(CL = Confidence Limits)

Calderon S.N., Newman A.H., Tortella F.C. (1991) J. Med. Chem. **34** 3159-3164
Newman A.H., Bevan K.A., Bowery N.G., Tortella F.C. (1992) J. Med. Chem. **35** 4135-4142
Tortella F.C., Musacchio J.M. (1986) Brain Res. **383** 314-318

27P THE EFFECTS OF DIHYDROPYRIDINES ON ELECTROSHOCK SEIZURES IN MICE

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Accumulating evidence suggests that voltage-sensitive L-type Ca^{2+} channels play a facilitatory role in the onset of experimental seizures. Epileptiform bursts are associated with the influx of calcium ions into nerve cells (Pumain et al, 1983) and a fall in extracellular calcium ion concentration often precedes the onset of ictal activity in animal seizure models (Heinemann et al, 1981). Dihydropyridine (DHP) compounds such as nimodipine (NIM) and amlodipine (AML) act as selective antagonists of calcium influx via L-type Ca^{2+} channels and, as such, possess anticonvulsant activity in many experimental seizure models (Larkin et al, 1992). The following studies were designed to determine the effects of NIM and AML on seizures induced by electroshock.

The studies were carried out in groups of adult male CF1 mice (25-35g). Drugs were administered intraperitoneally, NIM as a suspension in 0.5% Tween 80 solution (10 to 100mg/kg) and AML in sterile saline solution (1 to 40 mg/kg). Anticonvulsant profiles were determined using the maximal electroshock (MES) and minimal electroshock (Min-ES) tests. Plasma and brain NIM concentrations were determined by high performance liquid chromatography (Larkin et al, 1992a). Statistical significance was determined by the use of the Chi square test (MES seizures) and the Mann Whitney test (Min-ES seizures). Correlations were analysed by Pearson's product moment test.

NIM and AML produced dose-dependent reductions in the incidence of MES-induced seizures with ED_{50}'s of 87mg/kg (n=10) and 23mg/kg (n=10) respectively. Single dose NIM (75mg/kg) produced a significant (n=10, $p<0.05$) reduction in the occurrence MES-induced seizures for up to 12 hours. Single dose AML (20mg/kg) produced a significant (n=5, $p<0.01$) reduction in MES-induced seizures at 1, 12 and 24 hours only. While plasma and brain NIM concentrations correlated well ($r=0.677$, $p<0.01$), AML could not be extracted from brain tissue. Single doses of NIM (75mg/kg) and AML (20mg/kg) raised the seizure threshold, as determined by Min-ES, by 33% (n=30, $p<0.001$) and 38% (n=30, $p<0.001$) respectively.

In conclusion, both NIM and AML exhibit anticonvulsant effects against electroshock seizures.

Pumain, R., Kurcewicz, I. & Louvel, J. (1983) Science 222, 177-179
Heinemann, U., Konnerth, A. & Lux, H.D. (1981) Brain Res. 213, 246-250
Larkin, J.G., Binnie, C.D. & Brodie, M.J. (1992) J. Epilepsy 5, 38-48
Larkin, J.G., Thompson, G.G., Scobie, G., Forrest, G., Drennan, J.E. & Brodie, M.J. (1992a) Epilepsia 33, 760-769

28P INVESTIGATION OF THE ANTICONVULSANT EFFECTS AND MECHANISM OF ACTION OF VIGABATRIN IN EXPERIMENTAL SEIZURE MODELS

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Gamma-aminobutyric acid (GABA) neurotransmission is thought to play an important role in controlling seizure activity. GABA, an inhibitory neurotransmitter, is formed from the decarboxylation of glutamic acid by the enzyme glutamic acid decarboxylase (GAD) and is metabolised to the inactive compound, succinic semialdehyde, by the enzyme GABA-transaminase (GABA-T). The GABA analogue, vigabatrin (gamma-vinyl GABA) is a specific, enzyme-activated inhibitor of the catabolic enzyme GABA-T, which was designed specifically to increase the levels of GABA within the CNS and thus reduce ictal activity. The following studies examine the experimental anticonvulsant profile of vigabatrin and investigate the effects of the drug on selected enzyme activites.

The studies were carried out in groups of adult male CF1 mice (25-35g) and Sprague Dawley rats (150-250g). Vigabatrin was dissolved in sterile saline solution and administered intraperitoneally in doses of 25 to 200mg/kg. Chronically dosed animals were given once daily injections for one week. The anticonvulsant profile was determined using the pentylenetetrazol (PTZ) and maximal electroshock (MES) tests. Brain GABA-T activity was measured by radiometric assay (White and Faison, 1980). Brain GAD and GABA were measured by high performance liquid chromatography (Kochhar et al, 1989).

In mice, single (n=10) and chronic (n=6) dosing with vigabatrin were ineffective at lengthening the time to first myoclonic seizure induced by PTZ. Tonic seizures induced by MES were unaffected by treatment with single dose of vigabatrin (n=10). Chronically administered vigabatrin (50mg/kg) reduced the occurrence of MES-induced seizures by 60% (n=10, $p<0.01$). Vigabatrin produced a dose dependent reduction in brain GABA-T activity (n=6), an effect which was accentuated by chronic treatment. In rats, chronic vigabatrin administration reduced brain GABA-T activity by 59% (n=6, $p<0.005$) and brain GAD activity by 56% (n=6, $p<0.001$), but had no effect on mean brain GABA levels (n=6).

In conclusion, vigabatrin did not possess anticonvulsant activity against PTZ- or MES-induced seizures. At high dosage it appears to reduce GABA synthesis in addition to inhibiting its metabolism.

White, H.L. & Faison, L.D. (1980) Brain Res. 5, 115-119
Kochhar, S., Mehta, P.K. & Christen, P. (1989) Anal. Biochem. 179, 182-185

29P BICUCULLINE-RESISTANT INHIBITION BETWEEN PAIRED EVOKED EXTRACELLULAR POTENTIALS IN THE RAT HIPPOCAMPAL SLICE

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Paired-pulse effects in the normal hippocampus have been well characterised (e.g. Creager *et al.*, 1980) but paired-pulse effects in the disinhibited hippocampus have been less well studied. After observing that the GABA-A antagonist bicuculline appeared to increase paired-pulse inhibition we examined interactions between pairs of extracellularly-recorded potentials in the CA1 area of the rat hippocampal slice in the presence of this agent.

Slices were prepared 450 μm-thick from male Wistar rats (170-220g) in ACSF (in mM: KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10) saturated with 95% O_2 / 5% CO_2. For recording slices were submerged and superfused at 30°C. Electrodes were placed in the stratum radiatum for orthodromic stimulation and alveus for antidromic stimulation. Population potentials were recorded in the stratum pyramidale.

In 10 μM bicuculline methobromide the evoked orthodromic potential consisted of a short burst of population spikes. Paired-pulse interactions depended on the interpulse interval (ipi). Both facilitation and inhibition could be demonstrated. With orthodromic stimuli inhibition was increased (or facilitation reduced) compared to bicuculline-free controls in the same slices at ipis between 300 and 900 ms ($p<0.05$). The medium-latency (300 ms) paired-pulse inhibition in bicuculline was reduced by 200 μM 2-hydroxysaclofen (2HS) a GABA-B antagonist, e.g. with a supramaximal conditioning stimulus, control inhibition of 19 $\pm$ 7.5% (mean $\pm$ s.e. mean) became a non-significant facilitation of 2 $\pm$ 2.9% with the addition of 2HS ($p<0.05$ $n=6$). This medium-latency inhibition was probably therefore largely mediated by GABA-B receptor activation. The medium-latency inhibition was also significantly reduced by the GABA-B agonist baclofen. Baclofen reduced both the control (unpaired) and the conditioned (paired) test potential so stimuli were adjusted in the presence of baclofen to compensate for its depressant effect. Baclofen reduced the control more than the conditioned potential. For example, at 1μM baclofen, with a supramaximal conditioning stimulus, control inhibition of 26 $\pm$ 6.1% was reduced to 2 $\pm$ 2.8% calculated after stimulus readjustment ($p<0.01$ $n=7$). The similar effects of 2HS and baclofen probably reflect different sites of action in that baclofen will decrease GABA release from interneurones and 2HS will block the effects of released GABA. In addition to the GABA-B component a non-GABA mediated depression of postsynaptic excitability contributed to paired-pulse inhibition at this latency since a small but consistent (11.2 $\pm$ 1.04%, conditioning stimulus supramaximal, $n=22$) paired-pulse inhibition was demonstrated when an antidromic test stimulus was preceded by an orthodromic conditioning stimulus and this inhibition was not significantly changed by baclofen or 2HS. The results show that GABA-B mediated inhibition can be demonstrated using an extracellular recording paradigm and that a non-GABA mediated process contributes to paired-pulse inhibition under these conditions.

M.J. Higgins is supported by the Wellcome Trust.

Creager, R., Dunwiddie, T. & Lynch, G. (1980) *J.Physiol.* **299**, 409-424.

30P ORIGIN OF POST-DEPOLARIZATION HYPERPOLARIZATIONS EVOKED BY EXCITATORY AMINO ACIDS IN A GREASE-GAP RECORDING PREPARATION OF THE RAT HIPPOCAMPAL SLICE

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The grease-gap recording technique has been adapted to several brain areas for the quantitative study of excitatory amino acid pharmacology. A common observation in these preparations is that a hyperpolarization often follows the depolarizing response evoked by an agonist (post-depolarization hyperpolarization, PDH). These PDHs are insensitive to tetrodotoxin and it has been variously suggested that they may be due to: a) diffusion of the agonist through the slice which depolarizes tissue in the axonal compartment thus causing an "apparent" hyperpolarization, b) activation of the electrogenic Na^+/K^+ pump, and/or c) activation of Ca^{2+}-dependent K^+-channels (Evans, 1989). We have investigated these possibilities using the rat longitudinal hippocampal slice preparation as described by Martin *et al.* (1989).

Experiments were performed in medium containing 2 mM Ca^{2+} and no added Mg^{2+} which was perfused through both the somatic and axonal compartments of the slice chamber at a rate of 1.5 ml/min and 22-25°C. N-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) perfused through the somatic compartment for 90 s evoked PDHs in approximately 80% of slices tested and these were more prominent at concentrations towards the top of the respective dose-response curves (5-20 µM NMDA, 5-10 µM AMPA). Perfusion of the agonists through the axonal compartment evoked smaller, but reliable, deflections in the opposite direction (n=4). Perfusion of D-2-amino-5-phosphonopentanoic acid (AP5, 40 µM) through the axonal compartment did not block the the PDH evoked by perfusion of NMDA to the somatic compartment (97 ± 16% of control, mean ± s.e.mean), whereas in the same slices, perfusion of AP5 through the somatic compartment blocked both the depolarization and the PDH (7 ± 5% of control, n=6). Perfusion of Ca^{2+}-free medium through the somatic compartment caused a tonic hyperpolarizing shift in the baseline and selectively reduced the PDH evoked by NMDA (4 ± 9% of control, n=7) with little effect on the height of the depolarization. The effect of temperature was investigated in 7 slices. Raising the temperature of the bath by 5°C increased the height of the PDH evoked by NMDA, whereas, lowering the temperature by 5°C abolished the PDH and lengthened the depolarization. Perfusion of ouabain (1 µM for 15-25 min) through the somatic compartment reduced the PDH evoked by NMDA (46 ± 8% of control, n=4) without affecting the amplitude of the depolarization. Higher doses, or longer perfusion of ouabain caused a tonic depolarizing shift in the baseline and also reduced the depolarization. Experiments with carbachol (10-50 µM) proved inconclusive; in 2/6 slices carbachol markedly reduced the PDH evoked by AMPA, but only at concentrations which also reduced the amplitude of the depolarization.

We conclude that the PDHs evoked by excitatory amino acids in this preparation are not caused by diffusion of agonist through the slice. The evidence is consistent with them being caused by activation of the Na^+/K^+ pump, but we cannot exclude a contribution from Ca^{2+}-activated K^+-channels.

Evans R.H. (1989) in *The NMDA Receptor* eds. Watkins J.C. & Collingridge G.L. pp 65-75. Oxford: IRL.
Martin D., Bowe M.A. & Nadler J.V. (1989) J. Neurosci. Meth. 29, 107-114.

31P INDUCTION OF SPONTANEOUS TAIL-FLICKS IN THE RAT BY ANTAGONISM OF THE NMDA RECEPTOR RECOGNITION SITE AND BLOCKADE OF THE ASSOCIATED ION CHANNEL: A PHARMACOLOGICAL ANALYSIS

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Spontaneous tail-flicks (STFs), i.e., tail-flicks in the absence of extraneous stimulation, are elicited in rats by s.c. admi-nistration of (+)-MK 801, an open channel blocker at N-methyl-D-aspartate (NMDA) receptors (Millan, 1991). Herein, we examined whether NMDA receptors are genuinely involved in the mediation of STFs and whether dopamine D_1 and D_2 receptors, α_1- and α_2-adrenoceptors or serotonin (5-HT) receptors are involved in their modulation.

As previously (Millan, 1991), STFs were determined in rats loosely-restrained in opaque cylinders with the tail hanging freely. The ability of drugs to elicit STFs was determined 30 min or 60 min (NMDA recognition site antagonists) following their injection s.c.. Potency to induce STFs (Minimal Effective Dose versus vehicle in ANOVA) was compared to potency (Inhibitory $Concentration_{50}$ in nM) to displace the *in vitro* binding of [^{3}H]-(+)-MK 801 to NMDA receptors in homogenates of rat cortex (Canton *et al.*, 1989). For interaction studies, drugs were given, s.c., 30 min before (+)-MK 801 (0.08 mg/kg) or CPP (20.0 mg/kg).

The order of potency of channel blockers to elicit STFs and to displace [^{3}H]-(+)-MK 801 binding was: (+)-MK 801 (0.04 mg/kg/2.0 nM) > (±) MK 801 (0.08/3.7)> (-)- MK 801 (0.63/10.0) ≧ TCP (0.63/11.7) > PCP (5.0/60.0) > ketamine (20.0/676). The Pearson Product-Moment Correlation Coefficient was highly significant: r = +0.89. The order of potency of NMDA recognition site antagonists also corresponded well between induction of STFs and inhibition of [^{3}H]-(+)-MK 801 binding: CGP 37849 (10.0 mg/kg/45 nM) > CGS 19755 (20.0/204) ≧ CPP (20.0/251) > CGP 39551 (40.0/1096). NMDA (2.5 - 80.0 mg/kg) abolished induction of STFs by CPP but did not affect STFs elicited by (+)-MK 801. STFs evoked by (+)-MK 801 were blocked by the dopamine D_1 receptor antagonists, SCH 23390 (0.01-0.16 mg/kg) and SCH 39166 (0.04-0.63) but were not affected by the dopamine D_2 receptor antagonist, raclopride (2.5). While the α_1-adrenoceptor antagonist, prazosin (0.63 mg/kg), failed to modify (+)-MK 801-induced STFs, they were abolished by the α_2-adrenoceptor antagonists, RX 821002 (0.01-0.16) and idazoxan (0.04-2.5). Antagonists at 5-$HT_{1A/1B}$ (pindolol, 10.0 mg/kg), 5-$HT_{1C/2}$ (ritanserin, 2.5) and 5-HT_3 (ondansetron, 2.5) receptors failed to modify (+)-MK 801-induced STFs. Idazoxan and SCH 23390 also inhibited CPP-evoked STFs.

In conclusion, STFs can be evoked in rats by NMDA recognition site antagonists and open channel blockers, the actions of which can be distinguished by their respective sensitivity, and lack of sensitivity,to antagonism by NMDA. STFs are dependent upon dopamine D_1 (but not D_2) receptors and upon α_2- (but not α_1-) adrenoceptors for their expression. This model should help both identify novel NMDA receptor ligands and clarify the role of alpha-adrenoceptors and dopamine receptors in the modulation of actions mediated by NMDA receptors.

Canton, H., Colpaert, F. & Lehmann, J. (1989) *Neurosci.* Abstr. 15, 133.
Millan, M.J. (1991) *Eur J. Pharmacol.* 203, 315-318.

32P CHRONIC NICOTINE TREATMENT AND UNILATERAL LESION OF THE NUCLEUS BASALIS (NB) WITH AMPA ALTER RAT FRONTAL CORTEX PYRAMIDAL CELL SENSITIVITY TO NICOTINE

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Nicotine produces a number of well-defined and centrally-mediated behavioural effects. Chronic administration of nicotine to rats, unexpectedly, up-regulated cortical nicotinic receptors (Schwartz and Kellar 1983). Moreover, it has been shown that lesioning the nb, the main source of the cholinergic innervation to the frontoparietal cortex, increased the behavioural sensitivity of rats to nicotine (Hodges *et al.*, 1991). The aim of the present study was to compare the effects of chronic treatment with nicotine and of AMPA lesion of the nb on frontal cortex neurones' sensitivity to nicotine applied iontophoretically.

Nicotine (2 mg/kg s.c. in 0.9% NaCl daily for 10 days) was administered to Sprague-Dawley rats and a control group received vehicle alone. In a second groups of rats, the nb was lesioned unilaterally by injecting S-AMPA (2 injections of 0.93 µg in 0.5 µl) as described previously (Abdulla *et al.*, 1992); 5 naive rats served as control for this group. Twenty-four h after the last injection of nicotine or two weeks after lesion, the rats were anaesthetized with urethane (1.5 g/kg i.p.) and the sensitivities of pyramidal cells in frontal cortex to iontophorised nicotine and glutamate were determined. Each drug was applied with an ejection current of 30 nA for 20 s and for analytical purposes, the average responses of 3 applications (separated by 1 min recovery periods) were obtained. The control neuronal firing rate, monitored over 20 consecutive 1 s epochs was compared with the firing rate during drug administration and for 20 s after ceasing drug application by a Wilcoxon signed ranks test. The neurone was considered to be sensitive when the firing rate altered significantly ($P < 0.05$) either during, or within 20 s of, drug application. The two control groups responded similarly to nicotine and to glutamate and therefore data from these groups were pooled (n=235 neurones from 12 rats). Chronic nicotine significantly increased the proportion of cortical neurones responding to nicotine from 32.23% in the control group to 60.0% in the nicotine-treated group (n=185 neurones from 7 rats, $P<0.0001$). AMPA lesion of the nb (n=80 neurones from 5 rats) increased the proportion of frontal cortex neurones responding to nicotine to 53.75% ($P < 0.001$). Both chronic nicotine treatment and AMPA lesion of nb decreased the spontaneous firing rate of frontal cortex neurones ($P < 0.01$), reduced the latency to effect ($P < 0.05$) and prolonged the duration of nicotine's action ($P < 0.001$). The increase in the proportion of frontal cortex neurones responding to nicotine after chronic nicotine and lesioning the nb was similar ($P < 0.41$). The sensitivity of the frontal cortex neurones to glutamate was not changed after chronic nicotine administration or AMPA lesion of nb.

Chronic administration of nicotine, which is reported to upregulate cortical nicotinic receptors, increased sensitivity of cortical neurones to nicotine. Upregulation of nicotinic receptors could explain the lesion-induced increase in sensitivity to nicotine.

Schwartz, R.D. and Kellar K.J. (1981) Science **220**, 214-216.
Hodges, H., Allen, Y., Sinden, J., Lantos, P.L. and Gray, J.A. (1991) Neuroscience **45**, 609-623.
Abdulla, F.A., Dawe, G., Scorsa, A.M., Rispoli, V. and Stephenson, J.D. (1992) Funct. Neurol. **6**, 141-144.

33P NORADRENALINE AND 5-HYDROXY-TRYPTAMINE-INDUCED INCREASES IN INOSITOL (1,4,5)-TRISPHOSPHATE ACCUMULATION IN RABBIT AORTA: ROLE OF EXTRACELLULAR Ca^{2+}

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Inositol (1,4,5)-trisphosphate (IP_3) releases intracellular Ca^{2+} and causes contraction of vascular smooth muscle (Hashimoto *et al.*, 1986). Previous studies have shown that a number of vasoconstrictor compounds stimulate IP_3 production in vascular tissue, suggesting that IP_3 has a role in the pressor response to these compounds. The present study employed a radioreceptor assay (Challiss *et al.*, 1988) to study the time-course of IP_3 accumulation in rings of rabbit aorta following the application of the constrictor agents noradrenaline or 5-hydroxytryptamine (5-HT). The importance of extracellular Ca^{2+} in these responses was also examined.

Endothelium-denuded rings of rabbit thoracic aorta (approx. 5mm long) were incubated in oxygenated, warmed Krebs solution and intermittently gassed over a period 20 min. Drugs or vehicle solution were added and the reaction terminated at varying times between 5s and 20 min with 1M ice-cold trichloroacetic acid. Aliquots were taken and the acid extracted by repeated washing with water-saturated diethylether. The amount of IP_3 in the samples was determined by competition binding to bovine adrenal cortex microsomes in the presence of [^{3}H]IP_3. Noradrenaline (10 μM) caused a rapid increase in the amount of IP_3 in rabbit aorta, which peaked after 15 s at 7.19 $\pm$ 1.62 pmol/mg protein (n = 7), representing 328 % of the corresponding basal level. IP_3 returned to basal levels after 1 min exposure to noradrenaline, but increased again to a second peak of 4.96 $\pm$ 1.26 pmol/mg protein (n = 6) by 5 min (165 % of the corresponding basal level). 5-HT (100 μM) also caused a rapid increase in IP_3 levels, which peaked after 30 s at 8.26 $\pm$ 2.34 pmol/mg protein (n = 8; 264 % of the corresponding basal level). The 5-HT-stimulated increase in IP_3 returned to basal levels after 2 min exposure to 5-HT. The first noradrenaline -induced IP_3 increase was abolished by the α_1-adrenoceptor antagonist prazosin (1 μM), while the 5-HT-induced increase in IP_3 levels was abolished by the 5-HT_2 receptor antagonist ketanserin (1 μM). In Krebs solution containing 25.7 mM K^+ basal IP_3 levels increased from 2.91 $\pm$ 0.96 (n = 7) to 6.72 $\pm$ 0.88 pmol/mg protein (n = 6), an increase which was abolished by prazosin (1 μM). Neither noradrenaline nor 5-HT stimulated a further increase in IP_3 concentration in the presence of the high-K^+ Krebs solution. In Ca^{2+}-free Krebs solution, the first noradrenaline-induced increase in IP_3 was reduced and the second peak was abolished, while 5-HT did not increase IP_3 levels.

These results suggest that IP_3 may be involved in the contractile effects of both noradrenaline and 5-HT in rabbit aorta. Smooth muscle depolarization with K^+ also increased the basal level of IP_3 in the aorta, but this was most probably due to transmitter release from perivascular nerves. Extracellular Ca^{2+} appears to be necessary for IP_3 synthesis in response to 5-HT and noradrenaline.

Supported by the Wellcome Trust.

Challiss, R.A.J., Batty, I.H. & Nahorski, S.R. (1988) Biochem. Biophys. Res. Commun. **157**, 684-691.
Hashimoto, T., Hirata, M., Itoh, T., Kanmura, Y. & Kuriyama, H. (1986) J. Physiol. **370**, 605-618.

34P EVIDENCE FOR α_2-ADRENOCEPTOR BINDING SITES ON THE PORCINE THORACIC AORTA AND SPLENIC ARTERY

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The failure to observe a contractile response to an agonist cannot be taken as conclusive evidence against the existence of a particular receptor (see Dunn et al., 1991). For example, α_2-adrenoceptor binding sites have been detected on vascular smooth muscle from dog mesenteric artery (Shi et al., 1989) but complementary *in vitro* studies failed to reveal a functional response. The aim of this study was to determine α_2-adrenoceptor density and affinity in porcine thoracic aorta and splenic artery by radioligand binding.

Aortae and spleens from male pigs were obtained from a local abattoir. The tissue was homogenised in 50mM Tris buffer (pH 7.4) using a Polytron disrupter. The homogenate was centrifuged at 4°C, at 1,000g for 10 min, the supernatant taken and centrifuged at 40,000g for 20 min. The final pellet was resuspended in 50mM Tris (pH 7.4). In saturation studies membranes were incubated with the α_2-adrenoceptor antagonists ^{3}H-idazoxan (0.08 - 20nM) or ^{3}H-RX821002 (0.08 - 10nM) (Wilson et al., 1991) in 50 mM Tris buffer for 60 min at room temperature in a total volume of 0.5ml using 10μM yohimbine to define non-specific binding. Competition curves were constructed using a fixed concentration of either ^{3}H-idazoxan (1 -1.5nM) or ^{3}H-RX821002 (1 - 1.5nM) and increasing concentrations of the competing ligands yohimbine, rauwolscine and corynanthine. Bound radioactivity was separated from free by filtration under vacuum using a Brandel cell harvester. Radioactivity was determined using liquid scintillation counting at an efficiency of approximately 30%.

Saturation curves indicated the presence of a finite population of binding sites in both blood vessels, but the maximum number of binding sites (B_{max}) for ^{3}H-idazoxan was 50% of that for ^{3}H-RX821002 (Table 1). In aortic membranes labelled by both radioligands the affinity ratio of rauwolscine and corynanthine (~100) is consistent with the presence of α_2-adrenoceptor binding sites (Table 2; Wilson et al., 1991).

Table 1. Saturation studies. N=4 per saturation.

Tissue	^{3}H-Idazoxan		^{3}H-RX821002	
	K_D (nM)	B_{max} (fmol/mg)	K_D (nM)	B_{max} (fmol/mg)
Aorta	6.41±0.44	268.9±47.3	2.16±0.18	545.3±36.2
Splenic artery	5.82±0.89	58.6±14.0*	2.11±0.20	117.5±19.3*

*p<0.01, unpaired t-test.

Table 2 Competition studies in porcine aorta. N=4 per ligand.

	^{3}H-Idazoxan		^{3}H-RX821002	
	pK_i	Slope	pK_i	Slope
Yohimbine	8.35±0.13	-0.87±0.07	-	-
Rauwolscine	8.13±0.21	-0.63±0.05	8.41±0.02	-0.64±0.02
Corynanthine	5.83±0.04	-0.68±0.04	6.50±0.05	-0.81±0.02

The number of α_2-adrenoceptors in the splenic artery was significantly less than that in the aorta; it would be of interest to determine whether this is reflected in differences in α_2-adrenoceptor-mediated functional responses. Future studies will also be needed to address the apparent difference in the number of α_2-adrenoceptors labelled by ^{3}H-idazoxan and ^{3}H-RX821002.

Dunn, W.R., McGrath, J.C. and Wilson, V.G. (1991). Br. J. Pharmacol. **103**, 1484-1492.
Shi, A.G., Kwan, C.Y. and Daniel, E.E. (1989). J. Pharmacol. Exp. Ther. **250**, 1119-1124.
Wilson, V.G., Brown, C.M. and McGrath, J.C. (1991). Exp. Physiol. **76**, 316-346.

35P EVIDENCE FOR FUNCTIONAL α_2-ADRENOCEPTOR-MEDIATED CONTRACTIONS IN THE PORCINE ISOLATED THORACIC AORTA, BUT NOT IN THE SPLENIC ARTERY

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We have reported the presence of α_2-adrenoceptor binding sites on crude membrane preparations of porcine thoracic aorta and splenic artery (Wright *et al.*, this meeting). In this study we have examined the pharmacological characteristics of alpha-adrenoceptors mediating contractions to noradrenaline to determine whether the α_2-adrenoceptor binding sites have a functional correlate.

5mm segments of the porcine isolated thoracic aorta and splenic artery were stored overnight in Krebs-Henseleit saline at 37°C and 4°C, respectively. The following day the preparations were placed in Krebs-Henseleit saline, maintained at 37°C and gassed with 95%O2/5% CO_2, under a resting tension of 12 g wt. and 4 g wt., respectively. After two 'ranging' responses to either 60mM KCl (aorta) or 3μM noradrenaline (splenic artery) we examined (i) the effect of the agonists noradrenaline, phenylephrine (α_1- selective) and UK-14304 (α_2-selective), and (ii) the effect of the antagonists prazosin (α_1- selective) and rauwolscine (α_2- selective) against the noradrenaline-induced contractions (see Wilson *et al.*, 1991 for selectivity details). The pD_2 (concentration of agonist producing 50% of the maximum response) values for agonists and the pA_2 or log K_B values for antagonists were determined as previously described (Daly *et al.*, 1988).

Table 1: pD_2 and Emax values for agonists in the porcine isolated thoracic aorta and splenic artery (n=8-12 separate observations).

	(pD_2)	NA	(Emax)	(pD_2)	PHEN	(Emax)	(pD_2)	UK-14304	(Emax)
Thoracic aorta	5.71±0.09		1	>5.2*		>0.8*	6.24±0.11		0.51
Splenic artery	6.82±0.08		1	5.95±0.09		0.48	6.5*		0.08

* - estimated values because maximum response was not attained or responses were too small.

The results in Table 1 suggest that noradrenaline-induced contractions in the aorta are mediated by a combination of alpha1- and alpha2-adrenoceptors, but almost exclusively by alpha1-adrenoceptors in the splenic artery. This is supported by the findings with the selective antagonists. Both prazosin (10nM-1μM) and rauwolscine (0.1μM-10μM) produced concentration-dependent, non-parallel, rightward displacement of the NA concentration response curve (CRC), and a combination of 0.1μM prazosin and 1μM rauwolscine was more effective than either agent alone. However, in the splenic artery, prazosin and rauwolscine produced a concentration-dependent, parallel, rightward displacement of the NA CRC. Prazosin (pA_2 8.6, slope of Schild plot 0.95, n=5) was approximately 300-fold more potent than rauwolscine (log K_B 6.09, n=6), and 0.1μM prazosin and 1μM rauwolscine in combination was no more effective than 0.1μM prazosin alone.

These findings indicate that activation of α_2-adrenoceptor binding sites on the aorta, unlike those on the splenic artery (see: Wright *et al.*, this meeting), results in contraction. This may be a function of the 4-fold greater receptor density of these sites in the aorta.

Daly, C.J., McGrath, J.C. and Wilson, V.G. (1988). Br. J. Pharmacol., **95**, 437-484.
Wilson, V.G., Brown, C.M. and McGrath, J.C. (1991). Exp. Physiol., **76**, 317-346.

36P ZAMIFENACIN: A NOVEL GUT SELECTIVE MUSCARINIC RECEPTOR ANTAGONIST

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At least three peripheral muscarinic receptors (M_1, M_2 and M_3) have been identified based on the relative affinities of antagonists, including pirenzepine (M_1 selective) and AFDX-116 (M_2 selective). However, the selectivity of available agents is modest (~10 fold) and few compounds clearly differentiate between M_1 and M_3 receptors. In this study we report the receptor affinity profile of zamifenacin [(3R)-(+)-diphenylmethoxy-1-(3,4-methylenedioxyphenyl)piperidine] which is a novel and potent ileal selective muscarinic antagonist.
Studies were conducted at 32°C using Krebs solution gassed with 95% O_2 and 5% CO_2. Cumulative agonist dose response curves were constructed in the absence and presence of antagonists to determine pA_2 values (Arunlakshana and Schild, 1959).

Table 1 pA_2 values (mean ± S.E.M., n=6-8, slope Schild plots 0.87-1.03)

	G.Pig Ileum (M_3)		G.Pig Trachea (M_3)	G.Pig Atria (M_2)	Rabbit Vas Def (M_1)
	Acetylcholine	Carbachol	Carbachol	Carbachol	McN-A-343
Zamifenacin	9.27±0.08	9.09±0.18	8.06±0.14	7.14±0.13	7.38±0.13
Atropine	9.40±0.07	9.43±0.11	9.20±0.10	8.72±0.06	9.54±0.09
Pirenzepine	6.90±0.09	7.17±0.04	7.20±0.10	6.65±0.13	8.09±0.09
AFDX-116	6.40±0.07	6.82±0.06	6.88±0.05	7.50±0.07	6.74±0.16

Zamifenacin potently inhibited acetylcholine- and carbachol-induced contractions of the ileum with the potency being independent of the agonist used. Antagonism was surmountable and the slopes of the Schild plot were consistent with competitive antagonism. This action was stereoselective, with the (S)-enantiomer (UK-76,659, pA_2 6.35, slope 1.2) being nearly 1000 times less potent. Zamifenacin exhibited high selectivity over M_2 (~100 fold) and M_1 (~ 50 fold) receptors and, like p-F-HHSiD (Eglen *et al.*, 1990) also had lower affinity for tracheal M_3 receptors.
Carbachol-induced ^{86}Rb efflux from the isolated salivary gland can be used as an index of salivary secretion (Dehaye *et al.*, 1988). Guinea pig parotid salivary glands were finely chopped and superfused with Krebs solution at 37°C. Atropine potently inhibited carbachol-induced (10 μM) ^{86}Rb efflux (pIC_{50} 7.9±0.14, n=6), but zamifenacin (pIC_{50} 6.7±0.19, n=6) was 20 fold weaker in this respect. In radioligand binding studies zamifenacin had little interaction with a range of non-muscarinic binding sites at concentrations up to 1 μM. These data demonstrate that zamifenacin is a potent and selective muscarinic M_3 receptor antagonist which has functional gut selectivity over the trachea and salivary gland.

Arunlakshana, O. & Schild, H.O. (1959) Br.J.Pharmacol. 14, 48-58.
Dehaye, J.P., Marino, A., Soukias, Y., Poloczek, P., Winand, J. & Christophe, J. (1988) Eur. J. Pharmacol. 151 427-434.
Eglen, R.M., Comett, C.M. & Whiting, R.L. (1990) Naunyn Schmied. Arch. Pharmacol. 342, 394-399.

37P ZAMIFENACIN: A POTENT AND SELECTIVE MUSCARINIC M_3 ANTAGONIST *IN VIVO*

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Zamifenacin is a potent muscarinic receptor antagonist which *in vitro* has approximately 100- and 50-fold selectivity for the M_3 ileal over M_2 and M_1 receptors (Wallis et al, this meeting). Although zamifenacin is as potent as atropine as an inhibitor of acetylcholine- and carbachol-induced contractions of the guinea pig ileum, it is 20 fold weaker as an inhibitor of carbachol-induced ^{86}Rb efflux from the guinea pig salivary gland. These data are consistent with functional ileal selectivity over the salivary gland. The objective of these studies was to determine if the *in vitro* selectivity of zamifenacin could be demonstrated *in vivo*.

Male beagle dogs (11-14.5 kg) were fasted overnight, premedicated with piritramide 4 $mgkg^{-1}$ s.c. (Dipidolor, Janssen) and anaesthetised with chloralose/urethane (0.08/0.8 gkg^{-1} i.v.). Animals were prepared to record blood pressure, cardiac output and left ventricular pressure with small and large bowel motility being stimulated by intravenous infusions of cholecystokinin octapeptide sulphated (CCK-8s) at 75-100 $ngkg^{-1}min.^{-1}$ for 9 min. (Quinn et al, 1991). In studies investigating effects on pupil size, groups of 6 or 7 mice were injected intravenously via the tail vein and pupil size measured immediately before dosing and 15, 60 and 120 min. post dose via a graticule fitted to the eyepiece of a dissecting microscope. The effects on oxotremorine-induced tremor and salivation were determined by the method of Bevan et al (1988). Test compounds were injected intravenously via the tail vein, 10 min. later animals received an intravenous dose of oxotremorine (200 μgkg^{-1}) and after a further 5 min. animals were scored (scale 0-3) for salivary secretion and tremor.

In the dog, atropine (i.v., n=4) potently inhibited CCK-8s-induced small and large bowel motility (ED_{50} values 0.63 and 0.78 $\mu gkg^{-1}min.^{-1}$) and evoked a marked tachycardia over the same dose range (ED_{150} [dose to increase heart rate by 50%] 0.85 $\mu gkg^{-1}min.^{-1}$). Zamifenacin (i.v., n=3) also inhibited CCKs-stimulated gut motility (ED_{50} values 0.9 and 2.2 $\mu gkg^{-1}min.^{-1}$) but in the absence of effects on heart rate; only doses which were supramaximal for inhibition of gut motility increased heart rate (ED_{150} 80 $\mu gkg^{-1}min.^{-1}$). In the mouse, atropine induced a dose-related increase in pupil size with the dose to double pupil size (ED_{200}) being 3.3 μgkg^{-1} i.v.. Although zamifenacin induced a dose related increase in pupil size in the mouse it was nearly 300 times weaker (ED_{200} 1 $mgkg^{-1}$) than atropine in this respect. In the mouse, atropine inhibited oxotremorine-induced tremor (ED_{50} 100 μgkg^{-1}) and salivation (ED_{50} 40 μgkg^{-1}). Zamifenacin was 40-58 fold weaker than atropine in these models (ED_{50}s 4.0 and 2.3 $mgkg^{-1}$ for tremor and salivation).

Thus, zamifenacin inhibited stimulated gut motility in the anaesthetised dog with a potency similar to atropine but, unlike the latter, in the absence of effects on heart rate. This profile was consistent with the *in vitro* M_3/M_2 selectivity of zamifenacin. In contrast to its potent antimotility activity in the dog, zamifenacin was a weak inhibitor of salivary secretion in the mouse, consistent with the functional ileal selectivity over the salivary gland seen *in vitro*. Oxotremorine-induced tremor is believed to be a central M_1 mediated response (Bevan *et al.*, 1988) and the weak activity of zamifenacin is consistent with its weak M_1 antagonist activity *in vitro*. In addition, zamifenacin was a weak mydriatic agent in the mouse. In conclusion, zamifenacin is a novel M_3 selective muscarinic receptor antagonist which exhibits potent gut antimotility activity *in vivo*.

Bevan, K.A., Patterson, J.C. & Templeton, W.W. (1988) Br.J.Pharmacol. **93** 174P.
Quinn, P., Miner, W.D. & Wallis, R.M. (1991) Br.J.Pharmacol. **104** 153P.

38P *IN VIVO* SELECTIVITY OF THE NOVEL MUSCARINIC ANTAGONIST, ZAMIFENACIN, IN THE CONSCIOUS DOG

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Zamifenacin [(3R)-(+)-diphenylmethoxy-1-(3,4-methylenedioxyphenyl)piperidine] is a new competitive muscarinic antagonist which has been shown, in tissue bath experiments to, not only have marked selectivity for receptors of the M_3 subtype over both M_1 and M_2, but also to show some degree of selectivity for those M_3 receptors mediating contraction of guinea-pig ileum over receptors at other sites generally regarded as being of the M_3 subtype (Wallis et al, this meeting). We have therefore conducted some experiments in the conscious dog to try to assertain whether this in vitro activity translates to functional selectivity in vivo.

All experiments were conducted in male beagle dogs (n=4-8) each of which had been previously prepared with a chronically implanted miniature pressure transducer (Konigsberg) in the jejunum to measure intestinal motility and subcutaneous titanium electrodes allowing a lead II ECG signal to be recorded for determination of heart rate. All experiments were conducted after feeding the dogs to ensure a basal digestive motility pattern was present. In separate experiments pupil diameter was quantified, under constant illumination, with the aid of an opthalmoscope and wetness of the mouth, as an index of basal salivation, measured by placing a pre-weighed swab into the cheek jowl of the dog for one minute then reweighing before, and at various time points after, dosing.

Intravenous (0.01-1 $mgkg^{-1}$) or oral (0.3-3 $mgkg^{-1}$) administration of zamifenacin induced dose-related and marked inhibition (up to 85%) of digestive motility, calculated ED_{50} values (dose to reduce motility by 50%) being 0.06 and 0.3 $mgkg^{-1}$ after intravenous and oral dosing respectively. Peak inhibition after oral doses was seen at 1 hour and the duration of activity extended for at least 3 hours. In similar experiments atropine caused comparable inhibition of motility but was approximately 6 fold more potent with ED_{50} values of 0.01 (intravenous) and 0.04 (oral) $mgkg^{-1}$ respectively. Importantly, by either route of administration, zamifenacin caused no change in heart rate at any dose level tested (up to 1 $mgkg^{-1}$ i.v. and 10 $mgkg^{-1}$ p.o.) whereas atropine produced significant ($p<0.05$) tachycardia, an index of muscarinic antagonism at the M_2 receptor subtype (McRitchie & Merner 1991) at dose levels above 0.03 $mgkg^{-1}$ i.v. and 0.1 $mgkg^{-1}$ p.o.. Zamifenacin, over the dose range 1-10 $mgkg^{-1}$ p.o., did not alter pupil diameter whereas atropine produced significant ($p<0.05$) mydriasis after 0.3 $mgkg^{-1}$. Over the oral dose range 1-10 $mgkg^{-1}$ zamifenacin evoked a dose related dryness of the buccal mucosa, indicative of an inhibition of basal salivation, with an ED_{50} of 6 $mgkg^{-1}$, a dose level 20 times higher than that required to inhibit intestinal motility. In marked contrast, atropine was 100 fold more potent than zamifenacin on this parameter with an oral ED_{50} of 0.06$mgkg^{-1}$, very similar to the dose level inhibiting intestinal motility.

In agreement with the in vitro data (Wallis et al, this meeting), zamifenacin can exert an inhibitory effect on intestinal motility in vivo, after intravenous or oral administration, consistent with the actions of a muscarinicic M_3 antagonist. This effect occurs at a dose level much lower than that required to show an effect at cardiac M_2 sites and below doses evoking the typical anticholinergic effects of mydriasis and dry mouth.

McRitchie, B. & Merner, P.A. (1991) Br.J.Pharmacol. **104**, 154P

39P A DIRECT OPERATION MODEL-FITTING APPROACH TO THE ESTIMATION OF PARTIAL AGONIST AFFINITY BY THE INTERACTION METHOD

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Previous publications (Black *et al.*, 1985; Leff *et al.*, 1989) have shown how the operational model of agonism is used to estimate agonist affinities and efficacies when the receptor inactivation method (Furchgott, 1966) and the comparative method (Barlow *et al.*, 1967) are employed. The aim of this study was to develop an operational model-based approach to the analysis of partial agonists by the interaction method (Stephenson, 1956). Firstly, we derived the mathematical relationship which describes the interaction between a full agonist (A) and a partial agonist (B). Pharmacological effect is given by :

$$E = \frac{E_m([A]K_B + \tau_B[B][A_{50}])^n}{[A_{50}]^n (K_B + [B])^n + ([A]K_B + \tau_B[B][A_{50}])^n}$$

in which E_m is the maximum possible effect ; n determines the steepness of the occupancy-effect relation ; K_B is the dissociation constant of the partial agonist ; τ_B is the efficacy of the partial agonist ; $[A_{50}]$ is the midpoint location of the control full agonist E/[A] curve.
This model was used to analyse experimental E/[A] curve data generated for the interaction between pilocarpine (partial agonist) and carbachol (full agonist) at the M_3-muscarinic receptor which mediates contraction of the guinea-pig isolated trachea. Pilocarpine affinity estimates obtained by operational model-fitting were comparable with those obtained by use of the conventional null method (Stephenson, 1956) (see Table 1).

Table1. Comparison of pilocarpine affinity (mean $pK_B \pm$ s.e.; n=6) estimates obtained by operational model-fitting and the null method.

	10μM pilocarpine	30μM pilocarpine	100μM pilocarpine
Operational model	5.70 ± 0.06	5.71 ± 0.08	5.96 ± 0.06
Null method	5.77 ± 0.06	5.79 ± 0.09	6.03 ± 0.05

Use of the null method involves making a number of choices regarding the data to be analysed and the means by which to analyse them. Operational model-fitting not only eliminates the need for all these considerations but also uses the raw E/[A] curve data without transformation. Therefore, from the point of analytical simplicity, this approach appears to be favourable. When several different concentrations of partial agonist are used all the data can be simultaneously fitted to the operational model allowing the competitive nature of the interaction to be verified.
We conclude that operational model-fitting is a valid and analytically simple alternative to the conventional null method of analysing full/partial agonist interactions.

Barlow, R.B., Scott, N.C. & Stephenson, R.P. (1967). *Br. J. Pharmacol.*, **31**, 188-196.
Black, J.W., Leff, P., Shankley, N.P. & Wood, J. (1985). *Br. J. Pharmacol.*, **84**, 561-571.
Furchgott, R.F. (1966). *Adv. Drug Res.*, **3**, 21-55.
Leff, P., Prentice, D.J., Giles, H., Martin, G.R. & Wood, J. (1989). *J. Pharmacol. Methods*, **23**, 225-237.
Stephenson, R.P. (1956). *Br. J. Pharmacol.*, **11**, 379-393.

40P CONTRACTILE ACTIONS OF PURINERGIC P_2 RECEPTOR AGONISTS ON GUINEA-PIG ILEUM

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Guinea pig isolated ileum contracts in response to ATP (Moody & Burnstock, 1982). However, unlike responses to the P_{2x} receptor agonist α,β methylene ATP, responses to ATP are not blocked by tetrodotoxin and atropine, suggesting that the former acts at P_{2x} receptors located on cholinergic neurones, whereas the latter acts at a different receptor in smooth muscle. Subsequent work by Wiklund & Gustafsson, (1988a,b) suggested that the receptor on the smooth muscle resembles, but may not be identical to, the P_{2y} receptor. The purpose of this study was to investigate the characterisation of these receptors further.
Guinea pig ileum longitudinal smooth muscle was suspended in organ baths containing modified Krebs solution at 37°C and gassed with 5% CO_2 in oxygen. Agonists were administered in sequentially increasing doses at twenty minute intervals. Compounds tested were the selective P_{2X} receptor agonists α,β and β,γ methylene ATP, the selective P_{2y} receptor agonist, 2 methylthio ATP, and ATP. Two concentration-effect curves were constructed on each preparation, either one to α,β methylene ATP followed by one for one of the other compounds, or curves for one agonist in the absence and presence of a potential antagonist. The preparation contracted in response to α,β methylene ATP (0.3-100μM, n=4), 2 methylthio ATP (0.3-100μM, n=4) and ATP (10μM-1mM, n=4). Full concentration-effect curves were not obtained to any of the agonists, at the highest concentration tested they produced contractions of 452±91 mg, 372±59 mg,and 481±70 mg respectively (mean±s.e.m.). Due to the incomplete nature of the concentration-effect curves and because in individual experiments curves were not always parallel, no attempt was made to calculate potency ratios. However, α,β methylene ATP and 2 methylthio ATP were of similar potency, whilst ATP was approximately 30 times less potent. Responses to all concentrations of α,β methylene ATP were abolished by tetrodotoxin (300 nM, n=12). Atropine (300 nM, n=12) abolished responses to α,β methylene ATP at concentrations up to 30μM and reduced responses to 100μM by over 90%. Neither tetrodotoxin nor atropine had any significant effect on responses to 2 methylthio ATP (n=4 each) or ATP (n=4 each) ($p>0.05$). Surprisingly, β,γ methylene ATP produced no contraction in concentrations up to 100μM. However, at this concentration β,γ methylene ATP abolished responses to all concentrations of α,β methylene ATP, in contrast, responses to 2 methylthio ATP and ATP were not significantly reduced ($p>0.05$).
The finding that in the presence of atropine and tetrodotoxin the rank order of potency for contracting the ileum is 2 methylthio ATP > ATP >α,β methylene ATP is consistent with this response being mediated by a P_{2y}-like receptor. The high potency of α,β methylene ATP in producing atropine- and tetrodotoxin-sensitive contractions suggests the presence of a P_{2x} receptor on the cholinergic neurones. However, β,γ methylene ATP behaved as an antagonist rather than an agonist. It is therefore possible this receptor differs from the P_{2x} receptor as originally defined or alternatively that β,γ methylene ATP has low efficacy at the P_{2x} receptor and that the number of receptors or their transduction efficiency is low. Further work will be necessary to distinguish between these possibilities.

Moody, C.J. & Burnstock, G. (1982). Eur. J. Pharmacol., **77**, 1-9
Wiklund, N.P. & Gustafsson, L.E. (1988a). Eur. J. Pharmacol., **148**, 361-370
Wiklund, N.P. & Gustafsson, L.E.(1988b). Acta Physiol. Scand., **132**, 15-21

41P EFFECTS OF NEUROTRANSMITTERS ON VOLTAGE-DEPENDENT Ca^{2+} CHANNELS IN THE GUINEA-PIG BLADDER SMOOTH MUSCLE

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In urinary bladder, nerve stimulation releases ATP and ACh (Fujii, 1988). The mechanisms underlying receptor activation co-operate with the voltage sensitive Ca^{2+} channels to yield contraction. In the present study, we investigated the effects of these neurotransmitters on voltage-dependent Ca^{2+} channel current. A conventional whole-cell voltage-clamp technique was used. A holding potential of -60 mV was applied. Enzymatically (collagenase and pronase) isolated cells were normally superfused with a physiological saline solution containing 2.5 mM Ca^{2+} (pH, 7.4 at 25 °C). The pipette solution had the following composition: CsCl, 141; $MgCl_2$, 1.4; EGTA 0.1; HEPES/Tris, 10 ; ATP, 1; GTP, 0.1 (pH 7.2).

Ca^{2+} channel currents were regularly evoked by step depolarizations. ATP (0.1 mM) was applied by pressure ejection at the holding potential. The rapid application of ATP elicited a large inward current (1.6±0.9 nA, n=7), and reduced the subsequently evoked Ca^{2+} channel current. Since the large inward current flowing through ATP-activated non-selective cation channels involves some Ca^{2+} influx at negative membrane potentials (Inoue & Brading, 1990), the reduction of the subsequent Ca^{2+} current is probably due to Ca^{2+}-dependent inactivation (Schneider *et al.*, 1991). On the other hand, a rapid application of carbachol (1mM CCh) elicited little membrane current at the holding potential, but reduced the subsequent Ca^{2+} channel current (by 30±12 %, n=5) similarly.

Purinergic and muscarinic agonists were then applied during large depolarizations in order to eliminate Ca^{2+} entry. During 5 sec depolarizations to +80 mV, the Ca^{2+} channels were little inactivated, and inward currents were evoked by repolarizing the cell membrane. An application of α,β-methylene ATP at +80 mV elicited an outward current, but hardly affected the subsequent inward current on returning the membrane potential to 0 mV. On the other hand, application of CCh also elicited an outward current, but the subsequent Ca^{2+} channel current was markedly reduced (on average, by approx. 30 %, n=3).

These results suggest that the effects of purinergic agonists are solely due to opening non-selective cation channels. Thus, purinergic stimulation inactivates Ca^{2+} channels only if Ca entry occurs. If the reduction of the Ca^{2+} channel current upon muscarinic stimulation is through Ca^{2+}-dependent inactivation, the source of Ca^{2+} must be intracellular stores, because there is no evidence of receptor activated Ca^{+} entry at either -60 or +80 mV. The large outward current at +80 mV is probably due to depolarization-activated or Ca^{2+}-activated conductances, e.g. Ca^{2+}-activated non-selective cation channels or Ca^{2+}-activated Cl^- channels.

This work was supported by Bristol Myers Squibb Inc. N.J.B. is an Australian NH & MRC C.J. Martin Scholar.

Fujii, K. (1988). *J. Physiol.* **404**, 39-52.
Inoue, R. & Brading, A.F. (1990). *Br. J. Pharmacol.* **100**, 619-625.
Schneider, P., Hopp, H.H. & Isenberg, G. (1991). *J. Physiol.* **440**, 479-496.

42P DETRIMENTAL EFFECT OF GLIBENCLAMIDE DURING ISCHAEMIA AND REPERFUSION IN THE RAT ISOLATED HEART

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Activation of ATP-dependent K^+ channels in the ischaemic myocardium is reported to be cardioprotective (Cole *et al.*,1991).The use of sulphonylureas which are known to block this channel in myocytes (Fosset *et al.*,1988), has been controversially associated with an increased incidence of cardiovascular death (University Group Diabetes Program,1970).We have investigated the effect of glibenclamide (GLIB) in the Langendorff-perfused rat heart subjected to global, no-flow ischaemia and reperfusion.

Hearts from male Wistar rats (300-350g) were perfused at constant flow (10ml min^{-1}, 37°C) with a modified Krebs-Henseleit buffer containing 2mM glucose, with or without GLIB (10μM).After 2 hours of perfusion the preparations were subjected to 20 min of global, no-flow ischaemia followed by 20 min of reperfusion.Developed tension (DT), heart rate (HR) and the bipolar ECG were recorded. GLIB depressed DT after 20 min from 11.2 ± 0.4 to 8.9 ± 0.5g (mean ± SEM, n=9, $p<0.01$) and HR decreased from 253 ± 8 to 233 ± 5 beats min^{-1} ($p<0.05$); the vehicle (DMSO, 0.1% v/v) was without effect.After 20 min of ischaemia untreated hearts had developed a contracture to 13.6 ± 0.8g (n=10) vs 15.0 ± 0.9g in GLIB treated hearts.However in the latter the time taken for basal tension to double was reduced from 6.4 ± 0.4 min to 3.4 ± 0.5 min ($p<0.01$).Reperfusion induced ventricular fibrillation (VF) in 8/9 GLIB treated hearts vs 2/10 untreated ($p<0.01$).All hearts in VF reverted to normal sinus rhythm within 4 min.Reperfusion restored DT to 59 ± 2% of preischaemic levels in untreated hearts, however GLIB treated hearts exhibited significantly greater recovery of DT to 73 ± 3% of preischaemic levels ($p<0.01$).Basal tension rose following reperfusion from 2.0 ± 0.1g(pre-ischaemia) to 2.4 ± 0.2g in GLIB treated hearts ($p<0.05$) vs 1.4 ± 0.1g to 1.6 ± 0.2g in untreated hearts ($p<0.05$).GLIB decreased maximal lactate concentration in the first ml of reperfusate from 1.44 ± 0.11mM (n=7) to 1.11 ± 0.08mM (n=8, $p<0.05$), although the drug failed to attenuate maximal K^+ concentration in the first ml of reperfusate (1.44 ± 0.08mM [n=9] vs 1.22 ± 0.08mM untreated [n=9]). Cumulative lactic dehydrogenase release on reperfusion was increased in GLIB-treated hearts from 22 ± 3 U/ml (n=9) to 91 ± 7 U/ml (n=8, $p<0.01$).

These findings suggest that glibenclamide has a detrimental effect on the heart during ischaemia/reperfusion, possibly via an effect independent of ATP-dependent K^+ channel blockade.

Cole, W.C., McPherson, C.D., Sontag, D. (1991) *Circ. Res.* 69, 571-581
Fosset, M., DeWeille, J.R., Green, R.D. *et al.* (1988) *J.Biol.Chem.* 263, 7933-7936
University Group Diabetes Program (1970) *Diabetes* 19(suppl. 2), 747-772

S.A. Winter is supported by the SERC.

43P EFFECTS OF THE NITRIC OXIDE DONOR SIN-1 ON HAEMODYNAMICS, ISCHAEMIA-INDUCED ARRHYTHMIAS AND CARDIAC CYCLIC NUCLEOTIDES

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We are interested in the possible influence of cyclic GMP on cardiac arrhythmias. In previous studies with phosphodiesterase inhibitors we have not been able to selectively increase cardiac cyclic GMP, without also increasing cyclic AMP (Barnes & Coker, 1993). We have now examined the effects of the nitric oxide donor SIN-1 (3-morpholinosydnonimine-*N*-ethylcarbamide) which selectively increases cyclic GMP in endothelial cells (Schini & Vanhoutte, 1989). A Lead I ECG and carotid arterial blood pressure (BP) were measured in pentobarbitone-anaesthetized male Wistar rats (285 to 355 g, n = 12 per group) prepared for coronary artery occlusion (Coker & Ellis, 1987). Ten min after commencing i.v. drug infusion, the coronary artery was occluded and ischaemia-induced arrhythmias were monitored for 25 min. In separate groups (n = 5) of sham-operated rats, 15 min after starting drug administration, the hearts were freeze-clamped and cardiac cyclic nucleotides were measured as described by Kane *et al.* (1985).

Table 1. BP measured 10 min after starting drug infusion, ischaemia-induced ventricular fibrillation (VF) and mortality, and cardiac cyclic nucleotides.

	Systolic BP	Diastolic BP	VF	Mortality	Cyclic AMP	Cyclic GMP
	mmHg	*mmHg*	%	%	*pmol g^{-1}*	*pmol g^{-1}*
Control	136 ± 5	109 ± 5	92	75	506 ± 28	16 ± 1
SIN-1 10 μg kg^{-1} min^{-1}	73 ± 4*	48 ± 4*	75	50		
SIN-1 20 μg kg^{-1} min^{-1}	65 ± 4*	42 ± 4*	100	83	470 ± 49	14 ± 2
SIN-1 40 μg kg^{-1} min^{-1}	62 ± 3*	37 ± 3*	83	58	385 ± 45	17 ± 1

* $P<0.01$, compared with control group, ANOVA with modified t test (Bonferroni correction)

SIN-1 caused marked, dose-dependent reductions in arterial BP, but did not alter the incidence of VF or the mortality following coronary artery occlusion. In addition, the total number of ventricular premature beats that occurred in rats which survived the first 25 min of ischaemia was not significantly altered by SIN-1. In separate groups of sham-operated rats which received the two higher doses of SIN-1, there were no significant differences in cyclic AMP or cyclic GMP compared with the values in controls. Thus despite using doses which caused marked hypotension, SIN-1 did not have any affect on ischaemia-induced arrhythmias and no changes in cardiac cyclic nucleotides could be detected. These results suggest that even if coronary vascular cyclic GMP is increased, this cannot be detected by measuring *cardiac* cyclic nucleotides and does not influence ischaemia-induced arrhythmias.

This work was supported by the British Heart Foundation.

Barnes, C.S. & Coker, S.J. (1993) *Br. J. Pharmacol.*, in press (Proceedings of the Cambridge meeting)
Coker, S.J. & Ellis, A.M. (1987) *J. Cardiovasc. Pharmacol.*, **10**, 479-484.
Kane, K.A., Morcillo-Sanchez, E.J., Parratt, J.R., Rodger, I.W. & Shahid, M. (1985) *Br. J. Pharmacol.*, **84**, 139-145.
Schini, V.B. & Vanhoutte, P.M. (1989) *J. Cardiovasc. Pharmacol.*, **14** (Suppl. 11) S91-S94.

44P EFFECTS OF THE BRADYCARDIC AGENT UL-FS 49 IN THE ANAESTHETISED MINI-PIG

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A reduction in heart rate (HR) by selective 'sinus node' inhibitors such as UL-FS 49 (Kobinger & Lillie, 1984) may be expected to reduce cardiac *minute-* oxygen demand. Additionally, the bradycardia- associated reduction in arterial loading might also be expected to reduce cardiac oxygen demand *per beat*. To investigate this question Göttinger mini-pigs (10.5 -14 kg) were anaesthetised (pentobarbital) & ventilated. Left ventricular (LV) performance was characterised by end-systolic (ES) elastance (Ees =slope of ES pressure-volume relation following preload reduction) & vascular loading properties by arterial elastance (Ea= ES pressure/ stroke volume) obtained from pressure-volume loops (Millar & conductance catheter, respectively). Pressure-volume area (PVA) was used as an index of cardiac oxygen consumption per beat, & peripheral oxygen consumption (MVO_2) calculated (arterial- mixed venous oxygen content x CO). UL-FS 49 was infused (10 - 300 μgkg^{-1}) & the following additional parameters determined in the presence & absence of atrial pacing (baseline sinus rate + 10 bpm): stroke volume (SV), cardiac output (CO) mean arterial blood pressure (MABP), ventricular efficiency (VEFF= stroke work/PVA) & ejection fraction (EF).

SINUS RATE:	**HR**	**SV**	**CO**	**MABP**	**Ees**	**Ea**	**PVA**	**VEFF**	**EF**	**MVO_2**
	bpm	ml	l min^{-1}	mmHg	mmHg ml^{-1}	mmHg ml^{-1}	mmHg•l	%	%	ml min^{-1}
baseline	99 ± 5	7.7 ± 0.9	0.75 ± .06	94 ± 4	4.6 ± .9	16.6 ± 1.9	2.6 ± .4	30 ± 3	36 ± 4	36 ± 5
10 μg kg^{-1}	91 ± 4	7.8 ± 0.9	0.70 ± .07	88 ± 6	5.1 ± .9	15.8 ± 1.7	2.3 ± .5	32 ± 4	37 ± 4	41 ± 6
30	85 ± 4*	7.8 ± 0.9	0.66 ± .07*	83 ± 6*	5.2 ± 1.2	14.5 ± 1.5*	2.2 ± .5	35 ± 4	38 ± 4	42 ± 6
100	73 ± 4*	7.9 ± 1.0	0.57 ± .07*	70 ± 7*	5.3 ± 1.0	13.5 ± 1.5*	1.9 ± .5*	37 ± 4*	39 ± 3*	38 ± 6
300	56 ± 4**	8.4 ± 1.1*	0.46 ± .06*	54 ± 4**	6.1 ± 1.2	11.0 ± 1.3**	0.9 ± .2**	44 ± 4**	41 ± 4**	37 ± 7
PACING:										
baseline	110 ± 4	7.2 ± 0.8	0.80 ± .06	99 ± 4	5.3 ± 1.0	18.0 ± 1.8	2.5 ± .4	30 ± 3	35 ± 3	34 ± 3
10 μg kg^{-1}	110 ± 4	7.0 ± 0.9	0.76 ± .07	97 ± 6	5.6 ± 1.1	18.6 ± 2.1	2.3 ± .5	31 ± 4	35 ± 4	41 ± 5
30	110 ± 4	6.8 ± 0.8	0.74 ± .07	96 ± 7	5.8 ± 1.2	18.8 ± 2.1	2.3 ± .5	31 ± 4	34 ± 3	43 ± 6
100	110 ± 4	6.5 ± 0.7	0.71 ± .06	93 ± 5	5.5 ± 1.0	18.9 ± 1.5	2.3 ± .5	29 ± 4	34 ± 4	42 ± 4
300	110 ± 4	6.5 ± 0.7	0.70 ± .06	101 ± 4	5.9 ± 1.2	20.9 ± 1.6	2.5 ± .5	28 ± 4	32 ± 3	45 ± 5

Data are shown as the mean ± s.e. mean of 6 animals, with * $P < 0.05$;** $P < 0.01$ compared to baseline

UL-FS 49 caused a dose-related reduction in HR, CO & MABP, while MVO_2 was unaffected. Pacing of the hearts largely prevented these changes in CO and MABP. PVA showed a marked fall due to the reduction in arterial elastance, allowing ventricular efficiency & ejection fraction to increase. These data suggest that UL-FS 49 is highly selective and reduces cardiac oxygen consumption per unit time (reduced HR) & per beat (due to reduced arterial elastance) while maintaining MVO_2.

Kobinger,W. & Lillie,C. (1984) *Eur. J. Pharmacol.* 104, 9-18

45P ROLE OF CYCLIC GUANINE MONOPHOSPHATE (cGMP) IN THE SECRETION OF GROWTH HORMONE (GH) FROM RAT PITUITARY TISSUE *IN VITRO*

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Recent evidence suggests a role for cGMP in the control of the release of corticotrophin (Dayanithi et al.,1990) and luteinizing hormone (Wun et al.,1988) but the extent to which it contributes to other aspects of pituitary function is ill-defined. In these experiments the effects of a cGMP derivative and drugs which modulate intracellular cGMP concentrations on the secretion of GH by rat anterior pituitary segments in vitro were examined in the presence and absence of growth hormone releasing hormone (GHRH). In addition, in some experiments, the cGMP content of the segments was determined.

Anterior pituitary segments were collected post-mortem from adult male Sprague-Dawley rats (≈200g) and, following a 2h pre-incubation period, incubated in the presence or absence of various test substances using 1-60 min contact times. GH released into the medium was determined by enzyme linked immunosorbent assay (ELISA) and the tissue cGMP content was measured using a scintillation proximity assay. All results are expressed as mean±sem and the units are either ng/mg pituitary tissue (GH secretion) or fmol/mg pituitary tissue (cGMP content). GHRH (1-100nM) caused significant ($P<0.005$) concentration and time dependent increases in GH release. A submaximal concentration of GHRH (10nM) significantly increased GH release from 222.9±74.1 to 656.5±113.9 ($P<0.05$) within 60 min. The effects of GHRH were mimicked over the same time course by the stable cGMP derivative 8Br-cGMP (0.1-100μM) which at a concentration of 5μM produced maximal increases in GH release (control:351.6±38.7 vs 8Br-cGMP:1170±168.8, $P<0.01$), which were additive with those of GHRH (10nM). In contrast, the soluble guanylate cyclase activator, sodium nitroprusside (SNP, 3μM), which also stimulated basal peptide release (from 72.9±25.9 to 208.4±58.9, $P<0.05$), potentiated the GHRH-stimulated GH release by 2.9 fold (10μM, $P<0.01$), although at higher concentrations it failed to affect either basal or stimulated peptide secretion. The stimulatory effect of SNP (3μM) on basal GH release was associated with a time dependent increase in cGMP content of the tissue after 2min (from 19.0±1.3 to 44.1±7.9, $P<0.005$) and 5min (from 38.8±6.4 to 92.4±16.4, $P<0.005$). In contrast GHRH(10nM) was without significant effect on cGMP content. Zaprinast (0.25-1μM), a cGMP specific (Type V) phosphodiesterase (PDE) inhibitor, precipitated significant concentration dependent increases in basal GH release which were maximal at 0.1μM (control:29.2±2.2 vs zaprinast:238.0±61.6, $P<0.05$), but was without significant effect on the GHRH-stimulated release. In contrast, the responses to another Type V PDE inhibitor, dipyridamole (0.001-1.0 μM), were more variable. Thus, while it generally failed to influence basal GH secretion, in one instance 1μM precipitated a 1.7 fold increase ($P<0.05$). Similarly the responses to GHRH (10nM) were either decreased (0.01 μM) or increased (0.005 & 0.01μM) by this compound. Paradoxically an inhibitor of the soluble guanylate cyclase, LY83583, (0.1-50 μM) precipitated a concentration dependent increase in basal GH release which was maximal at 0.5μM (control:130.3±11.1 vs LY83583:352.3±81.6, $P<0.05$) and had an additive effect with GHRH (10nM).

These results suggest that cGMP contributes to the complex mechanisms regulating GH secretion by the anterior pituitary gland, however this pathway would appear not to be involved in GHRH-stimulated peptide release.

We are grateful to The Wellcome Foundation and Charing Cross &Westminster Medical School for financial support and to NIDDK for the reagents for the GH ELISA.

Dayanithi, G. & Antoni, F.A.(1990) J.Endocrinol. **125**(1), 39-44

Wun, W.S.A., Berkowitz, A.S. & Preslock, J.P.(1988) Mol.Cell.Endocrinol. **55**(2-3),173-182.

46P HYPOTHALAMIC CORTICOTROPHIN-RELEASING FACTOR-41 AND PROSTAGLANDIN E_2 RELEASE IN RESPONSE TO CONDITIONED MEDIA FROM ACTIVATED RAT SPLEEN CELLS

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Compounds which activate the immune and febrile responses have been shown to stimulate the release of hypothalamo-pituitary-adrenocortical (HPA) axis hormones and activate prostaglandins. Previous reports have shown that the pyrogenic interferon inducer polyinosinic:polycytidylic acid (poly-I:C) activates the HPA axis in a corticotrophin-releasing factor-41 (CRF-41)-dependent manner (Milton *et al.*, 1992a). However, poly-I:C fails to directly stimulate the release of CRF-41 or prostaglandin-E2 (PGE2) from the hypothalamus *in vitro* (Milton *et al.*, 1992b). In this study we sought to examine the effects of conditioned media from poly-I:C stimulated spleen cells on the release of CRF-41 and PGE2 from the intact rat hypothalamus incubated *in vitro*.

Spleens were removed from adult male Wistar rats and mechanically dissociated into a single cell suspension. After lysis of red blood cells and washing with RPMI 1640 culture medium, cells were incubated (10^7 cells ml^{-1}) in medium containing poly-I:C (100 μg ml^{-1}) for 16 hrs at 37°C. Medium was harvested from cells, centrifuged to remove debris, filtered through a 0.2 μm filter and diluted 1:10 with Krebs bicarbonate buffer to give conditioned medium for incubation with hypothalami. Rat hypothalami were removed from adult male Wistar rats after decapitation and incubated in a Krebs bicarbonate buffer for 1 hr, with three changes of medium. A 20 min incubation to establish basal release was followed by a 20 min incubation with test substance, a second 20 min basal incubation and a second 20 min test substance incubation. CRF-41 and PGE2 released into the medium were measured by two-site enzyme-amplified-immunometric assay and radioimmunoassay respectively. Results are expressed as mean ± s.e.m. from at least six determinations.

Results showed that conditioned medium from spleen cells incubated with poly-I:C contained 45 ± 7 pg ml^{-1} PGE2 and no detectable CRF-41 (< 10 pg ml^{-1}). Conditioned medium had no effect on CRF-41 release during the first stimulation (basal release 33 ± 5 pg ml^{-1}; conditioned medium 30 ± 4 pg ml^{-1}), however, the second incubation with conditioned medium stimulated a 7-fold increase in CRF-41 release (basal release 38 ± 6 pg ml^{-1}; conditioned medium 275 ± 49 pg ml^{-1}). PGE2 release from hypothalami was increased from 31 ± 7 pg ml^{-1} to 415 ± 98 pg ml^{-1} during the first incubation with conditioned medium and from 35 ± 7 pg ml^{-1} to 333 ± 80 pg ml^{-1} during the second incubation with conditioned medium. Dilution curves for conditioned media showed that CRF-41 release was stimulated, during the second incubation period, with both undiluted and a 1:10 dilution of conditioned medium, PGE2 release was stimulated, in a dose dependent manner, with dilutions of conditioned medium up to 1:10,000 causing significant increases in PGE2 release. Conditioned medium from spleen cells incubated in RPMI alone had no significant effect on CRF-41 or PGE2 release from the hypothalamus *in vitro*.

In conclusion, incubation of spleen cells with poly-I:C stimulates the production of substances which can activate both CRF-41 and PGE2 release from the intact rat hypothalamus incubated *in vitro*.

Milton, N.G.N., Hillhouse, E.W. & Milton, A.S. (1992a) *J.Endocrinol.*, 135, 69-75.

Milton, N.G.N., Self, C.H. & Hillhouse, E.W. (1992b) *J.Endocrinol.*, 132 (Supplement), Abstract 214.

47P PRE- AND POST-SYNAPTIC EFFECTS OF NEUROPEPTIDE Y (1-4)Aca(25-36) IN ANAESTHETISED, AREFLEXIC RATS

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Neuropeptide Y (NPY) has actions on the cardiovascular system through putative post-synaptic (Y_1) and pre-synaptic (Y_2) receptors. An analogue of NPY containing small N- and C-terminal fragments linked by ϵ-aminocaproic acid, NPY(1-4)Aca(25-36), has been shown to bind to NPY receptors with high affinity and to be an agonist at pre-synaptic receptors in vitro and at post-synaptic receptors in pithed rats (Beck et al, 1989). To study the selectivity of NPY(1-4)Aca(25-36) in vivo, we have compared its effects in anaesthetised, areflexic rats with NPY and the selective Y_1 receptor agonist Leu^{31}, Pro^{34} NPY (1-36) (LP-NPY) (Fuhlendorff et al, 1990).

Non-recovery experiments were carried out in pentobarbitone anaesthetised, bilaterally vagotomised, female AP strain rats which had been catecholamine depleted with syrosingopine as described previously (Haworth et al, 1992). Pressor responses were measured as an index of post-synaptic activity and inhibition of the increased pulse interval following electrical stimulation of the right vagus nerve as an index of pre-synaptic activity as described by Potter & McCloskey, 1992. Signals from a blood pressure transducer and e.c.g. electrodes were captured on a Compaq 386 computer and systolic and diastolic arterial pressure and pulse interval stored digitally for subsequent analysis using software developed by M Posnett, Research Engineering Laboratory, ZENECA Pharmaceuticals. NPY and LP-NPY were equipotent pressor agents, ED_{50} 1.4±0.4 and 1.4±0.5nmolkg^{-1} i.v. respectively (mean±s.e.mean, n=4), increasing systolic arterial pressure maximally by 74±4 and 78±6mmHg respectively. In contrast, NPY(1-4)Aca(25-36) increased systolic arterial pressure only slightly at 1nmolkg^{-1} (5±1mmHg, n=5) and by only 47±6mmHg at 100nmolkg^{-1}. NPY inhibited vagally-mediated bradycardia, ED_{50} 7.6±2.1nmolkg^{-1} i.v. (n=4) , while LP-NPY had little inhibitory effect at doses up to 160nmolkg^{-1} (maximum 15.1±6.7% inhibition, n=4). However, NPY(1-4)Aca(25-36) inhibited vagally-mediated bradycardia with similar potency to NPY (ED_{50} 10.6±1.6nmolkg^{-1} i.v., n=5). Inhibition of bradycardia by NPY(1-4)Aca(25-36) was rapid in onset and short in duration, reaching a maximum (86±3%) after 30s and disappearing by 10min after injecting 100nmolkg^{-1}. In contrast, the inhibitory effect of NPY (55nmolkg^{-1}) did not reach maximum (89±3%) until 4min post-injection and had not decreased by 10min.

These data suggest that NPY(1-4)Aca(25-36) is a selective agonist at the putative pre-synaptic (Y_2) receptor in rats and may be a useful tool for in vivo evaluation of potential antagonists of NPY at this receptor because of its short duration of action.

Beck, A., Jung, G., Gaida, W., Koppen, H., Lang, R. & Schnorrenberg, G. (1989) FEBS Letters 244, 119-122.
Fuhlendorff, J., Gether, U., Aakerlund, L. et al (1990) Proc.Natl.Acad.Sci.USA 87, 182-186.
Haworth, D., Culbert, J-A. & Thomson, D.S. (1992) Br.J.Pharmacol. 107, 22P.
Potter, E.K. & McCloskey, M.J.D. (1992) Neuroscience Letters 134, 183-186.

48P LOCALIZATION OF NEURONAL ACTIVITY MEDIATED BY NEUROPEPTIDE Y IN RAT BRAIN: THE USE OF C-FOS-LIKE IMMUNOREACTIVITY AS AN ANATOMICAL MARKER OF PEPTIDE RECEPTOR ACTIVATION

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Neuropeptide Y (NPY) is the most abundant peptide found in the mammalian brain. It is co-localised with other neurotransmitters such as noradrenaline (Lundberg and Hökfelt, 1986) and appears to act as a neuromodulator to affect their function. It has been shown to be involved in many physiological processes, both in the periphery and in the central nervous system (Michel and Buschauer, 1992). The purpose of the present study was to identify areas of neuronal activation following central NPY administration in rat brain. This was achieved by monitoring the protein product of the immediate-early gene, c-fos, which has previously been shown to be a useful marker of cellular activity within the CNS (Sagar *et al.*, 1988).

Male Wistar rats (250-310g) were anaesthetised and implanted with indwelling guide cannulae positioned 1mm dorsal to the planned injection site, the right lateral ventricle. The animals were then allowed to recover and handled daily for 6 days, after which physiological saline (1µl), with or without 1nmol NPY, was injected via an injection cannula inserted into and 1mm beyond the tip of the guide cannula. Three hours later the rats were reanaesthetised and perfused transcardially with paraformaldehyde solution (4% w/v). C-fos-like immunoreactivity (FLI) was detected in 100µm brain sections using immunohistochemistry.

Regions showing increased FLI following NPY administration are given in Table 1 (values are the mean (± SEM) number of immunopositive cells/0.03mm^2, n = number of animals). All regions listed showed significantly higher FLI in NPY treated brains ($p<0.05$, Mann-Whitney U test). No other brain areas examined showed any significant change in FLI.

Table 1

Brain Region	NPY (1nmol/µl)	n	Saline (1µl)	n
ventral hippocampus CA1 area	14.00 ± 2.55	5	5.20 ± 1.46	5
dorsal endopiriform nucleus	10.60 ± 1.66	5	5.40 ± 0.51	5
amygdalopiriform transition area	8.80 ± 3.22	5	0.80 ± 0.49	5
entorhinal cortex	6.80 ± 2.69	5	0.40 ± 0.24	5
accumbens nucleus	19.00 ± 2.61	4	6.75 ± 2.28	4
paraventricular thalamic nucleus	21.40 ± 3.50	5	10.00 ± 1.34	5

All regions of the rat brain which responded to NPY injection also contain either NPY-immunoreactivity or NPY receptors. However, the converse is not always true. Nevertheless FLI appears to provide a useful marker of NPY-induced cellular activity in mammalian brain.

Lundberg, J.M. and Hökfelt, T. (1986) Progress in Brain Research 68: 241-262
Michel, M.C. and Buschauer, A. (1992) Drugs of the Future 17: 39-45
Sagar, S.M., Sharp, F.R. and Curran, T. (1988) Science 240: 1328-1331n

49P BEHAVIOURAL EFFECTS OF FULL AND PARTIAL BENZAZEPINE D_1 DOPAMINE AGONISTS IN THE MPTP-TREATED MARMOSET

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The benzazepine SKF 38393 is a selective and partial efficacy D-1 dopamine (DA) agonist but which fails to alleviate motor deficits in MPTP-treated primates (Close et al., 1985; Nomoto et al., 1988). We now report on the behavioural effects, in the MPTP-treated marmoset of a range of D-1 DA receptor selective benzazepine analogues, with partial (SKF 83565, SKF83959 and SKF 75670) and full efficacies (SKF 80723 and SKF 82958) in stimulating the enzyme adenylate cyclase (AC).

Four to eight weeks after MPTP treatment (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; total dose 8-10 mg/kg over 5-7 days; sc) of 12 common marmosets (male and female), behavioural studies were undertaken using automated activity cages. SKF 38393 (7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine), SKF 80723 (6-Br analogue), SKF 83565 (6-Cl, 3-CH_3, 3'-Cl analogue), SKF 83959 (6-Cl, 3-CH_3, 3'-CH_3 analogue), SKF 82958 (6-Cl, 3-C_3H_5 analogue) and SKF 75670 (3-CH_3 analogue) were administered in doses of 2.5-10.0 mg/kg (in 1-5% DMSO/0.9% saline vehicle; ip). Locomotor activity was measured for 120 mins. Motor disability, grooming and oral activity were observer rated for 60 mins.

SKF 38393 and SKF 75670 further reduced locomotor activity and increased disability in MPTP-treated animals. SKF 83565 and SKF 82958 produced decreases in disability at high doses. Pronounced improvements in locomotion and a reversal of disability were observed with SKF 83959 and SKF 80723 (Table 1). Oral activity (tongue protrusions and vacuous chews) were increased by SKF 38393, SKF 83565, SKF 82958, SKF 83959 and SKF 80723. Grooming was increased with SKF 82958, SKF 83959 and SKF 80723 (table 1).

Table 1: Behavioural effects of SKF 80723 and SKF 83959 in MPTP-treated marmosets. Values are expressed as mean ± s.e.mean; (n = 4-6); *p<0.05; **p<0.01 compared to vehicle treatment (ANOVA and Duncan's multiple range test or Kruskal Wallis and Mann-Whitney U-test).

	SKF 80723			SKF 83959		
Dose (mg/kg)	*Locomotion (counts/120 min)*	*Oral activity (scores/60 min)*	*Grooming (scores/60 min)*	*Locomotion (counts/120 min)*	*Oral activity (scores/60 min)*	*Grooming (scores/60 min)*
vehicle	442 ± 157	0.8 ± 0.4	0.3 ± 0.1	561 ± 152	0.7 ± 0.4	0.8 ± 0.6
2.5	1877 ± 399	7.9 ± 1.0**	10.2 ± 1.7**	4556 ± 1353**	4.0 ± 0.5**	4.0 ± 1.5
5.0	1393 ± 284	5.8 ± 1.2*	6.3 ± 3.0	3243 ± 873*	5.9 ± 2.0*	8.5 ± 2.8*
10.0	2629 ± 1093**	11.0 ± 0.9*	6.8 ± 0.8*	5568 ± 988**	7.6 ± 1.2**	6.5 ± 1.7*

The antiparkinsonian effects of full (SKF 80723 and SKF 82958) and partial efficacy D-1 DA agonists (SKF 83959 and SKF 83565), may implicate a behavioural role for D-1 DA receptors uncoupled to the enzyme AC.

Close, S.P., Marriott, A.S. & Pay, S. (1985) Br. J. Pharmacol. 85, 320-322.
Nomoto, M., Jenner, P. & Marsden, C.D. (1988) Neurosci. Letts. 93, 275-280.

50P BEHAVIOURAL EFFECTS OF QUINPIROLE COMBINED WITH FULL AND PARTIAL EFFICACY D_1 DOPAMINE AGONISTS IN THE MPTP-TREATED MARMOSET

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The selective but partial efficacy, D-1 dopamine (DA) agonist SKF 38393 (7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) inhibits the antiparkinsonian activity of the D-2 DA agonist quinpirole (Nomoto et al., 1988). We now report on the behavioural effects in the MPTP-treated marmoset of the co-administration of quinpirole and a range of D-1 DA receptor selective benzazepine analogues, with partial (SKF 75670 and SKF 83959) and full (SKF 80723 and SKF 82958) efficacies in stimulating the enzyme adenylate cyclase (AC) (Arnt et al., 1992).

10 common marmosets (both sexes) were treated with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; total dose 8-10 mg/kg; sc) over a 5-7 day period. Four to eight weeks later, the animals were pretreated with domperidone (2 mg/kg in 10% sucrose solution; po) and were placed in automated locomotor activity cages. Thirty mins later, the animals recieved quinpirole (0.1 mg/kg in 0.9 % saline; ip). Twenty mins later, varying doses of SKF 80723 (6-Br analogue of SKF 38393; 0.125-1.0 mg/kg), SKF 83959 (6-Cl, 3-CH_3, 3'-CH_3 analogue; 0.25-1.0 mg/kg), SKF 82958 (6-Cl, 3-C_3H_5 analogue; 2-10 mg/kg) and SKF 75670 (3-CH_3 analogue; 2.5-5.0 mg/kg) (in 1-5% DMSO/0.9% saline vehicle; ip) were administered. Locomotor activity was measured for 120 mins. The animals were observer rated for motor disability, grooming and oral activity for 60 mins.

Administration of quinpirole increased locomotion and decreased motor disability, while grooming and oral activity were unaffected. SKF 75670 inhibited these quinpirole mediated effects in MPTP-treated animals (Figure 1a). SKF 82958, SKF 83959 and SKF 80723 had no overall effect on the actions of quinpirole. However, in individual animals both SKF 83959 and SKF 80723 were able to potentiate the locomotor effects of quinpirole (Figures 1b and 1c). Higher doses of SKF 83959 and SKF 80723 tended to induce seizures. Oral activity (tongue protrusions and vacuous chews) and grooming were unaffected by any of the D-1 DA agonists.

The ability of some full and partial efficacy benzazepine D-1 DA agonists to potentiate the antiparkinsonian effects of quinpirole, atleast in some animals may further indicate a behavioural role for D-1 DA receptors uncoupled to the enzyme AC.

Nomoto, M., Jenner, P. & Marsden, C.D. (1988) Neurosci. Lett. 93, 275-280.
Arnt, J., Hyttel, J. & Sanchez, C. (1992) Eur. J. Pharmacol. 213, 259-267.

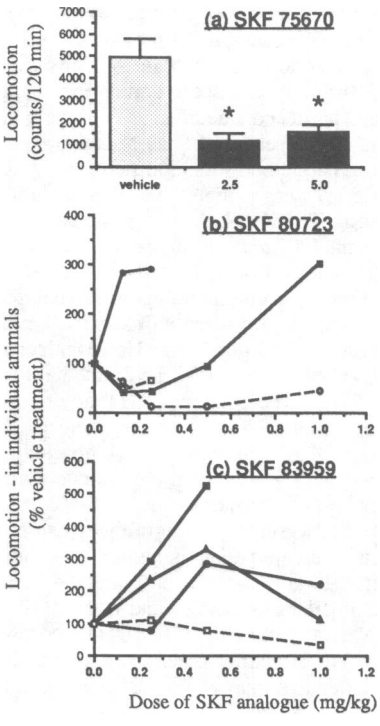

Figure 1: Effect of SKF 75670 (a), SKF 80723 (b) and SKF 83959 (c) on locomotor activity induced by quinpirole. (n=4-5); *p<0.05 (ANOVA and Duncan's multiple range test).

51P DOPAMINE D_4 RECEPTOR POLYMORPHISM IN CLOZAPINE-TREATED SCHIZOPHRENIC PATIENTS

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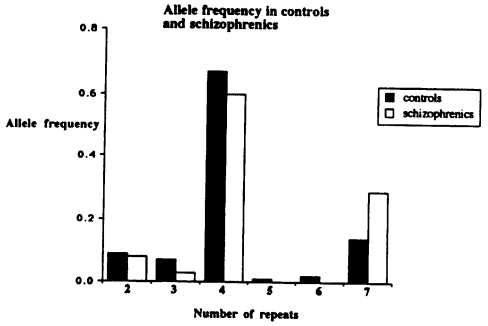
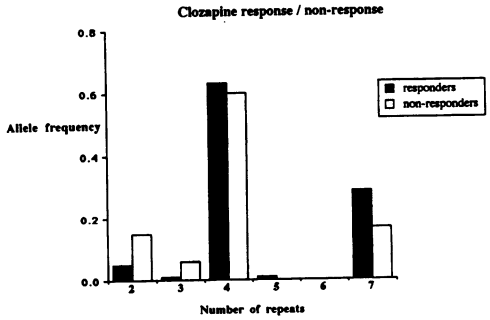
Dopamine receptors have remained the primary targets for drugs used in the treatment of schizophrenia. Drug binding studies have determined uniquely high affinity of the antipsychotic drug clozapine for the human dopamine D4 receptor. Following the cloning of the D4 receptor gene a recent report by Van Tol (1992) described the presence of a 48 base pair repeat polymorphism within the putative third cytoplasmic loop of this protein. Variant alleles with repeat numbers between two and eight were shown to exist, and were found to exhibit different affinities for clozapine. This suggest that such variable forms may underlie susceptibility to schizophrenia and/or its responsiveness to clozapine.

In order to test this hypothesis we have developed a novel PCR based method to rapidly and efficiently type a sample of control individuals and 64 schizophrenia patients divided into clozapine responders (41) and non-responders (23). We report the detection of alleles with repeats between two and seven. Southern hybridisation confirmed the identity of these polymorphisms as part of the D4 gene. To date the seventh homozygous variant appears only in schizophrenic patients and not in controls. For the clozapine patients analysis of the allelic frequencies of the polymorphisms do not seem to predict a pharmacogenetic substrate for variability in response to clozapine. The seventh homozygous repeat is in statistical excess in patients, but this is a low frequency association represented in a small number of patients.

Figures 1 & 2 show the frequency of polymorphisms in responders, non-responders, controls and schizophrenics.





Van Tol, H.M., Bunzow, J.R., Guan, H.C., Sunahara, R.K., Seeman, P., Niznik, H.B., Civelli, O. (1991) Nature 350, 610-614.

52P QUANTITATIVE ANALYSIS OF THE DISTRIBUTION OF [^{3}H]-PAROXETINE BINDING SITES IN RAT BRAIN SECTIONS

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Paroxetine is an antidepressant which selectively inhibits 5-HT uptake by CNS neurones and this may be a property important to its therapeutic efficacy. It has previously been reported that the binding of radiolabelled paroxetine provides a marker for neurones possessing 5-HT uptake sites in rat brain sections (De Sousa & Kuyatt 1987; Hrdina *et al.*, 1990; Battaglia *et al.*, 1991). Thus we are using this ligand currently to discern and define changes in 5-HT systems which occur following neural lesions. As a background for our studies we have produced a detailed map of the binding sites using quantitative autoradiography. Whilst our results concur with previous limited reports the present study has been more extensive detailing sites not mentioned before.

The method was modified from that described by Battaglia *et al.* (1991). Cryostat coronal sections (10μm) from male Wistar rats (200-250g) were mounted on chrome alum gelatine-coated glass slides and stored at -20°C. For labelling sections were preincubated for 15 min in Tris HCl buffer (50mM, pH7.7, 20°C) containing 120mM NaCl and 5mM KCl. Sections were then incubated in 0.25nM ^{3}H-paroxetine (20Ci/mmol) in the Tris buffer for 120 min at 20°C before rinsing for 2 x 60 min at 20°C. Background binding was determined by the inclusion of 4μM citalopram. Dried sections were apposed to Hyperfilm together with tritium standards for 8 weeks before developing. Autoradiographs were analysed using a Quantimet 970 analyser.

The highest density of specific binding was detected in the dorsal Raphé nucleus (340 ± 155 fmoles/mg tissue, mean ± s.e. mean, n = 3 rats, 4-6 sections/rat). However, other midbrain nuclei also exhibited moderately high binding including the pontine reticular (241 ± 144), Raphé pontis (170 ± 92), medial Raphé (83 ± 17) and rhabdoid (97 ± 23) nuclei. The central grey area (130 ± 63) was also fairly high. Within the pons medulla many nuclei were heavily labelled, in particular, the locus coeruleus (290 ± 109), facial nucleus (202 ± 51) and gigantocellular reticular nucleus (150 ± 51). Moderate levels were detected in the Raphé magnus (97 ± 31) and spinal trigeminal tract (62 ± 16). Throughout the hippocampus generally low levels were noted. In CA1, CA2 and CA3 densities of 36-38 fmoles/mg were detected with 5.9 ± 0.8 in the dentate gyrus and <1 in the fimbria. Low levels were also present throughout the hypothalamus (14-27 fmoles/mg) and thalamic nuclei (8-40), with the exception of the posterior paraventricular nucleus (75 ± 30) and lateral geniculate (61 ± 28). In the substantia nigra the densities ranged from 42 ± 9 (pars reticulata) to 67 ± 14 (ventral tegmental area) whilst in the globus pallidus, caudate putamen, ventral pallidum and claustrum the levels were all lower (18-27). The only region of the amygdala exhibiting moderate binding was the basolateral nucleus. In cerebrocortical regions the entorhinal cortex exhibited the highest binding (80 ± 36) with less in occipital (46 ± 27) and frontal (47 ± 10) cortices. However a distinct narrow band of high density was always observed on the mediolateral perimeter of the frontal cortex.

The structures considered here include some of those examined by Battaglia *et al.* (1991) and since the data are comparable our more extensive map of ^{3}H-paroxetine binding sites in rat brain should provide a firm basis for neural ablation studies.

Battaglia, G., Sharkey, K., Kuhar, M.J. & De Sousa, E.B. (1991) *Synapse* 8: 249-260.
De Sousa, E.B. & Kuyatt, B.L. (1987) *Synapse* 1: 488-496.
Hrdina, P.D., Foy, B., Hepner, A.G. & Summers, R.J. (1990) *J. Pharm. Exp. Ther.* 252: 410-418.

53P ENHANCEMENT BY TRICHLOROETHANOL OF AGONIST-INDUCED CURRENTS MEDIATED BY A CLONED MURINE 5-HT_3 RECEPTOR SUBUNIT (5-HT_3R-A) EXPRESSED IN *XENOPUS* OOCYTES

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Ethanol is reported to enhance 5-HT_3 receptor mediated currents recorded from murine NCB-20 hybridoma cells and rat nodose ganglion neurones (Lovinger and White, 1991). In behavioural studies, the 5-HT_3 receptor antagonist ondansetron reduces ethanol (EtOH) consumption in rats and man (Sellers *et al.*, 1992). A cDNA encoding a 5-HT_3 receptor subunit (5-HT_3R-A) has been cloned from NCB-20 cells (Maricq *et al.*, 1991). Here, we have investigated the action of trichloroethanol (TCEtOH) on 5-HT_3 receptor mediated currents recorded from voltage-clamped *Xenopus* oöcytes injected with *in vitro* RNA transcripts of the 5-HT_3R-A cDNA. In oöcytes voltage-clamped at -30mV, 5-HT (0·1-30μM) and the 5-HT_3 receptor agonist 2-Me-5-HT (1-300μM) evoked concentration dependant inward current responses with calculated EC_{50}s of 0·93 ± 0·04μM (n=3) and 13·6 ± 0·7μM (n=3) respectively. However, the maximal response evoked by 2-Me-5-HT (300μM) was only 66 ± 1% (n=3) of that produced by a maximally effective concentration (30μM) of 5-HT. TCEtOH (0·3-10mM) produced a concentration dependant enhancement of the amplitude of the current evoked by 0·5μM 5-HT. The estimated EC_{50} for TCEtOH was 4·4 ± 0·8mM (n=4), a concentration which potentiated the 5-HT-induced current to 816 ± 172% of control. 5mM TCEtOH reduced the EC_{50} for 5-HT and 2-Me-5-HT to 0·44 ± 0·02μM (n=3) and 4·7 ± 0·3μM (n=3) respectively. The maximal current induced by 5-HT (10μM) was not influenced by this concentration of TCEtOH. However, the current induced by 2-Me-5-HT (100μM) was increased to 107·0 ± 3·0% (n=3) of that evoked by a saturating concentration (30μM) of 5-HT. We have recently cloned, from N1E-115 neuroblastoma cells, an apparent splice variant of the 5-HT_3R-A cDNA(termed 5-HT_3R-A_S) which has a deletion of 6 amino-acid residues in the large cytoplasmic loop connecting the M3 and M4 domains (Hope *et al.*, 1993). In view of the differential sensitivity of the short and long forms of the γ_2 subunit of the $GABA_A$ receptor towards ethanol (Wafford *et al.*, 1991), we have further investigated the actions of TCEtOH on 5-HT currents recorded from oöcytes pre-injected with *in vitro* RNA transcripts of the 5-HT_3R-A_S clone. In such cells, currents evoked by 5-HT (3μM) and 2-Me-5-HT (10μM) were enhanced by 5mM TCEtOH. However, since recent studies have shown TCEtOH to modulate EtOH insensitive GABA responses, future experiments will evaluate the effect of EtOH itself.

We thank SmithKline Beecham for financial support.

Lovinger, D.M. and White, G. (1991). Mol. Pharmacol., **40**, 263-270.
Sellers, E.M., Higgins, G.A. and Sobell, M.B. (1992). Trends Pharmacol. Sci. **13**, 69-75
Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M. and Julius, D. (1991). Science, **254**, 432-437.
Hope, A.G., Downie, D.L., Lambert, J.J., Peters, J.A., Sutherland, L. and Burchell, B. (1993). Br. J. Pharmacol., in press
Wafford, K.A., Burnett, D.M., Leidenheimer, N.J. Burt, D.R., Wang, J.B., Kofuji, P., Dunwiddie, T.V.,*et al.* (1991). Neuron, **7**, 27-33.

54P 5-HT_3 RECEPTORS DO NOT MEDIATE THE RESPONSES OF THE GUINEA-PIG ISOLATED STOMACH TO VAGAL STIMULATION

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5-hydroxytryptamine (5-HT) has been proposed as a neurotransmitter in the vagal inhibitory innervation of the guinea-pig stomach (Bulbring and Gershon, 1967). Activation of neuronal 5-HT3-receptors causes depolarisation of efferent as well as afferent neurones, and neurotransmitter release (Humphrey & Feniuk, 1987). Ondansetron (ON, GR38032F) and metoclopramide (ME) are antagonists, and 2-methyl-5-HT a selective agonist, of 5-HT3 receptors (Butler et al., 1988). Nitric oxide (NO) is a mediator of vagally-induced non-adrenergic, non-cholinergic relaxation in the guinea-pig stomach (Desai et al., 1991)and here we investigate whether 5-HT3 receptors are also involved in this response.

Guinea-pig stomachs were cannulated through the duodenum by a plastic cannula (i.d. 5 mm), emptied of food and ligated. The oesophagus was ligated and the stomach mounted in Krebs' solution in a warmed (38 °C) 450 ml organ bath with the oesophagus and vagus nerve passed through a pair of ring electrodes. The gastric cannula was connected to a reservoir and sealed float recorder to record changes in gastric volume, and to a pressure transducer to measure intragastric pressure (Desai et al., 1991). Relaxations induced by vagal stimulation (VS, supramax. voltage, 10-16 Hz, 1 ms, 50-60 s) were recorded in the presence of atropine (3 μM) and guanethidine (5 μM), and contractions to VS in the presence of the NO synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME, 100 μM). Responses of the stomach were calculated as the % of the total stomach volume (basal + stimulated).

ME (1, 3, 10 or 30 μM, n=5-6 for each) did not affect relaxations induced by VS although 60 μM ME decreased them from 39±2 % (n=14) to 24±3% (n=12, $p<0.05$). Similarly, ON (1, 3, and 10 μM, n=5) did not affect the relaxations to VS, but 30 μM ON reduced them from 49±2 % (n=13) to 34±5 % (n=10). 2-methyl-5-HT (100 μM) initially induced a small relaxation of the stomach followed by desensitization such that further doses failed to elicit relaxations. This desensitization did not affect relaxations in response to VS (con., 39±1 %, n=9; desens., 39±2 %, n=9), and ON (30 μM) did not antagonise relaxations induced by 2-methyl-5-HT (con., 14±2 %, n=3; +ON, 12±3 %, n=3). Contractions induced by VS were also not inhibited by ON (30 μM) (con., -85±5 %, n=4; +ON, -83±7 %, n=4), while 2-methyl-5-HT induced only very small contractions (≤5 %). Desensitization to 2-methyl-5-HT did not lead to cross desensitization of contractions induced by VS.

Thus, relaxations to VS are not mediated via 5-HT3-receptors for they were unaffected by all except the highest doses of ME or ON, and desensitization to 2-methyl-5-HT did not lead to cross-desensitization of relaxations induced by VS. It is likely that the highest concentrations of ME and ON were inhibiting by effects at some other receptor, for these are potent and selective antagonists of the 5-HT3-receptor (Butler et al., 1988). It is also clear that 5-HT3-receptors do not mediate contractions in response to VS, for they were neither antagonised by ON, nor affected by desensitization to 2-methyl-5-HT.

This work was supported by Glaxo Group Research Limited.

Bulbring, E. and Gershon, M.D. (1967). *J. Physiol.*, **192**, *823-846.*
Butler, A., Hill, J.M., Ireland, S.J., Jordan, C.C. and Tyers, M.B. (1988). *Br. J. Pharmacol.*, **94**, *397-412.*
Desai, K.M., Zembowicz, A., Sessa, W.C. and Vane, J.R. (1991). *Proc. Natl. Acad. Sci. (USA)*, **88**, *11490-11494.*
Humphrey, P.P.A. and Feniuk, W. (1987). In: *Neuronal Messengers in Vascular Function.* (eds. Nobin, A., Owman, C. and Arnelko-Nobin, B.), Elsevier Science Publishers, pp.3-19.

55P EFFECT OF ANTAGONISTS AT BK_1, BK_2 AND INTERLEUKIN-1 RECEPTORS ON THE EXPRESSION OF BK_1 RECEPTORS IN THE LPS-TREATED RABBIT: *IN VIVO* STUDIES

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There are 2 main types of BK receptors, BK_2 and BK_1. The BK_2 receptor is predominantly constitutive whereas BK_1 receptors appear to be inducible and involved in chronic inflammatory syndromes such as hyperalgesia (Perkins et al,1992), airways hyperreactivity (Farmer et al,1992) and sepsis. The mechanism(s) involved in the up-regulation of BK_1 receptors is unclear, however a variety of agents including LPS and IL1 are particularly efficacious in vitro (e.g. rabbit aorta) and in vivo (e.g. rabbit blood pressure) (Deblois et al, 1991). We have investigated the effect of antagonists at BK_2 (CP-0127), BK_1 (des-Arg^{10}-Leu^9-kallidin, CP-0298) BK_2 and BK_1 (CP-0364), (Whalley et al, 1992) receptors and the interleukin 1 antagonist (IL1ra) Antril (Hannum et al, 1990) on the upregulation of BK_1 receptors in vivo using the LPS-treated rabbit blood pressure assay.

Male New Zealand rabbits were anaesthetized with pentobarbitone, 40 mg/kg i.v. and prepared with i.a. and i.v. cannulae for the recording of blood pressure and the infusion or injection of drugs respectively. Blood pressure responses were recorded to standard doses of BK, (0.8nmole) or des-Arg^9-BK, (8 nmole). LPS from E Coli, 10 ug, was then injected i.v. Animals then received a continuous i.v. infusion for 210 min of either saline (n=10), CP-0127, 1 nmole/kg/min, (n=6); CP-0298, 1 nmole/kg/min (n=3), CP-0364, 1 nmole/kg/min (n=4); CP-0127 + CP-0298 1 nmole/kg/min; Antril 10 mg/kg + 15 ug/kg/min(n=4) or Antril 10 mg/kg + 15 ug/kg/min + CP-0364 1 nmole/kg/min(n=4). Responses to BK and des-Arg^9-BK were produced at 30 min intervals during and after stopping the infusions. Rabbit mesenteric arteries (RbMA) were set up according to Marceau et al,(1991) and the effect of IL1ra (50ug/ml) against IL1-induced relaxation of Phenylephrine-induced contraction was tested.

In control animals responses to des-Arg9-BK but not BK increased with time and at 210 min appeared maximally induced. During the infusions, CP-0127 blocked BK_2 but not BK_1 and CP-0298 the BK_1 but not BK_2 responses. CP-0364, CP-0127 + CP-0298 and Antril + CP-0364 blocked both BK_2 and BK_1 responses. Antril alone had no effect on BK_2 or BK_1 responses. Within 30-60 minutes after stopping the infusions responses to BK and des-Arg^9-BK were the same as those seen in controls.Antril totally inhibited the relaxant response to IL1 on the RbMA.

These results indicate at least in the LPS treated rabbit, that neither BK_2, BK_1 or IL1 receptors alone or in combination, are involved in the upregulation of BK_1 receptors.

Farmer,S.G., Wilkins,D.E., Meeker,S.A., Seeds, E.A.M. and Page,C. (1992) Br.J.Pharmacol.**107**, 653-659.
Hannum,C.M. et al; (1990), Nature (London) **343**, 336-340.
DeBlois,D., Bouthillier,J and Marceau,F. (1991) Br.J.Pharmacol,**103**, 1057-1066.
Marceau,F., Petitclrec,D., Deblois,D., Pradellos,P. and Poubelle,P.E. (1991) Br.J.Pharmacol. **103**, 367-372.
Perkins,M.N., Campbell,E.A., Davis,A. and Dray,A. (1992) Br.J.Pharmacol, **197**, 237P.
Whalley,E.T., Loy,S.D., Modaferri,D., Blodgett,J.K., and Cheronis,J.C. (1992), Br.J.Pharmacol. **107**, 257P.

We wish to thank Synergen, Inc., Boulder, Colorado for the gift of Antril.

56P THE ENDOTHELIN-RECEPTOR ANTAGONIST PD142893 INHIBITS ENDOTHELIUM-DEPENDENT VASODILATATIONS INDUCED BY ENDOTHELIN/SARAFOTOXIN PEPTIDES

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Two receptors for the endothelin/sarafotoxin (ET/SX) family of peptides have been cloned and expressed. The ETA-receptor, which is selective for ET-1 or SX6b and on which SX6c is largely without effect, and the non-isopeptide-selective ETB-receptor. In the isolated perfused mesentery of the rat ET-1 and ET-3 produce endothelium-dependent dilatations, due to activation of non-selective ET-receptors, and vasoconstrictions, due to activation of ET-1-selective receptors (Warner et al., 1989). To characterise better the receptors mediating the effects of the ET/SX peptides in these vessels we have used ET-1 and SX6c as agonists and two ET-receptor antagonists, BQ123 (ETA-selective) and PD142893 (ETA/ETB non-selective).

The isolated perfused mesenteric bed of the rat was prepared as described before (Warner et al., 1989) and perfused at a constant flow of 5 ml min^{-1} with oxygenated (95% O_2:5% CO_2) Krebs' solution containing indomethacin ($5x10^{-6}$ M). Vasoconstriction was induced by infusion of methoxamine ($5x10^{-5}$ M). Vehicle, BQ-123 (10^{-6} M) or PD142893 (10^{-6} M) was then infused for 10 min before, and continued during, bolus injections of acetylcholine (ACh, 3-300 pmol), sodium nitroprusside (SNP, 30-3000 pmol) and ET-1 (0.3-300 pmol) or SX6c (0.3-300 pmol). Vasodilatations were calculated as the percentage reduction in the elevated perfusion pressure.

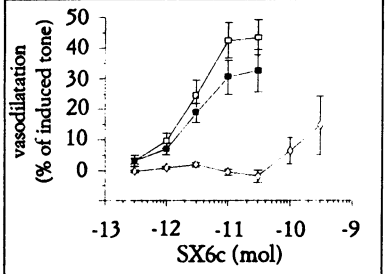

Figure 1. *Vasodilatations to SX6c.* □ *con.;* ■ *+BQ-123;* ◇ *+PD142893*

Vasodilatations to ET-1 or SX6c began at the same threshold dose (0.3 pmol), suggestive of an ETB-receptor-mediated response. 0.3-10 pmol of ET-1 caused principally vasodilatations, whereas doses of greater than 30 pmol produced primarily vasoconstrictions (e.g. 10, 30 and 100 pmol of ET-1 caused vasodilatations of 11±3, 4±1, and 1±1 %, and vasoconstrictions of 11±4, 27±6 and 33±9 mmHg, respectively, n=5-7). SX6c (0.3-30 pmol) produced dose-related vasodilatations (Fig. 1) which declined at higher doses, possibly due to receptor desensitization. BQ-123 (10^{-5} M) did not affect ET-1- or SX6b-induced vasodilatations (Figure 1, n=4-7) indicating an ETB-receptor-mediated response, but did antagonise ET-1-induced vasoconstrictions (e.g. vasoconstriction to 100 pmol ET-1 fell to 12±5 mmHg, n=5). PD142893 inhibited vasodilatations in response to both ET-1 and SX6c (Figure 1) and antagonised vasoconstrictions induced by ET-1 (e.g. 100 pmol ET-1 = 7±2 mmHg, n=5). Endothelium-dependent or -independent vasodilatations induced by ACh or SNP wer unaffected by BQ-123 or PD142893 (n=8-10).

Our results show that PD142893 but not BQ-123 inhibits vasodilatations to ET-1 or SX6c mediated by ETB-receptors within the isolated perfused mesentery of the rat. Thus, PD142893 is an antagonist of the endothelium-dependent vasodilatations induced by the ET/SX peptides.

This work was supported by the Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co. We thank Drs. A. Doherty and W. Cody of the Chemistry Department for providing the ET antagonists BQ-123 and PD142893 ((Ac-(D-Dip-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp)(D-Dip=3,3-diphenylalanine)) for these studies.

Warner, T.D. , de Nucci G. and Vane, J.R. (1989). *Eur. J. Pharmacol.*, **159**, *325-326.*

57P INFLUENCE OF AN ET_A-RECEPTOR ANTAGONIST ON REGIONAL HAEMODYNAMIC RESPONSES TO ENDOTHELIN-1 (ET-1) AND Ala[11,15]Ac-ET-1(6-21) IN CONSCIOUS RATS

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In an attempt to delineate the functional roles of ET_A- and ET_B-receptors, we examined the effects of an ET_A-receptor antagonist, (R)2-[(R)-2-[(S)-2-[1-hexahydro-1H-azepinyl)]carbonyl]amino-4-methyl-pentanoyl]amino-3[3-(1-methyl-1H-indonyl)]propionyl]amino -3- (-2-pyridyl) propionic acid) (FR 139317; Sogabe *et al.*, 1992) on the regional haemodynamic responses to ET-1, and the ET_B-receptor agonist [Ala[11,15]Ac-ET-1(6-21) (BQ3020; Molenaar *et al.*, 1992) in conscious Long Evans rats chronically instrumented with pulsed Doppler probes and intravascular catheters (surgery performed under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.)). Either ET-1(0.5 nmol kg^{-1} i.v.) or BQ3020 (10 nmol kg^{-1} i.v.) (doses matched for pressor effects) were given alone and then, at least 6h later, 5 min after FR139317(0.5 mg kg^{-1} i.v.). The results are summarised in Table 1.

Table 1: Cardiovascular changes (areas under or over curves AUC, $AOC_{0\text{-}5\ min}$) following ET-1 or BQ3020 in the absence (control) and presence of FR139317. Values are mean ± s.e. mean, [a] $P<0.05$ versus corresponding control, [b] $P<0.05$ versus ET-1 (Mann Whitney U-test).

	Mean arterial pressure		Renal conductance	Mesenteric conductance	Hindquarters conductance	
	AOC (mm Hg min)	AUC (mm Hg min)	AOC (% min)	AOC (% min)	AUC (% min)	AOC (% min)
ET-1	14 ± 1	126 ± 7	305 ± 17	245 ± 15	76 ± 29	120 ± 26
ET-1+FR	18 ± 3	40 ± 5[a]	198 ± 9[a]	195 ± 12[a]	67 ± 16	48 ± 16[a]
BQ3020	13 ± 1	113 ± 10	417 ± 12[b]	314 ± 22[b]	81 ± 37	92 ± 23
BQ3020+FR	15 ± 2	72 ± 9[a]	335 ± 9[a]	259 ± 25[a]	91 ± 27	57 ± 21[a]

Both peptides evoked initial transient depressor responses associated with hindquarters vasodilatations which were not affected by FR139317, consistent with their being ET_B-receptor-mediated. The subsequent rises in blood pressure and regional vasoconstrictions were all inhibited by FR139317. These results differ from those on anaesthetised rats in which the ET_A-receptor antagonist, BQ123, did not inhibit renal vasoconstrictor responses to ET-1 (Bigaud & Pelton, 1992; Cristol *et al.*, 1992). One possible interpretation of our results is that in all three vascular beds, both ET_A- and ET_B-receptors mediate vasoconstriction. However, the greater ($P<0.05$) inhibitory effect of FR139317 on the pressor (-66 ± 4%) and renal vasoconstrictor (-35 ± 1%) effects of ET-1, compared to BQ3020 (-36 ± 5 and -19 ± 3%, respectively), indicates a predominance of ET_A-receptor-mediated effects.

Bigaud, M. & Pelton, J.T. (1992) Br. J. Pharmacol.**107**, 912-918
Cristol, J.P., Warner, T.D., Thiemermann, C. & Vane, J.R. (1992) Br. J. Pharmacol.**107**, 278P
Molenaar, P., Kuc, R.E. & Davenport, A.P. (1992) Br. J. Pharmacol. **107**, 637-639
Sogabe, K., Nirei, H., Shoubo, M. *et al.*, (1992) Jap. J. Pharmacol. **58**, 105P

58P NERVE CONDUCTION AND BLOOD FLOW IN STREPTOZOTOCIN-DIABETIC RATS IS IMPROVED BY AN ANGIOTENSIN II BLOCKER

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Treatment with the angiotensin converting enzyme (ACE) inhibitor, lisinopril, prevents the development of nerve conduction velocity (NCV) deficits and resistance to hypoxic conduction failure (RHCF) in diabetic rats (Cameron *et al.*, 1992). ACE inhibitors not only prevent angiotensin II synthesis, but have other actions such as potentiating bradykinin-mediated vasodilation. To test the hypothesis that changes related to angiotensin II could contribute to diabetic neuropathy and to determine whether dysfunction could be reversed, the effect of 1 month treatment with an angiotensin II antagonist (ICI D8731, a biphenylmethoxy-quinoline derivative, 50 mg^{-1} kg^{-1} day^{-1} p.o.) following 1 month of untreated streptozotocin-induced (40 mg kg^{-1} i.p.) diabetes was examined. In acute experiments, the effect of ICI D8731 on sciatic nerve blood flow was also examined in non-diabetic and 2-month diabetic rats.

In vivo measurement of NCV (1-1.5 g kg^{-1} urethane anaesthesia i.p.) showed that the initial month of diabetes (n=10) caused a 20.3±2.4% (mean ± s.e. mean) reduction in sciatic motor NCV compared to an onset control group (n=23), which was completely reversed by ICI D8731 treatment (n=13, analysis of variance and Bonferroni-corrected Student's t-test, $P < 0.001$). A 10.9±1.8% reduction in sensory saphenous NCV was also completely corrected ($p<0.001$). RHCF was determined *in vitro*. Sciatic nerves were laid across stimulating (proximal end) and recording (distal end) electrodes in an organ bath under mineral oil gassed with N_2. Nerves were stimulated (1 Hz, 0.05 ms pulse-width, 10 ma) and compound action potential amplitude was monitored. The time for an 80% reduction in compound action potential amplitude was 21.7±0.7 min for control nerves. With diabetes this increased by 19.8±4.6% ($P < 0.05$) and 49.2±3.2% ($P < 0.001$) after 1 and 2 months respectively. With ICI D8731 the increase was limited to 22.4±6.8%, indicating that the effect of the first month of diabetes was not reversed, but that there was prevention of further RHCF ($p<0.01$).

In acute experiments (50-150 mg kg^{-1} inactin anaesthesia i.p.), sciatic blood flow was measured with a laser-Doppler probe (0.8 mm diameter), and ICI D8731 (1.5 ng kg^{-1} - 4.5 mg kg^{-1}) was administered cumulatively via a jugular vein cannula. For diabetic (n=13) and non-diabetic (n=9) rats, there were similar reductions in mean blood pressure at the highest dose (18% and 22% respectively, paired Student's t-tests, $P <0.05$). For non-diabetic rats, blood flow changes approximately mirrored those for blood pressure, with a maximum reduction of 21% ($P < 0.05$); there were no changes in sciatic vascular resistance. In contrast, for the diabetic group, blood flow increased by an average of 47% and vascular resistance decreased by 29% ($P < 0.05$) with ICI D8731 (2.25 - 45 µg kg^{-1}).

Thus, changes related to abnormalities in angiotensin II production and / or its actions may contribute to the reduction in blood flow which causes early neuropathic effects in diabetic rats.

We thank the British Diabetic Association and ICI for financial support.

Cameron N.E., Cotter M.A. & Robertson S. (1992) Diabetologia 35,12-18.

59P COMPARISON OF THE EFFECTS OF ω-3 AND ω-6 ESSENTIAL FATTY ACIDS ON NERVE FUNCTION IN DIABETIC RATS

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Evening primrose oil treatment prevents the development of diabetic neuropathy in rats (Cameron *et al.*, 1991). The putative ω-6 active component is γ-linolenic acid (GLA). A main aim was to test whether purified GLA (80% GLA, 20% linoleic acid) could prevent nerve conduction velocity (NCV) reductions over 2-month streptozotocin-induced (40 mg kg^{-1} i.p.) diabetes and whether the abnormalities which develop over 1 month of untreated diabetes could be reversed by a further month of treatment. Fish oil is rich in ω-3 essential components (e.g. eicosapentaenoic acid) has beneficial effects in vascular disease. As diabetic neuropathy has a vascular aetiology, a second aim was to examine potential fish oil effects on nerve function. Metabolic pathways for ω-3 and ω-6 fatty acids interact (Lands, 1992), thus, a third aim was to see whether this had any consequences for the treatment of nerve dysfunction.

Measurement of sciatic motor and saphenous sensory NCV was carried out *in vivo* (1-1.5 g kg^{-1} urethane anaesthesia i.p.). With 1-month diabetes (n=11) there were 19.4±2.5% (mean ± s.e. mean) and 15.5±1.7% deficits in motor and sensory NCV respectively (analysis of variance and Bonferroni-corrected Student's t-test, both $P < 0.001$) compared to an onset control (n=12) group. This was maintained over 2 months of untreated diabetes (n=20, motor deficit 23.3±2.7%, sensory deficit 14.7±1.6%, both $P<0.001$). Preventive GLA treatment (260 mg day^{-1} p.o.) over 2 months (n=25) reduced the motor NCV deficit to 2.2±1.3% (N.S. versus onset control group, $P < 0.001$ versus 2-month diabetic group). The sensory NCV deficit was completely prevented (0.0±0.8%, $P < 0.001$). Preventive fish oil treatment (10% w/w dietary supplement) had no significant effect on NCV (n=10; 16.1±0.8% motor deficit; 9.8±0.9% sensory deficit). Reversal GLA treatment for 1 month following 1-month untreated diabetes (n=11) significantly improved motor and sensory NCV (both $P < 0.001$) which were not significantly different from onset control values (1.9±1.5% motor deficit, 4.3±1.4% sensory deficit). Another reversal group of diabetic rats (n=10) were treated daily with a mixture of GLA and fish oil (260 mg GLA; 3.3% fish oil supplement containing 260 mg of essential ω-3 components). The motor NCV deficit was 13.8±0.8%, reduced compared to values for the 2-month diabetic group ($P < 0.05$) but not within the non-diabetic range ($P < 0.01$) and significantly worsened compared to reversal treatment with GLA alone ($P < 0.05$). For sensory NCV, results (8.0±1.4% deficit) were also intermediate between 2-month diabetic ($P < 0.05$) and control ($P < 0.01$) values but attenuation of the GLA effect was not significant.

Thus, it is likely that GLA is the main active constituent of evening primrose oil. Fish oil had little effect on diabetic neuropathy, however, it interfered with GLA's action, which may have therapeutic implications.

We thank the British Diabetic Association and Scotia for financial support. GLA and fish oil were obtained from Scotia.

Cameron N.E., Cotter M.A. & Robertson S. (1991) Diabetes 40, 532-539.
Lands W.E.M. (1992) F.A.S.E.B. J. 6, 2530-2536.

60P EFFECTS OF CHRONIC ILOPROST TREATMENT ON PERIPHERAL NERVE FUNCTION AND CAPILLARIZATION IN DIABETIC RATS

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A deficit in sciatic vasa nervorum prostacyclin production is found for chronic experimental diabetes (Ward *et al.*, 1989). This may contribute to reduced nerve blood flow; an important factor in the aetiology of diabetic neuropathy (Cameron *et al.*, 1991). The aim was to test whether compensatory treatment with the prostacyclin analogue iloprost (Schering, 83 ng kg^{-1} min^{-1} i.v. by osmotic minipump) could prevent the development of neurological changes over 2-month streptozotocin-induced (40 mg kg^{-1} i.p.) diabetes and whether the abnormalities which develop over 1-month of untreated diabetes could be reversed by a further month of treatment.

In experiments after the treatment period (1-1.5 g kg^{-1} urethane anaesthesia i.p.) sciatic motor and saphenous sensory nerve conduction velocity (NCV) were measured. One month of untreated diabetes (n=16) resulted in 20.7±1.4% (mean ± s.e. mean) and 14.5±1.7% reductions in motor and sensory NCV respectively compared to an onset control (n=20) group (analysis of variance and Bonferroni-corrected Student's t-test, $P<0.001$). This was maintained over 2-month untreated diabetes (n=20, motor deficit 24.6±2.0%, sensory deficit 13.8±1.3%, both $P<0.001$). With iloprost treatment, motor and sensory NCVs were in the control range, for both the preventive group (n=17) and the reversal group (n=24). *In vitro* measurement of sciatic nerve resistance to hypoxic conduction failure was accomplished by monitoring compound action potential amplitude while the preparation was bathed in mineral oil gassed with N_2 (35°C). The duration of hypoxia for an 80% reduction in compound action potential amplitude was progressively increased with diabetes duration (1-month, 18.5±4.6%, $P < 0.05$; 2-month, 56.8±3.4%, $P < 0.001$). This was limited to 30.6±5.4% ($P < 0.001$ compared to the 2-month diabetic group) for the preventive group and 28.3±3.2% ($P < 0.001$) for the reversal group. Frozen cryostat section of sciatic nerve were stained for alkaline phosphatase to visualize capillaries. Endoneurial capillary density was not significantly affected by diabetes, however, in preventive iloprost-treated nerves, capillary density was increased by 35.3±3.1% ($P < 0.001$) compared to the 2-month diabetic group and there was a smaller increase (15.9±2.9% , $P < 0.05$) with reversal treatment.

Thus, defective prostacyclin production may be important for the changes in experimental diabetic neuropathy. The iloprost dose used has a roughly equivalent vasodilator and anti-aggregant effect in rats to that employed (approximately 2 ng kg^{-1} min^{-1}) in clinical trials for peripheral vascular disease. Therefore, it is possible that iloprost could have therapeutic value in diabetic patients.

We thank the British Diabetic Association, Schering and Scotia for financial support. Iloprost and minipumps were supplied by Schering.

Cameron N.E., Cotter M.A. & Low P.A. (1991) Am. J. Physiol. 261, E1-E8.
Ward K.K., Low P.A., Schmelzer J.D. & Zochodne D.W. (1989) Brain 112, 197-208.

61P 'ALL-OR-NONE' CALCIUM RESPONSES TO BRADYKININ IN SINGLE BOVINE TRACHEAL SMOOTH MUSCLE CELLS

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The increase in intracellular calcium ion concentration ($[Ca^{2+}]_i$) required for the contraction of smooth muscle cells can be mediated either via the release of calcium from intracellular stores as a result of the formation and action of inositol-1,4,5-triphosphate (IP_3; Berridge & Irvine, 1989) or via an influx of calcium ions from the extracellular fluid. We have previously shown that bradykinin (BK) induces a concentration-dependent increase in the $[Ca^{2+}]_i$ in single bovine tracheal smooth muscle (BTSM) cells in the presence of extracellular calcium (Marsh & Hill, 1993). We now report that addition of BK to single BTSM cells, in the absence of extracellular calcium ions, produces an 'all-or-nothing' release of calcium ions from intracellular stores which is not concentration dependent.

Cultures of BTSM cells were established from explants of fresh tissue as described previously (Marsh & Hill, 1992). Image analysis was performed as described previously (Marsh & Hill, 1993) with the exception that, after loading with Fura-2 AM, experiments were performed in the absence of extracellular calcium but in the presence of 0.1mM EGTA. Bradykinin was also added in a calcium-free solution.

Bradykinin (10pM-10μM; n = 30-40 for each concentration of agonist) produced a monophasic increase in $[Ca^{2+}]_i$ (Table 1) which returned to basal levels (mean = 36 ± 2 nM; n = 90) within 90 sec.

Table 1. Calcium responses to BK in single BTSM cells. Numbers in parentheses indicate cells responding and observed.

Log [BK (M)]	-11	-10	-9	-8	-7	-6	-5
Increase in [Ca2+]i (nM)	170 ± 11	154 ± 21	202 ± 11	185 ± 10	135 ± 13	241 ± 22	193 ± 20
Frequency (%)	18 (7/38)	15 (5/34)	39 (14/36)	34 (13/38)	27 (9/33)	55 (22/40)	66 (20/30)

Table 1 indicates the percentage of cells responding to BK (frequency) and the increase in $[Ca^{2+}]_i$ (mean ± s.e.m, above basal) observed in BK-responsive cells, at each agonist concentration. There was an increase in recruitment of BK-sensitive cells with increasing concentration of agonist. Furthermore evaluation of data from BK-sensitive cells only reveals that the increase in $[Ca^{2+}]_i$ remains constant at approximately 180nM on addition of each concentration of BK where the slope of the linear regression line fitted did not differ significantly from zero.
These data suggest that, under calcium-free conditions, the BK-induced release of calcium from intracellular stores in single BTSM cells is not concentration-dependent and represents an 'all-or-none' response.

We would like to thank the AFRC for financial support of this work.

Berridge, M.J. & Irvine R.F. (1989) Nature, **341**, 197-205.
Marsh, K.A. & Hill, S.J. (1992). Br. J. Pharmacol., **107**, 443-447.
Marsh, K.A. & Hill, S.J. (1993). Br. J. Pharmacol, in press.

62P EFFECT OF HYPOXIA ON BASAL AND AGONIST-STIMULATED PHOSPHOLIPASE D ACTIVITY IN CULTURED PULMONARY ARTERY SMOOTH MUSCLE CELLS

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A major pathway involved in the regulation of cell growth and division in response to mitogens, involves the activation of protein kinase C (PKC). PKC activity is stimulated by sn 1,2-diacylglycerol, which is in turn derived from the agonist-stimulated breakdown of both inositol and choline-containing phospholipids by phospholipase C (PLC) and phospholipase D (PLD) (Plevin & Wakelam, 1992). Since hypoxia may regulate the growth and division of pulmonary artery smooth muscle cells (Dempsey *et al*, 1991) we have examined the effect of hypoxia upon PLD activity in sheep pulmonary artery smooth muscle cells in culture.

In all experiments confluent and quiescent sheep pulmonary artery smooth muscle cells were used (passages 3-9). PLD activity was assayed by measuring the accumulation of [^{3}H]phosphatidylbutanol in the presence of 30mM butanol in cells prelabelled with [^{3}H]palmitic acid (Plevin & Wakelam 1992). Protein kinase C activity was measured using the peptide PKC 19-31 (ser^{25}) as a substrate for PKC-mediated phosphorylation. Data is presented as mean±s.d.

ET-1(100nM) and 5-HT(30μM) stimulated a rapid increase in [^{3}H]phosphatidylbutanol accumulation which peaked between 1-2 min (% basal response ET-1=378±32 5-HT=253±17, n=3 &4). The PKC activator 12-0-tetradecanoylphorbol 13-acetate (TPA) (100nM) also stimulated [^{3}H]phosphatidylbutanol accumulation in a linear fashion up to 15min. EC_{50} values for ET-1, TPA and 5-HT-stimulated [^{3}H]phosphtatidylbutanol accumulation were 1.89±1.4 nM, 12.47±4.9nM, and 10.76±3.66μM, respectively (n=3). Pretreatment with the PKC inhibitor Ro-318220 (Davis et al., 1989) abolished both ET-1-and TPA-stimulated PLD activity (IC_{50} for Ro-318220 ; TPA =0.59±0.2μM , ET-1=7.25±2.3μM, n=3). Preincubation in low oxygen containing media (PO_2 = ~ 20 mmHg) for 30 minutes inhibited agonist-induced [^{3}H] phosphatidylbutanol accumulation (TPA from 802±196 to 347±119; ET-1 from 486±134 to 157±68; 5-HT from 313±87 to 108±19; n = 3). Basal accumulation of [^{3}H]phosphatidylbutanol also increased during 30 minutes of hypoxia by approximately 2 fold. This increase was abolished by 10μM Ro-318220. Incubation of cells in low oxygen containing media also stimulated basal PKC activity (from 695±86 to 1686±72 cpm, n=3) but not TPA-stimulated PKC activity (from 2951±275 to 3104±197 cpm, n=3).

It is concluded that hypoxia inhibits agonist-stimulated PLD activity in sheep cultured pulmonary artery smooth muscle cells. This is accompanied by increased basal PLD activity and may be mediated by activation of PKC.

We wish to thank G.Lawton of Roche products Ltd (Welwyn Garden City, Herts) for the kind gift of Ro-318220. This work was supported by the Wellcome Trust. Nicola Kellock was a recipient of a Nuffield Foundation Undergraduate Research Bursary.

Davis, P. Hill, C.H. Keech, C.H. Lawton, G. Nixon, T.S., Sedgewick, A.D., Wadsworth, S.E., et al. (1989) *FEBS Letts.* **259**, 61-63.
Dempsey, E.C., McMurty, I.V. & O'Brien, R. F. (1991) *Am. J. Physiol.* **260**, L136-L145.
Plevin, R. & Wakelam, M.J.O. (1992) *Biochem J.* **285**, 759-766.

63P 1,3,8-SUBSTITUTED XANTHINES ENHANCE THE INHIBITORY EFFECT OF FENOTEROL ON THE RAT UTERUS

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We have reported previously that the adenosine receptor antagonist, 8-phenyltheophylline, enhances the effects of fenoterol, but not those of forskolin, to inhibit field stimulation-induced contractions of the oestrogen-primed rat uterus (Gillman & Pennefather, 1991). We proposed that this alkylxanthine may modulate the coupling of the β-adrenoceptor to adenylate cyclase. The aim of the present study was to compare the effects of a number of other alkylxanthines on the response of the uterus of the oestrogen-treated rat to the β-adrenoceptor agonist, fenoterol, in order to determine the structural features associated with this enhancement.

Uterine horns were taken from rats treated with oestradiol cypionate 20 μg/kg s.c. 24 h previously. Preparations were field stimulated (55V, 2ms, 30 Hz, for 5s every 100s) to produce regular contractions. Log concentration-response curves to fenoterol (0.1nM-10μM) were constructed in the presence and absence of (i) the 1,3,8-substituted xanthines, 8-phenyltheophylline (8-PT) 1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine (PACPX), and 1,3-diethyl-8-phenylxanthine (DPX); and (ii) alkylxanthines lacking 8-substitution, namely theophylline, enprofylline and 3,7-dimethyl-1-(2-propynyl) xanthine (DMPX). Each compound was examined using paired preparations from 3-7 animals.

8-PT, PACPX and DPX (10μM) produced leftward shifts in the fenoterol log concentration-response curves of 86-fold (95% confidence limits 12, 801; df=15, n=6), 288-fold (95% confidence limits 118, 772; df=19, n=5), 4-fold (95% confidence limits 2, 130; df=17, n=3), respectively. In contrast theophylline (n=6), enprofylline (n=7) and DMPX (n=6), in concentrations of 10μM were without effect on responses to fenoterol. These data indicate that 8-substitution may be required for modulation by xanthines of the response to this β-adrenoceptor agonist. Although there is a correlation between the extent of this modulatory effect and the affinities of the alkylxanthines as adenosine A_1-receptor antagonists (see Bruns *et al.*, 1986) the mechanism underlying the action remains to be established.

This work was supported by a grant from the NH & MRC of Australia.

Bruns, R.F., Lu, G.H. & Pugsley, T.A. (1986) Mol. Pharmacol., **29**, 331-346.
Gillman, T.A. & Pennefather, J.N. (1991) Clin. Exp. Pharmacol. Physiol. Suppl **16**, 21.

64P ACTIVATION OF THE LARGE CONDUCTANCE CALCIUM-ACTIVATED K+-CHANNEL BY PINACIDIL IN HUMAN PREGNANT MYOMETRIUM

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We have previously demonstrated that potassium channel openers (including pinacidil) are potent relaxants of the pregnant human myometrium (Morrison *et al.*, 1993), an effect blocked by glibenclamide. Single channel studies have shown that the predominant channel type observed in excised inside-out patches from isolated human pregnant myometrium is a large conductance (212pS) calcium-activated potassium (BK_{Ca}) channel (Khan *et al.*, 1993). We have therefore investigated whether there is an interaction between the openers and this channel.

The effects of pinacidil on the activity of the BK_{Ca} channel were investigated in inside-out and outside-out membrane patches from isolated human myometrial cells obtained from elective Caesarian (non-labouring) sections (with Cambridge Regional Health Authority approval) at term gestation. For inside-out patches the electrode contained (in mM); 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES (pH 7.2). The bath (intracellular) solution consisted of (in mM); 140 KCl, 0.35 $CaCl_2$, 1 KEGTA (free calcium 50nM), 1 $MgCl_2$ and 10 HEPES (pH 7.2). These solutions were reversed for outside-out patch recordings. In 4 of 7 inside-out and 4 of 7 outside-out patches application of pinacidil (10-30μM) resulted in an increase in BK_{Ca} channel activity. This activation was due to an increase in the open state probability (P_o) of individual channels and an increase in the number of functional channels (N_f) observed. For example, in a single patch, P_o and N_f were 0.16 and 1 in the absence, and 0.67 and 4 in the presence, respectively, of 10μM pinacidil. Application to outside-out patches of charybdotoxin (50 - 100nM) inhibited the activity of the BK_{Ca} channel in the absence (n=7) and in the presence of pinacidil (n=4). Tolbutamide (200μM) had no effect on BK_{Ca} channel activity in outside-out patches in the absence (n=4) or the presence (n=2) of pinacidil.

In isometric tension studies (Morrison *et al.*, 1993) of human pregnant myometrium pinacidil (0.1 - 100μM) caused a concentration-dependent relaxation (n=12) of oxytocin (0.5nM) induced contractions. There was no effect of 100nM charybdotoxin on oxytocin-stimulated contractions. However, in the presence of this concentration of charybdotoxin, there was a significant ($P< 0.05$) reduction (n=4) in the relaxation elicited by pinacidil with pD2 values of 6.11 ± 0.10 (n=12) and 5.23 ± 0.17 (n=4) in the presence and absence of 100nM charybdotoxin respectively. These results indicate that pinacidil causes the relaxation of human pregnant myometrium, at least in part, through an increase in the activity of the charybdotoxin-sensitive BK_{Ca} channel.

Supported by Cambridge Health Authority, Glaxo & MRC.

Khan, R.N., Smith, S.K., Morrison, J.J. & Ashford, M.L.J. (1993). Proc. R. Soc. Lond.[B]. 251, 9-15
Morrison, J.J., Smith, S.K., Khan, R.N. & Ashford, M.L.J. (1993). Br. J. Pharmacol. 108, 34P

65P EXTRACELLULAR cGMP HAS A SPECTRUM OF ACTIVITY IN RODENT ISOLATED MAST CELLS SIMILAR TO THAT OF DISODIUM CROMOGLYCATE (DSCG)

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Many studies have linked the elevation of intracellular adenosine 3',5'-cyclic monophosphate (cAMP) with the attenuation of mast cell and basophil activation (Plaut et al.,1980). However, surprisingly little is known regarding the role that guanosine 3',5'-cyclic monophosphate (cGMP) plays in the functional modulation of these cell types. In the present study, the ability of cGMP and cAMP and their generally more efficacious 8-bromo and dibutyryl derivatives to inhibit immunologically induced histamine release is investigated, and compared with results obtained using the classical mast cell stabilising compound DSCG.

Throughout the study, rat (Sprague Dawley, 250-400g) peritoneal mast cells (RPMC) sensitised to the nematode *N. Brasiliensis* , or in some cases mouse (BKW, 30-40g) peritoneal mast cells (MPMC), were used. Cells were pretreated with the test drugs for periods between 0 and 30 min, before addition of the immunological stimuli (anti-rat IgE or concanavalin A). Histamine release and inhibition was determined as previously described (White and Pearce, 1982a). All values are means± S.E. of the mean, of at least 4 experiments.

At a concentration of 10mM, cAMP produced negligible inhibition of the unblocked histamine release (13.2±4.6%) from RPMC. At similar doses, both dibutyryl and 8-bromo cAMP were much more effective, producing maximal inhibition of 82.7±8.6% and 76.9±2.7% respectively, an effect relatively independent of preincubation period and in general keeping with previous work (White and Pearce, 1982b). Unexpectedly, however, cGMP produced a dose dependent inhibition of histamine release from RPMC having an IC_{50} of around 1mM and at 10 mM producing maximal attenuation of 80.7±3.2%. However, this inhibitory activity was strikingly reduced if the cells were preincubated with cGMP before addition of the secretory stimulus, and fell to 10.8±4.0% after a 10 min period. Such attenuation and marked tachyphylaxis closely resembles the effects of DSCG. Similar results were obtained with the cGMP derivatives tested, although tachyphylaxis was not as marked as that seen with the unmodified guanosine cyclic nucleotide.

The onset of tachyphylaxis for both cGMP and DSCG had parallel kinetics, with maximal down regulation of mast cell stabilisation occuring within 3-4 min. Both drugs underwent cross-tachyphylaxis, whereby cells incubated in DSCG (100µM, 10min) were then found to be refractory to the inhibitory effect of cGMP. Moreover concanavalin A induced histamine release from MPMC, which has been previously shown to be unaffected by DSCG (Pearce, 1986), was also unaltered by addition of cGMP.

In total these results demonstrate that many of the effects of extracellular cGMP and DSCG, on rodent mast cells, run in parallel. This suggests that they mediate mast cell 'stabilisation' through similar mechanisms, and indeed may share a common 'binding site' on the RPMC membrane.

Pearce FL (1986) *Pharmacology* **32**, 61-67.
Plaut M, Marone G, Thomas LL, Lichtenstein LM (1980) *Adv Cyclic Nucleotide Res* **12**, 161-172.
White JR and Pearce FL (1982a) *Immunology* **46**, 353-359.
White JR and Pearce FL (1982b) *Immunology* **46**, 361-367.

66P A FUNCTIONAL RECEPTOR FOR NERVE GROWTH FACTOR ON HUMAN PLACENTAL MAST CELLS

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Nerve growth factor (NGF) is an important neurotrophic factor which also influences the functions of non-neuronal cells, e.g. rodent mast cells (Johnston and Atterwill, 1992). NGF initiates its physiological roles by binding with a specific cell receptor, two forms of which have been identified: a high-affinity receptor located on neuronal cells; a low-affinity receptor (LNGFR), present on a range of cell types including neural cells, and suggested to be involved in the regulation of normal cell growth (MacGrogan *et al*, 1992).

Human placental tissue contains NGF and expresses the LNGFR (MacGrogan *et al*, 1992). In the present study, human placental mast cells (HPMC) were enzymatically dispersed from term placenta (Purcell and Hanahoe, 1991) and challenged (37°C, 15 mins) with NGF (0.001-10.0 $\mu g.ml^{-1}$) in the presence of phosphatidylserine (10 $\mu g.ml^{-1}$). Secretion of histamine was assayed fluorimetrically (Purcell and Hanahoe, 1991) and release expressed as a percentage of total histamine content of the cells, corrected for basal release. NGF induced histamine release from HPMC in a concentration dependent manner, with an EC50 of 0.1 $\mu g.ml^{-1}$ (11.6 ± 1.9%) and maximal secretion of 22.3 ± 3.4% of total histamine content at 3.0 $\mu g.ml^{-1}$. The secretory response had a half-life of 2 min and required 10 min for completion. NGF-induced histamine release from HPMC was dependent upon the presence of extracellular Ca^{2+} (≥1.0 mM); minimal amine output was observed in the absence of Ca^{2+} (2.7 ± 1.3%) and in the presence of 0.1 mM Ca^{2+} (2.8 ± 1.1%). Histamine release induced by NGF (0.1 $\mu g.ml^{-1}$) was non-cytotoxic and was reduced by omission of glucose from the incubation medium (from 13.7 ± 2.3% to 6.1 ± 2.8%) and totally blocked by preincubation (37°C, 20 mins) of the cells in the absence of glucose and with the metabolic inhibitors 2-deoxyglucose (5 mM; 1.1 ± 0.6%) and antimycin A (1 µM; 0.4 ± 0.4%). The secretory response was pH-dependent, being maximal at pH 7.2 and reduced under more acidic (pH 6.0, 4.6 ± 0.9%) or alkaline (pH 8.5, 8.3 ± 1.7%) conditions. The reaction was maximal at 37°C and reduced by extremes of temperature (21°C, 4.3 ± 2.5%; 45°C, 3.1 ± 1.2%).

This human *in vitro* mast cell model may be of great use in elucidating the role of the LNGFR in relation to the immature and mature CNS, where roles in neuronal development and NGF production and action in neural injury have been suggested (Altar *et al*, 1992). Furthermore, the mechanistic investigation of potential neurotoxins (Johnston and Atterwill, 1992) on neurotrophic factor action will be facilitated by this model.

This work was funded in part by a grant from the Dr Hadwen Trust for Humane Research. Data are the mean ± SEM of 3 experiments.

Altar, C., Armanini, M., Dugich-Djordjevic, M., Bennett, G., Williams, R. and Feinglass, S. (1992) *J Neurochem* **59**, 2167-2177
Johnston, H.B. and Atterwill, C.K. (1992) *Neurotoxicol* **13**, 155-160
MacGrogan, D. Saint-André, J.P. and Dicou, E. (1992) *J Neurochem* **59**, 1381-1391
Purcell, W.M. and Hanahoe, T.H.P. (1991) *Agents and Actions* **33**, 8-12

67P SUPPRESSION OF vWF-MEDIATED PLATELET AGGREGATION BY ASTENOSE (GM1077), A LOW ANTI-COAGULANT HEPARIN

R.X. Ni, H.W. Lopez and A.L. Willis. Department of Pharmacology, Glycomed Inc., Alameda, CA 94501, USA.

In the turbid flow conditions of the arterial circulation, von Willebrand Factor (vWF) interacts with platelet glycoprotein 1b to produce initial adhesion of platelets to the thrombogenic surface of the vascular subendothelium (Sadler, 1991). This is an early step in the production of acute thrombotic episodes such as those produced by vascular surgery in the arterial circulation. Platelet aggregation induced by ristocetin can mimic vWF-mediated platelet adhesion to the vascular wall. Moreover, heparin can bind with vWF, thus reducing ristocetin aggregation (Sobel *et al*, 1992).

Aggregation of citrated platelet-rich plasma (PRP) of guinea-pigs induced by ristocetin (Chronolog, 1.9 mg/ml, final concentration) was examined turbidometrically and heparin or GM1077 tested at 2-fold stepwise descending concentrations from 1000 µg/ml to 33.25 µg/ml. The steep, virtually "all-or-none" dose response curve for inhibition of ristocetin aggregation necessitated the use of "EC>70" values. These are the minimum concentrations ($\mu g.ml^{-1}$) of GM1077 or heparin producing >70% inhibition from control. Mean (± s.e.m.) EC>70 value for GM1077 was 105.5 ± 13.2, compared to 289.8 ± 75.9 for heparin ($P < 0.01$, n = 16), a 2.8-fold difference.

Ex-vivo anti-aggregatory effects of heparin or GM1077 were also compared in guinea-pigs. The compound or aqueous vehicle (0.8 ml/kg) was injected via cardiac puncture into methoxyflurane-anaesthetized guinea pigs at a range of doses from 0.08 to 160 mg/kg. Fifteen minutes after injection, the animals were again anaesthetized and blood withdrawn via the abdominal aorta into 0.1 vol of 3.8% sodium citrate. Aggregation induced by ristocetin (1.9 mg/ml) was then examined turbidometrically in stirred (1,000 r.p.m., 37 °C) 400 µl samples of PRP.

Aggregation response to ristocetin (1.9 mg/ml) was completely inhibited by both heparin and GM1077 at 160 $mg.kg^{-1}$, but effects of GM1077 persisted down to 10 mg/kg ($P < 0.05$, n=5 or more), an effect approximately four-fold more potent than that of heparin. Finally, in pentobarbital-anaesthetized guinea-pigs, intravascular injection of GM1077 at 160 mg/kg (10 min before challenge) significantly ($P < 0.05$) attenuated the severe (>90%) thrombocytopenic response to retrograde carotid injection of ristocetin (150 mg/kg).

Thus, Astenose™ (GM1077) is more potent as an inhibitor of vWF-mediated platelet activation than heparin and might have useful anti-thrombotic activity in the arterial circulation, particularly with regard to vascular surgery.

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Sobel, M., McNeill, P.M., Carlson, P.L., Kermode, J.C., Adelman, B., Conroy, R. & Marques, D. (1992) J. Clin. Invest., 87, 1787-1793.

68P HISTAMINE MODULATES EPILEPTIFORM ACTIVITY IN THE RAT ENTORHINAL CORTEX SLICE

R.J. Melrose, M.H.T. Roberts, A.R. Sizer & S.K. Long[1], Department of Physiology, University of Wales, Cardiff, CF1 1SS, and [1]Solvay-Duphar, 1380 DA Weesp, The Netherlands.

5-hydroxytryptamine (5-HT) and noradrenaline reduce epileptiform activity induced by bicuculline in cells of the rat entorhinal cortex (Sizer & Roberts, 1990). We show here that histamine has similar properties. Slices of rat entorhinal cortex (Jones & Heinemann, 1988), were placed in an interface chamber perfused with artificial c.s.f containing 4.5mM K^+. Intracellular current clamp techniques were used to record from the superficial layers. The membrane potential (Vm) of these cells was -76.7mV (±1.19 s.e.mean, n=23) and the resistance (Rm) measured with -0.4nA current injection was 36.9MΩ (±2.81, n=13). With 10µM histamine ΔVm was -0.42mV (±0.19, n=12, n.s. Students t test) and ΔRm was 0.22MΩ (±0.757, n=13, n.s). In only one cell did Vm change noticeably (-2mV). Four cells showed a slight increase in Rm (3.6MΩ ±0.89), 6 a slight decrease (-1.9MΩ ±0.29) and with 3 cells no change could be seen in full current voltage relationships plotted between +0.4 and -0.8nA of injected current. Changes in spike accommodation caused by 10µM histamine were also examined by injecting a 500ms pulse of depolarising current sufficient to evoke 7 or 8 sodium spikes. Six of the eleven cells tested showed a very slight increase in accommodation (from a time interval between 3rd and 4th spikes of 34.5ms ±7.4 to 43.5ms ±10.1); two cells showed a small reduction (64ms to 42.5ms), and three showed no change.

The effects of histamine on epileptiform activity were far more consistent and pronounced. The epileptiform activity, induced by 30 min superfusion with 5µM bicuculline, occurred spontaneously and consisted of an initial depolarising shift of about 20-40mV lasting 100ms followed by typically 3-4 smaller depolarisations (after-discharges). These "bursts" of depolarisation occurred at intervals of 4-8 seconds. Histamine (10µM) reliably reduced the epileptiform bursts to approximately 80% of control (n=12), and was rarely without effect (n=2). Histamine acted preferentially on the after-discharges. The initial depolarising shift appeared to be unaffected by the application of 10µM histamine. Histamine also increased the rate of spontaneous activity (n=11). This effect was only seen in conjunction with an attenuation of the spontaneous burst duration. Similar but smaller effects on epileptiform activity have been observed with 1 and 5µM concentrations of histamine.

Jones, R.S.G. & Heinemann, U. (1988) J.Neurophysiol. 59(5), 1476-1496.
Sizer, A.R & Roberts, M.H.T. (1990) J.Physiol. 427, 47P.

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69P THE INCREASE IN *IN VIVO* [3H]-NITRENDIPINE BINDING DURING ETHANOL WITHDRAWAL CORRELATES WITH RATINGS OF HANDLING-INDUCED CONVULSIVE BEHAVIOUR

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Calcium channel blockers of the dihydropyridine class (e.g. isradipine, nitrendipine) are effective in preventing both the behavioural and electrophysiological manifestations of the ethanol withdrawal syndrome. (Littleton et al,, 1990, Whittington et al., 1991). Previous work has shown an increase in in vitro [3H]-nitrendipine binding following chronic ethanol treatment (Dolin et al., 1987). In the present study, the in vivo binding of [3H]-nitrendipine was examined 30 min and 3h into ethanol withdrawal.

Male TO mice (35-40g) were made dependant on ethanol by a period of 14 days inhalation of ethanol vapour (6 - 10 mg/100ml). On the day of withdrawal, in vivo binding of [3H]-nitrendipine was measured by the method of Supavilai and Karobath (1984). Mice were given 4 μCi of ligand via the tail-vein in a volume of 0.1ml. 15 minutes after this injection, the mice were killed and the brain homogenised to a concentration of 20 mg/ml in ice-cold 50mM pH7.4 Tris-buffer. The radioactivity in 1 ml of crude homogenate was counted, as was the activity in filter papers after 1 ml of the homogenate had been filtered. Non-specific binding was determined by incubating 1 ml of the crude homogenate for 1 hour at room temperature in the presence of 10 μM nimodipine; this was then filtered and counted. All measurements were made in triplicate. Each treatment group contained 8 mice. Specific binding was determined as the number of counts in filtered homogenate minus the number of counts of non-specific binding. To overcome variations in the amount of ligand injected or metabolism, specific binding was expressed as a percentage of the counts in 1 ml of the crude homogenate. Control mice showed specific binding of 8.2 ± 1.1 %, Mice withdrawn from ethanol for 30 minutes showed binding of 7.95 ± 1.1 %. At 3h into withdrawal the binding was increased to 12.5 ± 1.3 %; this increase was significantly different from control values ($P < 0.05$, Student's t-test).

Ratings were made of handling-induced convulsive behaviour (on a scale of 0 to 5; based on Green et al., 1990) every hour during the withdrawal, until injection of the ligand. When the increase in binding was plotted against the behavioural ratings, there was a significant, positive, correlation (Spearman rank correlation) between the percentage in vivo binding of tritiated ligand and the handling score at 3h. ($P < 0.05$). These data provide further evidence for the role of dihydropyridine binding sites during withdrawal from long-term exposure to ethanol.

Dolin S. et al., (1987) Neuropharmacol., **26**, 275-279
Green A.R. et al., (1990) Psychopharmacol., **102**, 239-242
Littleton J. M. et al., (1990) Psychopharmacol., **100**, 387-392
Supavilai P. and Karobath M. (1984) J. Neural Trans., **60**, 149-167
Whittington M.A. et al. (1991) Br. J. Pharmacol., **103**, 1669-1676

70P NITRIC OXIDE INDUCES CHANGES IN GENE EXPRESSION IN THE GRANULE CELLS OF THE HIPPOCAMPAL DENTATE GYRUS

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Long term potentiation of synaptic transmission in the hippocampus is generally mediated through the N-methyl-D-aspartate (NMDA) receptor complex. Long-term potentiation can induce sustained changes in granule cell gene expression, causing a substantial increase in proenkephalin (Penk) mRNA levels while markedly reducing the levels of prodynorphin (Pdyn) mRNA (Morris et al, 1988). Recently it has been suggested that nitric oxide (NO) may act as an intercellular mediator of glutamate actions (Garthwaite et al, 1989). This study has attempted to determine whether administration of NO can mimic the effects of NMDA receptor stimulation on hippocampal gene expression. Male wistar rats (180-200g) were given a unilateral injection into the molecular layer of the dentate gyrus using standard stereotaxic techniques. All drug combinations were delivered in a total volume of 75nl physiological saline, given over a 3 min period. The animals were sacrificed 24 hours later and 20μm cryostat sections of the brain were processed for in-situ hybridisation using ^{35}S-dATP-labelled oligonucleotide probes specific for Penk mRNA and Pdyn mRNA (Morris and Hunt, 1991). Labelled slides were dipped in photographic emulsion, exposed for 21 days, developed and photographed for quantification. The hybridisation signal in the region of the injection was expressed as a percentage of the signal in the equivalent region on the contralateral side. Whereas vehicle injection altered the Pdyn hybridisation signal by -2.6%±3.1% and the Penk hybridisation signal by -6.6%±1.6%, NMDA (50μM) injection reduced the Pdyn mRNA signal by 27.5% + 3.1% ($n=4$, $p<0.05$ compared to vehicle injection, one-tailed Mann-Whitney U-test). In a single animal, NMDA increased Penk mRNA signal by 46.3%. No significant changes were seen when the NMDA receptor antagonist aminophosphovaleric acid (APV, 100μM) was co-injected with NMDA. Sin-1 molsidomine (1mM), a releaser of NO, also reduced the Pdyn mRNA signal and increased the Penk mRNA signal by 47.3%± 21.0% and 222.3%+181.0% respectively ($n=3$, both $p<0.05$ relative to vehicle injection) . Again co-injection of 100μM APV appeared to attenuate this effect: (Pdyn mRNA signal decreased by 9.1%±4.8%, Penk mRNA signal increased by 13.0%±11.0%). Injection of 8-bromocyclicGMP (500μM) induced the same pattern of changes as sin-1 molsidomine. These results indicate that NO release can cause long-term changes in hippocampal gene expression similar to those seen after NMDA receptor stimulation. These changes may be due to a NO-induced increase in endogenous glutamate release.

This work was supported by the MRC and the Wellcome Trust. We are grateful to Dr.J.Garthwaite for the gift of sin-1 molsidomine.

Morris, B.J., Feasey,K.J., ten Bruggencate, G., Herz, A., & Hollt, V. (1988) *Proc. Natl.Acad.Sci. USA* 85, 3226-3230.
Garthwaite, J., Garthwaite, G., Palmer, R.M.J. and Moncada, S. (1989) *Eur.J. Pharmacol.* 172, 413-416.
Morris,B.J. and Hunt,S.P. (1991) *Neurosci.Lett.* 125, 201-204

71P A CENTRALLY LOCATED ADENOSINE A_1 RECEPTOR PROTECTS AGAINST KAINIC ACID NEUROTOXICITY

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Previous studies have shown that the adenosine agonist R-phenylisopropyl-adenosine (R-PIA) is neuroprotective against intrahippocampal quinolinic acid administration (Connick & Stone, 1989) or systemic kainic acid administration (MacGregor & Stone, 1992). In the present study adenosine A_1 antagonists were used to determine whether the neuroprotection was mediated by central or peripheral receptors.

Neurotoxicity was assessed by the increase in [^{3}H] PK11195 binding to hippocampal P_2 membranes (Eshleman & Murray, 1989; MacGregor & Stone, 1992). [^{3}H] PK11195 is a ligand for peripheral benzodiazepine binding sites, found in glial cells, and has been shown to be a sensitive marker for reactive gliosis and hence an indirect marker for neuronal damage (Benavides *et al,* 1987).

Male Wistar rats (180-220g) were pretreated with 200 $\mu g\ kg^{-1}$ clonazepam i.p. 10 minutes before being injected with 10 mg kg^{-1} kainic acid i.p. and were left to recover for 7 days.

This dose of kainic acid in the absence of R-PIA gave a 272 $\pm$ 28% (n=12) (mean $\pm$ s.e. mean, significance v kainate group) increase in [^{3}H] PK11195 binding compared to controls ($p<0.001$). Coadministration of 25 $\mu g\ kg^{-1}$ R-PIA i.p. abolished this increase (127 $\pm$ 12% control, n=9 $p<0.001$). R-PIA is also significantly neuroprotective when administered up to 2 hours before or after the kainate injection. If the selective A_1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX) 250 $\mu g\ kg^{-1}$ was administered i.p. at the same time as kainate and R-PIA, the neuroprotection was abolished (281 $\pm$ 24% control n=9), and when administered with kainate in the absence of R-PIA it caused a significant increase in binding (416 $\pm$ 48% control, n=6 $p<0.05$).

The A_1/A_2 antagonist 8-(p-sulfophenyl)-theophylline (20mg kg^{-1}) which, unlike CPX or R-PIA, cannot cross the blood brain barrier (Baumgold *et al.*, 1992) did not significantly alter the neuroprotective action of R-PIA (142 $\pm$ 16% n=6), although again in the absence of R-PIA it significantly enhanced the kainate neurotoxicity (394 $\pm$ 21% control n=4, $p<0.001$). In neither case did the adenosine antagonists cause neurotoxicity on their own.

These results are taken to indicate that R-PIA is acting at a centrally located adenosine A_1 receptor to prevent kainate induced neurotoxicity.

Benavides, J., Fage, D., Carter, C. & Scatton, B. (1987) Brain Res. **421,** 167-172.
Baumgold, J., Nikodijevic, O. & Jacobson K.A. (1992) Biochem. Pharmacol. **43,** 889-894.
Connick, J.H. & Stone, T.W. (1989) Neurosci. Letts. **101,** 191-196.
Eshleman, A.J. & Murray, T.F. (1989) J. Neurosci. **53,** 494-502.
MacGregor, D.G. & Stone, T.W. (1992) Neuroreport **3,** 536-538.

72P CENTRALLY ACTING CHOLINOCEPTOR DRUGS ALTER SENSORIMOTOR DEFICIT INDUCED BY UNILATERAL α-AMINO-3-OH-ISOXOZOLE PROPIONIC ACID (AMPA) LESION OF NUCLEUS BASALIS (nb)

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Basal forebrain cholinergic neurones project to wide areas of the cortex (Bigl *et al.*, 1982) and may therefore be involved in the regulation of many cortical functions. The aim of the present study was to investigate the effects of acute injections of two centrally acting cholinoceptor agonists, arecoline and nicotine, in rats with unilateral lesions of nb on sensorimotor functions of rats.

The nb was lesioned by injecting S-AMPA (0.93 µg in 0.5 µl, Abdulla *et al.*, 1992); a group of sham-operated animals (vehicle injection) served as controls. The sensorimotor tests were carried out 1 wk later and consisted of a series of tests measuring orientation towards external stimuli applied to either side of the body (Dunnett *et al.*, 1985). These included: number of full 360° turns in each direction over 3 min in an open field, direction of rotation after tail pinch and placement on 45° grid over 4 trials, and the responses to light pin-prick, snout brushing, whisker touching and ammonia olfactory stimulation applied to one side of the body.

Unilateral AMPA lesion of nb significantly increased turning toward the lesioned side in rats placed in the open field, after tail pinch and on 45° grid ($P<0.001$). Responses to pin-prick, snout brushing, whisker touching and ammonia olfaction of the lesioned side were significantly higher than on the unlesioned side ($P<0.001$). Sham-operated rats did not show any lateral bias in any of the tests. Nicotine (0.05 mg/kg i.p., 15 min before testing) given to lesioned rats, changed the turning bias induced by the open field, by tail pinch and on the 45° grid toward the unlesioned side (contralateral-ipsilateral bias, $P<0.001$) and decreased the lateral differences in response to pin-prick. Hexamethonium (1.0 mg/kg i.p., a peripheral nicotinic antagonist) and ondansetron (0.01 mg/kg i.p., a $5HT_3$ antagonist to block nicotine-induced dopamine release, Carboni *et al.*, 1989) failed to block the effects of nicotine. Nicotine's effects were prevented by mecamylamine (1.0 mg/kg, centrally acting nicotine antagonist), a dose without effect when given alone. Amphetamine (up to 1.0 mg/kg, i.p) did not affect the lesion-induced ipsilateral motor bias and contralateral sensory neglect. The observed effects of nicotine are therefore due to action at central nicotinic receptors. Arecoline (0.5 mg/kg i.p., 15 min before testing) given to lesioned rats, abolished the turning bias induced by tail pinch and the 45° grid tests, significantly reduced ipsilateral turning in the open field and the pin-prick response. Arecoline, nicotine and amphetamine failed to decrease the lesion-induced increase in response to snout brushing, whisker touching and ammonia olfaction. Sham operated rats showed no responses to the three drugs in the doses used. The results confirm that the basal forebrain cholinergic system plays a role in sensorimotor cortical functions, but that the roles of muscarinic and nicotinic receptors are probably different.

Bigl, V., Woolf, N.J. and Butcher, L.L. (1982) Brain Res. Bull **8,** 727-749.
Abdulla, F.A., Dawe, G., Scorsa, A.M., Rispoli, E. and Stephenson, J.D. (1992) Funct. Neurol. **6,** 141-144.
Carboni, E., Acquas, E., Frau, R. and Di Chiara, G. (1989). Eur. J. Pharmacol. **164,** 515-519.
Dunnett, S.B., Toniolo, G., Fine, A., Ryan, C.N., Bjorklund, A. and Iversen S.D. (1985) Neuroscience **16,** 787-797.

73P THE SELECTIVE 5-HT$_3$ RECEPTOR ANTAGONIST, WAY 100289, ENHANCES SPATIAL MEMORY IN RATS WITH IBOTENATE LESIONS OF THE FOREBRAIN CHOLINERGIC PROJECTION SYSTEM

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The results of several behavioural studies have suggested that selective 5-HT3 receptor antagonists can reverse the cognitive impairment induced by the muscarinic receptor antagonist, scopolamine (Costall et al, 1990). In the present study we have examined the effect of the 5-HT3 antagonist, WAY100289 (Bill et al., 1992), in comparison with the cholinergic agonists, nicotine and arecoline, on cognitive deficits induced by partial lesioning of central cholinergic projections. Male Sprague-Dawley rats were trained to locate a hidden platform in a Morris water maze by twice daily exposure to the maze on each of 9 consecutive days. The effects of s.c. administration of arecoline (1 mg/kg), nicotine (0.1 mg/kg) and WAY100289 (0.003-1.0 mg/kg) on acquisition were examined. Rats were then lesioned by stereotaxic injections of ibotenic acid (10 μg/μl) into the nucleus basalis and medial septal area. Control rats were sham-operated. At the end of behavioural testing, rats were killed and tissue samples taken from the frontal cortex and dorsal and ventral hippocampus for the assay of ChAT activity by the method of Fonnum (1975). Rats were re-tested to examine (a) the effects of lesioning on retention, and (b) the effects of the drug treatments on deficits induced by the lesions. Two further experiments examined:- (a) reversal learning, with the platform placed in the quadrant opposite to its original position, and (b) minimal cue position learning, with the platform placed in the quadrant adjacent to blank walls in the laboratory. Long-term memory in each case was assessed by probe trials with the platform removed, 24h after the end of training. Swim path was monitored by a video image analysing system and search parameters (latency, distance, speed, heading angle and time in each sector) were analysed by ANOVA, with group means compared by Newman-Keul's test. Both arecoline and nicotine improved acquisition in normal rats, i.e. latency to find the platform was reduced significantly ($p<0.01$ and $p<0.05$, respectively), whereas WAY100289 had no significant effect. Lesioned rats displayed task-related impairments in training rather than in probe trials, consistent with deficits in learning rather than long-term memory. In retention there was no overall difference in latency between treatment groups, although the latency of lesioned rats was increased in the first two trials ($p<0.01$). In reversal learning, the latency of lesioned rats was significantly ($p<0.01$) greater than controls and drug-treated groups. Rats treated with arecoline, nicotine or WAY100289 (all doses) did not differ significantly from controls. Similar results were obtained in the minimal cue experiment, i.e. lesioned rats had a significantly increased latency which was restored to control values by all drug treatments. Lesioned rats were significantly ($p<0.025$) impaired relative to controls, whereas animals treated with arecoline, nicotine or the top dose of WAY100289 had a significantly lower latency ($p<0.01$). Significant drug effects on latency were accompanied by significant improvements in search strategy (heading angle and time spent searching the appropriate region of the maze) without any significant effects on swim speed, indicating that they reflected cognitive, rather than motor stimulant, effects. In the frontal cortex, dorsal and ventral hippocampus, ChAT activity was significantly reduced to 76% ($p<0.001$), 86%($p<0.01$) and 77% ($p<0.001$) of control values respectively. The finding that WAY100289 can significantly reverse cognitive deficits induced by cholinergic lesions indicates that this 5-HT3 receptor antagonist may be effective in the treatment of dementia.

Bill, D.J., Fletcher, A., Glenn, B.D. & Knight, M. (1992) Eur. J. Pharmacol., 218, 327-334.
Costall, B., Naylor, R.J. & Tyers, M.B. (1990) Pharmac. Ther., 47, 181-202.
Fonnum, F. (1975) J. Neurochem., 24, 407-409.

74P THE GABA$_B$ ANTAGONIST CGP 36742 ENHANCES SPATIAL LEARNING PERFORMANCE AND ANTAGONISES BACLOFEN-INDUCED AMNESIA IN MICE

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A variety of evidence has prompted the suggestion that antagonism of GABA$_B$ receptors in mammalian brain will improve cognition (Bowery 1989) but until recently no brain penetrating antagonists have been available. With the advent of CGP 36742 (3-aminopropyl-n-butyl phosphinic acid) which crosses the blood brain barrier Mondadori and colleagues (1992) have now been able to demonstrate an improvement in cognitive performance in a passive-avoidance test in mice, in social learning in rats and in a colour spatial task in monkeys. To extend and confirm these observations we have examined this antagonist on spatial learning performance in mice and on the deficit in performance produced by (-)baclofen. An eight-arm radial maze task was employed as described by Fagioli *et al.* (1991).

Male CD1 mice, 8 per group, (25-30g) were tested on each of 10 consecutive days, 15 min after injection (i.p.) of CGP 36742 (1, 10 or 100mg/kg), (-)baclofen (2 or 4 mg/kg), hyoscine (1mg/kg) or saline. CGP 36742 (10mg/kg) was also administered in combination with (-)baclofen (4 mg/kg) or hyoscine (1mg/kg). Results were analysed using a one-way analysis of variance (ANOVA). CGP 36742 (10 and 100mg/kg but not 1mg/kg) significantly enhanced the radial maze task performance by 17-34% and 20-31% respectively (occurrence of first error $p<0.05$) when compared to control animals 5-10 days after commencement of the study. By contrast, (-)baclofen (2 and 4mg/kg) produced a significant impairment (16-20% and 20-30%, $p<0.05$ respectively) of performance. This depressant effect was completely reversed when administered in combination with CGP 36742 whereas the depressant action of hyoscine was unaltered. At 2 and 4mg/kg (-)baclofen produced no significant muscle relaxation which might have contributed to its apparent amnesic action. This was determined using a rota-rod test (accelerating from 8 to 16 rpm over 360 s period) 15 and 30 min after injection. The mean (± s.e.m.) number of falls from the rod after 2mg/kg was 3.37 ± 0.69 (15 min) and 2.62 ± 0.45 (30 min) and after 4mg/kg 3.50 ± 0.68 (15 min) and 2.87 ± 0.51 (30 min). The number of falls for control animals was 3.0 ± 0.63 and 2.50 ± 0.49 after 15 and 30 min respectively.

The present findings support the previous report that CGP 36742 has positive effects on cognitive function and also show that the decrease in learning produced by a GABA$_B$ agonist can be prevented by this antagonist.

We thank CIBA-Geigy for CGP 36742.

Bowery N.G. (1989) Trends in Pharmacol. Sci. 10: 401-407.
Mondadori, C., Preiswerk G. and Jaekel J. (1992) Pharmacol. Communications 2: 93-97.
Fagioli S., Rossi-Arnaud C. and Ammassari-Teule M. (1992) Psychopharmacology 105: 209-212.

75P APPARENT DESENSITISATION TO ADENOSINE OF HIPPOCAMPAL PYRAMIDAL CELLS

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The binding of purines to neuronal membranes is known to be dependent on guanine nucleotides and divalent cations (Goodman et al., 1982). We have previously shown that the presynaptic inhibitory effect of adenosine requires magnesium ions (Bartrup and Stone, 1988) and now report a study of adenosine effects postsynaptically in the hippocampus.

Hippocampal slices 450 μm thick were prepared from male Wistar rats (170-210 g) and superfused at 30°C with ACSF (in mM: KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10) saturated with 95% O_2 / 5% CO_2. Orthodromic extracellular recordings were made in the CA1 pyramidal cell layer following stimulation of Schaffer collateral fibres in stratum radiatum. Stimulation of the alveus was used for antidromic stimulation. In antidromic stimulation experiments, $CaCl_2$ was omitted or reduced to 0.24 mM and in some experiments $MgSO_4$ increased to 2 mM.

Adenosine and baclofen induced a dose-dependent reduction in the amplitude of orthodromic potentials. Maximum effects were seen at 20 and 5 μM respectively. In nominally Ca^{2+} free medium, bursts of multiple population spikes were obtained in response to antidromic stimulation. Adenosine had little effect on the secondary spike amplitude reducing it by 8.96% ±4.25 (mean ± s.e.m., n.s., Student's t-test, n=11) at 200 μM, 21.88% ± 3.52 ($p<0.001$, n=8) at 500 μM and 10.46% ± 3.94 (n.s., n=5) at 1mM. With continued superfusion the inhibitory effect of adenosine declined: at 2 mM the response was lost after 3 minutes. Raising the cation concentrations partially restored sensitivity to adenosine. For example, adenosine at 200 μM reduced the secondary population spike by 74.60% ± 7.26 ($p<0.01$, n=3) in 0.24 mM calcium and by 90.42% ± 5.42 ($p<0.01$, n=4) in calcium-free medium with 0.8 mM added magnesium (to a total of 2 mM). Inhibition by all submaximal concentrations of baclofen tested was reduced in calcium-free medium but at 100 μM, baclofen still reduced the secondary spike size by 87.96% ± 5.10 ($p<0.001$, n=4); at concentrations of 500 μM or above the potential was abolished and no sign of recovery was observed during maintained superfusion for up to 45 minutes. If slices were superfused in calcium-free medium with adenosine at 2 mM for 10 minutes, by which time its inhibitory effect had disappeared, the addition of baclofen 500 μM was still able to abolish the secondary evoked potential with no recovery during 30 minutes superfusion.

The results indicate that the omission of calcium caused a loss of adenosine sensitivity postsynaptically on pyramidal cells probably due to desensitization, and that there is no comparable loss of responsiveness to baclofen.

Bartrup, J.T. & Stone, T.W. (1988) Brain Res., **463**,374-379.
Goodman, R.R., Cooper, M.J., Gavish, M. & Snyder, S.H. (1982) Mol. Pharmacol., **21**,329-335.

76P DISPARITY BETWEEN TOLERANCE TO THE SEDATIVE EFFECTS OF NITRENDIPINE AND *IN VIVO* BINDING OF NITRENDIPINE, FOLLOWING CHRONIC DILTIAZEM TREATMENT

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We have reported previously that cross tolerance can occur to the sedative effects of the dihydropyridine calcium channel antagonists, isradipine and nitrendipine (Clavell et al., 1992). We now examine whether cross-tolerance occurs with two calcium channel antagonists of separate classes; nitrendipine and diltiazem (a benzothiazepine). In addition, the in vivo binding of [3H]-nitrendipine following acute and chronic treatment with diltiazem was examined.

Male TO mice (30-35g) were injected i.p. twice daily for 13 days with diltazem, 100 mg/kg, or saline vehicle. On day 14, 24h after the last injections, pairs of mice were given nitrendipine, 50 mg/kg, or diltiazem, 100 mg/kg, or saline, then 30 minutes later the locomotor activity was recorded (by infra-red beam crossings) for 30 min. 14 mice were used in each treatment group, results in table are mean ± s.e.m. The results (Table 1) showed that the effect of either diltiazem or nitrendipine in decreasing locomotor activity was reduced after chronic treatment with diltiazem, demonstrating that cross-tolerance occurred between diltiazem and nitrendipine.

In vivo binding of [3H]-nitrendipine was measured by the method of Supavilai and Karobath (1984). Mice (8 per treatment group) were given 4 μCi of ligand via the tail-vein in a volume of 0.1 ml, 20% EtOH. 15 min later, the mice were killed and the brain homogenised to a concentration of 20 mg/ml in ice-cold 50mM pH7.4 Tris-buffer. The radioactivity in 1ml of crude homogenate was counted, as was the activity in filter papers after 1ml of the homogenate had been filtered. Non-specific binding was determined by incubation of 1ml of the crude homogenate for 1h at room temperature in the presence of 10 μM nimodipine, followed by filtration and counting. All measurements were made in triplicate. Specific binding was determined by the number of counts in filtered homogenate minus the number of counts of non-specific binding. To correct for variation in the amount of ligand injected or its metabolism by the mice, the specific binding was then expressed as a percentage of the counts in 1 ml of the crude homogenate.

Table 1. sal = saline, dilt = diltiazem 100 mg/kg, nit = nitrendipine 50 mg/kg, statistics by Student's nonpaired t-test.

Chronic / Acute Drug	Locomotor Activity counts (mean ± S.E.)	in vivo binding (as %)
Sal/Sal	9064 ± 276	3.4 ± 1.1
Sal/Dilt	1823 ± 413 * ($p<0.01$ c.f. sal/sal)	8.6 ± 1.5 * $p<0.05$ c.f sal/sal
Dilt/Sal	8734 ± 600	4.8 ± 0.8
Dilt/Dilt	4201 ± 828 * ($p<0.01$ c.f. sal/dilt)	13.5 ± 3.3 * $p< 0.05$ c.f. dilt/sal
Dilt/Nit	4626 ± 259 * ($p<0.01$ c.f. sal/nit)	-
Sal/Nit	3093 ± 413 * ($p<0.01$ c.f. sal/sal)	-

The in vivo binding was significantly increased after either acute or chronic treatment with diltiazem, suggesting that tolerance to the sedative effect of diltiazem was not due to tolerance to the effect on dihydropyridine binding.

Clavell D.M.J., Watson, W.P., Ripley, T.L. and Little, H.J (1992) Br. J. Pharmacol., 104, 450P
Supavilai P. and Karobath M. (1984) J. Neural Trans., 60, 149-167

77P DIFFERENCE IN ACUTE INTERACTION WITH ETHANOL OF TWO STRUCTURALLY RELATED DIHYDROPYRIDINES: NITRENDIPINE AND FELODIPINE

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Calcium channel blockers of the dihydropyridine class (e.g. nitrendipine) potentiate the acute effects of ethanol, including ataxia, sedation and general anaesthesia. (Dolin and Little, 1986). The dihydropyridines also prevented the behavioural and electrophysiological manifestations of the ethanol withdrawal syndrome. (Whittington et al., 1991). Felodipine, another dihydropyridine calcium channel blocker, has been shown to be ineffective against the ethanol withdrawal syndrome at a dose which caused the same degree of displacement of tritiated isradipine from CNS tissue as an effective dose of nitrendipine (Watson et al., 1990). In the present study we compare the effects of these two dihydropyridines, nitrendipine and felodipine, on the general anaesthetic and ataxic actions of ethanol.

Male TO mice (30-35g) were injected i.p. 30 minutes before all tests with either nitrendipine, 10 or 50 mg/kg, felodipine, 2 or 10 mg/kg, or vehicle (0.5% Tween-80 in distilled water). General anaesthesia was determined by the loss of righting reflex; dose-response curves to ethanol (2.5 - 3.6 g/kg i.p. as 20% ethanol/water) were performed within these groups (n = 10). The number of mice having lost their righting reflex 10 minutes after injection of ethanol was determined and the quantal dose response data plotted. ED_{50} of ethanol for each dose of drug is shown in the table. Ataxia was measured, by a rotarod, on separate groups (n = 16) of mice. Ethanol, 1.75 g/kg, was given i.p. to pretreated mice; the mean time each treatment group spent on the rotarod 30 minutes later is shown in the table.

Felodipine, 2 mg/kg, showed little or no potentiation of ethanol's effects in either test. As reported previously, nitrendipine 50 mg/kg potentiated considerably the general anaesthetic and ataxic effect of ethanol. The effect of felodipine, 10 mg/kg, was similar in magnitude to that of nitrendipine 10 mg/kg. As felodipine 10 mg/kg caused much greater displacement of tritiated dihydropyridines than nitrendipine 10 mg/kg, these results show that the acute interactions of dihydropyridines and ethanol, may be distinct from the binding displacement characteristics in central nervous system tissue previously shown in ex-vivo binding studies.

* $p < 0.05$ (Ataxia results used student's t-test, anasthesia Lichfield and Wilcoxon, 1949)

Drug	ED50 (g/kg) (95%conf.limits)		Time on Rotarod (mean + S.E.)
Tween Vehicle	3.50	(3.44 - 3.55)	171 ± 9.3
Nitrendipine 10 mg/kg	3.19 *	(3.13 - 3.26)	125 ± 26.0
Nitrendipine 50 mg/kg	2.73 *	(2.63 - 2.83)	74 ± 18.5 *
Felodipine 2 mg/kg	3.47	(3.11 - 3.89)	173 ± 8.0
Felodipine 10 mg/kg	3.29 *	(3.16 - 3.31)	102 ± 18.6 *

Dolin, S. and Little, H.J. (1986) Br. J. Pharmacol., **88**, 909-914
Litchfield, J.T. and Wilcoxon, F. (1949) J. Pharmacol. Exp. Ther., **96**, 94-113
Watson, W.P. et al. (1992) Br. J. Pharmacol., **105**, 160P
Whittington, M.A. et al. (1991) Br. J. Pharmacol., **103**, 1669-1676

78P POTASSIUM-STIMULATED CALCIUM INFLUX AND ITS INHIBITION BY NIMODIPINE IN CULTURED ASTROCYTES AND NEURONES

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Calcium plays an important role in the regulation of cellular function in the central nervous system. It enters the cell through both receptor-operated channels following activation of cell membrane receptors and voltage-sensitive channels responsive to alterations in membrane potential. The 1,4-dihydropyridines, of which nimodipine is an example, selectively block calcium influx through the subclass of voltage sensitive channels denoted L-type. The central effects of these compounds promote them as putative antiepileptic agents. The following study was designed to investigate the effect of nimodipine on depolarisation-induced calcium influx into primary cultures of astrocytes and neurones.

Primary cultures of astrocytes were derived from the cortices of newborn Sprague Dawley rats by the method of Bender and Hertz (1984) and maintained in Dulbecco's modified Eagle medium for 38 days prior to use. Primary cultures of granular cell neurones were derived from the cerebella of 7-day old Sprague Dawley rats by the method of Courtney et al (1990) and maintained in minimal essential medium for 10 days prior to use. Individual cultures were washed and then incubated for 60s with serum-free medium containing 0.5μ Ci $^{45}Ca^{2+}$. Depolarisation was then induced, where applicable, with 55mM KCl. The effect of nimodipine was assessed in cultures with 1μM nimodipine for 60 mins. Calcium influx was determined by liquid scintillation counting and the protein concentration was analysed by the Biorad method.

Results for all cultures are illustrated in Table 1.

Table 1. Mean (s.e. mean) calcium influx (nmol mg protein^{-1})

	n	Cortical astrocytes	n	Cerebellar neurones
Control	6	3.50 (0.41)	6	1.42 (0.08)
Control + Nimodipine	6	4.57 (0.80)	6	1.38 (0.21)
Depolarised	6	15.33 (2.03)*	6	6.10 (1.11)**
Depolarised + Nimodipine	6	2.39 (0.24)	6	2.30 (0.10)

* $p<0.02$, ** $p<0.01$ by Mann Whitney U-test. (Depolarised vs control)

In conclusion, nimodipine inhibits calcium influx into both astrocytes and neurones following potassium depolarisation, but is without effect on calcium influx in unstimulated cells.

Bender, A.S. & Hertz, L. (1984) J. Neurochem. 43, 1319-1327
Courtney, M.J., Lambert, J.J. & Nicholls, D.G. (1990) J. Neurosci. 10, 3873-3879

79P SELECTIVE INHIBITION BY H7 OF A FORM OF PROTEIN KINASE C PRESENT IN RAT MIDBRAIN

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There are now known to be at least ten different isoforms of the protein kinase C (PKC) family of phospholipid-dependent serine/threonine protein kinases (Nishizuka, 1992). These may be divided into two groups, the Ca^{2+}-dependent A series and the Ca^{2+}-independent B series. There is evidence that different isoforms vary in their enzymatic properties and may also exhibit different pharmacological properties (Ryves *et al,* 1991). We have previously shown that various PKC activators can differentially modulate $^{45}Ca^{2+}$ influx through L type Ca^{2+} channels into pituitary cells (MacEwan *et al,* 1990) and that PKC inhibitors differ in their ability to inhibit phorbol ester-induced hormone release from these cells (Johnson & Mitchell, 1989).

To further examine such differences in PKC pharmacology, an *in vitro* assay was used to measure the histone IIIS thiophosphorylation activity of cytosol from rat midbrain, this tissue having been shown to contain all the well-characterized PKC isoforms (Scott Young III, 1989). The phosphatidyl serine-dependent kinase activity, after partial purification on DEAE cellulose, was determined in the presence of either 100 μM or low free Ca^{2+} (< 3nM), in a mixed micelle assay. PKC activity was induced by phorbol 12,13 dibutyrate (PDBu) and mezerein, which differ in their effect on Ca^{2+} channels in the GH_3 clonal pituitary cell line (MacEwan *et al,* 1990), and the effects of the kinase inhibitors H7 (Johnson & Mitchell, 1989) and Ro 31-8220 (Davis *et al,* 1989) were studied. The IC_{50} values for the inhibitors are expressed as mean ± s.e.m. Ro 31-8220 inhibited both the Ca^{2+}-dependent and -independent PDBu-induced activity with similar potencies (IC_{50} values 190 ± 10 and 180 ± 30 nM respectively) but H7 was slightly more potent on the Ca^{2+}-dependent activity (IC_{50} 29 ± 6 μM compared to Ca^{2+}-independent activity IC_{50} 43 ± 11 μM). When the activity was evoked by mezerein, however, this difference was more pronounced, H7 being highly potent on the Ca^{2+}-dependent activity (6 ± 1μM) compared to the Ca^{2+}-independent activity (IC_{50} 28 ± 4 μM). There was also a small difference in the potency of Ro31-8220 on mezerein-induced activity, Ca^{2+}-dependent activity again being more sensitive than the Ca^{2+}-independent (IC_{50} values of 120 ± 10 nM and 210 ± 30 nM respectively). Both of these values, however, are in the range for PKC inhibition (Davis *et al,* 1989). The proportion of the total activity that was Ca^{2+}-dependent was smaller when activity was induced by mezerein (19%) than with PDBu (40%) even though the amount of total activity was the same. This is consistent with previous evidence that certain PKC activators can activate some A series isoforms in the absence of Ca^{2+} (Ryves *et al,* 1991). It appears that mezerein is able to selectively cause Ca^{2+}-dependent activation of a form of PKC present in midbrain that is potently inhibited by H7.

Davis, P.D., Hill, C. H., Keech, E., Lawton, G., Nixon, J.S., Sedgwick, A.D., Wadsworth, J. *et al* (1989) *FEBS Lett.* 259, 61-63.
Johnson, M.S. & Mitchell, R. (1989) *Biochem. Soc. Trans.* 17, 751-752.
MacEwan, D.J., Mitchell, R., Johnson, M.S. & Thomson, F.J. (1991) *Brit. J. Pharmacol.* 102, 258P.
Nishizuka, Y. (1992) *Science* 258, 607-614.
Ryves, W.J., Evans, A.T., Olivier, A.R., Parker, P.J., Evans, F.H. (1991) *FEBS Lett.* 288, 5-9.
Scott Young III, W. (1989) *J. Neuroendocrinol.* 1, 79-82.

80P THE EFFECTS OF PROTEIN KINASE C INHIBITORS ON CONSTITUTIVELY-ACTIVE MAP KINASE FROM RAT HIPPOCAMPUS

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The MAP kinases (mitogen-activated protein kinases), otherwise known as ERKs (extracellular signal-regulated kinases) are a family of serine/threonine kinases that are rapidly activated by mitogens (Cobb *et al,* 1991). A consensus target motif for phosphorylation by MAP kinases has been defined and recognised within the amino acid sequences of cytoskeletal proteins, other kinases and transcription factors (Clark-Lewis *et al,* 1991). In hippocampal neurons, phosphorylation and increased activity of MAP kinase is brought about by NMDA receptor activation (Bading & Greenberg, 1991). MAP kinase is also activated by phorbol esters, presumably by means of their ability to promote protein kinase C (PKC) activity (Cobb *et al,* 1991).

In order to assess some of the pharmacology of MAP kinases, we have examined the effects of a number of widely-used protein kinase inhibitors on MAP kinase activity from hippocampus cytosol. Tissue was homogenised in ice-cold 20 mM Tris HCl pH 7.4 with 12 mM EDTA, 50 mM β-mercaptoethanol, protease inhibitors and 2.5 mM sodium orthovanadate, 62.5 mM β-glycerophosphate and 200 nM okadaic acid. Five μl samples of a crude cytosolic fraction were assayed in 25 μl assays also containing 16 mM Tris HCl pH 7.4, 40 mM β-mercaptoethanol, 160 nM okadaic acid and 50 μM ATP [γ-^{35}S] (specific activity 1285 Ci/mmol; 0.35 μCi/tube). After 40 min at 30°C, with or without the selective peptide substrate APRTPGGRR (2 mM; Clark-Lewis *et al,* 1991), the reaction was stopped with ice-cold trichloroacetic acid and the thiophosphorylated peptide collected by spotting onto phosphocellulose paper and extensive washing in 75 mM H_3PO_4. The constitutive kinase activity could be immunoprecipitated by a protein G-sepharose coupled monoclonal antibody (Zymed Z033) to the p44 and p42 species of MAP kinase and was inhibited by pretreatment of the cytosolic extract with sweet potato acid phosphatase (0.3 - 10U) in the absence but not in the presence of 10 mM pyrophosphate.

A number of protein kinase inhibitors showed the following IC_{50} values (mean ± s.e.mean, n = 6 - 9 separate determinations): staurosporine, 1.5 ± 1.2 μM; K252a, 5.8 ± 1.4 μM; Ro 31-8220 (Davis *et al,* 1989), 3.8 ± 0.9 μM; H7, 66 ± 1 μM; HA 1004, 7.0 ± 2.5 μM; chelerythrine, 69 ± 5 μM. It is important to consider that higher concentrations of these compounds used in some experiments on cellular responses may well influence MAP kinase as well as PKC. Interestingly, the 10-fold greater potency of HA 1004 than H7 seen here has not been observed for any of the other kinases investigated (Hidaka *et al,* 1984).

Bading, H. & Greenberg, M.E. (1991) *Science* 253, 912-914.
Clark-Lewis, I., Sanghera, J.S. & Pelech, S.L. (1991) *J. Biol. Chem.* 266, 15180-15184.
Cobb, M.H., Boulton, T.G. & Robbins, D.J. (1991) *Cell. Regul.* 2, 965-978.
Davis, P.D., Hill, C.H., Keech, E., Lawton, G., Nixon, J.S., Sedgwick, A.D., Wadsworth, J. *et al* (1989) *FEBS Lett.* 259, 61-63.
Hidaka, H., Inagaki, M., Kawamoto, S. & Sasaki, Y. (1984) *Biochemistry* 23, 5036-5041.

81P CHARACTERIZATION OF THE PROTEIN KINASE C ACTIVITY FROM THE αT3-1 GONADOTROPE-DERIVED CELL LINE

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The mouse clonal pituitary gonadotrope cell line, αT3-1 (Windle *et al*, 1990) was used as a model system to investigate the role of protein kinase C in receptor-secretion coupling since there is evidence that different aspects of cellular signalling in pituitary cells including gonadotropes may be controlled by specific PKC isoforms (MacEwan and Mitchell, 1991; Johnson *et al*, 1992; Thomson *et al*, 1993). In this study we have examined the properties of PKCs (partially-purified on DEAE cellulose) from αT3-1 cell cytosol using a histone IIIs kinase assay in the presence of phosphatidylserine/Nonidet P-40 mixed micelles (Johnson *et al*, 1992).

Activators and inhibitors of PKC were included in the assay which was carried out either in the absence (< 3 nM) or presence (100 μM) of Ca^{2+}. Phorbol 12,13-dibutyrate (PDBu) induced kinase activity with an EC_{50} of 313 ± 66 nM in the absence of Ca^{2+} and 76 ± 16 nM in the presence of Ca^{2+}, whilst the related compound mezerein had respective EC_{50} values of 112 ± 11 nM and 53 ± 17 nM. The synthetic diglyceride, 1,2-dioctanoyl *sn*-glycerol, gave EC_{50} values of 22 ± 3 μM for Ca^{2+}-independent activity and 6.9 ± 2.2 μM for activity in the presence of Ca^{2+}. Inhibitors of PKC were examined on basal PKC activity and on activity induced by PDBu (1 μM) with or without Ca^{2+}. Staurosporine, K252a, Ro 31-8220 and H7 had IC_{50} values of 249 ± 23 nM, 1.1 ± 0.2 μM, 3.0 ± 0.2 μM and 71 ± 3 μM respectively on basal kinase activity. With PDBu in the absence of Ca^{2+}, the corresponding values were 96 ± 77 nM, 10 ± 4 μM, 191 ± 13 nM and 38 ± 18 μM respectively and with Ca^{2+} (after subtraction of PDBu-induced, Ca^{2+}-independent activity) the values were 382 ± 143 nM, (K252a, not determined), 113 ± 11 nM and 20 ± 4 μM.

There were clearly variations in the IC_{50} values for the inhibitors on PKC activity from different fractions of the assay. Thus staurosporine inhibited PDBu-induced Ca^{2+}-independent activity particularly well, K252a was most effective on basal kinase activity and Ro 31-8220 and H7 were most effective on Ca^{2+}-dependent PDBu-induced PKC activity. The inhibition of basal kinase activity by these compounds may reflect involvement of kinases other than PKC or of the ζ isoform of PKC which has been shown to be present in these cells (R. Clegg, personal communication). PKC ζ shows constitutive activity in the absence of phorbol esters and is much more sensitive to K252a than other isoforms of PKC (Gschwendt *et al*, 1992). The PDBu-induced Ca^{2+}-independent activity may reflect ε and/or δ PKC which is reported to be particularly resistant to K252a (Gschwendt *et al*, 1992) and the Ca^{2+}-dependent activity may be due to α and β PKC.

Gschwendt, M., Leibersperger, H., Kittstein, W. and Marks, F. (1992) *FEBS Lett.* 307, 151-155.
Johnson, M.S. Thomson, F.J., Avery, J.L., MacEwan, D.J. and Mitchell, P.R. (1992) *J. Physiol.* 446, 283P.
MacEwan, D.J. and Mitchell, R. (1991) *FEBS Lett.* 291, 79-83.
Thomson, F.J., Johnson, M.S., MacEwan, D.J. and Mitchell, R. (1993) *J. Endocrinol.* 136, 105-117.
Windle, J.J., Weiner, R.I. and Mellon, P.M. (1990) *Mol. Endocrinol.* 4, 587-603.

82P CHARACTERIZATION OF THE CANNABINOID BINDING SITE IN THE GUINEA-PIG

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Recently, evidence has accumulated that the effects of cannabinoids are mediated by specific cannabinoid receptors. In particular, a functional cannabinoid receptor has been cloned (Matsuda et al.,1990) and high affinity, stereospecific cannabinoid binding sites have been demonstrated in rat brain (Devane et al., 1988; Westlake et al., 1991). Cannabinoids show a high potency and stereospecificity as inhibitors of the electrically-evoked twitch in the guinea-pig myenteric plexus and mouse vas deferens suggesting that cannabinoid receptors are present in these tissues (Pertwee et al., 1992). To examine this further, we have studied the binding of cannabinoids in homogenates of guinea-pig brain and myenteric plexus.

A P_2 preparation of guinea-pig brain minus the cerebellum was prepared by the method described by Devane et al. (1988). The homogenate was used at a final concentration of 1 mg tissue ml^{-1}. The cannabinoid binding sites were labelled with [^{3}H]-CP-55,940 (102 Ci $mmol^{-1}$; Du Pont). Specific binding was determined as the difference in the counts obtained in the presence of 500 nM Win 55212-2 and Win 55212-3, the active and inactive enantiomers respectively (Pacheco et al., 1991). Incubations were carried out for 60 min at 25°C. Bound and free ligand were separated by filtration over GF/B or GF/C glass fibre filters which had been pre-soaked in 1% polyethylenimine for one hour.

In homogenates of guinea-pig brain, [^{3}H]-CP-55,940 labelled a single class of binding sites with a K_D of 0.13 ± 0.04 nM and a binding capacity of 487 ± 92 fmol mg^{-1} protein (mean ± s.e.mean; n=4). These values are comparable to those reported for the rat (Devane, et al. 1988; Westlake et al.,1991). The levels of binding in the brain were ten times those in the myenteric plexus longitudinal muscle preparation.

Supported by a grant from The Wellcome Trust.

Devane, W.A., Dysarz, F.A., Johnson, M.R. et al. (1988). Mol. Pharmacol. 34, 605-613.
Matsuda, L.A., Lolait, S.J., Brownstein, M.J. et al. (1990). Nature 346, 561-564.
Pacheco, M., Childers, S.R. et al. (1991). J. Pharmacol. exp. Ther. 257, 170-183.
Pertwee, R.G., Stevenson, L.A., Elrick, D.B. et al. (1992). Br. J. Pharmacol. 105, 980-984.
Westlake, T.M., Howlett, A.C., Ali, S.F. et al. (1991). Brain Res. 544, 145-149.

83P THE EFFECT OF DELTA-9-TETRAHYDROCANNABINOL ON THE DEGREE OF CATALEPSY INDUCED IN RATS BY INTRAPALLIDAL INJECTIONS OF MUSCIMOL, BACLOFEN OR PROSTAGLANDIN E_2

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When given peripherally, muscimol, baclofen and PGE_2 can interact synergistically with (–)-delta-9-tetrahydrocannabinol (THC) to produce catalepsy in mice (Pertwee, 1992). The present investigation was directed at establishing if such interactions occur when the drugs are injected into the globus pallidus which is rich in cannabinoid receptors and contains sites at which GABA and THC can act to induce catalepsy (Pertwee & Wickens, 1991; Pertwee, 1992). Experiments were performed with unanaesthetized adult male Sprague-Dawley rats using methods described by Pertwee & Wickens (1991). Injections (0.5 µl) were made bilaterally into the posterior medial region of the globus pallidus through chronically implanted cannulae. The stereotaxic coordinates were A 6.0, L 2.5 and V 1.0 (König & Klippel, 1963). Injection sites were verified histologically. Catalepsy was measured 15 min after drug administration by placing a rat with both forelegs over a 9 cm high horizontal bar and both hindlegs on the bench and noting how long it remained in this position (descent latency). Rats with descent latencies of more than 90 s were classified as "high scoring". THC (30 µg) and PGE_2 (100 ng) were dispersed in Tween 80 (60 µg) and saline (the dose injected through each cannula is shown in parentheses). Muscimol (25 ng) and (±)-baclofen (50 ng), were dissolved in saline. The significance of differences between the effects of treatments ($P > \text{or} < 0.05$) has been evaluated by the Mann-Whitney U-test or by the Fisher exact probability test (n=6).

The median descent latency after THC + muscimol (554 s) was significantly greater (U-test) than after Tween + muscimol (31 s) (mean ± s.e. = 808 ± 236 and 45 ± 13 s respectively) whilst there was no significant difference between the median responses to THC (5 s) and to Tween (2 s), each injected by itself, (mean ± s.e. = 5 ± 1 and 2 ± 1 s respectively) or between the median responses to THC + baclofen (423 s) and to Tween + baclofen (43 s) (mean ± s.e. = 584 ± 221 and 215 ± 135 s respectively) or between the median responses to THC + PGE_2 (558 s) and to Tween + PGE_2 (10 s) (mean ± s.e. = 641 ± 274 and 9 ±3 s respectively). However, there was a significant difference between the proportions of "high scoring" rats in the THC + baclofen (100%) and Tween + baclofen groups (33.3%) (exact probability test). The proportions of "high scoring" rats in the THC + PGE_2 (66.7%) and Tween + PGE_2 groups (0%) were also significantly different. We conclude that in the globus pallidus, THC interacts synergistically with muscimol and probably also with baclofen and PGE_2. Our data support the hypothesis that psychotropic cannabinoids act at least in part by enhancing the actions of GABA and prostaglandins (Pertwee, 1992).

König, J.F.R. & Klippel, R.A.(1963) *The Rat Brain.* Baltimore: Williams & Wilkins.
Pertwee, R.G (1992) in *Marijuana / Cannabinoids*, eds. Murphy, L. & Bartke, A. pp. 165-218. Boca Raton: CRC Press.
Pertwee, R.G. & Wickens, A.P. (1991) Neuropharmacology **30**, 237-244.

84P THE USE OF SHORT-TERM DSP-4 LESIONING TO QUANTIFY PRESYNAPTIC α_2-ADRENOCEPTORS IN VARIOUS REGIONS OF RAT BRAIN

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Although neuronal lesioning has been used to study the synaptic location of central α_2-adrenoceptors, this approach has generally failed to demonstrate a reduction in receptor binding after noradrenergic denervation (e.g. Dooley et al., 1983). The probable explanation is that binding studies were performed ≥10 days after lesioning when postsynaptic α_2-adrenoceptor proliferation had occurred to compensate for the loss of noradrenergic input. Recently, Payvandi et al. (1990) quantified presynaptic α_2-adrenoceptors in rat cortex 3 days after lesioning with DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine). We have now used this approach to quantify presynaptic α_2-adrenoceptors in various regions of rat brain.

Male CD rats (200-220g) were injected with DSP-4 (100mg kg^{-1} ip) 30 min after zimeldine (10 mg kg^{-1} ip). Three days later, cortices, hippocampi, cerebella and hypothalami were removed and divided. α_2-Adrenoceptors were quantified using [^{3}H]-idazoxan (0.1-8 nM) defined by 5µM phentolamine. Binding was performed on single tissues, except for hypothalami which were pooled from 3 rats. Brain monoamines were measured by h.p.l.c. with electrochemical detection (Heal et al., 1989).

Table 1 Effects of short-term DSP-4 lesioning on brain α_2-adrenoceptors and noradrenaline concentrations

	[^{3}H]-Idazoxan binding†			Noradrenaline concentration††	
	Control	DSP-4	Reduction	Control	DSP-4
Cortex	356 ± 9	284 ± 16***	20%	210 ± 7	17 ± 7***
Hippocampus	342 ± 13	281 ± 15**	18%	219 ± 11	23 ± 13***
Cerebellum	139 ± 4	106 ± 5***	24%	177 ± 11	14 ± 8***
Hypothalamus	710 ± 45	435 ± 11***	39%	1774 ± 67	301 ± 63***

Results: mean ± s.e. mean (n = 4-10); † fmol mg $protein^{-1}$; †† ng g wet $weight^{-1}$; **p<0.01; ***p<0.001

DSP-4 depleted noradrenaline by 83-93% (Table 1) with minimal effects on 5-HT (0-9%) or dopamine (0-12%). It also reduced α_2-adrenoceptor number in all 4 regions with the greatest effect in the hypothalamus. DSP-4 treatment did not alter Kd values for [^{3}H]-idazoxan binding. The results for cortex agree well with our earlier study (Payvandi et al., 1990). Overall, the data show that presynaptic α_2-adrenoceptors can be measured after DSP-4 lesioning. In general, they comprise approximately 20% of the total population, but twice this percentage in the hypothalamus.

Dooley, D.J., Bittiger, H., Hauser, K.L., Bischoff, S.F. & Waldmeier, P.C. (1983) Neuroscience **9**, 889-898.
Heal, D.J., Prow, M.R. & Buckett, W.R. (1989) Br. J. Pharmacol. **96**, 547-556.
Payvandi, N., Elliott, J.M. & Heal, D.J. (1990) Br. J. Pharmacol. **100**, 346P.

85P A MICRODIALYSIS STUDY OF THE NEUROCHEMICAL EFFECTS IN RAT STRIATUM OF RX821039, A SELECTIVE LIGAND FOR NON-ADRENOCEPTOR IDAZOXAN BINDING SITES

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Some imidazolines, eg. idazoxan, bind to non-adrenoceptor idazoxan binding sites (NAIBS; Michel & Insel, 1989) as well as α2-adrenoceptors. A function for NAIBS is still unclear but evidence exists of involvement in depression (Piletz et al., 1991), and chronic treatment with some monoamine oxidase inhibitors (MAOIs) downregulates NAIBS in rat brain (Olmos et al., 1993). Using microdialysis we have examined some neurochemical effects of a NAIBS selective ligand, RX821029 (2-(1,3-benzodioxanyl)-2-imidazoline; Hudson et al., 1992). Chloral hydrate anaesthetised male Wistar rats (270-310g) had concentric dialysis probes (perfused with artificial CSF at 2.67 μl min^{-1}) stereotaxically implanted into the striatum (bregma;L,3.0;V,-7.6 mm). Dialysates were collected every 15 min and assayed by HPLC-ECD for dopamine (DA) and its metabolites (DOPAC & HVA) and 5HIAA (a serotonin metabolite). When a stable DA baseline was established rats were injected (i.p.) with either saline or RX821029 (10 mg kg^{-1}). Mean basal release values (per 15 min sample) for DA (pg) and the metabolites (ng) are shown in brackets in the table.

Table 1. The effect of RX821029 on neurochemical output in rat striatum.

	Treatment		Collection time (min) post administration								
			0	15	30	45	60	75	90	105	120
DA	saline	(6.3±1.0)	98±4	99±5	91±5	96±10	96±10	85±6	90±9	100±11	90±10
	RX821029	(5.9±1.6)	96±3	108±4	107±9	116±4	131±5*	129±6**	121±6*	105±4	108±6
DOPAC	saline	(6.9±0.3)	103±1	105±1	105±1	107±3	104±3	105±3	106±3	106±2	102±1
	RX821029	(6.3±1.0)	100±0	98±3	94±4*	90±5*	83±6*	78±6**	75±4**	75±4**	75±3**
HVA	saline	(5.6±0.3)	106±1	112±1	114±2	118±2	119±2	122±3	125±3	125±3	125±4
	RX821029	(5.2±0.7)	107±1	110±3	112±3	115±5	114±6	113±7	111±6	110±6	108±5*
5HIAA	saline	(1.9±0.2)	102±1	107±1	109±1	111±1	111±1	113±2	117±3	117±2	118±1
	RX821029	(1.9±0.2)	103±1	102±3	102±3	100±4*	98±5*	98±7	97±6*	99±7*	100±6*

Data represent mean±s.e.mean (% basal release), n=4. 2-Way ANOVA revealed that RX821029 significantly increased DA output ($p=0.0001$) and decreased DOPAC, HVA & 5HIAA output ($p<0.0001$) relative to saline controls. **($p<0.01$) and *($p<0.05$) are significant changes from controls at individual timepoints (unpaired t-test).

RX821029 clearly has central effects. One possible explanation for increasing DA whilst decreasing metabolite output is that RX821029 may modulate MAO activity, supporting the postulated regulatory link between MAO and NAIBS. Again, this may have functional implications but further studies are required.

Hudson, A., Mallard, N., Nutt D.J. & Chapleo, C. (1992) Fundam. Clin. Pharmacol., 6 (Suppl 1), 45s.
Michel, M. & Insel, P. (1989) Trends. Pharmacol. Sci. 10, 324-344.
Olmos, G., Miralles, A., Barturen, F. & Garcia-Sevilla, J.A. (1993) Br. J. Pharmacol., 108, 597-603.
Piletz, J.E., Halaris, A., Saran, A. & Marler, M.R. (1991) Arch. Gen. Psychiat. 48, 813-820.

86P DIFFERENTIAL LABELLING OF DOPAMINE RECEPTORS IN RAT BRAIN *IN VITRO* AND *IN VIVO*: COMPARISON OF [3H]-PIRIBEDIL AND [3H]-N,n-PROPYLNORAPOMORPHINE

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Piribedil is a dopamine agonist of unusual structure and which produces atypical behavioural responses (Butterworth et al., 1975). It is extensively metabolised *in vivo* to the corresponding catechol derivative and there is debate over the nature of the active moiety. Previously, we showed [3H]-piribedil to accumulate *in vivo* in rat substantia nigra and nucleus accumbens, but not striatum, employing a technique of brain tissue oxidation (Hall et al., 1983). We now report on the autoradiographic distribution of [3H]-piribedil in brain slices *in vitro* and following its systemic administration to rats compared to that of [3H]-N,n-propylnorapomorphine ([3H]-NPA).

Male Wistar rats (200-250 g) were employed. Frozen brain sections (12-20 μm) were cut on a cryostat at the level of striatum and substantia nigra. The characteristic of the *in vitro* binding of [3H]-NPA was described previously (Dubois et al., 1985); the same incubation conditions were used to establish optimal [3H]-piribedil binding parameters. The specific binding of [3H]-piribedil (10 nM; 52 Ci/mmol) and of [3H]-NPA (0.25 nM; 51 Ci/mmol) was defined using 10^{-5} M sulpiride and represented 65-70% of the total binding. For the *in vivo* binding, rats were injected with [3H]-piribedil or [3H]-NPA (50 μCi) into the tail vein, killed 30 min after and frozen brain sections prepared. Sections labelled *in vitro* and those obtained by *in vivo* binding were apposed to tritium Hyperfilm for 6-8 weeks and subsequently analysed by computerized densitometry.

In vitro, the highest density of [3H]-piribedil and [3H]-NPA binding sites were observed in the striatum, nucleus accumbens, olfactory tubercle, islands of Calleja and the superficial grey layer of the superior colliculus. A lower density of binding sites was present in the substantia nigra. *In vivo*, [3H]-NPA binding sites were observed in the striatum, nucleus accumbens, olfactory tubercle and islands of Calleja. A low density of [3H]-NPA binding sites was found in the substantia nigra. No binding was observed in any of these areas after administration of [3H]-piribedil (Table 1).

Table 1: Specific binding of [3H]-piribedil and [3H]-NPA (both *in vivo* and *in vitro*) to rat brain slices. Specific binding is expressed in fmol/mg tissue. ST= striatum, NA=nucleus accumbens, OT=olfactory tubercle, SN=substantia nigra, ND=not detectable. (n = 3)

	In vitro				*In vivo*			
Specific binding	**ST**	**NA**	**OT**	**SN**	**ST**	**NA**	**OT**	**SN**
[3H]-piribedil	35.0±6.7	23.4±4.9	18.2±1.2	9.2±0.1	ND	ND	ND	ND
[3H]-NPA	23.6±10.4	17.4±5.6	22.4±5.4	11.1±2.1	9.4±0.3	5.0±0.5	8.6±0.8	2.8±0.4

These data suggest that piribedil and NPA selectively interact with dopamine receptors *in vitro* with a similar pattern of distribution. In contrast only [3H]-NPA appears to label dopamine receptor sites *in vivo*. The difference observed between the *in vitro* and the *in vivo* binding of [3H]-piribedil may be due to its extensive metabolism *in vivo*.

Butterworth, R. F., Poignant J. C. & Barbeau A. (1975) Adv. Neurol. 9, 307-327.
Hall M.D., Jenner P. & Marsden C.D. (1983). Eur. J. Pharmacol. 87, 85-94.
Dubois A. & Scatton B. (1985). Neuroci. Lett. 57, 7-12.

87P CHARACTERISATION OF MONOAMINE EFFLUX IN RAT LOCUS COERULEUS SLICES USING FAST CYCLIC VOLTAMMETRY

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Fast cyclic voltammetry at carbon fibre microelectrodes (FCV) has been used to monitor stimulated monoamine efflux *in vivo* and *in vitro*. We now report the use of the technique in slices of the locus coeruleus (LC) and the characterisation of the released amine. Slices (350μm) of LC were prepared from male Wistar rats (100-150g) and superfused with oxygenated artificial cerebrospinal fluid (ACSF) at 32°C at 1.0 ml/min. Monoamine efflux was evoked using trains of electrical stimulation (30 pulses, 0.2 ms, 10 mA, 100 Hz, every 5 min) at bipolar stimulating electrodes and monitored using FCV as previously described (Palij & Stamford, 1992). Experiments lasted for 2½ h and consisted of 30 stimulations. Drugs were administered, via the ACSF, after the sixth train (S_6).

Electrical stimulation evoked the release of a substance which generated a voltammogram indistinguishable from those produced by the catecholamines, noradrenaline (NA) and dopamine (DA) but distinct from that produced by 5-hydroxytryptamine (5HT). Removal of Ca^{2+} (2 mM) from the superfusate and replacement with Mg^{2+} (from 2 to 4 mM) produced a rapid and readily-reversible reduction in efflux (Control: 93.5 ± 3.4%, Zero Ca^{2+}: 25.1 ± 4.6%, $P<0.001$). Peak amine efflux was equivalent to 0.31 ± 0.04 μM NA (mean ± s.e.m., n=28) and decayed with a half-life ($t_{½}$) of 2.93 ± 0.28 s (n=16). The efflux and $t_{½}$ were stable for the duration of the experiments, declining to 87.2 ± 4.5% and 93.1 ± 5.0% (mean of S_{25}-S_{30} expressed as a percentage of the mean of S_1-S_6, n=4) respectively. Table 1 shows the effects of various drugs upon monoamine efflux.

Drug (μM)	Desipramine (0.05)	Fluvoxamine (0.5)	GBR 12909 (0.3)	Pargyline (2.0)	Ro 4-1284 (1.0)
Efflux	*188.8 ± 4.6****	*160.0 ± 7.1****	*98.3 ± 5.3*	*124.9 ± 4.6***	*15.8 ± 2.2****
Half-life	*405.0 ± 55.9***	*238.8 ± 36.1**	*115.2 ± 1.2**	*Not measured*	*Not measured*

*Mean of S_{25}-S_{30} as a percentage of S_1-S_6 ± s.e.m. (n=4). *P<0.05, **P<0.01, ***P<0.001 vs controls.*

Voltammograms recorded after the uptake blockers desipramine, fluvoxamine and GBR 12909 (1-[2-[bis(4-fluorophenyl) methoxy]ethyl]-4-[3-phenylpropyl]piperazine) were indistinguishable from those of NA or DA, but distinct from 5HT. Furthermore amine efflux was significantly and reversibly inhibited, in a concentration-dependent fashion, by the α_2 agonist bromoxidine (66.0 ± 1.9 % reduction at 1 μM, $P < 0.01$) but not by the $5HT_{1B}$ agonist trifluoromethylphenylpiperazine (TFMPP) (23.5 ± 12.0 % at 1 μM). The monoamine oxidase inhibitor pargyline increased NA efflux while Ro 4-1284 (Pletscher et al, 1962) irreversibly reduced efflux. The results are consistent with the released substance being NA although the effect of fluvoxamine suggests that some released NA may be taken up by 5HT uptake. The data suggests that FCV can monitor NA release and reuptake in slices of the LC.

Palij, P. & Stamford, J.A. (1992) *Brain Res.* **587**, 137-146.
Pletscher, A., Brossi, A. & Gey, K.F. (1962) *Int. Rev. Neurobiol.* **4**, 275-306.

88P EFFECTS OF GBR 12935 ON DOPAMINE EFFLUX AND UPTAKE IN THREE STRIATAL SUBREGIONS

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Previous studies have suggested that the nucleus accumbens (NAc) has fewer dopamine (DA) transporter sites than the caudatoputamen (CPu) (Missale et al, 1985). In this study we used fast cyclic voltammetry (FCV) to examine the effects of the DA uptake blocker GBR 12935 (Janowsky et al, 1986) on stimulated DA efflux and uptake half-life ($t_{½}$) in three anatomical subdivisions of the striatum: NAc, CPu and the putative clusters of A10 terminals in the medial axis of the CPu. (Davidson & Stamford, 1992).

All experiments were conducted in 350 μm brain slices from male Wistar rats (100-150g) superfused with artificial CSF at 32°C. NAc slices were taken at approx. +10.5 mm vs interaural line (Paxinos & Watson, 1986) and recordings made in the NAc core. All other slices were taken from approx. +9.2 mm interaural and recordings made from the dorsolateral CPu or from clusters in the medial axis. DA efflux was evoked using 0.1 ms, 10mA pulses applied singly (1p) or in 5 pulse (5p) trains at 50 Hz applied every 10 mins via a bipolar tungsten electrode. DA efflux was recorded by FCV at a carbon fibre microelectrode sampling at 2 Hz. Experiments consisted of 9 cycles of alternate 1p & 5p (18 stimulations, 3 hours). GBR 12935 (0.2 μM) was administered immediately after the third cycle.

DA efflux on 1p stimulation did not differ significantly between CPu (147 ± 24 nM, mean ± s.e.m., n=15), NAc (90 ± 17 nM, n=8) and the clusters (212 ± 56 nM, n=14). However, uptake $t_{½}$ was significantly shorter in CPu (1007 ± 100 ms, n=12) than in NAc (1557 ± 179 ms, n=8, $P < 0.01$) and the clusters (1772 ± 368 ms, n=10, $P < 0.05$).

	CPu			*NAc*			*Clusters*		
	Control		***GBR 12935***	***Control***		***GBR 12935***	***Control***		***GBR12935***
1p	96 ± 14	**	363 ± 71	102 ± 10	**	226 ± 31	85 ± 6	ns	101 ± 9
5p	85 ± 14	*	462 ± 104	103 ± 19	*	288 ± 54	107 ± 17	ns	154 ± 50
t½	104 ± 15	*	418 ± 95	103 ± 15	*	246 ± 45	105 ± 5	ns	219 ± 33

*Means ± s.e.m. (n=3/4). ns = not significant, *P < 0.05, **P < 0.01 (control v GBR).*

Table 1 (above) shows the effects of GBR 12935 on 1p and 5p DA efflux and on uptake $t_{½}$ on the 9th cycle expressed as a percentage of pre-drug values.GBR 12935 significantly increased DA efflux and $t_{½}$ in CPu and NAc, but not in the putative A10 clusters. The lack of effect of GBR 12935 in the clusters suggests either that DA uptake is less important or that the uptake has different pharmacological characteristics. Further studies are in progress to test this assertion.

Davidson C & Stamford JA (1992) *Br. J. Pharmacol.* **107**, 213P
Janowsky A et al (1986) *J. Neurochem.* **46**, 1272-1276
Missale C et al (1985) *J. Neurochem.* **45**, 51-56.
Paxinos G & Watson C. (1986) *The rat brain in stereotaxic coordinates.*, London, Academic Press.

89P REPEATED ECS DOES NOT AFFECT D_1-RECEPTOR-STIMULATED ADENYLATE CYCLASE *IN VITRO* WHEN ASSESSED USING RAT STRIATAL SLICES

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The effect of repeated electroconvulsive shock (ECS) on dopamine D1 receptor number in rat striatum is contentious, with reports of an increase (Fochtmann et al., 1989), a decrease (Klimek and Nielsen, 1987) and, in our hands, no change (Martin et al., 1988). Recently, we reported that this treatment potentiates D1 receptor function when determined by dopamine-stimulated adenylate cyclase in rat striatal homogenates (Martin et al., 1990). We have now extended these investigations by examining the effects of ECS on D1-receptor mediated adenylate cyclase activity in rat striatal slices because Newman et al. (1986) have observed that ECS had differential actions on this enzyme when measured in homogenates and slices.

Male CD rats (100-125 g) received either an ECS (200V, 2s) or halothane anaesthesia only 5 times over 10 days. Striata were removed 24 h later and the adenylate cyclase response to DA (10E-6 - 10E-3M) was determined in the absence (DA) and presence (DA + SULP) of sulpiride (10E-5 M) and to SKF 38393 (10E-8 - 10E-4M) (Lazareno et al., 1985).

Table 1 Effects of repeated ECS on dopamine-stimulated adenylate cyclase in striatal slices.

		DA		DA + SULP		SKF 38393	
Treatment	Combined Basal	10E-5M	10E-4M	10E-5M	10E-4M	10E-7M	10E-5M
Halothane x 5	21.8 ± 1.2	23.5 ± 2.2	32.3 ± 2.7	30.1 ± 1.0*	41.1 ± 0.7*	27.1 ± 2.7	39.5 ± 2.3
ECS x 5	23.3 ± 1.1	25.6 ± 3.3	33.4 ± 2.4	29.6 ± 1.9	43.3 ± 1.5**	27.2 ± 4.6	37.5 ± 4.6

Values are means ± s.e.mean (n = 17-18 for basals and 5-9 for others) cAMP produced in pmoles/mg protein. Significantly different from DA *p<0.05, **p<0.01.

As shown in Table 1, dopamine potentiated adenylate cyclase activity and this effect was enhanced by the presence of sulpiride. This enzyme was also stimulated by the D1 partial agonist, SKF 38393. Repeated ECS had no effect on either basal adenylate cyclase activity or the enhancements produced by any of the dopaminergic agents. These results confirm our earlier findings obtained with homogenates (Martin et al., 1990) that repeated ECS does not alter dopamine (D1+D2) stimulation of adenylate cyclase, but fails to reproduce the ECS-induced enhancement of selective D1 receptor activation. Therefore, repeated ECS does not affect dopamine-stimulated adenylate cyclase when measured in striatal slices. In addition, the differential effects of ECS in homogenate and slice preparations complements the earlier findings of Newman et al. (1986) and indicates that neuroanatomical integrity can be an important determinant of the observed effects of ECS on adenylate cyclase.

Fochtmann, L. J., Cruciani, R., Aiso, M. & Potter, W. Z. (1989) Eur. J. Pharmacol. 167, 305-306.
Klimek, V. & Nielsen, M. (1987) Eur. J. Pharmacol. 139, 163-169.
Lazareno, S., Marriott, D. & Nahorski, S. R. (1985) Brain Res. 361, 91-98.
Martin, K. F., Needham, P. L., Atkinson, J., Cowan, A., Heal, D. J. & Buckett, W. R. (1988) Br. J. Pharmacol. 95, 896P.
Martin, K. F., Browning, J. G., Redfern, J., Heal, D. J. & Buckett, W. R. (1990) Br. J. Pharmacol. 100, 351P.
Newman, M. E., Salomon, H. & Lerer, B. (1986) J. Neurochem. 46, 1667-1669.

90P INDUCTION OF jun-B mRNA IN THE RAT STRIATUM AND NUCLEUS ACCUMBENS AFTER ACUTE ADMINISTRATION OF HALOPERIDOL

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Immediate early genes (IEG's) appear to play an important role in transducing extracellular stimuli into altered patterns of gene expression and, therefore, into long term changes in cellular function. The IEGs c-fos and zif-268, which are thought to activate gene transcription, are induced in the rat striatum after acute administration of haloperidol (Dragunow et al, 1990;Nguyen et al, 1992). Another IEG, jun-B, has been proposed to have a repressive action on downstream genes,but there is currently no evidence as to whether this IEG is induced by haloperidol treatment. In this study we have used in situ hybridisation to measure the levels of c-fos mRNA and jun-B mRNA in the rat striatum and nucleus accumbens after acute administration of haloperidol. Male wistar rats (180-220g) were treated with haloperidol (1mg/kg) or vehicle(physiological saline) i.p. After 30 minutes the animals were killed. Their brains were processed for the detection of mRNA encoding c-fos and jun-B by in situ hybridisation using ^{35}S-labelled oligonucleotide probes (Morris, 1989). The c-fos and jun-B hybridisation signals were quantified using the MCID image analysis system. The hybridisation signal for c-fos mRNA was dramatically increased in the striatum following haloperidol administration (table1) confirming previous results. However, we also observed an induction by haloperidol of jun-B mRNA in both accumbens and striatum. (table 1).

Table1:

		c-fos		jun-B	
	n	Saline	Haloperidol	Saline	Haloperidol
Striatum	3	17±2	56±5*	16±10	111±20*
Accumbens	3	20±4	50±14	33±11	157±27*

Values are mean optical density± S.E.M. * $p < 0.05$ as compared to control values (ANOVA).
Table 1: Effect of acute administration of haloperidol on c-fos and jun-B mRNA levels

These results suggest that haloperidol, an antipsychotic drug with potent dopamine receptor antagonist properties, can induce jun-B mRNA in the rat striatum and accumbens. It is tempting to speculate that the induction of jun-B mRNA may be related to the long-term decrease that is observed in the expression of certain genes in the striatum following haloperidol treatment.

We gratefully acknowledge the support of the Wellcome Trust.

Dragunow,M.,Robertson,G.S.,Faull,R.L.M.,Robertson,H.A. & Jansen,K. (1990) *Neuroscience* 37, 287-294
Morris,B.J.(1989) *J. Comp. Neurol.* 290, 358.
Nguyen,T.V., Kosofsky,B.E., Birnbaum,R., Cohen,B.M., Hymens,S.E.(1992) *Proc. Natl. Acad. Sci. U.S.A.* 89, 4270-4274.

91P THE EFFECT OF 5-HT AGONISTS ON ETHANOL PREFERENCE AND FOOD INTAKE IN FEMALE SPRAGUE-DAWLEY RATS

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Recent work suggests that serotonin (5-HT) is involved in the control of alcohol consumption, thus compounds which inhibit 5-HT re-uptake reduce ethanol intake (for review see Sellers *et al.* 1992). The present study was undertaken to examine the effects of several 5-HT receptor agonists on ethanol, water and food intake in the rat.

Female Sprague-Dawley rats (Bantin & Kingman, UK) weighing 250-300g were injected once per day with 1.4g/kg of ethanol in saline for 12 days, then housed singly and given a choice between 5% ethanol and water, available for 15h daily (from 17.30-08.30h) with food constantly available for a period of 19 days. Rats displaying a consistent preference for 5% ethanol over water were subsequently given a choice between 10% ethanol and water for 19 days and those animals ingesting 60% or more of their total fluid as ethanol were used for subsequent drug studies. Drugs were administered at 17.30h and intake data were analysed by ANOVA followed by Dunnett's t-test. The 5-HT re-uptake inhibitor, fluoxetine, at 5 and 10mg/kg i.p. significantly reduced 15h ethanol intake from a control value of 21.6±1.9g (mean ± s.e. mean) to 13.2±2.7g and 12±0.6g respectively ($P<0.001$, Dunnett's t-test). A dose of 1mg/kg significantly increased 24h food intake ($P<0.05$), while 10mg/kg significantly reduced food intake ($P<0.05$). Fluoxetine had no effect on 15h water intake. The $5\text{-HT}_{1B/1C}$ receptor agonist, mCPP (1-(3-chlorophenyl)piperazine), at 5.0 and 10.0mg/kg i.p. significantly reduced ethanol ingestion from a control value of 14.7±2.5g to 10.3±1.6g and 8.3±1.7g ($P<0.05$ and $P<0.01$) and significantly reduced 24h food intake ($P<0.05$). mCPP had no effect on water intake. TFMPP (1-(3-trifluoromethyl)phenyl)piperazine), a $5\text{-HT}_{1B}/5\text{-HT}_{1C}$ receptor agonist, at 5.0 and 10.0mg/kg i.p., significantly reduced ethanol consumption from a control value of 14.4±1.3g to 9.4±0.9 and 8.1±1.5g ($P<0.01$) with no effect on food or water intake. The selective 5-HT_{1B} receptor agonist CGS12066B (Neale *et al.*, 1987) (0.1-3.0mg/kg i.p.) had no effect on ethanol, water or food intake. The 5-HT_2 receptor agonist, DOI (1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane), at 1.0 and 5.0mg/kg s.c. significantly reduced ethanol intake from a control value of 23.6±0.9g to 10.6±1.2g and 10±1.3g ($P<0.01$) and increased water intake from a control value of 5.1±1.6g to 14.6±2.7g and 14±1.2g ($P<0.01$), with no effect on food intake.

The present study describes a method whereby rats may be induced to ingest large quantities of ethanol and show a consistent preference for ethanol over water. The results obtained support the hypothesis that increasing central 5-HT neurotransmission leads to a reduction in ethanol preference in the rat. In addition, these data show that compounds with activity at 5-HT_{1C} and 5-HT_2 receptor subtypes selectively reduced ethanol intake.

This work is supported by Glaxo Group Research.

Neale R.F., Fallon S.F., Boyar W.C. et al. (1987) *Eur. J. Pharmac.* 136, 1-9
Sellers E.M., Higgins, G.A. & Sobell, M.B. (1992) *Trends Pharmac. Sci.* 13, 69-75

92P EVIDENCE THAT D-FENFLURAMINE RELEASES 5-HT SELECTIVELY FROM TERMINALS OF DORSAL RAPHÉ NEURONS

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Immunocytochemical data suggest that several 5-HT-releasing agents including p-chloroamphetamine (PCA) and d-fenfluramine (FEN) can produce a degeneration of terminals of 5-HT-containing axons arising from the dorsal raphe (DR) but not the median raphe (MR) nucleus (Molliver et al., 1990). Since this neurodegenerative effect is selective for DR fibres it was of interest to investigate whether the acute 5-HT-releasing action of such drugs might also be selective for DR fibres. Here we have studied the effect of a PCA lesion on the acute 5-HT-releasing action of FEN.

5-HT lesions were induced in male Sprague-Dawley rats (180-250 g, 5-9 per group) by two injections of PCA (10 mg/kg i.p. x2, 24 h apart). This regime has previously been shown to induce a substantial lesion of terminals of DR but not MR 5-HT neurons (Molliver et al., 1990; Series and Molliver, unpublished data). Controls received saline vehicle. Two weeks after injection, rats were anaesthetized with chloral hydrate, and microdialysis probes (4 x 0.4 mm) were implanted stereotaxically into the frontal cerebral cortex and perfused with artificial CSF. Dialysate samples were collected every 20 min and analysed for 5-HT and 5-HIAA by HPLC-EC as previously described (Sharp et al., 1990). When 5-HT release was steady, 3-4 h after probe implantation, rats received either a systemic injection of FEN (10 mg/kg i.p.) or a 20 min pulse of iso-osmotic CSF containing 100 mM KCl via the dialysis probe. 5-HT release was followed for a further 2 h.

Basal levels of 5-HT were not affected by PCA pretreatment (controls, 11.08 ±1.36 fmol/20 min; PCA, 14.85 ±4.79 (not significant, Student's t-test)). PCA, however, had a substantial effect on both basal 5-HIAA (controls, 5892.2 ±469.5 fmol/20 min; PCA, 867.7 ±261.5 fmol/20 min ($p<0.001$, t-test)) and potassium-evoked release of 5-HT (peak response in controls, 137.4 ±22.6 fmol/20 min; PCA, 54.0 ±6.6 fmol/20 min ($p<0.01$, t-test)). In controls, FEN produced an immediate increase in 5-HT release which had almost returned to basal within 2 h (5-HT release summed over 2 h, 549.6 ±166.2 fmol). The 5-HT response to FEN was almost abolished by PCA-pretreatment (49.8 ±19.2 fmol/2 h; a reduction of 90.9% compared with controls ($p<0.001$, t-test)). Since previous results (Molliver et al., 1990) suggest that the 5-HT terminals lesioned by PCA are principally derived from DR neurons, our finding of a near total loss of FEN-evoked 5-HT release in cortex after PCA pretreatment is consistent with the hypothesis that the acute 5-HT-releasing effect of FEN is selective for DR terminals. This finding is relevant to basic and clinical studies using FEN as a probe of central 5-HT function.

Molliver, M.E., Berger, U.V., Mamounas, L.A., Molliver, D.C., O'Hearn, E. & Wilson, M.A. (1990) *Ann. N. Y. Acad. Sci.* 600, 640-661.
Sharp, T., Bramwell, S.R. & Grahame-Smith, D.G. (1990) *Neuroscience* 39, 629-637.

93P DIFFERENTIAL LEVELS OF 5-HT/5-HTIAA BETWEEN HEMISPHERES IN THE RAT FRONTAL CORTEX AND HIPPOCAMPUS

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Functional asymmetry in the brain has been demonstrated to occur in many behavioural paradigms and species (see Bradbury *et al.*, 1985). In the rat, studies of laterality have invariably been restricted to an analysis of circling behaviour and neurochemical asymmetry within the nigrostriatal dopamine system (see Glick, 1983). The present study was designed to investigate levels of 5-hydroxytryptamine (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in cortical and limbic areas in both the left and right hemispheres of the rat brain.

Female Hooded-Lister rats (250-300g, Bradford bred) were killed by cervical dislocation. The frontal cortex, hippocampus, striatum, amygdala and septum from each hemisphere were dissected and homogenised in 100-200 µl 0.2 M perchloric acid containing N-methyl-5-HT and 3,4-dihydroxybenzylamine (100 pg μl^{-1} each). The homogenate was centrifuged at 15,600 g for 5 min. The supernatant was analysed for the content of 5-HT, 5-HIAA and noradrenaline (NA) using HPLC-ECD according to the method of Barnes *et al* (1992).

5-HT levels in the frontal cortex from the left (L) hemisphere were nearly 4 fold greater than in the right (R) hemisphere whereas in the hippocampus the 5-HT levels were elevated 150% in the right hemisphere compared to the left. Both brain areas showed reduced levels of 5-HIAA in the right hemisphere. The 5-HIAA/5-HT ratio was higher in the right frontal cortex but in the left hippocampus (Table 1). No such bilateral differences were observed in tissues from striatum, amygdala and septum. NA levels in the left and right hemispheres of each brain region were not significantly different.

Table 1. Levels of 5-HT, 5-HIAA, 5-HIAA/5-HT ratio and NA in the left and right hemispheres of the rat brain.

Area	Frontal Cortex		Hippocampus		Striatum		Amygdala		Septum	
	L	R	L	R	L	R	L	R	L	R
5-HT	95±16	25±5**	169±28	255±21*	489±74	391±42	347±68	270±71	86±21	79±27
5-HIAA	674±50	398±50**	826±63	428±49*	1070±160	878±81	1295±148	1368±128	1353±101	1280±80
5-HIAA/5-HT	7.7±0.9	21±6.5	5.4±0.9	1.7±0.2*	2.2±0.1	2.4±0.6	5.0±1.9	8.4±3.7	15±5	12±4
NA	145±25	127±34	469±36	423±22	20±3.3	20±2.2	503±64	310±68	241±57	154±36

Data represent the mean ± S.E.M. of five determinations (pg mg^{-1} wet weight tissue). Significant differences in neurotransmitter levels and 5-HIAA/5-HT ratio between right and left hemispheres are indicated as *P<0.05, **P<0.01 (Student's t test).

The results indicate a laterality of 5-HT and 5-HIAA levels in the frontal cortex and hippocampus of the rat. Further studies are required to investigate if drug treatment or behavioural modulation has differential effect on the neurochemical levels in each hemisphere. The existence of laterality has important implications to an analysis of forebrain 5-HT function using the intracerebral microdialysis technique.

Barnes, N.M., Costall, B., Ge, J., Kelly, M.E. & Naylor, R.J. (1992) Eur. J. Pharmacol., **218**, 15-25

Bradbury, A.J., Costall, B., Domeney, A.M. & Naylor, R.J. (1985) Neuropharmacology, **24**, 1163-1170

Glick, S.D. (1983) In Hemisyndromes, Psychobiology, Neurology, Psychiatry (Myslobodsky M.S., Ed.), pp 7-26, Academic Press, London.

94P ALTERED GLUCOSE USE IN ADRENERGIC PROJECTION AREAS OF ADULT RAT BRAIN FOLLOWING *IN UTERO* EXPOSURE TO METHYLENEDIOXYMETHAMPHETAMINE

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Monoaminergic cell bodies develop relatively early in the mammalian brain, and it has been postulated that amongst other things they may provide trophic effects in the growth of other neuronal systems (Lauder et al., 1981). Psychostimulant drugs of abuse are known to interact with monoaminergic neurones in the adult brain, but their effects upon prenatal cerebral development are largely unknown. The purpose of the present study was to examine cerebral function in adult rats following their repeated exposure whilst *in utero* to the psychostimulant methylenedioxymethamphetamine (MDMA; "Ecstasy").

Pregnant, time-mated Sprague-Dawley rats were injected (s.c.) with either MDMA (20mg/kg; n=4) or saline (0.2ml; n = 4) twice daily on gestational days 13-16 inclusive. After the litters had been weaned, the dams were sacrificed and the efficacy of MDMA treatment assessed with [^{3}H]-paroxetine radioligand binding in membrane preparations from forebrain as described by us previously (McBean et al., 1990). At 4-6 weeks of age, local cerebral glucose utilization (LCGU; µmol/100g/min) was measured in 5 MDMA-treated and 5 control male offspring (at least one from each litter) using the 2-deoxyglucose autoradiographic technique (Sokoloff et al., 1977). Adjacent brain sections were processed for autoradiographic [^{3}H]-paroxetine radioligand binding (Sharkey et al., 1991). Data, presented as mean ± s.e. mean, were analysed using Student's t-test with significance set at $P < 0.05$.

Paroxetine binding was reduced in dams treated with MDMA (B_{max} = 16.6 ± 2fmol/mg tissue) when compared to control females (B_{max} = 34.4 ± 0.7). In contrast, paroxetine binding in brain sections adjacent to those used for LCGU autoradiography from the mature offspring, revealed neither qualititative nor quantitative changes in serotonin uptake sites between control and MDMA treated rats. Exposure to MDMA *in utero* did however alter cerebral glucose use, although statistically significant changes were largely limited to the locus coeruleus, where LCGU was increased from 51 ± 2 in controls to 70 ± 3µmol/100g/min in MDMA-treated rats, and primary projection areas from the coeruleus such as globus pallidus (from 42 ± 2 to 53 ± 3µmol/100g/min) and septal nucleus (from 49 ± 2 to 61 ± 4µmol/100g/min). In contrast there was no evidence of any effects of MDMA in either serotonergic or dopaminergic cell groupings, or their primary projection areas.

This study would suggest that repeated exposure to MDMA in utero results in the subsequent manifestation of cerebral dysfunction (as it is reflected in altered rates of glucose utilization) in the rat brain at 4-6 weeks after birth. The mechanism which underlies the apparent vulnerability of central noradrenergic systems to MDMA exposure remains to be determined, but we could find no evidence that MDMA produces the same serotonergic toxicity found in adult brain.

Lauder, J.M., Wallace, J.A. & Krebs, H. (1981) *Adv. Exp. Med. Biol.* 133, 477-506.
McBean, D.E., Sharkey, J., Ritchie, I.M. & Kelly, P.A.T. (1990) *Neuroscience* 38, 271-275.
Sharkey, J., McBean, D.E. & Kelly, P.A.T. (1991) *Psychopharmacology* 105, 113-118.
Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patlak, C.S., Pettigrew, K.D. *et al.* (1977) *J. Neurochem.* 28, 897-916.

95P THE DEVELOPMENT OF METHYLENEDIOXYMETHAMPHETAMINE NEUROTOXICITY IN THE NEONATAL RAT BRAIN

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Although methylenedioxymethamphetamine (MDMA), induces a highly selective loss of serotonergic (5-HT) nerve terminals in adult rat brain (Battaglia et al., 1987), we have not been able to demonstrate any depletion of 5-HT uptake sites when individuals are exposed *in utero* (Kelly et al., this meeting). The purpose of this study was to investigate whether prenatal resistance to MDMA is related to the ontogeny of 5-HT neurones, which are known to continue the development of terminal processes into early postnatal life (D'Amato et al., 1987).

Neonatal male rats were injected (s.c.) with either MDMA (20mg/kg) or saline (0.2ml) twice daily on four consecutive days, commencing on postnatal days 10, 20, or 25. All animals were sacrificed on postnatal day 40. Saturation analysis of [^{3}H]paroxetine labelled 5-HT uptake sites was performed by incubating homogenate of frontal cortex in 5ml buffer containing [^{3}H]paroxetine (concentration range = 3-300pM) for 2 h at 22°C as described previously (Battaglia et al., 1987). Nonspecific binding was defined by the presence of 4µM citalopram in the incubation medium. Data were analysed using the non-linear, least-squares curve fitting programme "Ligand" (Munson & Rodbard, 1980). The efficacy of our MDMA treatment protocol was assessed using adult male rats. Statistical analysis was performed using the Scheffe test for multiple comparisons.

TABLE 1: Data presented as mean±s.e.mean (K_D, pM; B_{MAX}, fmol/mg tissue). *, Significantly different from control ($P < 0.05$).

Age	CONTROL			MDMA		
Days	K_D	n	B_{MAX}	K_D	n	B_{MAX}
10	38.0±2.0	6	36.8±1.0	40.8±4.7	6	35.2±1.7
20	39.3±1.6	5	37.8±1.1	40.7±2.1	6	34.3±1.5
25	33.4±0.6	6	30.4±0.8	38.1±4.2	5	16.6±0.8*
Adult	27.3±3.8	5	23.5±2.3	25.8±8.3	5	3.5 ±1.2*

In keeping with previous studies (Battaglia, 1987), [^{3}H]-paroxetine binding was reduced by 85% in adult rat brain 14 days after MDMA (Table 1), but there was no evidence for any significant effect when animals were treated with MDMA on days 10 or 20. In contrast, animals treated on day 25 with MDMA displayed a significant decrease in [^{3}H]-paroxetine binding (-45%) (Table 1).

These studies would suggest that the susceptibility of serotonergic neurones to the toxic effects of MDMA develops postnatally in rats, and coincides in time with the adult pattern of 5-HT terminal distribution (D'Amato et al., 1987) although the possibility of regrowth at earlier times cannot be discounted.

Battaglia, G., Yeh, S.Y., O'Hearn, E., Molliver, M.E., Kuhar, M.J. *et al.* (1987) *J. Exp. Pharmac. Exp. Ther.* 242, 911-916.
D'Amato, R.J., Blue, M.E., Largent, B.L., Lynch, D.R., Ledbetter, D.J. *et al.* (1987) *Proc. Natl. Acad. Sci. (USA)* 84, 4322-4326.
Munson, P.J. & Rodbard, D. (1980) *Analyt. Biochem.* 107, 220-239.

96P THE EFFECT OF 5-HT$_3$ RECEPTOR ANTAGONISTS ON THE UPTAKE OF [^{3}H]5-HT INTO RAT CORTICAL SYNAPTOSOMES

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The 5-HT$_3$ receptor antagonists ondansetron, zacopride and tropisetron have recently been identified as possessing antidepressant activity in a learned helplessness paradigm in the rat (Martin *et al.*, 1992). The structural similarity of tropisetron to cocaine may indicate that it exerts part of its antidepressant activity via inhibition of 5-HT uptake. The present study compares the ability of 5-HT$_3$ receptor antagonists with reference 5-HT uptake inhibitors fluoxetine and cocaine to inhibit [^{3}H]5-HT uptake into synaptosomes prepared from rat cortex.

Female Hooded-Lister rats (250-300g, Bradford bred) were killed by cervical dislocation. The cerebral cortex was dissected, homogenised in 20 volumes of ice-cold 0.32 M sucrose and centrifuged at 1000 g at 4°C for 10 min. The pellet was discarded and the supernatant recentrifuged at 48,000 g for 10 min. The pellet was resuspended in ice-cold 0.27 M sucrose to form the crude synaptosomal preparation. To initiate [^{3}H]5-HT uptake, 250 µl of the crude synaptosomal preparation was added to 750 µl Krebs buffer (mM; NaCl 118, KCl 4.75, KH_2PO_4 1.20, $MgSO_4.7H_2O$ 1.20, $CaCl_2.6H_2O$ 2.50, $NaHCO_3$ 25.0, glucose 11.11, and 10µM pargyline; gassed for 60 min with 95%CO_2/5%O_2; pH 7.4) containing (final concentration) 0.1-0.2 nM [^{3}H]5-HT (25.4 Cimmol^{-1}, NEN) in the presence or absence of competing compound (10^{-4} to 10^{-9}M, six concentrations). Uptake was allowed to proceed at 37°C for 6 min before termination by rapid filtration through Whatman GF/B filters followed by immediate washing for 13 sec with ice-cold Krebs buffer. Protein content was determined by the method of Bradford (1976).

[^{3}H]5-HT uptake was inhibited by up to 80-90% by the uptake inhibitors and 5-HT$_3$ receptor antagonists. Fluoxetine was the most potent agent tested, tropisetron and cocaine had a similar but 10 fold reduced potency to inhibit uptake, MDL 72222 and zacopride were a further 10 times less potent, whereas ondansetron was the least potent agent tested (see table 1).

Table 1. The effect of fluoxetine, cocaine and 5-HT$_3$ receptor antagonists on the uptake of [^{3}H]-5-HT into cortical synaptosomes

Compound	pIC50	Compound	pIC50
fluoxetine	7.3±0.1	MDL 72222	5.61±0.11
cocaine	6.45±0.15	(R/S)-zacopride	5.39±0.05
tropisetron	6.57±0.04	ondansetron	4.32±0.09

Results are the means+S.E.M.s of 3 determinations.

Tropisetron was found to be as potent as cocaine to inhibit the synaptosomal uptake of [^{3}H]5-HT but in concentrations considerably in excess of those known to antagonise at 5-HT$_3$ receptors. (R/S) zacopride and ondansetron were only effective at concentrations unlikely to be achieved *in vivo*. It is concluded that the reported antidepressant activity of the 5-HT receptor antagonists is not generally related to an ability to inhibit 5-HT uptake. But 5-HT uptake inhibition should be considered in the use of high-dose/concentrations of tropisetron in *in vitro* and *in vivo* assays.

Bradford, M.M. (1976) Anal. Biochem. **72**, 248-254.
Martin, P., Gozlan, H. & Puech A.J. (1992) Eur. J. Pharmacol. **212**, 73-78.

97P INTERACTION OF ANTIDEPRESSANTS WITH 5-HT UPTAKE SITES AND NEUROTRANSMITTER RECEPTORS IN POST-MORTEM HUMAN BRAIN

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Inhibition of monoamine reuptake is an important mechanism in antidepressant drug action. Tricyclic antidepressants (TCAs) inhibit 5-HT and NA reuptake, with varying selectivity. Recently more selective 5-HT reuptake inhibitors (SSRIs) have been introduced as antidepressants. Ligand binding studies in rat brain indicate that SSRIs have less interaction with neurotransmitter binding sites than TCAs (Johnson, 1991). In this study we have compared the interaction of 4 SSRIs and 3 TCAs with 5-HT uptake sites and a number of neurotransmitter binding sites in human brain tissue obtained at post-mortem.

Displacement by antidepressants of specific binding was performed in the following assays: 5-HT uptake sites ([^{3}H] paroxetine, 50pM, putamen), 5-HT_{1A} ([^{3}H] 8-OH DPAT, 0.4nm, parietal cortex), 5-HT_2 ([^{3}H] ketanserin, 0.4nM, parietal cortex), 5-HT_3 ([^{3}H] BRL 43694, 2nM, thalamus), α_1 NA([^{3}H] prazosin, 0.1nM, parietal cortex), α_2 NA([^{3}H] rauwolscine, 2nM, parietal cortex), and mACh ([^{3}H] N-methylscopolamine, 0.2 nM, parietal cortex). K_i values (nM, mean ± SEM, n=3, calculated using LIGAND) are shown in Table 1.

Table 1: K_i values for antidepressant displacement of specific binding

	5-HT uptake	5-HT_{1A}	5-HT_2	5-HT_3	α_1 NA	α_2 NA	mACh
Paroxetine	0.1±0.01	>10000	>10000	>10000	>10000	>10000	300±70
Fluoxetine	2.2±0.5	>10000	270±40	>10000	>10000	>10000	8000±1200
Fluvoxamine	3.6±0.9	>10000	>10000	>10000	6500±500	6600±260	>10000
Sertraline	0.5±0.03	9000±400	5900±250	>10000	760±130	1800±240	7700±1200
Amitriptyline	7.2±0.2	700±40	28±3	5100±2600	5±0.6	152±8	10±5
Nortriptyline	32±1	800±40	43±8	>10000	9±1	580±50	27±10
Dothiepin	16±2	1800±100	500±13	>10000	260±50	1430±80	67±44

The TCAs had affinity for all the binding sites studied, except for 5-HT_3 (for nortriptyline and dothiepin). SSRIs were more potent than TCAs at 5-HT uptake sites, with a rank order paroxetine > sertraline > fluoxetine > fluvoxamine. Paroxetine, the most selective of the SSRIs, had affinity for mACh sites. Fluoxetine had affinity for 5-HT_2 and mACh sites, whereas fluvoxamine had weak affinity for α_1 NA and α_2 NA sites. Sertraline had affinity for α_1 NA and α_2 NA sites and weaker affinity for 5-HT_{1A}, 5-HT_2 and mACh sites. The ratio of the K_i values at mACh and 5-HT uptake sites for SSRIs was at least 2-3 orders of magnitude greater than for TCAs. The present results in human brain confirm the greater selectivity of SSRIs compared to TCAs previously demonstrated in rat brain (Johnson, 1991).

Johnson, A.M. (1991) in Selective Serotonin Re-uptake Inhibitors ed Feighner JP & Boyer WF pp 37-70. Chichester: J. Wiley & Sons.

98P MODULATION BY DIVALENT CATIONS OF CURRENT RESPONSES MEDIATED BY A CLONED MURINE 5-HT RECEPTOR (5-HT_3R-A) EXPRESSED IN HEK 293 CELLS

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Several reports indicate the group IIa divalent cations Ca^{2+} and Mg^{2+} (Nash and Wallis, 1981; Peters *et al.*,1988) and the group IIb divalent Zn^{2+} (Lovinger,1991) to suppress depolarizing or inward current responses mediated by 5-HT_3 receptors. In this study, the effects of Ca^{2+}, Mg^{2+} and Zn^{2+} upon current responses recorded from HEK 293 cells stably expressing the cloned 5-HT_3 R-A homo-oligomeric 5-HT_3 receptor (Maricq *et al.*, 1991) were examined.

Currents evoked by ionophoretically applied 5-HT were recorded using the whole-cell recording mode of the patch-clamp technique under the experimental conditions previously described (Peters *et al.*, 1988). The 5-HT-induced currents had a reversal potential of -3.5 ± 0.6 mV (n = 10) and rectified inwardly, the chord conductance increasing e-fold for a 151 mV hyperpolarization. Reduction of the external concentrations of Ca^{2+} ($[Ca^{2+}]_o$) and Mg^{2+} ($[Mg^{2+}]_o$), each to 0.1 mM, from their standard values of 1 and 2 mM respectively enhanced the amplitude of the response to 5-HT (recorded at -60 mV) to 165.9 ± 7.8% (n = 14) of control. In subsequent experiments, the divalent deficient solution containing 0.1 mM Ca^{2+} and 0.1 mM Mg^{2+} was used as standard, all measurements of 5-HT evoked current amplitude being expressed as a percentage of those obtained in this solution. With $[Mg^{2+}]_o$ set at 0.1 mM, increasing $[Ca^{2+}]_o$ from 0.1 to 1.0 and 10.0 mM reduced the amplitude of the 5-HT induced response to 76.4 ± 8.6% (n = 4) and 36.9 ± 10.1% (n = 5) of control respectively. A depressant action of Mg^{2+} was observed with $[Ca^{2+}]_o$ set at 0.1 mM, the response to 5-HT being reduced to 85.8 ± 2.6% (n = 4) and 38.2 ± 4.5% (n = 5) of control in the presence of 1.0 and 10.0 mM Mg^{2+} respectively. The effects of Ca^{2+} and Mg^{2+} were voltage-independent and occurred in the absence of any significant shift in the reversal potential of the 5-HT-induced response. Zn^{2+} (0.3-300 μM) exerted a biphasic effect upon the 5-HT-induced response, low concentrations (1-10 μM) potentiating and higher concentrations depressing response amplitude. For example, in the presence of 3 μM Zn^{2+} the response was enhanced to 135.3 ± 4.9% (n = 4) of control, whereas 300 μM Zn^{2+} reduced the current to 29.2 ± 8.7% (n = 3) of control.

A similar biphasic effect of Zn^{2+} upon AMPA/kainate receptor operated cation conducting channels has been reported (Resendren *et al.*, 1990). However, previous studies of the effect of Zn^{2+} upon 5-HT_3 receptors present in NCB-20 hydridoma cells (from which the 5-HT_3 R-A was cloned) reported its antagonist action but did not detect enhancement (Lovinger, 1991). If this difference is confirmed, it may be indicative of differing subunit composition between the 5-HT_3 R-A and the 5-HT_3 receptor native to the NCB-20 cell.

CHG is a Glaxo Research Scholar. We thank Drs. G. Kilpatrick and H. Rogers for helpful discussion.

Lovinger, D.M. (1991) J. Neurophysiol. **66**, 1329-1337.
Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M. and Julius, D. (1991) Science **254**, 432-437.
Nash, H.L. and Wallis, D.I. (1981) Br. J. Pharmacol. **73**, 759-772.
Peters, J.A., Hales, T.G. and Lambert, J.J. (1988) Eur. J. Pharmacol. **151**, 491-495.
Resendren, F-A., Lory, P., Pin, J-P. and Nargeot, J. (1990) Neuron **4**, 733-740.

99P CHARACTERISATION OF 14-0-ETHYL-5-METHYLNALTREXONE: A NOVEL OPIOID RECEPTOR ANTAGONIST

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Although three types of opioid receptor, are recognised, namely μ, δ and κ, there have been suggestions that subtypes of these receptors exist. In order to characterise such subtypes there is a need for selective antagonists. Recently we have synthesised a novel opioid antagonist, 14-O-ethyl-5-methylnaltrexone, which appears to differentiate the actions of certain μ-agonists *in vivo*. In this communication we present these results and compare the *in vivo* activity with the antagonist activity towards opioid receptors *in vitro*.

The antinociceptive effects of μ-opioids were determined in the mouse (Cox Standard, 20-22g) abdominal constriction test, using acetic acid (0.6% i.p.) as previously described (Zimmerman *et al.*, 1987). Drugs were administered s.c. 30 min before the test. Five animals were used per dose and the data given as ED_{50} values with 95% confidence limits. The antagonist action of 14-O-ethyl-5-methyl-naltrexone *in vitro* was determined in the mouse vas deferens preparation (mvd, CSI strain, 25-30g) and the myenteric-plexus longitudinal muscle preparation (mplm) of the guinea-pig ileum (male Dunkin-Hartley, 300-400g) using normorphine (μ), [D-Pen^2,D-Pen^4]enkephalin (δ) and ethylketocyclazocine (κ) as agonists (Traynor *et al.*, 1987). Values are means ± sem of six experiments.

At a dose of 2.5mg/kg s.c. 14-O-ethyl-5-methylnaltrexone shifted the antinociceptive ED_{50} (μg/kg) of fentanyl from 18 (7.5-28)to 290 (110-610) and of sufentanyl from 0.82 (0.73-0.92) to 18 (3.0-40) but the ED_{50} for morphine was not significantly altered (600 (450-790) and 1369 (640-2300)). However in the mouse vas deferens preparation 14-O-ethyl-5-methylnaltrexone readily antagonised the. agonist action of normorphine affording a Ke value of 4.3±0.3nM. The compound was nonselective and also acted as an antagonist at both κ-opioid (Ke 9.2±1.2nM) and δ-opioid (Ke 69.6±16nM) receptors. Similarly in the mplm preparation 14-O-ethyl-5-methylnaltrexone afforded Ke values of 2.83±1.04nM at μ-receptors and 5.04±1.97nM at κ-receptors but also showed partial agonist activity (ED_{50} 4886±219nM) which was not reversed in the presence of 30nM naloxone. Thus whilst 14-O-ethyl-5-methylnaltrexone appears to be unable to antagonise morphine in *vivo*, at least in the mouse abdominal constriction assay, this cannot be readily explained on the basis of actions at opioid receptors *in vitro*.

Acknowledgement: We thank Dr. J.D. Leander (Lilly Research Laboratories, Indianapolis) for performing the *in vivo* studies.

Traynor, J.R., Corbett, A.D. and Kosterlitz, H.W. (1987) Eur.J.Pharmacol., 137, 85-89.
Zimmerman, D.M., Leander, J.D., Reel, J.K. and Hynes, M.D. (1987) J.Pharmacol.Exp. Ther., 241, 374-378

100P MORPHINE-6-GLUCURONIDE: COMPARISON WITH MORPHINE BY LIGAND-BINDING AND ISOLATED TISSUE BIOASSAY

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Morphine-6-glucuronide (M-6-G) is a minor morphine metabolite which may contribute significantly to analgesia (Osborne *et al.*, 1988). In support of this M-6-G has an increased potency and a longer duration of action than morphine in animal models of nociception (Frances et al., 1992). To determine whether these effects are due to improved activity at opioid receptors we have studied the action of morphine and M-6-G using isolated tissue preparations and ligand-binding assays.

Binding assays were performed in brain homogenates from male CSI mice (25g) in Tris buffer (50mM, pH 7.4) by the method of Gillan *et al.*, 1980) using [^{3}H]DAMGO ([D-Ala^2,MePhe^4,Glyol^5]enkephalin ,1nM), [^{3}H]DPDPE ([D-Pen^2,D-Pen^5]enkephalin, 1nM) and [^{3}H]U69593 ((5α,7α,8β)-(-)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacatamide, 1nM) to label μ-, δ- ,and κ-opioid binding sites respectively. The potencies of morphine and M-6-G were assessed using field stimulated vasa deferentia of the mouse (mvd, CSI, 25-30g) and rat (Wistar, 200-250g) and the myenteric-plexus longitudinal muscle (mplm) of the guinea-pig ileum (male Dunkin-Hartley, 300-400g) by methods previously described (Traynor et al, 1987). All values are means ± sem of at least 3 experiments.

Morphine and M-6-G were seen to have similar affinity for μ-opioid binding sites (Ki, 6.22± 0.86nM and 7.50±1.48nM respectively). However M-6-G had a higher affinity for opioid δ-binding sites (Ki 75.1±15.6nM compared with 218±41.6nM for morphine), but a very much reduced affinity for κ-opioid binding sites (850±118nM compared with 84.7± 4.0nM for morphine). In the mplm preparation M-6-G (IC_{50} 58.0±4.3nM) was approximately twice as potent as morphine (IC_{50} 130 ± 7.2nM), though no difference was seen in the mvd (IC_{50} M-6-G 104±17nM, IC_{50} morphine 173±63nM). In both tissues the agonist action of M-6-G was antagonised by naloxone with Ke values of 3.0± 0.8nM (mplm) and 2.6±0.9nM (mvd) confirming an action*via* μ-opioid receptors. In the field stimulated rat vas deferens both morphine and M-6-G acted as partial agonists, reaching a maximum of 30% inhibition of the electrically induced twitch and were able to antagonise the action of the μ-opioid agonist DAMGO. In contrast to the binding studies the equilibrium dissociation constant (Ke) for M-6-G measured by this method (194±28nM) suggested a higher affinity for the μ-opioid receptor than morphine (Ke 1007±188nM). In conclusion M-6-G has a different opioid receptor binding profile to morphine and a higher affinity for μ-opioid receptors under physiological conditions. These effects may contribute to the improved activity of the metabolite over the parent compound.

Gillan, M.G.C., Kosterlitz, H.W., and Paterson, S. J. (1980). Br. J. Pharmacol., 70, 481-490.
Frances, B., Gout, R., Monsarrat, B., Cros, J. & Zajac, J-M. (1992) J. Pharmacol. Exp.Ther., 262, 25-31.
Osborne, R., Joel, S., Trew, D. & Slevin, M. (1988) Lancet, 1, 828.
Traynor, J.R., Corbett, A.D. and Kosterlitz, H.W. (1987) Eur.J.Pharmacol., 137, 85-89.

101P ENDOGENOUS OPIOID INHIBITION OF SUPRAOPTIC NUCLEUS (SON) OXYTOCIN CELL ACTIVITY IN LATE PREGNANT RATS

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Late pregnant rats develop endogenous opioid inhibition of magnocellular secretory oxytocin neurones since i.v. injection of naloxone (NLX) increases oxytocin secretion in the final week of gestation but not earlier in pregnancy or post partum (Douglas et al 1993). Endogenous opioids are present in oxytocin and vasopressin cells and their terminals in the neural lobe and act locally to inhibit oxytocin secretion. We have previously reported that in late pregnant rats oxytocin nerve terminals become desensitised to opioid actions (Douglas et al 1993). We now report studies on the central component of the opioid inhibition of oxytocin neurones in late pregnancy from experiments measuring SON oxytocin neurone firing rate and Fos protein expression (an indicator of activated neurones).

Virgin and 21 day pregnant rats were anaesthetised with urethane (1.25g/kg b.w. i.p.) and the SON exposed by ventral surgery. Oxytocin neurones were recorded extracellularly and identified antidromically by neural stalk stimulation and by an excitatory response to i.v. cholecystokinin (CCK 20 μg/kg, Leng et al 1992). Thirty minutes later, NLX was injected (2mg/kg, i.v.) followed after 10 min by CCK again. NLX does not affect basal or CCK excitation of oxytocin neuronal firing rate but does potentiate CCK stimulation of oxytocin secretion (Leng et al 1992) in virgin rats, indicating action only at the neural lobe. In 21 day pregnant rats NLX had no effect on basal firing rate but strongly potentiated CCK excitation of oxytocin neurones (by 2.6 fold, $p<0.05$ Mann Whitney, n=4), indicating endogenous opioid inhibition of the oxytocin neurone response to i.v. CCK in late pregnancy, possibly through presynaptic mechanisms. Conscious virgin and 21 day pregnant rats were injected with either vehicle (VEH) or NLX (5mg/kg; 4ml/kg, i.p.). They were decapitated after 90 min, the brains were frozen and cryostat sections subsequently processed by immunocytochemistry for Fos protein (Luckman et al 1993). The mean ± s.e. number of Fos positive nuclei per μm^2 SON was 0.26 ±0.10 in virgin rats given NLX (n=6), 0.39 ± 0.10 in pregnant rats given VEH (n=6), and 1.32 ± 0.26 in pregnant rats given NLX (n=6, $p<0.05$, ANOVA).

The data indicate that endogenous opioids inhibit oxytocin neurones in late pregnant but not virgin rats. Thus the opioid inhibition revealed by NLX in pregnant rats is probably due to endogenous opioid actions on oxytocin neurone cell bodies or on their inputs.

Funded by an AFRC link grant.

Douglas, A.J., Dye, S., Leng, G., Russell, J.A. & Bicknell, R.J. (1993) *J.Neuroendocrinol.* (in press)
Leng, G., Dyball, R.E. & Way, S.A. (1992) *Exp. Brain Res.* **88**:321-325.
Luckman, S. Antonijevic, I., Leng, G., Dye, S., Douglas, A.J., Russell, J.A. & Bicknell, R.J. (1993) *J.Neuroendocrinol.* **5**:7-12.

102P MORPHINE TOLERANCE/DEPENDENCE INCREASES CHOLECYSTOKININ (CCK) BINDING IN THE RAT SUPRAOPTIC NUCLEUS (SON)

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Magnocellular oxytocin neurones in the SON appear to co-produce CCK. These neurones are directly excited by CCK, this being reversed by morphine. Chronic i.c.v. infusion of morphine leads to tolerance and dependence in oxytocin neurones (Pumford *et al.* 1991). CCK may be an endogenous opioid antagonist involved in the development of tolerance. We have used autoradiography to quantify CCK binding within the SON in morphine-treated rats to seek evidence for a role of CCK in morphine tolerance of oxytocin neurones. Under ether anaesthesia female Sprague-Dawley rats were fitted with an i.c.v. cannula to infuse morphine sulphate (up to 50μg/h) or vehicle (1μl/h) from a subcutaneous osmotic mini-pump. After 5 days the rats were decapitated, their brains rapidly removed and frozen onto cryostat chucks on dry ice. A further group of rats was given 2% w/v NaCl to drink for 48h as a positive control, since it has previously been shown that this treatment increases CCK binding within the SON (Day *et al.*, 1989); a control group was given water. 10μm transverse brain sections on gelatinised slides were incubated with [^{125}I]CCK8 with or without excess unlabelled CCK8 to determine Total Binding (TB) and Non-Specific Binding (NSB) respectively; specific CCK binding was defined as (TB-NSB). The sections were then apposed to Hyperfilm [^{3}H] for 4 days. Films were then processed, tissue sections fixed and the autoradiographs of SON profiles viewed under a microscope (objective x10) for quantification using a Joyce-Loebl μMagiscan image analyser. Results were expressed as mean density of silver deposit over the measured area of dorsal SON (202 x 133μm). Each film processed contained sections from at least one animal from each treatment group; the untreated group measurement on each film was used to calculate relative values from the measurements for each treatment group on the film, to overcome variability between films.

Specific [^{125}I]CCK8 binding in the SON increased by 2.4 fold ($P<0.01$) after i.c.v. morphine treatment (n=6, mean relative value ± s.e. mean: 1.37 ± 0.18) compared with the vehicle treated group (n=6, 0.57 ± 0.1). There was no difference between the morphine-treated group and the salt loaded group (n=3, 1.48 ± 0.15); in both, binding was significantly increased ($P<0.05$) relative to the untreated controls. The increase in specific CCK8 binding within the SON, which may reflect an increase in receptor density, after chronic morphine treatment indicates that increased activity of endogenous CCK mechanisms within the SON could play a role in the development of morphine tolerance and dependence in oxytocin neurones in the rat.

Supported by the A.F.R.C.

Day, N.C., Hall, M.D. & Hughes, J. (1989) *Neuroscience* 29, 371-383.
Pumford, K.M., Leng, G. & Russell, J.A. (1991) *J. Physiol.* 440, 437-454.

103P ELECTROPHYSIOLOGICAL EFFECTS OF SELECTIVE δ-OPIOID RECEPTOR AGONISTS IN RAT VENTROMEDIAL HYPOTHALAMUS *IN VITRO*

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Electrophysiological studies on ventromedial hypothalamus (VMH) slices from guinea-pig brain *in vitro* have suggested that the inhibition of neuronal firing in this area, by opioid agonists, is largely mediated by the μ-receptor (Charpak *et al.*, 1988). However autoradiographic studies in rats have shown the presence of δ-opioid receptors in the VMH (Desjardins *et al.*, 1990) and it is therefore possible that these receptors are important in this species. The availability of selective stable peptide δ-agonists and a selective non-peptide δ-antagonist, naltrindole, have allowed us to investigate whether activation of δ-receptors produces functional responses in the VMH. Sprague-Dawley rats were killed by decapitation, hypothalamic blocks were dissected and coronal slices (350μm) containing VMH were cut using a vibratome. Slices were perfused with artificial cerebrospinal fluid at 33°C gassed with 95%O_2/CO_2 and extracellular recordings were made with a glass pipette filled with 3M NaCl (DC tip resistance 10MΩ). Drugs were applied in the perfusate for a period of 90 sec and responses were measured as changes in the firing rate of single neurons. The effects of three selective δ-opioid receptor agonists, [D-Pen^2, D-Pen^5]enkephalin (DPDPE, 4-400nM), [D-Ser^2, Leu^5, Thr^6]enkephalin (DSLET, 12-400nM) and D-Ala^2 deltorphin I (DELT I, 4-400nM), on the neuronal firing sustained by 0.5μM quisqualic acid were studied. Sensitivity to a non-opioid depressant was established using GABA (10-100 μM).

Of the cells tested 77% had their firing rate depressed by DSLET (n=47), 61% by DPDPE (n=33) and 59% by DELT I (n=27). Dose response curves for each agonist carried out on separate neurons allowed estimation of the concentrations causing a 75% inhibition of firing (ID_{75}) and these were 144±29nM (DPDPE n=10), 61±14nM (DSLET n=9) and 33±4nM (DELT I n=5). The μ-agonist [D-Ala^2 Me Phe^4 Gly-ol^5]enkephalin (DAGOL, 12nM-4μM) in half of the cells tested was unable to inhibit firing by more than 70%. Preliminary results show that the depressant effect of both DSLET and DPDPE are completely reversed by the highly selective non-peptide δ-antagonist naltrindole (10nM, n=3). This dose of naltrindole had no effect on the inhibition following addition of GABA (n=4) or the μ-selective agonist DAGOL (n=2). These results support the existence of functional δ-receptors in rat VMH.

TJC holds a SERC-CASE award with MSD

Charpak, S., Dubois-Dauphin, M., Raggenbass, M. and Dreifuss, J.J., 1988, Brain Res., 450, 124-130.
Desjardins, G.C., Brawer, J.R. and Beaudet, A., 1990, Brain Res., 536, 114-123.

104P EXCITATION BY MORPHINE WITHDRAWAL INDUCES *c-fos* EXPRESSION IN MAGNOCELLULAR OXYTOCIN NEURONES

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Magnocellular oxytocin (OXT) neurones are inhibited by acute morphine but develop tolerance and dependence during intracerebroventricular (i.c.v.) morphine infusion over 5d (Bicknell et al. 1988). Withdrawal induced acutely by naloxone (NLX) increases OXT neurone firing rate 3.5 fold in dependent rats but has no effect in controls, and as a result oxytocin secretion is greatly increased. Vasopressin neurones are unaffected. We have studied whether withdrawal activates the early onset gene, *c-fos*, and stimulates production of Fos in dependent OXT neurones. To establish morphine dependence, virgin female Sprague Dawley rats, n=8, were implanted with a lateral i.c.v. cannula, under ether anaesthesia, to infuse morphine sulphate (up to 50μg/h) from a subcutaneous osmotic minipump; controls were given vehicle (1μl/h). In the first study after i.c.v. morphine infusion for 5d a femoral vein was cannulated, under urethane anaesthesia (1.25g/kg i.p.), for injection 2h later of NLX (5mg/kg) or vehicle. The rats were decapitated 0.5h after NLX, the brains removed, frozen on dry ice and stored at -70°C. 20μm cryostat sections through the hypothalamus were hybridised with ^{35}S-labelled oligonucleotide *c-fos* mRNA probe and exposed on film for 3 weeks (Hamamura et al 1991). Autoradiographs were viewed by microscope (x10 objective) and grain density above background over the supraoptic nucleus (SON) was measured with a Joyce Loebl image analyser. In the SON *c-fos* mRNA was barely detected in vehicle injected rats (n=4, mean grain density ± s.e. 0.8 ± 0.8% field area) whereas NLX induced *c-fos* mRNA expression (n=4, 13.3 ± 1.8%, $p<0.001$, t-test). In the second study after i.c.v. morphine or vehicle infusion for 5d conscious rats were given either s.c. NLX or vehicle(n=6 per group). The rats were decapitated 90min later and the brains removed, frozen on dry ice and stored at -70°C. 15μm cryostat sections through hypothalamus were immuno-cytochemically processed using Fos antibody (Oncogene Sciences) and peroxidase labelled second antibody detected by the glucose oxidase-nickel-DAB method(Shu et al 1988). Sections were viewed at x10 mag and the density of Fos positive nuclei in the SON calculated by counting and by measuring the area of each SON profile with the image analyser. In the i.c.v. vehicle group after i.v. vehicle or NLX, and in the i.c.v. morphine group after i.v. vehicle, the density of Fos positive neurones in the SON was <1 neurone/ $10^4\mu m^2$ but in the morphine/NLX group the density was 22.6±3.30(mean± s.e,$p<0.0001$,ANOVA). Activation of the *c-fos* gene in SON neurones during morphine withdrawal may be confined to oxytocin neurones, and a consequence of their increased firing rate.

Supported by AFRC.

Bicknell, R.J., Leng, G., Lincoln, D.W., & Russell, J.A.(1988) *J.Physiol.*396:297-317.
Hamamura, M., Leng, G., Emson, P.C., Kiyama, H.,(1991) *J.Physiol.* 444:51-63.
Shu, S., Ju, G. & Fan, L.(1988) *Neurosci. Lett.* 85:169-171.

105P VASA DEFERENTIA OBTAINED FROM MICE PRETREATED WITH DELTA-9-TETRAHYDROCANNABINOL (THC) SHOW TOLERANCE TO DELTA-9-THC, WIN 55212-2 AND CP 55,940

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When obtained from mice pretreated with delta-9-tetrahydrocannabinol (THC), vasa deferentia have been found to show tolerance to the inhibitory effect on electrically-evoked contractions of the cannabinoid, CP 55,940 (Pertwee et al., 1992). It was concluded, therefore, that the mouse vas deferens might be suitable as a model for studying the mechanisms responsible for cannabinoid tolerance. The aim of the present experiments was to investigate this possibility further by establishing whether *in vivo* pretreatment with THC would also induce tolerance to itself and to WIN 55,212-2, a compound which has cannabimimetic properties and which, like THC and CP 55,940, is known to bind avidly to the putative cannabinoid receptor (Jansen et al., 1992).

Male MF1 mice were injected once daily for 2 days with THC (20 mg/kg i.p.). Control animals received injections of Tween 80 (40 mg/kg i.p.). Mice were killed 24 h after the second injection and their vasa deferentia removed. Tissues were also obtained from untreated mice. Vasa deferentia were mounted in 4 ml siliconized organ baths, at an initial tension of 0.5 g. The baths contained Mg^{++}-free Krebs solution kept at 37°C and bubbled with 95% O_2 and 5% CO_2. Tissues were stimulated supramaximally with 0.5 s trains of 3 pulses (train frequency 0.1 Hz; pulse duration 0.5 ms). Isometric contractions were recorded. All drugs were mixed with two parts of Tween 80 by weight and dispersed in saline.

THC (10 nM) and WIN 55,212-2 (3.16 nM) produced significantly less inhibition of the twitch response (Student's t test; $P < 0.001$) in vasa deferentia obtained from mice treated with THC (5.4 ± 5.1% and 23.2 ± 4.9% respectively; mean ± se; n=8) than in those obtained from Tween-treated animals (42.4 ± 5.5% and 77.2 ± 4.4% respectively; n=8). The inhibitory effects of 10 nM THC and 3.16 nM WIN 55,212-2 in tissue obtained from untreated mice were respectively 42.7 ± 4.9% (n=6) and 74.7 ± 4.4% (n=8). We also confirmed the inhibitory effect of CP 55,940 (0.316 nM) to be significantly less ($P < 0.001$) in tissue from THC treated mice (1.3 ± 6.5%; n = 8) than in tissue from animals treated with Tween (40.2 ± 4.9%; n=8). Our finding that *in vivo* pretreatment with THC can induce tolerance both to its own inhibitory effect on the vas deferens and to that of other cannabimimetic drugs, supports the idea that the mouse vas deferens could be of use in elucidating the mechanisms underlying cannabinoid tolerance. Further experiments are now required to establish whether THC also induces tolerance to inhibitors of the twitch response that are not cannabimimetic.

We thank Sterling Winthrop for WIN 55,212-2, Pfizer for CP 55,940, NIDA for THC & the Wellcome Trust for support.

Jansen, E.M., Haycock, D.A., Ward, S.J. & Seybold, V.S. (1992) Brain Research **575**, 93-102.
Pertwee, R.G., Stevenson, L.A., Fernando, S.R. & Corbett, A.D. (1992) Br. J. Pharmacol. **107**, 365P.

106P CHANGES IN OPIOID SYSTEMS IN THE RAT SPINAL CORD FOLLOWING PERIPHERAL CARRAGEENAN INFLAMMATION

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In the intact halothane anaesthetized rat three hours after the injection of carrageenan into the hind paw the evoked neuronal responses of convergent dorsal horn nociceptive neurones are altered; some neurones show enhanced responses whilst others show decreases. Concurrently, the spinal potency of morphine is enhanced whilst naloxone produces complex effects (Stanfa *et al.*, 1992). The levels of the endogenous opiate peptides dynorphin and enkephalin are increased in the spinal cord following inflammation (Dubner & Ruda, 1992). This electrophysiological study uses the mixed peptidase inhibitor kelatorphan (which protects the enkephalins from breakdown) and the kappa opiate receptor antagonist nor-binaltorphimine (nBNI) to investigate the roles of enkephalin and dynorphin respectively in spinal nociceptive events during inflammation.

The effects of intrathecal kelatorphan (5, 50, 250μg) were examined on the C-fibre evoked responses of dorsal horn neurones in normal animals and animals 3 hours post-carrageenan. There was no difference in the potency of kelatorphan or in the maximum inhibition of the response in either case even with the highest dose of 250μg (43±9% inhibition of the C-fibre response in normal animals (n=5); 39±12% in carrageenan animals (n=4)). These inhibitions were reversed to 90±6% of control by 1μg of naloxone. These findings suggest that 3 hours after the onset of inflammation there is no change in the inhibitory effects exerted by spinal enkephalins.

The effects of two intrathecal doses of the kappa antagonist nBNI (10 & 100μg) were examined on the neuronal responses in both groups of animals. nBNI enhanced the responses of some neurones (5/10 cells in normals; 4/10 cells in carrageenan), whilst reducing that of the others. The magnitude of the change produced by 100μg of nBNI was significantly greater in the carrageenan animals implying a greater release / role of spinal dynorphin. However there was no relationship between the effects of nBNI and the direction of change in the neuronal activities induced by carrageenan. Thus dynorphin is unlikely to be a causal factor in these neuronal changes.

Dubner, R. & Ruda, M.A. (1992) *Trends Neurosci.* 15, 96-103.
Stanfa, L.C., Sullivan, A.F. & Dickenson, A.H. (1992) *Pain* 50, 345-354.

107P NALOXONE PRETREATMENT CAN BLOCK NSAID ANALGESIA IN HEALTHY SHEEP

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Non steroidal anti inflammatory drugs (NSAIDs) have been commonly used as mild analgesics for many years. Vane (1971) proposed that they worked by inhibiting prostaglandin production. This property is now one of the definitions of this diverse group of drugs. Prostaglandins have been shown to sensitise tissues to bradykinin and histamine resulting in severe pain although they did not cause pain when given on their own (Ferreira, 1972). The NSAIDs should thus reduce pain caused by inflammation but have no effect in the absence of inflammation. Flunixin meglumine is a NSAID widely used in animals as an analgesic. It is believed to act, like other NSAIDs, through inhibition of prostaglandin synthetase; it will certainly reduce thromboxane A_2 levels in the plasma of the horse (Semrad et al. 1985).

12 sheep were given flunixin at the clinically effective dose of 2.2mg/kg into a jugular vein and the response to a mechanical noxious stimulus applied to one leg measured every five minutes for one hour. The experiments were repeated at a later date with naloxone 0.2mg/kg iv given five minutes before flunixin 2.2mg/kg iv. As controls, the sheep were given saline 2ml iv, naloxone 0.2mg/kg iv alone and dexamethasone (an anti-inflammatory glucocorticoid) 0.1mg/kg iv on separate occasions at least a week apart.

Flunixin raised the sheep's thresholds significantly ($P< 0.5$) from 4.59 ± 0.27N (mean $\pm$ s.e. mean) to a maximum of 8.31 ± 0.94N at 30 minutes after the flunixin was injected. Saline, dexamethasone and naloxone had no effect. However, naloxone given five minutes before flunixin prevented any rise in thresholds.

Since there was no peripheral inflammation it seems unlikely that the NSAID was acting by inhibition of prostaglandin production in the periphery. NSAIDs have been shown to have central effects in man and animals and to increase the rate of firing in descending monoaminergic pain modulation pathways, at the level of the thalamus or periaqueductal grey (Carlsson et al., 1986). Our results indicate that flunixin may be acting at this level and may produce hypoalgesia by a descending system which includes an opioidergic link.

Carlsson, K-H., Helmreich, J. and Jurna, I. (1986) *Pain*, **27**, 373 - 390
Ferreira, S.H. (1972) *Nature New Biology*, **240**, 200 - 203
Semrad, S.D., Hardee, G.E., Hardee, M.M. and Moore, J.M. (1985) *Am J Vet Res*, **46**, 2474 - 2479
Vane, J.R. (1971) *Nature New Biology*, **231**, 232 - 235

108P PHARMACOLOGY OF MORPHINE 6 GLUCURONIDE IN MAN: DOSE RESPONSE RELATIONSHIP IN COMPARISON TO MORPHINE

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Morphine and its active metabolite morphine 6 glucuronide (M6G) show differing affinities for the mu 1 and mu 2 receptor subtypes (Hucks et al 1992). The systemic bioavailability of subcutaneously (s/c) administered M6G in man is greater than 90 % by 12 hours (Hanna et al 1991). This study compares the pharmacological prophile of s/c M6G with that of morphine in human volunteers.

Six healthy volunteers received s/c morphine sulphate (0.1, 0.15, 0.3 mg/kg), and M6G (0.07, 0.15, 0.2 mg/kg) on 6 non consecutive days in accordance with a randomised double blind cross over design. Cardio-respiratory parameters (expired minute volume, end tidal CO2, respiratory rate, oxygen saturation, non invasive blood pressure, pulse rate) and non respiratory parameters (pupil size, sedation, nausea, vomiting and euphoria) were assessed before and for 6 hours after dosing. Respiratory response to CO_2 was assessed by exposure of the subjects to a 4 minute 5.5% CO_2 challenge 20 minutes and 40 minutes before and 20, 40, 60, 120, 180 and 240 minutes after drug administration. Changes from preadministration values, and differences in response to M6G and morphine were assessed using the paired Wilcoxon Rank test.

Subjective side effects occurred within 20 minutes of injection in all subjects following all doses of M6G and morphine, and these were qualitatively different with the two compounds. No volunteers vomited after M6G, and the incidence of nausea was low (1 report). Of the six subjects 1/6, 3/6 and 5/6 vomited during the low, intermediate and high dose morphine limbs respectively. There were no clinically significant differences in cardiovascular parameters, oxygen saturation or pupil size recorded following M6G or morphine. Maximal respiratory depression occurred within 2 hours of drug administration in all subjects. Mean(SE) maximal reduction in expired minute volume from baseline was significant ($p<0.05$) for the three morphine doses [34.7(5.6)%, 26.7(2.3)% and 36.1(3.1)%] but not for the three M6G doses [5.5(4.2)%, 17.1(4.5)%, 7.7(2.2)%]. These changes were accompanied by significant ($p <0.05$) increases from baseline values in endtidal CO_2 and reductions in respiratory rate for all doses of morphine, which did not occur with any dose of M6G. There was a linear response in mean(SE) maximum reduction in expired minute volume during CO_2 challenge for morphine [34.6(4.2)%, 37.2(4.6)% and 47.6(5.8)%] but not for M6G [14.8(4.4)%, 22.6(4.6)% and 9.7(3.9)%].

M6G caused significantly less respiratory depression, nausea and vomiting than morphine administered to normal volunteers at doses believed to equipotent with respect to analgesic efficacy in man. At these doses morphine appears to exhibit a dose/response relationship for depression of respiratory response to CO_2 challenge whereas M6G does not.

Hucks, D. Thompson P.I., McLoughlin L., Joel S.P., Patel N., Grossman A., Rees L.H., & Slevin M.L. Br. J. Cancer, 65, 122-126.
Hanna, M.H., Zilkha T.R., Peat S.J.,D'Costa P. & Fung C. (1991) Proceedings of Europain. Abstract No. 57.

109P INVESTIGATION OF A PUTATIVE P_{2X}-PURINOCEPTOR ANTAGONIST IN GUINEA-PIG ISOLATED VAS DEFERENS

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It has recently been proposed that pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) is a selective antagonist of P_{2X}-purinoceptor-mediated contractions in the rabbit isolated vas deferens (Lambrecht *et al.*, 1992). We have now further examined the ability of PPADS to inhibit selectively purine-mediated electrical and mechanical responses of the guinea-pig isolated vas deferens.

For both electrical and mechanical recording, the vas deferens was maintained in an organ bath at 35 °C in a physiological salt solution bubbled with 95% O_2, 5% CO_2. Mechanical responses were evoked by field stimulation of the sympathetic nerves using trains of pulses at 4 Hz for 20 s (pulse width 0.5 ms, supramaximal voltage). We have previously shown that the biphasic neurogenic contractions are due to the combined action of adenosine 5'-triphosphate (ATP) and noradrenaline, the initial phasic component being mainly purinergic and the secondary, tonic phase being mainly noradrenergic (Sneddon *et al.*, 1982). Concentration-response curves were obtained by addition of α,βmethylene-ATP or noradrenaline directly to the bath. Membrane potential and excitatory junction potentials (e.j.p.s) were recorded using standard intracellular microelectrode techniques. The e.j.p.s recorded in this tissue are due to the action of ATP on P_{2X}-purinoceptors (Sneddon *et al.*, 1982). In all experiments PPADS was allowed to equilibrate for at least 15 minutes. All data presented below refer to the mean ± s.e.mean and are compared using Student's t-test for either paired or unpaired data as appropriate (* = P<0.05; ** = P<0.01).

Table 1 The effects of PPADS on mechanical and electrical responses of guinea-pig isolated vas deferens

Concentration of PPADS(M)	Phasic Contraction(g)	Tonic Contraction(g)	E.j.p. Magnitude(mV)	Membrane Potential(mV)
Control	1.7 ± 0.4 (n=6)	1.3 ± 0.6 (n=6)	13.7 ± 0.6 (n=51)	-64.8 ± 0.6 (n=51)
10^{-7}	1.7 ± 0.3 (n=6)	1.4 ± 0.4 (n=6)	12.3 ± 1.4 (n=10)	-61.5 ± 1.0 (n=10)**
3×10^{-7}	1.8 ± 0.3 (n=6)	1.5 ± 0.3 (n=6)	9.3 ± 1.4 (n=10)*	-61.6 ± 2.5 (n=10)
10^{-6}	1.5 ± 0.3 (n=6)	1.4 ± 0.3 (n=6)	6.0 ± 1.1 (n=17)**	-58.2 ± 1.7 (n=17)**
3×10^{-6}	0.5 ± 0.2 (n=6)*	1.3 ± 0.2 (n=6)	3.6 ± 0.6 (n=21)**	-57.4 ± 1.7 (n=21)**
10^{-5}	0.2 ± 0.1 (n=6)**	1.4 ± 0.3 (n=6)	1.8 ± 0.7 (n=12)**	-55.0 ± 1.8 (n=12)**
3×10^{-5}	0.4 ± 0.1 (n=6)**	1.3 ± 0.3 (n=6)	2.2 ± 0.6 (n=5)**	-58.4 ± 2.3 (n=5)**

Table 1 shows that PPADS caused a significant, concentration-dependent reduction in the initial phasic component of the neurogenic contraction, but had no effect on the tonic component. PPADS also caused a significant and concentration-dependent depolarization and decrease in e.j.p. magnitude. The depolarization was unaffected by suramin (10^{-4}M), showing that PPADS is not a partial agonist at P_{2X}-purinoceptors. Contractions to the P_{2X}-purinoceptor agonist α,βmethylene-ATP were significantly inhibited by 3×10^{-5}M PPADS, whilst responses to exogenous noradrenaline were not reduced. These results indicate that PPADS can act as a selective P_{2X}-purinoceptor antagonist in guinea-pig vas deferens, but its ability to depolarise smooth muscle cells shows that it also has other actions.

Lambrecht, G., Friebe, T., Grimm, U., Windscheif, U., Bungardt, E., Hildebrandt, C., Baumert, H.G., Spatz-Kumbel, G. & Mutschler, E. (1992) *Eur. J. Pharmacol.* 217, 217-219.
Sneddon, P., Westfall, D.P. & Fedan J.S. (1982) *Science*, 218, 693-696.

110P HETEROGENEITY OF HIGH AFFINITY BINDING SITES FOR [^{3}H]-αβ-METHYLENE-ATP IN RAT TISSUES

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Tritiated αβ-methylene-ATP (αβmeATP) labels high and low affinity sites in rat vas deferens (Bo & Burnstock, 1990) and it is thought that the high affinity sites for the radioligand represent functional P_{2X} receptors (Bo et al., 1992). In the present study we have further characterised the binding sites for this radioligand and present evidence for heterogeneity of the high affinity binding site. Assays were conducted as described previously (Bo et al.,1992: Michel et al., 1993) using 50mM Tris, 3mM $CaCl_2$ buffer (pH 7.4). Incubations were for 40 min at 4°C and were terminated by vacuum filtration. Non specific binding was defined with 30μM αβ-methylene-ATP. Data are presented as the mean±SEM of 3-4 experiments.

In saturation studies (n=3) the radioligand bound to both high (Kd = 1.1±0.2 nM) and low (Kd=150±30 nM) affinity sites in vas deferens. When a low radioligand concentration (1 nM) was utilised the high affinity sites were selectively labelled since ≅ 90% of the binding sites displayed high affinity for αβmeATP (see table) and for βγ-methylene-ATP, ATPγS and βγ-imido-ATP (data not shown). The high affinity sites for αβmeATP were not homogeneous, however, since both ATP, 2-me-S-ATP, and, to a lesser extent, diadenosine pentaphosphate identified a lower proportion of the sites with high affinity (see table) suggesting that there are two populations of the high affinity αβmeATP binding site, one which also displays high affinity for ATP and 2-me-S-ATP and one which displays low affinity for these compounds. The heterogeneity of the high affinity binding sites identified by ATP and 2-me-S-ATP was not affected by the inclusion of the guanine nucleotides GTPγS (1μM) or GTP (10 μM) in competition studies (data not shown). In rat spleen and striatum >85% of the sites displayed high affinity for αβmeATP. As in the vas deferens, ATP and 2-me-S-ATP identified heterogeneity of the high affinity αβmeATP binding sites since these compounds only identified 25-30% of the sites with high affinity (e.g. % Sites with high affinity for ATP : striatum 27±5%, spleen 30±4 % n=3-4).

	pIC50 (1)	% Site (1)	pIC50 (2)	% Sites (2)
αβmeATP	8.5 ± 0.1	91 ± 4	5.9 ± 0.5	9 ± 4
ATP	7.3 ± 0.3	44 ± 6	4.9 ± 0.6	56 ± 6
2-me-S-ATP	7.1 ± 0.3	53 ± 8	4.5 ± 0.4	47 ± 8
Ap(5)A	7.5 ± 0.2	67 ± 9	5.2 ± 0.3	33 ± 9

(1) and (2) refer to the high and low affinity sites, respectively, for each ligand in rat vas deferens. Values are mean ±SEM. n=3-4 % Sites is the % of the total specific binding of 1nM [^{3}H]αβmeATP that each site comprised. 2-me-S-ATP = 2-methyl-thio-ATP. Ap(5)A = diadenosine pentaphosphate.

These data indicate that high affinity [^{3}H]αβmeATP binding sites in several rat tissues are heterogeneous since two populations of the high affinity site can be distinguished by ATP, 2-me-S-ATP and, to a lesser extent, diadenosine pentaphosphate. Further studies will be required to identify whether this heterogeneity represents agonist affinity states of the P_{2X} receptor or is due to the presence of subtypes of the P_{2X} receptor.

Bo, X., & Burnstock, G. (1990). Br. J. Pharmacol., 101, 291-296
Bo, X., Simon, J., Burnstock, G., & Barnard, EA. (1992). J. Biol. Chem. 267, 17581-17587.
Michel, AD., Brown, H., Sewter, C., Kennedy, I., & Humphrey, PPA. (1993). Br. J. Pharmacol., Proc. Suppl., 108, 44P.

111P IMMUNOCYTOCHEMICAL DETECTION OF NITRIC OXIDE SYNTHASE-CONTAINING NERVES IN THE MOUSE ANOCOCCYGEUS MUSCLE

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In the mouse anococcygeus, non-adrenergic non-cholinergic (NANC) relaxations are reduced by inhibitors of nitric oxide synthase (NOS), although responses to nitrovasodilator drugs are unaffected. Such observations have led to the proposal that the L-arginine:NO pathway is involved in NANC neurotransmission and, more particularly, in the presynaptic generation of the neurotransmitter (Gibson et al., 1990). If this hypothesis is correct, then NOS should be detectable within nerves in the anococcygeus, and should disappear following procedures which produce functional NANC denervation. Here, we show that this is indeed the case.

Immunofluorescence studies were carried out on whole mount preparations of the mouse anococcygeus, using antisera to the general neuronal marker, protein gene product (PGP; human; 1:1000), and to NOS (rat brain; 1:300); sites of antibody-antigen interaction were detected by incubation with fluorescein-conjugated goat antiserum to rabbit IgG (1:100). The specificity of the technique, and of the antiserum to NOS, has been described (Springall et al., 1992). Immunofluorescence in free-floating tissues was visualised using a confocal microscope (Bio-Rad MRC-600).

In control tissues (n=6), a dense network of PGP-positive nerve fibres was observed. The density of these was reduced, but not abolished, in muscles from mice pre-treated with 6-hydroxydopamine (6OHDA; 2 x 50 mg kg^{-1} on day 1, 2 x 100 mg kg^{-1} on day 4, muscles removed on day 5; n=4); 6OHDA has been shown to destroy sympathetic nerves in the mouse anococcygeus but to leave the NANC nerves intact (Gibson & Wedmore, 1981).

A network of NOS-positive fibres was present in control tissues (n=4), with a density similar to that of PGP in 6OHDA-treated muscles; no NOS immunofluorescence was detected in smooth muscle cells. NOS-positive nerve fibres were still present in muscles from animals pre-treated with 6OHDA (n=4).

Cold-storage of the mouse anococcygeus (4°C; 72h) has been shown to produce complete functional denervation, although contractile and relaxant responses to directly-acting drugs is little affected (Gibson et al., 1992). In such cold-stored tissues, very few PGP- or NOS-positive fibres were observed (n=2 for PGP; n=4 for NOS).

Thus, NOS is detectable in nerve fibres, but not muscle cells, in the mouse anococcygeus. The NOS-positive fibres are resistant to 6OHDA, but disappear on cold-storage. These results support the hypothesis that NOS is involved in the presynaptic generation of the NANC tansmitter.

The support of the MRC is appreciated.

Gibson, A., Babbedge, R., Brave S.R., Hart, S.L., Hobbs, A.J., Tucker, J.F., et al., (1992) *Br. J. Pharmacol.*, 107, 715-721.
Gibson, A., Mirzazadeh, S., Hobbs, A.J. & Moore, P.K. (1990) *Br. J. Pharmacol.*, 99, 602-606.
Gibson, A. & Wedmore, C.V. (1981) *J. Auton. Pharmacol.*, 1, 225-233.
Springall, D.R., Riveros-Moreno, V., Buttery, L., Suburo, A., Bishop, A.E., Merrett, M., et al., (1992) *Histochemistry*, in press.

112P CHARACTERISATION OF α_1-ADRENOCEPTORS IN ISOLATED ANOCOCCYGEUS MUSCLE OF RAT

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Isolated anococcygeus muscle has been known for some time to contract forcefully to noradrenaline (NA) via an action at α_1-adrenoceptors (Gillespie, 1972). Recent investigations in several tissues have demonstrated subtypes of α_1-adrenoceptors based upon pharmacological distinctions (α_{1A} and α_{1B}; Morrow and Creese, 1986) as well as molecular cloning studies ($\alpha_{1A/D}$, α_{1B} and α_{1C}; Schwinn and Lomasney, 1992). However, α_1-adrenoceptors in rat anococcygeus muscle have not been classified in this regard.

Anococcygeus muscles (male Sprague-Dawley rats, 250-400 g) were suspended in 10 ml baths (Krebs' solution containing cocaine, 30μM, corticosterone, 30μM, propranolol, 1μM, idazoxan, 0.3μM, indomethacin, 10μM, nitrendipine, 1μM), for measurement of isometric contractile force (10 mN resting). Two cumulative E/[A] curves were constructed on each tissue, with 45 min antagonist equilibration before the second curve. Binding studies using [^{3}H]-prazosin were performed on membranes from anococcygeus muscles, prepared in Krebs' solution (with additions as above), using 10 concentrations of displacer in competition studies. In alkylation studies, tissues were exposed to chloroethylclonidine (CEC; 10-100μM, 20 min) or phenoxybenzamine (PBZ; 0.03-0.3μM, 15 min) and then washed (40 min).

E/[A] curves typical of mass-action kinetics with parallel antagonist-induced shifts were obtained only in the presence of nitrendipine (1μM). This allowed satisfactory affinity estimation (pA_2 values; table 1). *S*(+)-niguldipine (to 1μM) was without effect on responses to NA. CEC (100μM) produced a slowly reversible partial agonism (α = 10-15%) and, after washout, a slight shift (3-fold) in the NA E/[A] curve. PBZ inactivated responses to NA with 0.3μM causing complete inactivation, although prior exposure to CEC rendered tissues less susceptible to inactivation by PBZ. [^{3}H]-Prazosin saturation binding (1pM - 5nM) was consistent with a single population of receptors (K_D = 0.07nM, B_{max} = 0.16 pmol/mg protein); competition studies (80% specific) yielded affinity estimates shown below (pK_i; table 1).

Table 1. Affinity estimates of compounds from contractile ([a] pA_2) and binding ([b] pK_i) studies in rat anococcygeus.

	prazosin	YM-617	abanoquil	5Me-urapidil	WB 4101	phentolamine	oxymetazoline	spiperone
pA_2	8.9 (0.1)	10.3 (0.2)	10.4 (0.1)	8.9 (0.1)	9.0 (0.1)	8.0 (0.2)	8.2 (0.2)	7.3 (0.2)
pK_i	9.5 (0.1)			8.9 (0.2)	10.0 (0.2)	8.8 (0.1)	8.7 (0.1)	8.0 (0.2)

[a] mean estimates (s.e. mean), n = 6-12, from Schild analysis, slopes not different from 1 ($p < 0.05$); [b] means (s.e. mean), n = 3-5.

The pharmacological profile obtained has some characteristics of α_{1A}-adrenoceptors (low CEC sensitivity; high affinity for 5Me-urapidil, oxymetazoline; low affinity for spiperone), but the low affinity of prazosin and WB 4101 in functional studies, and insensitivity to *S*(+)-niguldipine, precludes such a classification. Binding data reflect high affinity prazosin sites, and functional α_1-adrenoceptors may not be labelled sufficiently by the low ligand concentrations employed. In conclusion, rat anococcygeus muscle contracts to NA via a novel population of α_1-adrenoceptors, which resembles that described in rabbit and dog tissues, designated α_{1L} by Muramatsu *et al.* (1990).

Gillespie, J.S. (1972) *Br. J. Pharmacol.*, **45**, 404-416.
Morrow, A.L. & Creese, I. (1986) *Mol. Pharmacol.*, **29**, 321-330.
Muramatsu, I., Kigoshi, S. & Oshita, M. (1990) *Br. J. Pharmacol.*, **101**, 662-666.
Schwinn, D.A. & Lomasney, J.W. (1992) *Eur. J. Pharmacol, Mol. Pharmacol. Section*, **227**, 433-436.

113P FURTHER EVIDENCE FOR DEPRESSANT EFFECTS OF ANOXIA OR SIMULATED ISCHAEMIA ON NEURO-EFFECTOR TRANSMISSION

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Day & Vane (1963) reported that oxygen deprivation blocked electrically-induced isotonic contractions of guinea-pig ileum at a time when responses to directly-acting agents were scarcely altered. In order to extend their observations, a pharmacological model of neuronal ischaemia (Reiner et al., 1990) was used to study the effects of anoxia and ischaemia on neuro-effector transmission in guinea-pig small intestine and rat anococcygeus muscle.

Contractions of myenteric plexus-longitudinal muscle preparations, elicited by supramaximal electrical field stimulation or drugs, were displayed on a Grass Polygraph. In 7 preparations, sodium cyanide (NaCN; 0.1-3.0 mM for 30-60s) caused a concentration-dependent, readily-reversible, inhibition of the electrically-evoked contractions (IC_{50} 0.3 ± 0.02 mM). Gassing with 95% N_2/5% CO_2 instead of 95% O_2/5% CO_2 had a similar, rapid and reversible depressant effect in the same preparations (n=4), except that it was preceded by a brief potentiation (up to 20%). The effect of anoxia on smooth muscle made to contract isometrically was different from that under isotonic conditions; although the peak tensions induced by carbachol (control, 3.8 ± 0.6 g; n=5) were unaffected by NaCN (1 mM; 3.5 ± 0.4 g), the second (maintained) phase of the isometric contractile response was almost completely, but reversibly, abolished. Identical results were obtained with histamine or KCl as agonist and when under anoxic conditions. Isometric contractions were similarly altered in glucose-free, oxygenated-Krebs solution. In contrast, blockade of glycolysis by iodoacetic acid (IAA; 0.05-0.5 mM) irreversibly abolished all contractile responses. Over the range 0.01-20 Hz, the higher the stimulus frequency, the more rapid was the decline in evoked tension; at low frequencies of stimulation (0.01-0.2 Hz), however, there was an initial potentiation (up to 40% at 0.2 Hz) of the contractions, which may have been due to a prejunctional facilitation of transmission. Subthreshold concentrations of IAA (0.03-0.06 mM) caused previously threshold concentrations of NaCN (0.1-0.2 mM) to inhibit electrically-evoked contractions profoundly (> 70%) and irreversibly. In contrast, NaCN (1 mM) or anoxia had an exclusively postjunctional site of action in reversibly depressing adrenergically-mediated contractions of rat anococcygeus muscle preparations (n=3).

It is concluded that intestinal neuro-effector transmission was susceptible to anoxia and hypoglycaemia and that prejunctional, as well as postjunctional, events had been affected. Thus, transient ischaemic episodes may be important in the aetiology of Inflammatory Bowel Disease.

Day, M. & Vane, J.R. (1963). *Br. J. Pharmacol.* **20**, 150-170
Reiner, P.B., Laycock, A.G. & Doll, C.J. (1990). *Neuroscience Letters* **119**, 175-178

114P TACHYKININ RECEPTORS IN RABBIT URINARY BLADDER ARE OF THE NK_{2a} SUBTYPE

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Tachykinins are widely distributed in neurones of the peripheral autonomic nervous system. They are potent vasodilators and secretogogues (Erspamer, 1981) and in addition contract smooth muscle (Bury & Mashford, 1977). The biological actions of tachykinins are mediated by at least three receptor types known as NK_1, NK_2 and NK_3. Receptor antagonists of high affinity and selectivity have recently been developed (for review see Watling, 1992). As non-cholinergic, non-adrenergic transmission is a large and important component of both excitatory and inhibitory nervous control of bladder function in the rabbit, it was decided to investigate the nature of neurokinin receptors in this tissue.

Male Dutch rabbits were used for two experimental procedures. Urinary bladder smooth muscle strips were suspended in small organ baths. Their contractile activity was measured with isometric transducers, and recorded on flat-bed recorders. Oxygenated Krebs' solution at 37°C was continuously perfused at 2 ml min^{-1} over the tissue. Each drug was in contact with the tissue for 10 s only and dose intervals of 20-45 min adopted with higher doses to avoid receptor desensitization. For radioligand binding assays crude urinary bladder membrane preparations were prepared by the method of Lee *et al.* (1986). Binding reactions were performed in Tris-HCl buffer (pH 7·4) containing 3 mM $MnCl_2$, 0·02% BSA, 2 μg ml^{-1} chymostatin, 4 μg ml^{-1} leupeptin, 40 μg ml^{-1} bacitracin, and 2 μg ml^{-1} phosphoramidon to inhibit peptidase activity. These compounds did not interfere with ligand binding under these conditions. 150 μg samples of membrane protein were incubated at room temperature for 90 min with 0·5 nM [^{125}I] NKA in the presence or absence of antagonists. Non-specific binding was defined using 1 μM NKA or 10 μM [β–Ala^8] NKA (4-10) an NK_2-selective agonist. The bound drug was separated by rapid filtration through Whatman GF/C filters pre-wetted with 0·3% polyethylenimine and 0·5% Triton X-100.

Agonists	EC_{50} values (nM)	pK_i values from receptor binding	Antagonists	pA_2 values	pK_i values from receptor binding
NKA	277 ± 27	8·89 ± 0·06	MEN 10,207	7·45 ± 0·18	7·03 ± 0·04
[βAla^8] NKA (4-10)	285 ± 44	7·41 ± 0·03	MEN 10,376	-	7·45 ± 0·04
NPK	1110 ± 230	-	MEN 10,208	7·86 ± 0·31	-
NPγ	1570 ± 690	-	L-659,877	6·38 ± 0·16	6·46 ± 0·06

[Sar^9 Met $(O_2)^{11}$]-SP was a partial agonist with an ED_{50} of ~10^{-5} M; senktide inactive at 10^{-4} M; R 396 has a pK_i of ~5·0. Results are given as means ± S.E.M.

It is recognised that identification of receptor subtypes using the order of potency of agonists and antagonists is improved if receptor binding data are compared (Lee *et al.*, 1981). As NKA is the most active agonist and MEN 10,376 has a higher binding affinity than L-659,877 and R 396, it is concluded that NK_{2a} receptors are present in rabbit urinary bladder.

Erspamer, V. (1981) *Trends Neurosci*, 4, 267-269.
Bury, R.W. & Mashford, M.L. (1977) *Aust. J. Exp. Biol. Med. Sci.* 55, 671.
Watling, K.J. (1992) *Trends Pharmacol. Sci.* 13, 266-269.
Lee, C.M., Campbell, N.J., Williams, B.J. & Iversen, L.L. (1986) *Eur. J. Pharmacol.* 130, 209-217.

115P INFLUENCE OF STREPTOZOTOCIN-INDUCED DIABETES ON RESPONSES TO SUBSTANCE P AND NEUROKININ A AND PEPTIDASE ACTIVITY IN RAT URINARY BLADDER

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Bladder dysfunction is a major consequence of diabetes (Ellenberg, 1980). The aim of the present study was to determine whether the sensitivity of the bladder to tachykinins or the activity of peptidases that may degrade tachykinins released from the peripheral terminals of the sensory nerves supplying the bladder are modified in experimental diabetes.

Virgin female Sprague Dawley rats (155-240 g) received either streptozotocin 60 mg/kg i.v. in 0.5 M citrate buffer, or an equivalent volume of buffer, two weeks prior to use; all rats also received oestradiol cypionate 20 μg/kg s.c. 48 and 24 h before use. Four preparations (1 mm x 6 mm) from each animal were obtained from the bladder dome region. Log concentration response curves to neurokinin A and substance P were constructed in the absence and presence of phosphoramidon (1 μM) to block endopeptidase 24.11, amastatin (1 μM) to block aminopeptidases, and captopril (10 μM) to block angiotensin converting enzyme. A second concentration response curve to each tachykinin was constructed after the addition of atropine (0.1 μM).

The responses to both tachykinins were atropine-insensitive and increased in diabetes. In tissues from diabetic, but not from control animals, responses to substance P were enhanced (3.1-fold; 95% confidence limits=1.35, 7.69; df=32) in the presence of peptidase inhibitors. Responses of preparations from both control and diabetic animals to neurokinin A were unaffected by peptidase inhibitors.

These results indicate (i) that the receptors mediating the stimulant effects of substance P and neurokinin A on the dome region of the rat bladder are not located on cholinergic neurones, (ii) that diabetes leads to amplification of the responses of this bladder re gion to these tachykinins, and (iii) that diabetes may enhance the levels or activity of a peptidase inactivating substance P. The enzyme may be angiotensin converting enzyme since this inactivates substance P but not neurokinin A (Hooper *et al.*, 1985).

Ellenberg, M. (1980) Ann. Intern. Med. **92**, 321-323.
Hooper, N.M., Kenny, A. J. & Turner, A. J. (1985) Biochem J. **231**, 357-361.

116P EFFECTS OF VERAPAMIL AND TRIFLUOPERAZINE ON VAGALLY-MEDIATED AIRWAY CONSTRICTION IN ANAESTHETISED NORMAL AND ALBUMIN-SENSITIZED GUINEA-PIGS

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Abnormal Ca^{2+} handling in airway smooth muscle may contribute to the airway hyperreactivity associated with asthma (Black et al., 1989). The calcium antagonist verapamil and the calmodulin antagonist trifluoperazine (TFP) each attenuate substantially vagally-mediated constriction in guinea-pig trachea *in vitro*, but are less effective in trachea isolated from albumin-sensitized guinea-pigs, an allergic model of asthma (De Jonckheere and McCaig, 1992). In this study the effects of verapamil and TFP on vagally-induced airway constriction *in vivo* have been compared in normal and albumin-sensitized guinea-pigs.

Guinea-pigs, untreated (UT) or albumin-sensitized (AS, sensitizing injections of albumin ip and sc plus twice weekly inhalation challenge for 3 weeks) were anaesthetised with sodium pentobarbitone (60 $mgkg^{-1}$ ip), intubated and artificially ventilated (50 breaths min^{-1}, stroke volume 8 $mlkg^{-1}$). Increases in airoverflow pressure (AP), obtained from a pressure transducer attached to a side arm of the tracheal cannula, were used as an index of airway constriction. The distal end of the left or right vagus nerve, sectioned high in the neck, was stimulated (25V for 10s at 2min intervals, frequencies 2-30 Hz). Frequency-response curves were obtained after 30 min and again commencing 5 min after iv injection (via a jugular vein) of verapamil or TFP, in increasing doses.

Resting AP was 14 ± 0.9 mmH_2O (n=11) and 13 ± 0.6 mmH_20 (n=16) in UT and AS groups, respectively, and vagal stimulation elicited frequency-dependent increases in AP up to a maximum at 30 Hz, which were reproducible over time. Verapamil, 1 μgkg^{-1}, had no effect on vagal responses, but at 10 or 50 μgkg^{-1} the frequency-response curve was shifted to the right and the maximum response reduced, to a similar degree in both groups (e.g. verapamil 50 μgkg^{-1} reduced response at 30 Hz by $52\pm6\%$ in UT and $62\pm6\%$ in AS animals). TFP, 1,10 and 50 μgkg^{-1}, also reduced vagal response amplitude, the effect being similar in both groups (e.g. TFP 50 μgkg^{-1} reduced response at 30 Hz by $55\pm14\%$ in UT and $70\pm9\%$ in AS guinea-pigs).

These results indicate that verapamil and TFP attenuate vagally-mediated airway constriction *in vivo* as well as *in vitro*. In contrast to the findings *in vitro*, however, there was no reduction in the attenuating effect in sensitized animals *in vivo*, suggesting that any alteration in Ca^{2+} handling at the cellular level may be masked *in vivo*.

Black,J.L., Marthan,R., Armour,C.L. & Johnson,P.R.A. (1989). J. Allergy Clin. Immunol. **84**, 440-447.
De Jonckheere,S.& McCaig,D.J. (1992). Br. J. Pharmac. **106**, 2P.

117P CLENBUTEROL TREATMENT IN THE RAT INCREASES DIAPHRAGM PROTEIN CONTENT BUT DECREASES RESPONSE TO TETANIC STIMULATION

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The potential to protect against or reverse muscle atrophy due to denervation or disuse has been advanced through investigation of clenbuterol (CLEN). Yet, in therapeutic use this β-adrenoceptor agonist will reach both affected muscles and normal muscles. We have studied the effects of CLEN on the function of normal rat diaphragms. Male Sprague Dawley rats (19d) were used as described previously (Maltin *et al.*, 1986). After being accustomed to a semisynthetic diet (PW3; Pullar & Webster, 1977), at 28 days of age one group (CONTRx) was kept on PW3 and the other (CLENRx) was fed PW3 containing CLEN (2mg/kg diet). After 8,9 or 10 days treatment rats were killed and the left phrenic nerve-hemidiaphragms were set up in Krebs' buffer (95% O_2 / 5% CO_2 at pH 7.4). Right hemidiaphragms were frozen immediately for determination of RNA and protein (Maltin *et al.*, 1989), left diaphragms were frozen after study. Stimuli were delivered to phrenic nerve or muscle at tetanic frequencies from Grass S5 Stimulators and responses were recorded isometrically (Statham FTO3 Transducers; Grass P7 Polygraphs). After 8-10 days on their respective diets there was no change in body weight (g)(+/- s.e.mean) between groups. However, diaphragm weights and protein contents were significantly increased in CLENRx rats and a trend to an increase in total RNA: the RNA/protein ratio remained unchanged (Table 1).

Diet (n)	Body weight (g)	Weight of Diaphragms (mg)	Total RNA [μg]	Total protein [mg]	RNA /Protein
CONTRx (6)	144.6 (4.1)	271.5 (21.4)	382.5 (83.5)	14.5 (4.9)	20.6 (7.6)
CLENRx (6)	145.3 (4.5)	339.0** (34.4)	499.1 (60.0)	23.9** (4.1)	20.9 (4.9)

Table 1. Values given are means with standard errors in parentheses, significant differences were assessed by a two tailed t-test; $P<0.01^{**}$

Contractions to nerve stimulation (0.7ms) or to muscle stimulation (2.0ms) at 20Hz were not significantly different. However, those to 30Hz to 100Hz were significantly less in CLENRx compared to CONTRx rats (see Table 2).

Responses:	To NERVE STIMULATION: (mg / mg Wet Weight)						To Muscle Stim. in (+)-Tubocurarine 2μM		
Diet (n)	20Hz	30Hz	40Hz	60Hz	80HZ	100HZ	20Hz	60Hz	100Hz
CONTRx (18)	123 (10)	208 (15)	257 (16)	323*** (18)	356 (19)	362*** (16)	84 (7)	325 (19)	397** (19)
CLENRx (18)	108 (14)	151** (14)	182** (15)	218*** (18)	262** (21)	250*** (24)	85 (10)	268* (18)	268** (18)

Table 2. Values and statistical analyses as for Table 1, $P,0.05^{*}$, $P<0.01^{**}$, $P<0.001^{***}$

Thus, diaphragms from CLENRx rats stimulated at > 20 Hz either, indirectly or directly, (or by addition of 65mM KCl; not shown), develop less tension than in CONTRx rats. This is consistent with previous studies in which, despite increases in muscle weight and protein, fibre type shifts towards fast fibres may increase fatiguability. Although chronic administration of clenbuterol improves contractile properties of denervated muscle (Zeman *et al.*, 1987), normal diaphragm may be less able to sustain the same maximal tension to high frequency stimuli following 8 to 10 days treatment with the drug.

Maltin, C.A., Delday, M.I., & Reeds, P.J. (1986) Bioscience Reports **6**, 293-299.
Maltin, C.A., Hay, S.M., Delday, M.I., Lobley, G.E. & Reeds, P.J. (1989) Biochem. J. **261**, 965-971.
Pullar J.D. & Webster, A.J.F.(1977) Br. J. Nutr. **37**:355-363.
Zeman, R.J., Ludeman, R. & Etlinger, J.D. (1987) Am. J. Physiol. **252**, E152-155.

Supported by The Canadian Spinal Research Organization

118P COMPARISON OF GLUCOSE UPTAKE BY DIAPHRAGM OF NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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In recent years it has been proposed that insulin resistance may be a cause of hypertension (Reaven, 1988). Soleus muscle of patients with type 2 diabetes mellitus, another syndrome of insulin resistance, has been shown to be less responsive to insulin than normal muscle (Dohm *et al.*, 1988), and Reaven and colleagues (1989) have demonstrated insulin resistance in adipocytes from spontaneously hypertensive rats (SHR). We have shown that SHR are glucose intolerant when compared with Wistar rats and that this intolerance is ameliorated by captopril treatment (Crabbe *et al.*, 1991). These factors have led us to compare glucose uptake in an *in vitro* skeletal muscle preparation of SHR and Wistar rats.

We have adapted the method of Kirby and Turner (1975) for measuring glucose uptake by hemidiaphragms from male Wistar rats or SHR (both University of Bath strain) weighing 200-250g. Diaphragms were excised, washed in ice cold, glucose-free Krebs-Henseleit solution (KHS), and incubated in 3ml of glucose-containing (15mM) KHS in a shaking (150 strokes.min^{-1}), heated (37°C) water bath for two hours. Each flask already contained appropriate incubation additions to the KHS (10μU.ml^{-1}-100mU.ml^{-1} human insulin or vehicle) and was gassed with moist 95%O_2/5%CO_2. 100μl samples were taken at t=10min, t=20min, and every 20min thereafter for subsequent glucose estimation using a colourimetric glucose oxidase assay. A second set of experiments was performed to determine the effect of captopril (1-30μM) on insulin-stimulated (1mU.ml^{-1}) glucose uptake.

Insulin-stimulated glucose uptake by hemidiaphragms between 10 and 120 minutes was concentration-related and linear. There was no significant difference between the rates of uptake in the two strains. For example at 100μU.ml^{-1} insulin the rate of uptake was 0.55 ± 0.09μmol.min^{-1}g^{-1} tissue wet weight in Wistar rats and 0.54 ± 0.04μmol.min^{-1}g^{-1} in SHR (mean ±sem, n=4). Likewise, the total insulin-stimulated glucose uptake after two hours was similar (80.76 ± 15.06μmol.g^{-1} in Wistar rats and 79.04 ± 9.02μmol.g^{-1} in SHR, at 100μU.ml^{-1} insulin, n=4). Captopril (1-30μM) did not alter insulin-induced glucose uptake in either strain of rat.

The results show that isolated diaphragm of SHR is not insulin resistant under these conditions. Since it has been demonstrated that this same strain of rat is glucose intolerant (Crabbe *et al.*, 1991) it seems that a systemic input is necessary to render the animals insulin resistant. Perhaps, therefore, reduced insulin sensitivity in SHR is not purely a defect of insulin signalling, but is dependent on other factors. These factors remain obscure, but might include glucagon and other glucose regulating hormones.

DSTC is a SERC-funded scholar.

Crabbe, D.S.T., Redfern, P.H., Woodward, B., & Williams,K.I. (1991) *Br. J. Pharmac.* **104**, 127P.
Dohm, G.L., Tapscott, E.B., Pories, W.J., Dabbs, D.J., Flickinger, E.G., *et al.* (1988) *J. Clin. Invest.* **82**, 486-494.
Kirby, M.J., & Turner, P. (1975) *Postgrad. Med. J.* **51**(Suppl. 1), 73-76.
Reaven, G.M. (1988) *Diabetes* **37**, 1595-1607.
Reaven, G.M., Chang, H., Hoffman, B.B., & Azhar, S. (1989) *Diabetes* **38**, 1155-1160.

119P TREATMENT OF MOLT-4 CELLS WITH CsA FOLLOWING DFMO PRETREATMENT

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Cyclosporine A is well known as an immunosuppressive agent used extensively in the clinical management of allograft rejection. Its immunosuppressive action has been well documented (Borel et al., 1977) and is attributed to inhibition of $CD4^+$ T-helper lymphocyte activation and production of lymphokines. In addition, CsA has undergone evaluation as a potential anticancer agent and selective cytostatic and cytolytic effects on malignant T cells, including freshly isolated human T-leukaemia/lymphoma cells, have been shown in various studies *in vitro* (Totterman *et al.*, 1982). We have reported previously that CsA inhibits the development of the leukaemic phase in rats injected with the Roser T-cell leukaemia. CsA inhibits the activity of ornithine decarboxylase (ODC), the rate limiting enzyme of polyamine biosynthesis, in a murine T-cell lymphoma. Furthermore, Saydjari *et al* (1986) reported inhibition of growth of two animal tumours *in vitro* by CsA and α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC. This inhibition could be reversed by addition of the diamine, putrescine, which suggests that both drugs may be depleting intracellular polyamine content. It was found that when used in combination the drugs had greater antitumour activity than either drug alone suggesting an additive or synergistic interaction. We have found dose-dependent inhibition of growth of MOLT-4 human T leukaemia cells by CsA and DFMO alone, however we also found that, in our model, simultaneous addition of the drugs was no more effective than monotherapy. Previous studies using DFMO in combination with other cytotoxic or anticancer drugs had shown that DFMO can be more effective when given as a pretreatment. In this study MOLT-4 cells were pretreated with DFMO for 48h before addition of CsA. This combination treatment was more effective at decreasing protein content than either drug alone and appeared to give an additive effect. ($^*p<0.01$ compared to controls $^+p<0.01$ compared to DFMO alone)

Protein content (mg/well) at 48h after addition of CsA or vehicle.

Table 1.	Control	DFMO 2.5mM	CsA 1μg/ml	CsA 2.5μg/ml	DFMO+CsA 2.5mM 1μg/ml	DFMO+CsA 2.5mM 2.5μg/ml
(n=3)	0.47 ± 0.02	0.39 ± 0.01*	0.42 ± 0.03	0.39 ± 0.02*	0.36 ± 0.01*+	0.31 ± 0.01*+

The combination treatment had no greater effect on intracellular polyamine levels than DFMO alone. It may be that there is an interaction between the drugs which affects the uptake of one or other of the drugs. Further work is therefore required to help clarify the mechanisms involved.

Borel, JF., Feurer, C., Magnee, C. & Stahelin, H. (1977) Immunology **32**, 1017-1925.
Totterman, T H., Danersund, A., Nilsson, K. & Killander, A. (1982) Blood **59**, 1103-1107.
Saydjari, R., Townsend, CM.Jr., Barranco, SC., James, E. & Thompson, JC. (1986) J.NaH.Cancer Inst **77**, 1087-1092.

120P CHARACTERISTICS OF PHOSPHOLIPASE D ACTIVATION BY LHRH IN THE αT3-1 GONADOTROPE-DERIVED CELL LINE

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The mouse gonadotrope-derived clonal cell line, αT3-1 (Windle *et al*, 1990) was used as a model system to study receptor activation of phospholipase D (PLD). Induction of PLD activity in αT3-1 cells was assessed in response to phorbol 12,13-dibutyrate (PDBu; 1 μM) or LHRH (100 nM). Cells were preincubated with 5 μCi/ml [9,10 ^{3}H] palmitate for 2 h, to allow labelling of membrane phospholipids. Butan-1-ol (30 mM) was used as the nucleophilic acceptor in the transphosphatidylation reaction carried out by PLD (Pai *et al*, 1988) and the stable reaction product phosphatidylbutanol (PtdBut) was then separated by thin layer chromatography before the label incorporated was quantified by scintillation counting.

Time course studies carried out with quiescent αT3-1 cells demonstrated significant accumulation of PtdBut only after 10 or more min stimulation with LHRH. From 10 min, the rate of PtdBut accumulation in response to LHRH was approximately constant, up to 40 min (the longest time assessed). In PDBu-stimulated αT3-1 cells, there was no detectable PtdBut accumulation until 15 min and it was 20 min before accumulation approached the level of PtdBut achieved with LHRH at 10 min. After 20 min, the rate of PDBu-stimulated PtdBut accumulation reduced and by 40 min was minimal. At 30 min the stimulus-induced formation of PtdBut was approximately 10-fold and 4-fold over basal for LHRH and PDBu respectively. Basal values ranged from 900-1500 dpm per assay. The delay in LHRH receptor-induced formation of PtdBut is consistent with previous observations on Ptdethanol formation (Netiv *et al*, 1991). The protein kinase C inhibitor Ro 31-8220 (Davis *et al*, 1989) completely inhibited the PtdBut formation elicited by 30 min incubations with PDBu or LHRH with IC_{50}s of 62 ± 30 nM and 460 ± 180 nM respectively. The highly-selective tyrosine kinase inhibitor lavendustin A (Hsu *et al*, 1991) inhibited approximately 70% of the response to LHRH with an IC_{50} of 133 ± 17 nM. Lavendustin A (0.1 - 3 μM) had no effect on the response elicited by PDBu. In addition, the tyrosine phosphatase inhibitor, pervanadate (1 mM; Uings *et al*, 1992) caused PtdBut formation to approximately 5-fold of basal levels after 30 min incubation.

To assess whether LHRH could induce tyrosine phosphorylation of proteins, quiescent cells were incubated for 3 - 60 min with the LHRH agonist [D-Ser(tBu)^6, DesGly^{10}]LHRH ethylamide (buserelin; 100 nM), and cell extracts separated on 7.5% homogeneous gels by SDS-PAGE (PhastSystem, Pharmacia). Western blots of the gels on polyvinylidene difluoride membranes were probed with a mouse monoclonal anti-phosphotyrosine antibody (4G10, UBI) with or without 1 mM phosphotyrosine. Compared with saline-treated controls, buserelin induced numerous tyrosine phosphorylations on proteins of molecular mass 63 to > 170 kDa, but especially on 66, 76 and 119 kDa proteins. Phosphorylation of proteins > 170 kDa increased continuously over 3 - 60 min whereas other phosphorylations were maximal at 10 min. These results suggest that both PKC and a tyrosine kinase are involved in PLD activation by the LHRH receptor.

Davis, P.D., Hill, C.H., Keech, E., Lawton, G., Nixon, J.S., Sedgwick, A.D., Wadsworth, J. *et al* (1989) *FEBS Lett.* 259, 61-63.
Hsu, C.-Y.J., Persons, P.E., Spada, A.P., Bednar, R.A., Levitzki, A. & Zilberstein, A. (1991) *J. Biol. Chem.* 266, 21105-21112.
Netiv, E., Liscovitch, M. & Naor, Z. (1991) *FEBS Lett.* 295, 107-109.
Pai, J.-K., Siegel, M.I., Egan, R.W. and Billah, M.M. (1988) *Biochem. Biophys. Res. Commun.* 150, 355-364.
Uings, I., Thompson, N., Randall, R., Spacey, G., Bonser, R., Hudson, A. & Garland, L. (1992) *Biochem. J.* 281, 597-600.
Windle, J.J., Weiner, R.I. & Mellon, P.M. (1990) *Mol. Endocrinol.* 4, 587-603.

121P EVENING PRIMROSE OIL INHIBITS HISTAMINE SECRETION FROM RODENT MAST CELLS

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Evening primrose oil is derived from a wild flower, the seeds of which are very rich in essential fatty acids (EFAs), e.g linoleic acid (LA), gamma linolenic acid (GLA). In the body, LA is normally metabolized to GLA by Δ^6-desaturase, and GLA is converted to dihomoGLA (DGLA). Free arachidonic acid (AA), produced from DGLA by Δ^5-desaturase, may be metabolized by cyclo- and lipooxygenase to produce an array of lipid mediators. Patients with atopic eczema have below normal levels of GLA, DGLA, AA and PGE1, indicative of a deficiency in Δ^6-desaturase. Recent studies have shown a beneficial effect of dietary EPO on atopic conditions; administration of EPO supplies GLA which bypasses the enzyme deficiency and enters the linoleic pathway (Isseroff, 1988). In addition to low levels of EFAs, patients with atopic eczema also exhibit increased numbers of mast cells and serum IgE levels.

In the present study, rat peritoneal mast cells (RPMC) were challenged with compound 48/80 (Purcell and Hanahoe, 1989) in the absence and presence of EPO (1-1000 $\mu g.ml^{-1}$; solubilized in DMSO ≤1%). Secretion of histamine was measured fluorimetrically (Purcell and Hanahoe, 1989) and release expressed as a percentage of total histamine content of the cells, corrected for basal release. Compound 48/80 induced histamine release from RPMC, with an EC50 of 0.5 $\mu g.ml^{-1}$ and maximal release of 88.0 ± 6.0% of total histamine content at 10 $\mu g.ml^{-1}$. Co-addition of EPO with compound 48/80 (0.5 $\mu g.ml^{-1}$) inhibited histamine release from RPMC in a concentration dependent manner (Table 1). Incubation of RPMC with EPO for 15 mins (37°C) prior to challenge, enhanced this inhibitory effect by 18%; pretreatment for up to 60 mins had no further effect. EPO had no significant effect on the basal release of histamine. The mode of action of EPO on mast cells remains unclear. However, in addition to limited detergent properties of compound 48/80 on the oil, EPO may be associated with modulation of regulatory lipid products involved in degranulation, e.g. PGE1 inhibits histamine release from mast cells via stimulation of adenylate cyclase, resulting in increased cAMP formation (Loeffler *et al*, 1971).

Table 1 Effect of EPO on compound 48/80 (0.5 μg/ml) induced histamine release from RPMC (mean ± SEM, n=3)

EPO ($\mu g.ml^{-1}$)	Histamine Release (%)	Inhibition (%)
0	50.4 ± 1.6	-
1	48.8 ± 1.9	3.2
10	44.6 ± 2.1	11.5
100	40.1 ± 3.2	20.4
1000	31.1 ± 2.3	38.3

Isseroff, R.R. (1988) *J Amer Acad Dermatol* **19**, 1073-1080
Purcell, W.M. and Hanahoe, T.H.P. (1989) *Int Archs Allergy Appl Immunol* **90**, 382-394
Loeffler, L.J., Lovenberg, W. and Sjoerdsma, A. (1971) *Biochem Pharmacol* **20**, 2287-2297

122P INDUCTION OF HISTAMINE RELEASE BY H_2O_2+NaI FROM UTERINE AND PERITONEAL MAST CELLS AND ITS INHIBITION BY THEOPHYLLINE AND ANTIOXIDANTS

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H_2O_2 modulates a variety of cellular functions, including induction of histamine release (HR) from rat peritoneal mast cells (RPMC)(Ohmori et al. 1979, Vercelloti et al 1991). We have studied the effects of H_2O_2+NaI on HR from RPMC and rat uterine mast cells (RUMC) and its modulation by antioxidants (mannitol,MT and glutathione,GSH) as well as theophylline (TH, an inhibitor of HR from RPMC and RUMC).

RUMC were obtained by collagenase digestion and RPMC by direct lavage. The results of pilot experiments showed that H_2O_2 or NaI per se had no effect, whereas H_2O_2+NaI caused marked HR. In subsequent experiments, RPMC or RUMC were incubated at 37 °C for 30 min with H_2O_2+NaI (1 mM H_2O_2 immediately added after the various concentration of NaI in mM : 0.1,1,10,20 and 40 for RUMC; 0.1,0.5,1,5 and 10 for RPMC). HR was marked and dose-dependent, reaching a maximum of 90%. H_2O_2+NaI stimulation of HR from RUMC and RPMC was not calcium dependent and not changed by 2-deoxyglucose and antimycin A. The results of studies with TH and antioxidants are shown in the table below as % inhibition of HR (mean±sem, n=3).

	GSH(M, 0 Min)		MT (M, 0 Min)			TH(M, 10 Min)		
	10^{-4}	10^{-3}	10^{-4}	10^{-3}	10^{-2}	1.25×10^{-5}	5×10^{-5}	10^{-4}
RUMC	32.1±1.3	ND	-1.6±0.4	-3.2±2.7	-1.8±2.9	9.8±8.4	12.1±4.5	38.3±6.9
RPMC	62.1±0.4	82.7±0.7	4.1±1.4	24.6±0.9	34.9±2.4	44.4±4.9	48.6±2.4	62.9±3.4

Cells were incubated with GSH, MT or TH at the time indicated, followed by a further 30 min incubation with H_2O_2+NaI (1 mM/40 mM for RUMC, 1 mM/1 mM for RPMC). Stimulated HR was 87.4±2.1% and 89.5±2.4% for RUMC and RPMC, respectively (ND=not determined).

The present results show that H_2O_2+NaI was a potent stimulant of HR from RUMC or RPMC, which responded differently to various inhibitors. It remains to be resolved whether H_2O_2+NaI in relation to HR may play a role in uterine function.

Ohmori, H et al (1979) Biochem.Pharmacol. **28**, 333-334.
Vercelloti, G.M. et al (1991) J. Lab. Clin. Med. **117**, 15-24.

123P THE ISOPROPYL ESTER OF PALMITOYL CARNITINE CAN SUPPRESS AGONIST- INDUCED POSITIVE INOTROPIC RESPONSES IN THE ISOLATED PERFUSED RAT HEART

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It has been shown that the esterification of acyl carnitines produces potent and long acting coronary vasodilators, which have no concomitant effect on contractility of the heart (Criddle *et. al.*, 1990; 1991). In this study we demonstrate an additional property of one of these esters, the isopropyl ester of palmitoyl carnitine (P1Pi), to suppress agonist-induced positive inotropic effects using isolated perfused rat hearts.

Hearts from male Wistar rats were perfused via the aorta at 10ml/min, using 5.9mM K^+ Krebs-Henseleit gassed with 95% O_2, 5% CO_2, at 37 C. Bolus dose-response curves to the positive inotropic agents prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 0.1-30nmol.), noradrenaline (NA; 0.1-10nmol.), or isoprenaline (ISO; 0.01-1nmol.) were followed by either a 20min. time-matched control (TMC) perfusion or the addition of 10^{-8}M P1Pi to the Krebs-Henseleit perfusate. The appropriate dose-response curve was then repeated in the same preparation.

The positive inotropic effect of $PGF_{2\alpha}$ at all doses was significantly reduced by 10^{-8}M P1Pi when compared with TMC experiments ($p<0.01$, $n=4$ for each, Student's t-test). In test preparations, the increase in developed tension (DT) produced by 30nmoles $PGF_{2\alpha}$ (the optimum dose for a positive inotropic response) was reduced from 149.3±7.9% to 93.0±6.2% with 10^{-8}M P1Pi, compared with the same dose in TMC's which produced 149.5±18.7% 1st. curve, 143.3±14.9% repeat curve (data shown as %basal DT, mean±s.e.mean). The positive inotropic response to NA was biphasic, and was accompanied by a dose-related increase in heart rate. The second phase of the positive inotropic effect of NA at all doses was significantly reduced by 10^{-8}M P1Pi when compared with TMC's ($p<0.01$, n=4-5). In test preparations the second phase increase in DT produced by 1nmole NA (the optimum dose for the second phase positive inotropic effect) was reduced from 134.0±6.3% to 98.0±3.9% with 10^{-8}M P1Pi, compared with TMC's, which produced 125.4±5.9% 1st. curve, 132.0±7.9% repeat curve (%basal DT, mean±s.e.mean). The same concentration of P1Pi showed no effect on NA-induced increases in heart rate. The initial positive inotropic response to NA showed a similar time course to the positive inotropic effect of ISO. These responses, and responses to ISO, were only slightly reduced with 10^{-8}M P1Pi, and this was not significant at a 1% level, when compared to time-matched controls (n=4-5).

In conclusion, P1Pi at a concentration which has no effect on basal developed tension, can reduce or eliminate the positive inotropic responses to $PGF_{2\alpha}$ and NA, but does not significantly affect NA-induced increases in heart rate, the positive inotropic effect of ISO or the initial phase of the positive inotropic effect to NA which shows a similar time course to the ISO response. These results suggest that P1Pi can produce a selective inhibition of receptor-mediated positive inotropic effects.

Supported by Sigma-Tau, Rome.

Criddle,D.N., Dewar,G.H., Wathey,W.B. & Woodward B. (1990) *Br.J.Pharmacol.* 99, 477-480.
Criddle,D.N., Dewar,G.H., Rad-Niknam,M., Wathey,W.B. & Woodward,B. (1991) *J.Pharm.Pharmacol.* 43, 636-639.

124P EFFECT OF AMIODARONE AND BRETYLIUM ON EARLY AND LATE COMPONENTS OF THE DELAYED RECTIFIER POTASSIUM CURRENT IN GUINEA-PIG ISOLATED VENTRICULAR CELLS

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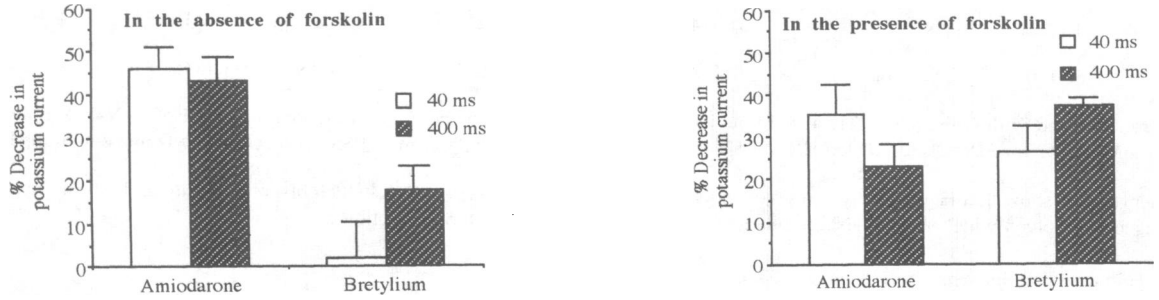
The differential blocking effects of amiodarone and bretylium on the early and late components of the delayed rectifier potassium current (see Sanguinetti and Jurkiewicz 1990) in cardiac muscle were investigated in the presence and absence of forskolin.

Single cells were isolated from guinea pig ventricular muscle. The delayed rectifier potassium current was studied under voltage clamp conditions (balanced salt solution with 2.5 mM Ca at 36°C; microelectrodes containing 75 mM BAPTA to suppress the cytosolic Ca transient) and measured as an outward tail current upon repolarization to a holding potential of -40 mV following depolarizations to +40 mV. The current at 40 ms was taken as a measure of the early component and that at 400 ms as a measure of the late component.

The figure shows that amiodarone (10μM) reduced both components of the tail current to a similar extent (46±5% decrease at 40 ms and 43±6% decrease at 400 ms, $p>0.05$, n=6). Bretylium (3μM) showed differential block of the late component (2±9% decrease at 40 ms and 18±6% decrease 400 ms, $p<0.05$, n=6). In the presence of forskolin (1μM) to activate cAMP dependent protein kinase, the inhibitory effect of amiodarone on the late but not the early component was significantly attenuated (35±7% decrease at 40 ms, $p>0.05$ compared with absence of forskolin, and 23±5% decrease at 400 ms, $p<0.05$, n=5). In contrast, the blocking effect of bretylium on both components was enhanced in the presence of forskolin (26±6% decrease at 40 ms, $p<0.05$; 37±2% decrease at 400 ms, $p<0.01$, n=6).



The results show that the two class III agents studied affect the delayed rectifier potassium current in different ways. The therapeutic significance of these differences and the possibility of differential drug effects on potassium channel subtypes remain for future study.

Sanguinetti M.C. and Jurkiewicz N.K. (1990) Journal of General Physiology **96**, 195-215.

125P CONTRACTILE MECHANISMS OF THE NOVEL PHOSPHODIESTERASE INHIBITOR, HN-10200, IN ISOLATED RAT VENTRICULAR CARDIOMYOCYTES

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Extensive efforts to find alternatives to the conventional therapy of congestive heart failure have resulted in the development of several new cardiotonic agents, which combine positive inotropic effects with vasodilating activity to achieve maximum improvement in cardiac performance. Whilst the actions of these agents have not been elucidated fully, these compounds have been shown to derive their inotropic and vasodilating actions, at least in part, from the selective inhibition of PDE isoenzymes which are specific for cyclic AMP (PDE III and PDE IV) (Colucci *et al.*, 1986; Nicholson *et al.*, 1991). The effectiveness of these drugs at increasing intracellular levels of cyclic AMP varies considerably and there is evidence that a potentially more useful drug would have less of its effect mediated through cyclic AMP (Hohl and Li, 1991).

The effects of HN-10200 (2-[3-methoxy-5-methylsulfinyl-2-thienyl]-1H-imidazo-[4,5-c]-pyridine hydrochloride), a novel cardiotonic agent, were assessed in ventricular cardiomyocytes isolated from adult Sprague-Dawley rats (200-250g) (n=4-6). Contractile function, as measured by mechanical shortening, and biochemical systems involving cyclic AMP were investigated. Comparisons were made between HN-10200 and a number of compounds having different selectivity for PDE isoenzymes, namely, sulmazole (PDE III inhibitor), Ro 20-1724 (PDE IV inhibitor), and 3-isobutyl-1-methylxanthine (non-selective PDE inhibitor). In the presence of isoprenaline (10 μM), the PDE inhibitors increased levels of cyclic AMP to a lesser extent than in the presence of forskolin, at an equimolar concentration. IBMX, sulmazole and HN-10200 ($3x10^{-4}$M), produced 3.1, 2.2 and 1.9 fold increases in cyclic AMP over basal levels (0.672 pmol/mg protein), respectively, in the presence of isoprenaline, by comparison to the 13.4, 13.4 and 10.4 fold increases, respectively, in the presence of forskolin. HN-10200 and sulmazole had similar concentration-dependent profiles for the accumulation of cyclic AMP, but had lower potencies than that of IBMX (concentrations of forskolin required to increase cyclic AMP by 4 pmol/mg protein were 13±3 mM, 14±3 mM and 3±0.6 mM, respectively). HN-10200 exerted a concentration-dependent positive contractile effect, which was not affected by the α- or β-adrenoceptor antagonists, phentolamine and propranolol, or the histamine receptor antagonists, pyrilamine (H_1) and cimetidine (H_2). The efficacy of HN-10200 was greater than that of IBMX and sulmazole; maximum stimulations were 54%, 41% and 38% greater than the basal levels, respectively. Potencies were of the opposite order, in that the EC_{50} values were 93 μM, 64 μM and 16 μM for HN-10200, IBMX and sulmazole, respectively. Ro 20-1724 did not effect contractile activity.

In conclusion, HN-10200 increased contractile activity in a concentration-dependent manner over the range, 10^{-8} to 10^{-4}M, whereas the effect of this compound on levels of cyclic AMP was only evident at concentrations $>10^{-4}$M. These results indicate that the positive inotropic effect of HN-10200 is only partially mediated through cyclic AMP. Furthermore, the mechanism of action of HN-10200 did not involve stimulation of adrenoceptors or histamine receptors.

Colucci, W.S., Wright, R.F. & Braunwald, E. (1986) New Engl. J. Med., **314**, 290-299.
Hohl, C.M. & Li, Q. (1991) Circ. Res., **69**, 1369-1379.
Nicholson, C.D., Challis, R.A. & Shahid, M. (1991) TIPS, **12**, 19-27.

126P EFFECT OF GLIBENCLAMIDE AND RP49356 DURING MYOCARDIAL ISCHAEMIA IN RAT: UNEXPECTED PREVENTION OF VENTRICULAR FIBRILLATION BY THE DRUG COMBINATION

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Since the ATP-dependent K^+ current ($I_{K(ATP)}$) in cardiac muscle (Noma, 1983) is activated when ATP levels decline (i.e. during ischaemia), $I_{K(ATP)}$ has been purported to carry an important component of K^+ efflux during ischaemia, leading to the hypothesis that blockade of the channel may be antiarrhythmic (Kantor *et al.*, 1990); however this hypothesis remains controversial (Uprichard & Lucchesi, 1989). We have tested this hypothesis using a blocker of the channel (10 μM glibenclamide; GL) and an opener of the channel (10 μM RP49356; RP) and a combination of the two drugs (GL+RP; 10 μM of each) in a coronary collateral deficient species. Isolated rat hearts (n=8/group), perfused with solution containing (in mM) NaCl 118.5, $NaHCO_3$ 25.0, KCl 3.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4 and glucose 11.1 (pH 7.4, 37°C) for 5 min, were randomised to solution containing vehicle (1 % DMSO) or one of the above 3 solutions. Hearts were then subjected to 30 min left regional ischaemia. Coronary flow, QT interval and the incidence of ischaemia-induced ventricular fibrillation (VF) were measured. $I_{K(ATP)}$ blockade by GL is modulated by ADP levels, which rise in ischaemia (Venkatesh *et al.*, 1991) so parallel experiments were perfomed using the above solutions plus 20 μM ADP.

After 10 min of ischaemia GL and GL+RP tended to prolong QT interval (197±39 and 203±20 msec respectively versus 154±12 msec in controls), whereas RP significantly shortened QT interval to 123±6 msec ($p<0.05$). GL and GL+RP significantly decreased coronary flow in the uninvolved zone during ischaemia (8±0.9 and 9±0.5 ml/min/g respectively versus 13±1.2 ml/min/g in controls; $p<0.05$), whereas RP caused a slight increase in flow (16±1.0 ml/min/g). In the presence of ADP the effects of GL and of GL+RP on QT interval and coronary flow were reversed, whereas the effects of RP were unaffected. These effects are all consistent with modulation of $I_{K(ATP)}$. GL alone had no effect on the incidence of VF (88 % versus 100 % in controls), but the incidence of sustained VF (VF lasting for >2 min) was significantly reduced (14 % versus 88 % in controls) indicating a defibrillatory effect. RP had no effect on VF incidence (100 %) or on the ability of VF to sustain (63 %). Surprisingly, GL+RP significantly reduced the *incidence* of VF to 25 % ($p<0.05$), i.e. had an antifibrillatory effect. Furthermore, when ADP was added to GL+RP, VF was completely abolished ($p<0.05$). In conclusion, agents demonstrating effects attributable to block and opening of $I_{K(ATP)}$ had no effects on the incidence of VF. However, unexpected antifibrillatory effects were observed with the combination of these drugs. Specific $I_{K(ATP)}$ blockade does not appear to be an effective antifibrillatory mechanism in isolated rat heart.

(S.A.R. is a recipient of the A.J. Clark Award from the British Pharmacological Society)

Kantor *et al.* (1990). Circ. Res. 66, 478-485.
Noma, A. (1983). Nature 305, 147-148.
Uprichard & Lucchesi. (1989). J. Mol. Cell. Cardiol. 21, S89.
Venkatesh *et al.*. (1991). Circ. Res. 69, 623-637.

127P CARDIOVASCULAR PROFILE OF GR60139 IN ANAESTHETIZED DOGS

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That dihydropyridines (DHPs) are more vascular selective than verapamil (V) and diltiazem (D) is supported by much evidence obtained from "in vitro" and "in vivo" animal studies (Opie, 1987). The new 1,4 DHP GR60139 (GR) has been suggested as a potential compound for treatment of ischaemic heart disease from "in vitro" studies showing a less vascular-selective calcium antagonist activity than the parent compound lacidipine (L). The overall aim of this work was to examine the haemodynamic effects induced by GR in anaesthetized open-chest dogs in comparison with L, and the non DHP calcium entry blockers V and D.
Male Beagle dogs (9-12 kg) anaesthetized with Pentobarbital sodium (25 mg/kg i.v. + 4 mg/kg/h i.v.) were used. Two electromagnetic flow probes were placed around the ascending aorta and the left circumflex coronary artery, while a double-tip catheter was introduced into the left ventricle via the left carotid artery. GR (1, 3, 10 mg/kg), L (0.003, 0.01, 0.03, 0.1 mg/kg), V (0.03, 0.1, 0.3, 1 mg/kg) and D (0.1, 0.3, 1 mg/kg) were administered intravenously as a single bolus. Dose-response regression analysis was performed to evaluate ED_X values (doses inducing, at the peak effect, x % reduction or increase with respect to basal value).

Table 1 Cardiovascular effects in anaesthetized dogs: ED_{25} values (mg/kg)

	Mean Blood Pressure	Heart Rate	Coronary Vascular Resistance
GR60139	1.1(0.8-1.5)	2.2(1.9-2.7)	< 1.0
Lacidipine	0.006(0.004-0.008)	> 0.1	0.005(0.001-0.01)*
Verapamil	0.10(0.07-0.13)	0.94(0.16-1.93)	0.13(0.05-0.26)*
Diltiazem	0.12(0.05-0.28)	> 1.0	< 0.1

Numbers in brackets are the 95% confidence limits; * = ED_{50}; (n = 4-8).

GR induced a dose-related hypotensive effect, but unlike L, caused a concomitant dose-related reduction in heart rate (Table 1). Importantly, GR at doses which markedly slowed the sinus rate, did not delay anterograde atrio-ventricular conduction. This is in clear contrast with V and D. In addition, at equibradicardic doses GR was clearly less negative inotropic than V and D. All drugs decreased coronary vascular resistance confirming a direct action on the coronary vasculature.
In conclusion these experiments indicate that GR is less vascular selective than the classical DHPs, such as L, and is endowed with a selective activity on the sinus node.

Opie, L.H. (1987) Cardiovasc. Drugs Ther. **1**, 411-430.

128P RECEPTOR BINDING CHARACTERISTICS OF THE SLOW ACTING DIHYDROPYRIDINE CALCIUM ANTAGONIST, LACIDIPINE

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Lacidipine is a new dihydropyridine calcium antagonist with a slow onset and long duration of antihypertensive activity (Micheli et al, 1990). The present study aimed to explore whether a direct relation exists between the pharmacodynamic and the receptor binding characteristics of lacidipine.

A [^{3}H]-lacidipine binding assay has been set up in guinea pig (GP) heart and cerebral cortex membranes. 50-100µg membrane proteins were incubated for 4 h at 25°C in a 50 mM TrisHCl buffer solution, pH 7.4, containing 1 mM $CaCl_2$. Specific binding (determined using 1µM of nifedipine) was saturable and consistent with a single class of non interacting binding sites. It accounted for 40-50% of total binding at 0.5nM of [^{3}H]-lacidipine in GP heart and for 30-40% in GP cerebral cortex membranes. Equilibrium saturation analysis of [^{3}H]-lacidipine binding showed a pK_D of 9.43 ± 0.03 (K_D = 0.37 nM) and a B_{max} of 1.47 ± 0.27 pmol/mg protein in heart membranes (Values ± SEM; n = 9). In cerebral cortex membranes the pK_D amounted to 9.29 ± 0.08 (K_D = 0.51 nM) and the B_{max} to 0.77 ± 0.15 pmol/mg protein. Furthermore, pK_D values obtained from non-equilibrium, kinetic studies, were in good agreement with equilibrium studies (9.84 and 9.90 in cardiac and cerebral cortex membranes, respectively). The pharmacological profile of [^{3}H]-lacidipine binding has been determined by studying a series of compounds in competition experiments: dihydropyridine drugs showed nanomolar affinity values (e.g. Nifedipine 0.51 and 0.59 nM, amlodipine 5.6 and 3.9 nM), verapamil 134 and 91nM, diltiazem 250 and 347nM and flunarizine 1380 and 955nM in heart and brain membranes, respectively, which agrees with literature evidence for other dihydropyridine calcium antagonists (e.g. Glossmann et al, 1985). Kinetic studies (n=3) showed that both the rate of association (k_{on}=9.34 ± 1.33 x 10^6 L/mol/min) and dissociation (k_{off}=1.33 ± 0.08 x 10^{-3} min^{-1}) were significantly slower than for isradipine (59 ± 26 x 10^6 L/mol/min and 3.21 ± 0.60 x 10^{-3} min^{-1}, respectively), (Student *t*-test; P<0.05). As for other dihydropyridines, k_{off} of [^{3}H]-lacidipine binding was enhanced by diltiazem (2.83x10^{-3} min^{-1}) or verapamil (4.21x10^{-3} min^{-1}), while the presence of nifedipine did not significantly modify (P>0.05) the rate of dissociation of [^{3}H]-lacidipine from the receptor (k_{off}=1.91x10^{-3} min^{-1}). These results indicate that the inhibition of [^{3}H]-lacidipine binding by phenylalkylamine and benzothiazepine drugs occurs through an allosteric mechanism.

In conclusion, lacidipine binds to the dihydropyridine calcium antagonist receptor with pharmacological characteristics analogous to isradipine, but with significantly slower binding kinetics, both in GP brain and heart membranes. Although the high lipophilicity will certainly contribute to the pharmacodynamic characteristics, at least part of the slow onset and long duration can be explained on the basis of the interaction of lacidipine with the dihydropyridine receptor.

Glossmann H et al, (1985), Arzneim. Forsch **35**: 1917-1935.
Micheli, D. et al, (1990), J. Cardiovasc. Pharmacol. **15**: 666-675.

129P ENDOTHELIN-1 INCREASES THE SEVERITY OF ISCHAEMIA-INDUCED ARRHYTHMIAS IN ANAESTHETISED RATS

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Plasma levels of endothelin-1 (ET-1) are elevated with a variety of cardiovascular diseases such as acute myocardial infarction (Stewart et al. 1991), vasospastic angina pectoris (Toyo-oka et al. 1991) and heart failure (Margulies et al. 1990). However the exact role of endogenous endothelin under pathophysiological conditions is not yet known. This study investigates the effects of ET-1 on the incidence and severity of arrhythmias induced by a period of myocardial ischaemia in open-chest anaesthetised rats.

Male Sprague-Dawley rats were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.) and prepared for left coronary artery ligation using the technique described by Clark et al. (1980). Saline or ET-1 (0.047 and 0.094 nmole kg^{-1} min^{-1}) were infused commencing 5 min before occlusion (using a low-sited ligature) and maintained for the duration of the experiment. The total number of ventricular ectopic beats (VEBs), the number of beats occuring as ventricular tachycardia (VT), and the incidence and duration of VT and ventricular fibrillation (VF) during the 30 min occlusion period were analyzed. The results are presented in table 1.

Table 1

Group	n	VEBs	VT	VT Duration (sec)	VT %Incidence	VF Duration (sec)	VF %Incidence	%Mortality
Saline(control)	9	556±75	255±32	18±3	100	3±3.0	33	22
ET1(0.047nmole)	9	515±93	236±61	18±4	100	24±7.2*	100**	44
ET1(0.094nmole)	9	1029±182*	768±179**	62±14**	100	22±11*	88*	33

*p<0.05, **p<0.01 compared to control group (Mann Whitney or Fisher tests as appropriate), mean±s.e.mean.

Infusion of ET-1 (0.094 nmole kg^{-1} min^{-1}) resulted in a significant increase in the total number of VEBs, and in beats occuring as VT. There was a marked increase in the incidence of ventricular fibrillation with both doses of ET-1. Changes in heart rate and blood pressure were not significant during ET-1 infusion. These results demonstrate a pro-arrhythmic effect of ET-1 under conditions of myocardial ischaemia.

Clark, C., Foreman, K.I, Kane, K.A., McDonald, F.M. & Parrat, J.R. (1980) J. Pharmacol. Methods 3, 357
Margulies, K.B., Fredric, L., Hildebrand, J.R., Lerman, A., Pervella, M.A. et al. (1990) Circulation 82, 2226-30
Stewart, D.J., George, K., Kevin, B., Costello & Cernacek, P. (1991) J. Am. Coll. Cardiol. 18, 38-43
Toyo-oka, T., Aizaw, T., Suzuki, N., Hirata, Y., Miyauchi, T. et al. (1991) Circulation 83, 476-83

130P THE ET_A RECEPTOR ANTAGONIST, FR139317, DOES NOT REDUCE INFARCT SIZE IN A RABBIT MODEL OF ACUTE MYOCARDIAL ISCHAEMIA AND REPERFUSION

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Plasma levels of ET-1 are elevated in patients with acute myocardial infarction (Miyauchi *et al.*, 1989). Ischaemia and reperfusion enhance the coronary vasoconstrictor response to ET-1 in the rat isolated perfused heart (McMurdo *et al.*, 1991). Monoclonal antibodies to ET-1 reduce infarct size after coronary artery ligation (1h) and reperfusion (24h) in anaesthetised rats (Watanabe *et al.*, 1990). Here, we study the effects of the ET_A receptor antagonist, FR139317 (Sogabe *et al.*, 1992) on i) ET-1 induced coronary vasoconstriction in the isolated perfused heart of the rabbit, (ii) the pressor response to ET-1 in anaesthetised rabbits and (iii) infarct size in a rabbit model of ischaemia and reperfusion.

NZW rabbits (2-3 kg) were given heparin (100 U kg^{-1}, i.v.) and anaesthetised with sodium pentobarbitone (30 mg kg^{-1}, i.v.). After thoracotomy, the hearts were excised and perfused at constant flow (25 ml min^{-1}) with warmed (37°C), oxygenated (95% O_2, 5% CO_2) Krebs' solution. Changes in coronary perfusion pressure (CPP) induced by ET-1 (1-100 pmol) were recorded in hearts treated with vehicle (20% DMSO) or FR139317 (1 μM). Resting CPP was 29 $\pm$ 1 mm Hg (n=16). ET-1 increased CPP in a dose-dependent fashion so that 30 pmol ET-1 increased CPP by 22 $\pm$ 8 mm Hg and 100 pmol by 47 $\pm$ 10 mm Hg (n=8). The increase in CPP produced by 30 and 100 pmol of ET-1 was attenuated by FR139317 to 3 $\pm$ 1 and 8 $\pm$ 3 mm Hg, respectively ($p<0.05$; n=8).

In a separate series of experiments, rabbits were anaesthetised (as above) and subjected to 1h occlusion of the first antero-lateral branch of the left coronary artery (LAL) followed by 2h of reperfusion. Ten min prior to LAL occlusion, rabbits were treated with a continuous infusion of either vehicle (20 % DMSO; n=5) or FR139317 (0.2 mg kg^{-1} min^{-1}, i.v.; n=6). This infusion of FR139317, when started 30 min prior to injection of ET-1, attenuated the rise in blood presssure produced by ET-1 (1 nmol kg^{-1}, i.v.) from 47 $\pm$ 5 mm Hg (control, n=8) to 8 $\pm$ 2 mm Hg ($p<0.05$; n=4). Evans blue solution (4 ml of 1% w/v) was used to determine the area at risk and nitro-blue tetrazolium (NBT; 0.5 mg ml^{-1}) to determine infarct size (Thiemermann *et al.*, 1989). In vehicle and FR139317 treated animals, the area at risk was 34 $\pm$ 5% and 33 $\pm$ 3% of the left ventricle, respectively. FR139317 had no effect on infarct size, expressed as % area at risk (vehicle: 55 $\pm$ 7%; FR139317: 49 $\pm$ 7%; $p>0.05$).

Thus, FR139317 attenuates the vasoconstrictor effects of ET-1 in the isolated perfused heart of the rabbit and the ET-1 induced rise in blood pressure in the anaesthetised rabbit. However, FR139317 did not affect infarct size following LAL occclusion (1h) and reperfusion (2h). These findings demonstrate that the coronary vasoconstrictor effects of ET-1 in the rabbit are primarily due to the activation of the ET_A receptor. In addition, we demonstrate that antagonism of ET_A receptors with FR139317 does not reduce infarct size after coronary artery ligation followed by a brief period of reperfusion. *We thank Parke-davis Pharmaceutical Research for funding this work, and the Medicinal Chemistry Department for providing FR139317.*

Miyauchi, T. et al. (1989). *Lancet* **2**, 53-54.
McMurdo, L. et al. (1991). *Br. J. Pharmacol.* **104**, 323P.
Sogabe, K. et al. (1992). *J. Vasc. Res.* **29**, 201-202.
Thiemermann et al., (1989). *Br. J. Pharmacol.* **97**, 401-408.
Watanabe, T. et al. (1990). *Nature* **348**, 673.

131P REGIONAL HAEMODYNAMIC RESPONSES TO THE ET_B-RECEPTOR-SELECTIVE AGONIST, BQ3020, IN CONSCIOUS RATS

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[$Ala^{1,3,11,15}$]ET-1 can cause vasoconstriction *in vivo* (Bigaud & Pelton, 1992; Gardiner *et al.*, 1992), so we investigated responses to [$Ala^{11,15}$]Ac-ET-1(6-21) (BQ3020) (Molenaar *et al.*, 1992), since it should be metabolised less rapidly. Male Long Evans rats were instrumented chronically with renal, mesenteric and hindquarters flow probes and intravascular catheters; all surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.). Increasing i.v. bolus injections of BQ3020 (0.15-10 nmol kg^{-1}) were given at 30 min intervals.

Table 1: Changes in heart rate (HR), mean arterial blood pressure (MAP) and vascular conductance (VC) in conscious, Long Evans rats (n=8) following injection of different doses of BQ3020. Values are mean ± s.e. mean; * P<0.05 for change (Friedman's test).

	0.15 nmol kg^{-1}			0.3 nmol kg^{-1}		
	15 s	1 min	3 min	15 s	1 min	3 min
Δ HR (beats min^{-1})	20 ± 9	7 ± 10	0 ± 9	13 ± 8	-14 ± 5*	3 ± 13
Δ MAP (mm Hg)	3 ± 2	7 ± 2 *	5 ± 1*	5 ± 3*	10 ± 4*	7 ± 3*
Δ Renal VC (%)	-7 ± 3*	-16 ± 2	-11 ± 2*	-12 ± 3*	-25 ± 3*	-17 ± 3*
Δ Mesenteric VC (%)	-47 ± 1*	-38 ± 2*	-25 ± 2*	-55 ± 3*	-49 ± 4*	-37 ± 2*
Δ Hindquarters VC (%)	21 ± 8*	14 ± 9	4 ± 6	30 ± 13*	4 ± 6	7 ± 8
	1 nmol kg^{-1}			10 nmol kg^{-1}		
	15 s	1 min	3 min	15 s	1 min	3 min
Δ HR (beats min^{-1})	36 ± 12*	-23 ± 18	-33 ± 14	77 ± 7*	49 ± 11*	-14 ± 11
Δ MAP (mm Hg)	4 ± 4	16 ± 3*	23 ± 4*	-32 ± 2*	15 ± 2	27 ± 2*
Δ Renal VC (%)	-14 ± 4*	-44 ± 4*	-36 ± 4*	-53 ± 7*	-95 ± 1*	-84 ± 2*
Δ Mesenteric VC (%)	-64 ± 4*	-66 ± 3*	-59 ± 4*	-11 ± 15	-47 ± 8*	-71 ± 3*
Δ Hindquarters VC (%)	41 ± 11*	6 ± 8	-23 ± 4*	131 ± 16*	-6 ± 12	-11 ± 12

The results are summarised in Table 1, BQ3020 (0.15 to 1 nmol kg^{-1}) caused dose-dependent hypertension, renal and mesenteric vasoconstrictions, but an initial hindquarters vasodilatation. However, only the 10 nmol kg^{-1} dose of BQ3020 caused an initial hypotension in association with the hindquarters vasodilatation, probably because there was no significant mesenteric vasoconstriction at that time. These results are consistent with a greater potency of BQ3020 than [$Ala^{1,3,11,15}$]ET-1 (Gardiner *et al.*, 1992) possibly due to slower metabolism. It appears that at 10 nmol kg^{-1}, BQ3020 activates additional mesenteric vasodilator mechanisms, as does ET-1 (unpublished observations).

Bigaud, M. & Pelton, J.T. (1992) Br. J. Pharmacol. **107**, 912-918.
Gardiner, S.M., Kemp, P.A., Bennett, T. & Davenport, A.P. (1992) Br. J. Pharmacol. **107**, 415P.
Molenaar, P., Kuc, R.E. & Davenport, A.P. (1992) Br. J. Pharmacol. **107**, 637-639.

132P PHARMACOLOGICAL CHARACTERIZATION OF HUMAN ET_A AND ET_B RECEPTORS EXPRESSED IN CHINESE HAMSTER OVARY (CHO) CELLS

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Two subtypes of Endothelin (ET) receptors exist: ET_A (ET-1 > ET-3) and ET_B (ET-1 = ET-3). Selective ligands for ET_A (BQ123 [cyclo-D-Asp, Pro, D-Val, Leu, D-Trp]; Ihara *et al.*, 1992) or ET_B receptors (sarafotoxin S6c; Williams *et al.*, 1991) support this classification. In this study, we have characterized human cloned ET receptors (hET), expressed by transfection in Chinese hamster ovary (CHO) cells.

Open reading frames for hET receptors were cloned by reverse transcriptase - polymerase chain reaction into the mammalian expression vector pcDNA1. Following transfection into CHO-DUKX11 cells by calcium phosphate precipitation, stable cell lines were created by limited dilution cloning. Expression of each receptor subtype was stable for at least 30 cell passages. Cell membranes (30-100μg protein ml^{-1}) were incubated for 3hr at 25°C in buffer containing TRIS (50mM), BSA (0.1% w/v), $CaCl_2$ (1mM), bacitracin (100μg ml^{-1}), [^{125}I]-ET-1 (15-25pM) and varying concentrations of unlabelled ET ligands. Specific binding of [^{125}I]-ET-1 (~ 90% of total binding) was defined as that inhibited by unlabelled ET-1 (1μM).

Table 1: IC_{50} values (nM) for inhibition by ET receptor ligands of [^{125}I]-ET-1 binding to membranes from CHO cells transfected with hET_A or hET_B receptors. (Data are geometric means with 95% confidence limits, from 3-10 experiments)

Ligand	hET_A	hET_B
ET-1	0.20 (0.12 - 0.35)	0.09 (0.03 - 0.28)
ET-3	4.9 (1.06 - 22.9)	0.08 (0.02 - 0.31)
BQ123	14.5 (4.3 - 49.0)	> 10000
Sarafotoxin S6c	1160 (490 - 2750)	0.32 (0.10 - 0.97)

The inhibitory ligand profiles are consistent with the selective expression of either hET_A or hET_B receptors. Scatchard analyses of saturation isotherms showed that [^{125}I]-ET-1 bound to hET_A and hET_B receptors with Kd's (mean ± s.e.m.) of 20.5 ± 1.8 pM and 25.1 ± 4 pM (n=3), respectively. B_{max} values were 71.0 ± 5.5 fmol/mg protein (hET_A) and 179 ± 20 fmol/mg protein (hET_B).

In conclusion, we have demonstrated the stable expression of hET_A and hET_B receptors in CHO cells. This system will be of value in the identification and characterization of novel ET receptor ligands with affinity for hET_A and hET_B receptors.

Ihara, M., *et al.* (1992). Life Sci., 50, 247-255.
Williams, D.L., *et al.* (1991). Biochem. Biophys. Res. Commun., 175, 556-561.

133P IgE PEPTIDE III (Asp-Ser-Asp-Pro-Arg) INHIBITS CELL-MEDIATED INFLAMMATION

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A pentapeptide derived from human IgE, Asp-Ser-Asp-Pro-Arg (Hamburger, 1975) suppresses allergic rhinitis and conjunctivitis (Kalpaxis & Thayer, 1991) and reduces immune complex-induced colitis in mice (Hahn *et al*, 1990). We have examined the actions of the peptide (Sigma Chemical Co. Ltd) on cell-mediated immune colitis induced by the hapten trinitrobenzene sulphonic acid (TNB) in rats (Morris *et al*, 1989), carrageenan-induced rat paw oedema (Winter *et al*, 1962) and rat adjuvant-arthritis (Pearson, 1956). Daily i.p. injections of the peptide reduced colitic features (scored 0 - 5) and colonic tissue myeloperoxidase activity (Bradley *et al*, 1982) in rats killed 7 days after induction of colitis with 0.25 ml intrarectal instillation of 80 $mg.ml^{-1}$ TNB solution in 50% aqueous ethanol.

Table 1:

	Vehicle (ml)	Pentapeptide ($mg.kg^{-1}$)			
Parameter	0.2	0.4	0.7	1.4	5.0	10.0
Colitic score	4.1±0.3	3.6±0.7	2.8±0.5*	2.6±0.7*	3.0±0.5*	2.8±0.6*
MPO activity	0.21±0.02	0.15±0.04	0.10±0.04*	0.11±0.02*	0.35±0.11	0.32±0.07

Asterisks indicate differences from vehicle-treated controls ($p < 0.05$, Mann-Whitney U-test). Results are presented as mean ± s.e.m., N = 6.

Daily administration (0.7 or 1.4 $mg.kg^{-1}$, i.p.) of the pentapeptide significantly ($p < 0.05$) reduced the mean adjuvant-induced knee joint swelling in the non-injected limbs on the 18th day to 30.3 ± 7.9 % and 31.3 ± 9.5 % of the mean inflamed control responses respectively. Intraperitoneal administration of the peptide (0.7 - 2.8 $mg.kg^{-1}$) 2 h prior to induction of carrageenan-induced paw oedema had no significant ($p < 0.05$) inhibitory effect on either the maximal oedema responses attained during 6 h or total oedema response (monitored as area under the time-course curve). In comparison, indomethacin (2.5 $mg.kg^{-1}$, p.o.) significantly ($p < 0.05$) suppressed the maximal oedema response to 50.0. ± 0.01 % of the mean control (vehicle-treated) response. The selective inhibition of the cell-mediated inflammatory responses indicates a modulatory action for the pentapeptide and possibly its parent peptide in cell-mediated inflammatory conditions.

Bradley, P.P., Priebat, D.A., Christensen, R.D. & Rothstein, G. (1982). J. Invest. Dermatol. **78**: 206 - 209
Hahn, G.S., Howes, C.N., Al-Haboubi, H & Zeitlin, I.J. (1990). J. Allergy Clin. Immunol. **85 (1/2)**, 298
Hamburger, R.N. (1975) Science, **189**, 389 - 390
Kalpaxis, J.G., & Thayer, T.O. (1991) Ann. Allergy **66(5)**, **393 - 398**
Morris, G.P., Beck, P.L., Herridge, M.S. *et al* (1989) Gastroenterol. **96**, 795.
Pearson, C.M. (1956) Proc. Soc. Exp. Biol. **91**, 95-101.
Winter, C.A., Risley, E.A. & Nuss, G.W. (1962) Proc. Soc. Exp. Biol. (N.Y) **111**, 544-547.

134P COMPARATIVE STUDIES OF THE ANTI-ANGIOGENIC ACTIVITY OF CPD 14 AND SURAMIN IN A RAT SPONGE MODEL

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Suramin is currently being evaluated for the treatment of certain types of cancer. Experimental evidence has shown that suramin blocks growth factor/receptor interactions and inhibits the activity of enzymes that are critical for cell growth and proliferation, leading to its anti-proliferative and anti-metastatic actions. We have previously reported that suramin potentiates the anti-angiogenic effect of hydrocortisone in a rat sponge model (Hu & Fan, 1992). Further studies in our laboratories have identified a suramin analogue CPD 14 (s-carbamide of 1-m-aminobenzoyl-amino-8-naphthol-3,6-disulphonic acid), which is 5 to 10-fold less toxic in mice than suramin. Here we used the rat sponge model to test whether CPD 14 can also inhibit angiogenesis *in vivo*.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (180-200g) and neovascularisation was assessed by using ^{133}Xe clearance to measure blood flow through the implants every two days over a period of 14 days. Test substances (50 µl) were applied to the sponges through the cannulae daily 24h before ^{133}Xe measurement. The effects of test substances on angiogenesis were confirmed by histological studies.

Daily local administration of 10, 100, 1000 µg suramin produced no apparent effect on sponge-induced neovascularisation. In contrast, this sponge-induced neovascularisation was inhibited by daily treatment with 100 µg CPD 14. The 6 min ^{133}Xe clearance values obtained on Day 8, 10, 12, 14 for control group and treated groups were 19.8±1.0, 28.2±0.9, 32.5±1.4, 35.3±2.3 % ($n = 6$) and 14.2±1.6, 16.1±1.6, 19.5±2.0, 20.8±1.9 % respectively ($n = 6$, $P < 0.01$). Histological studies of sponge sections stained with haematoxylin & eosin or the endothelial cell marker, *Bandeirea simplicifolia* lectin I, isolectin B_4, showed that both cellular infiltration and neovascularisation were profoundly inhibited by CPD 14 as compared to controls. However, lower doses of 10 µg CPD 14 produced no apparent effect on sponge-induced neovascularisation.

Daily administration of 6 pmol (100 ng) basic fibroblast growth factor (bFGF) into the sponges elicited an intense neovascularisation. This neovascular response was inhibited by co-injection of 3 mg suramin. However, there is a narrow therapeutic window for suramin, 1 mg was not effective but 10 mg proved to be toxic. A single intravenous injection of 100 mg kg^{-1} suramin on Day 1 after sponge implantation produced no apparent effect, whereas 200 mg kg^{-1} totally blocked the bFGF-induced neovascularisation. Higher doses led to severe toxicity. Parallel study showed that the bFGF-elicited neovascularisation was inhibited by co-administration of 1, 3 mg, but not 0.1 mg CPD 14. The systemic effect of CPD 14 was also investigated. A single intravenous injection of 200 mg kg^{-1}, but not 100 mg kg^{-1}, totally blocked the bFGF-induced neovascularisation.

Thus, these results suggest that the suramin analogue CPD 14 possesses anti-angiogenic activity. This together with its lower toxicity offers an opportunity of widening the suramin therapeutic window.

This work was supported by the Wellcome Trust.

Hu, D.E. & Fan, T.-P.D. (1992) *Br. J. Pharmacol.*, **107**, 163P.

135P DuP 532 BLOCKS THE ANGIOGENIC EFFECT OF ANGIOTENSIN II IN RATS

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It is now recognised that angiogenesis is controlled by the balance of angiogenic inducers and inhibitors. Experiments have shown that these factors produce their effects via activation of specific receptors. Thus manipulation of growth factor activities at the receptor level would be an attractive strategy in future management of angiogenic disorders. We have previously shown that daily administration of 1 nmol angiotensin II (AII) caused an intense neovascularisation in a rat sponge model (Hu & Fan, 1991). The angiogenic effect of AII was totally abolished by losartan (DuP 753), a specific AT1 receptor antagonist, but not by the AT2 antagonist CGP 42112A (Fan & Hu, 1992). Here we tested if the angiogenic activity of AII could be manipulated by other specific AT1 receptor antagonist DuP 532 or specific AT2 receptor antagonist PD 123319. Since AII has been shown to induced the production of the angiogenic factor, basic fibroblast growth factor (bFGF), we also examined if the angiogenic activity of AII can be modified by an bFGF antibody.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (180-200g) and neovascularisation was assessed by using ^{133}Xe clearance to measure blood flow through the implants every two days over a period of 14 days. To exclude acute vascular effects of the test substances, they were injected (50 µl) to the sponges through the cannulae 24h before ^{133}Xe measurement. The effects of test substances on angiogenesis were confirmed by histological studies.

Daily doses of 1 nmol AII induced significantly higher clearance values than the control group, e.g. the 6 min ^{133}Xe clearance value of the control group on Day 6, 8, 10, 12 were 15.7±0.4, 20.6±1.0, 29.9±0.7, 36.9±1.0% (n = 8) while AII increased it to 22.6±1.2, 30.2±0.9, 39.2±1.0, 42.7±0.6%, respectively (n = 8, $P < 0.01$). Histological studies of sponge sections stained with haematoxylin & eosin or an endothelial cell marker *Bandeirea simplicifolia* lectin I, isolectin B_4 showed increased cellularity and vascularity in the test sponges as compared to controls. The AII-induced neovascular response was inhibited by co-administration of 100 nmol DuP 532. The 6 min ^{133}Xe clearance values obtained on Day 6, 8, 10, 12 were 15.5±1.1, 22.0±1.9, 31.9±1.1, 34.4±2.0 (n = 6, $P < 0.05$). Histological studies showed that both the cellular infiltration and neovascularisation in the AII-treated sponges were profoundly suppressed by DuP 532. In contrast, the AII-induced neovascular response was not inhibited by co-administration of 100 nmol PD 123319. In the absence of exogenous AII, neither antagonist had any effect.

Angiotensin converting enzyme inhibition by local daily administration of captopril (1, 10 or 100 µg) or lisinopril (40 µg) or by daily intraperitoneal injection of captopril (100 mg kg^{-1}) or lisinopril (40 mg kg^{-1}) did not significantly change sponge-induced angiogenesis. Co-administration of 1000 ng DG2, a specific antibody to bFGF (a gift from Dr. T.M. Reilly, Du Pont Merck, USA), can totally block the bFGF (100 ng)-induced neovascularisation, but did not modify the AII-induced angiogenesis.

Thus, we propose that the angiogenic response elicited by AII in rats is mediated by AT_1 receptors and this angiogenic activity can be blocked at the receptor level. These data suggest that receptor modulation of angiogenesis would provide an effective strategy for treatment in angiogenic diseases.

This work is supported by the Wellcome Trust.

Fan, T.-P.D. & Hu, D.E. (1992) *FASEB* **6(4)**, A937-4
Hu, D.E. & Fan, T.-P.D. (1991) *Br. J. Pharmacol.*, **104**, 83P.

136P ZENECA ZD7155: A NOVEL, POTENT AND ORALLY-EFFECTIVE ANGIOTENSIN II RECEPTOR ANTAGONIST

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ZENECA ZD7155 (5,7-diethyl-1-[2'-(1H-1,2,3,4-tetrazol-5-yl)biphenyl-4-ylmethyl]-1,2,3,4-tetrahydro-1,6-naphthyridin-2-one hydrochloride) is a novel compound which antagonises the pharmacological actions of angiotensin II (AII). This study concerns the in vitro effects of ZD7155 in guinea-pig tissues and its in vivo effects in the rat.

In guinea-pig adrenal gland membranes, ZD7155 displaced (^{125}I)-AII from its binding sites in a concentration-related manner, with an IC50 of 3.8 nM (95% confidence limits 3.0 - 4.8 nM, n=7). Similarly, the compound was a potent antagonist of AII-mediated contractions in isolated guinea-pig ileum. At a concentration of 0.2 nM, ZD7155 caused a >10-fold shift in the AII dose-response curve with a marked depression of the maximal response (n=17). In contrast, ZD7155 at a concentration of 1.0 µM had no significant effect on agonist responses to acetylcholine or histamine in the same preparation (dose ratios of 1.60 ± 0.17 and 1.38 ± 0.06, respectively; n=4). Hence the compound was a specific antagonist of AII responses in vitro.

Conscious male Charles River Wistar rats were prepared with indwelling carotid artery and jugular vein cannulae. Continuous AII infusion at 1.0 µg kg^{-1} min^{-1} i.v. increased mean arterial pressure by 46.7 ± 2.0 mmHg and cumulative i.v. dosing of ZD7155 inhibited this response with an ID50 of 0.19 ± 0.02 mg kg^{-1} (n=22). In further experiments, rats prepared as above were given intermittent AII infusions of 1.0 µg kg^{-1} min^{-1}. ZD7155 dosed at 3.0 mg kg^{-1} p.o. inhibited the angiotensin II pressor responses by 96.0 ± 2.2% and 65.6 ± 6.1% at 1 and 12 hrs after dosing, respectively (n=6).

Conscious male Alderley Park Wistar rats were prepared with a partial occlusion of the left renal artery, using a platinum clip of 0.25 mm i.d. After 12-14 days, carotid artery cannulae were implanted and, the following day, blood pressure measurements were taken before and for up to 24 hours after administration of ZD7155 (1.0 mg kg^{-1} p.o.). Mean arterial pressure was reduced from 189.3 ± 2.2 mmHg at the start of the experiment to 132.2 ± 7.5 mmHg, 8 hrs after dosing the compound (n=13). Blood pressure at 24 hrs after dosing (129.2 ± 4.7 mmHg) was reduced compared with rats prepared as above but treated with dosing vehicle alone (170 ± 4.7 mmHg, n=10).

These results are consistent with the conclusion that ZD7155 is a potent, long-acting and orally effective AII receptor antagonist. Hence ZD7155 may have therapeutic potential in the treatment of hypertension and heart failure.

137P EFFECTS OF FLOSEQUINAN ON CONTRACTIONS OF RAT AORTIC STRIPS ELICITED BY A VARIETY OF AGONISTS

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Flosequinan (F) is a novel vasodilator currently being used to treat congestive heart failure. The putative mechanism of action is considered to depend on reducing generation of inositol 1,4,5 triphosphate stimulated by vasoconstrictor agents in vascular smooth muscle (Lang and Lewis 1992). The work presented here was undertaken in order to explore the vascular activity of F in rat aortae exposed to a variety of agonists. Rat aortae were removed, spirally cut and mounted in Kreb's solution at 37°C gassed with 95% O_2, 5% CO_2. Contractions were measured with isotonic transducers. Cumulative dose response curves to noradrenaline (n=6), phenylephrine (n=6), angiotensin II (n=23), 5-HT (n=6) and BHT-920 (n=6) were constructed and repeated in the presence of F:1μM, 10 μM and 50 μM.

F caused a rightward shift of the dose-response curves for all agonists (Table 1). With angiotensin II, F reduced the maximum response by 11% and 40% at 10 μM and 50 μM. With BHT-920 there was an 80% depression of the maximum response with 50 μM. F does not displace ligand binding to α_1, α_2, angiotensin AT_1 and $5HT_1$ or $5HT_2$ receptors (Sim *et al* 1988) so the results indicate effects which may occur beyond the receptor. Noradrenaline, phenylephrine, angiotensin II and 5HT all increase the generation of inositol phosphates (Legan *et al* 1985, Griendling *et al* 1987) and effects on the activity of these agonists by F would be expected if the drug reduces this generation. Contractions to BHT-920 are entirely dependent on extracellular calcium but are also affected by F. This suggests that additional mechanisms, possibly involving receptor-operated calcium channels, may also play a part in the mode of action of this drug.

Table 1

Effect of flosequinan (F) on EC50 values for different agonists

	Noradrenaline (nM)	Phenylephrine (nM)	Angiotensin II (nM)	5HT (μM)	BHT-920(μM)
Control	0.91	7.59	3.31	1.05	5.37
F 1μM	2.45*	19.1**	3.8	1.20	8.71
F 10μM	5.25**	49.0**	8.91	1.62	56.2**
F 50μM	12.0**	269.0**	20.4**	2.88**	>1000**

*p<0.05; **p<0.01 using analysis of variance and Dunnets test. EC50 = concentration giving 50% of maximal contraction

Lang, D. & Lewis, M.J. (1992) *Eur. J. Pharm.* **226**,259-264
Sim, M.F. *et al.* (1988). Abstract of the 12th Scientific Meeting of the International Society of Hypertension, Kyoto
Legan, E., Chernow, B., Parillo, J. & Roth, B.J. (1985) *Eur. J. Pharmacol.* **110**,389-390
Griendling, K.K., Berk, B.C., Ganz, B., Gimbrone, M.A. & Alexander, R.W. (1987) Hypertension. 9,(Suppl III), 181-185

138P EFFECTS OF cGMP-INCREASING AGENTS ON PLC-MEDIATED PHOSPHATIDYLINOSITOL HYDROLYSIS INDUCED VIA α_1-ADRENOCEPTORS IN RAT AORTA

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In vascular smooth muscle, noradrenaline (NA)-induced contractions, mediated via alpha$_1$-adrenoceptors, are the result of activation of phospholipase C (PLC) and hydrolysis of phosphatidylinositol (PI) (Fox et al., 1985). Such contractions can be inhibited by acetylcholine (ACh) and sodium nitroprusside (SNP), which increase the synthesis of guanosine monophosphate (cGMP). The precise mechanism of inhibition by these agents is unknown, but it is likely that this effect is mediated by cGMP.

This study investigated this possibility by measuring the effects of these drugs on the cGMP levels and on the PLC-mediated PI-response, and also the effect of the permeant cGMP analogue 8-bromo cGMP (8-Br-cGMP) on PI-response. The PLC-mediated PI-response was monitored by measuring ^{3}H-inositol phosphates (IP). The effect of NA (6.2 μM) in presence and absence of inhibitory agents were investigated in aortic rings (2-3 mm), prelabelled with myo-^{3}H-inositol (8 μCi.ml^{-1}, 37°C, 2 h). Aortic rings were incubated with drugs (1 h) in Krebs buffer containing lithium chloride (10 mM). The reaction was terminated by adding a mixture of chloroform and methanol (1:2 v/v). After homogenisation of the tissue, the inositol phosphates were extracted and separated by ion exchange chromatography. The cGMP was measured by radioimmunoassay.

ACh (1 x 10^{-5} M) and SNP (1 x 10^{-6} M) increased cGMP levels (ACh, from 150 ± 10.9 to 575 ± 21.8 pmol.mg^{-1} tissue, mean ± s.e. mean, n=8, P<0.001, SNP from 150 ± 10.9 to 5100 ± 60.2 pmol mg-1 tissue, n=8, P<0.001). NA (6.2 μM) increased accumulation of ^{3}H in aortic rings (from 63.7 ± 14.3 to 470.8 ± 17.9 d.p.m. mg^{-1} tissue, n=8, P<0.001). This response was abolished in the presence of alpha$_1$-adrenoceptor antagonist prazosin (1 x 10^{-6} M) (from 470.8 ± 17.9 to 58.2 ± 4 d.p.m. mg^{-1} tissue, n=8, P<0.001). NA (6.2 μM)-induced PI-response was inhibited by ACh (1 x 10^{-5} M) (from 502.2 ± 30.9 to 323.5 ± 33.8 d.p.m. mg^{-1} tissue, n=8, P<0.01), SNP (1 x 10^{-6} M) (from 635.4 ± 27.9 to 351.4 ± 17.1 d.p.m. mg^{-1} tissue, n=10, P<0.001) and by 8-Br-cGMP (0.3 mM) (from 635.4 ± 27.9 to 357.2 ±19.8 d.p.m. mg^{-1} tissue, n=8, P<0.001).

These results suggest that vasorelaxant agents such as ACh and SNP which increase the synthesis of cGMP, inhibit alpha$_1$-adrenoceptor-mediated contraction of aorta at least partly by inhibition of PLC-mediated PI-response via cGMP. This inhibitory effect of cGMP was confirmed using the permeant cGMP analogue, 8-Br-cGMP.

Fox, A.W., Abel, P.W. & Minneman, K.P. (1985). Eur. J. Pharmacol. 116, 145-162.

139P 5-HT$_4$ RECEPTOR MEDIATED FACILITATION OF THE PERISTALTIC REFLEX IN THE ISOLATED GUINEA-PIG ILEUM

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The 5-HT$_4$ receptor has been implicated in the facilitatory effect of 5-hydroxytryptamine (5-HT) in the peristaltic reflex in the isolated guinea-pig ileum (Craig & Clarke, 1991, Buchheit & Buhl, 1991). In this study we have investigated the role of the 5-HT$_4$ receptor in the facilitation of the peristaltic reflex using both indole and benzamide agonists and the 5-HT$_4$ receptor selective antagonist SDZ 205-557 (Buchheit *et al.* 1992).

Male or female guinea-pigs were killed by cervical dislocation and segments of ileum about 5 cm in length and from a distance of 5-30 cm from the ileo-ceacal junction were cannulated at the oral and aboral ends and secured horizontally in 25 ml perspex baths containing Krebs-Henseleit solution containing 1μM methysergide and 2μM ondansetron maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2. The oral ends were connected to peristaltic pumps and the aboral ends were connected to narrow vertical tubes with an outlet about 3 cm above the level of fluid in the baths. The peristaltic response was initiated by pumping the Krebs-Henseleit solution inside the lumen. By changing the rate of pumping a reasonably uniform rate of peristalsis in all of the tissues was obtained before addition of the agonists. The drugs were added to the serosal side and any decrease in the threshold for peristalsis was measured and calculated as the percentage of the minimum theshold in the two minute period before the addition of the drug. Four tissues were used to evaluate four different concentrations and the pEC_{50} values and EC_{50} ratios determined. Each tissue was used twice, once for evaluating the effect of 5-HT and secondly, after washing for evaluating the effect of 5-HT in the presence of SDZ 205-557 or the effect of another agonist, with a resting period of at least an hour between treatments. SDZ 205-557 was equilibrated for 45 min before initiation of the peristalsis. 5-HT, other indole and substituted benzamides reduced the threshold and the interval between the peristaltic contractions. In the presence of SDZ 205-557 (1μM) the concentration response curve to 5-HT was dextrally shifted from which a pA_2±s.e.mean value of 7.38±0.30 (n=7) was calculated.

Table 1. The potency of 5-HT$_4$ receptor agonists causing facilitation of the peristaltic response in the isolated guinea-pig ileum.

Agonist	pEC_{50}±s.e.mean	EC_{50} ratio	n	Agonist	pEC_{50}±s.e.mean	EC_{50} ratio	n
5-HT	7.36±0.06	1	37	Renzapride	6.09±0.17	16	5
5-Methoxytryptamine	7.01±0.17	2.8	6	(S) Zacopride	5.99±0.11	45	5
5-Carboxamidotryptamine	5.43±0.06	124	4	(R) Zacopride	5.61±0.13	43	6
2-Methyl-5-HT	<5	>200	4	Metoclopramide	4.80±0.15	135	5

The presence of methysergide and ondansetron excludes agonist action at the 5-HT$_1$, 5-HT$_2$ or 5-HT$_3$ receptors. The pA_2 value of 7.38 for SDZ 205-557 to antagonise the effect of 5-HT and the rank order of potency for the agonists to facilitate peristalsis strongly suggest an involvement of a 5-HT$_4$ receptor.

Buchheit, K-H. & Buhl, T. (1991). Eur. J. Pharmacol. **205**, 203-208.
Buchheit, K-H., Gamse R. & Pfannkuche H-J. (1992). Naunyn-Schmiedeberg's Arch. Pharmacol. **345**, 387-393.
Craig, D. A. & Clarke D. E. (1991). Br. J. Pharmacol. **102**, 563-564.

140P MONOPHOSPHORYL AND DIPHOSPHORYL LIPID A INDUCE NITRIC OXIDE SYNTHASE

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Bacterial lipopolysaccharide (LPS) or endotoxin evoke a variety of toxic, pharmacological, and immunomodulatory effects in several species including man. Most of the biological effects of LPS may be mediated by the diphosphoryl lipid A (DPL) moiety of the LPS molecule. Like LPS, lipid A is extremely toxic in most species. Removal of the glycosidic phosphate from the reducing end of lipid A yields monophosphoryl lipid A (MPL) which shares the adjuvant properties of lipid A and LPS, but has less toxic side effects (Galanos et al., 1985). Nitric oxide (NO) mediates some of the biological actions of LPS. Here we compare the effects of DPL, MPL and LPS on the induction of NO synthase (NOS) in vitro and in vivo.

J774.2 macrophages and rat aortic smooth muscle cells (SMC) were cultured to confluence in 96-well plates. Nitrite accumulation as an indicator of NO formation was measured by the Griess reaction (Gross et al, 1991) in the supernatant of cells exposed to LPS (Sigma), DPL (Caiichi Chem. Co.), or MPL (Sigma); (0.001 - 10 μg/ml). In anaesthetized rats, mean arterial blood pressure (MAP) and heart rate (HR) were measured via a carotid arterial cannula. Rats were treated with LPS or MPL (10 mg/kg; i.v. bolus). 180 min after LPS or MPL, calcium-independent (induced) NOS activity was measured by the conversion of ^{3}H-arginine to ^{3}H-citrulline in lung homogenates (Mitchell et al., 1991).

In J774.2 macrophages, LPS, DPL, or MPL (10 μg/ml) induced the synthesis of nitrite after a lag phase of 6 h; 7.6±0.3, 4.8±0.2, and 3.8±0.1 nmol/well/24h, respectively (n=4). In SMC, LPS, but not DPL or MPL (0.001 - 100 μg/ml), caused a significant nitrite production, which was increased by interferon-γ (IFN; 50 ng/ml). In the presence of IFN, DPL or MPL also induced SMC to release nitrite (after a lag phase of 12 h). Thus, in the presence of IFN, LPS, DPL or MPL (10 μg/ml) caused nitrite formation of 7.4±0.1, 3.4±0.1, and 3.0±0.1 nmol/well/72h, respectively (n=4). NO formation was due to the induction of NOS for it was inhibited by (i) N^G-methyl-L-arginine (10^{-3} M), an inhibitor of NO synthesis (ii) the protein synthesis inhibitor cycloheximide ($4x10^{-6}$ M) and (iii) dexamethasone (10^{-6} M), an inhibitor of NOS induction.

In anaesthetized rats, LPS caused a fall in MAP (from 127±5 to 71±5, 77±10 and 61±7 mmHg, at 5, 120 and 180 min, respectively, $p<0.01$; n=8). HR increased from 434±19 (control) to 506±10 beats/min at 180 min after LPS ($p<0.01$, n=8). MPL caused a fall in MAP (from 115±5 to 92±5, 101±10 and 60±7 mmHg, at 5, 120 and 180 min, respectively, $p<0.05$; n=3) and an increase in HR from 398±9 (control) to 475±53 beats/min (180 min; $p<0.05$, n=3). At 180 min, an elevation of the calcium-independent NOS activity was observed in lung homogenates of LPS- (161±5 pmol, n=4) or MPL- (30±2 pmol, n=3) treated rats (control: 10±3 pmol, n=3).

Thus, LPS, DPL or MPL induce a calcium-independent NOS in J774.2 and SMC in vitro and in the anaesthetized rat (order of potency: LPS>>DPL>MPL). The lipid A moiety of LPS may largely account for the induction of NOS. (YH is a scholar of the Japan Society for the Promotion of Science. This work was supported by a grant of Glaxo Group Research Ltd.)

Galanos C., Luderitz O., Rietschel E.T. et al. (1985). Eur. J. Biochem. 148, 1
Gross S.S., Jaffe E.A., Levi R., Kilbourn, R.G. (1991). Biochem. Biophys. Res. Comm. 187, 823
Mitchell J.A., Sheng H., Forstermann U. & Murad F. (1991). Br. J. Pharmacol. 104, 289

141P SELECTIVITY OF NG-NITRO-L-ARGININE-P-NITROANILIDE TO PREFERENTIALLY INHIBIT NEURONAL NO SYNTHASE CAN BE EXPLAINED BY DIFFERENTIAL HYDROLYSIS TO NG-NITRO-L-ARGININE

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Nitric oxide (NO) is released by endothelial cells and certain neurones by distinct constitutive, calcium-dependent isoforms of NO synthase (NOS). Analogues of L-arginine, such as NG-monomethyl-L-arginine (L-NMMA) and NG-nitro-L-arginine (NO2Arg) are inhibitors of all isoforms of NOS isolated to date. NO2Arg is a more potent inhibitor of the endothelial and neuronal isoforms of NOS than L-NMMA. In addition to NO2Arg, NO2Arg containing dipeptides, such as NO2Arg-Arg, are also potent inhibitors of NOS *in vitro* and *in vivo* after metabolism to free NO2Arg (Thiemermann et al., 1991). Recently NG-nitro-L-arginine-p-nitroanilide (NAPNA) was described as a selective inhibitor of the neuronal isoform of NOS (Babbedge et al., 1992). The aim of this study was to elucidate the relative potencies of NO2Arg, L-NMMA and NAPNA in crude enzyme preparations of endothelial and neuronal isoforms of NOS. In addition, we investigated whether differences in enzymatic hydrolysis of NAPNA by endothelial and neuronal cells to NO2Arg explain the selectivity of NAPNA as an inhibitor of the neuronal isoform of NOS. Crude preparations of the neuronal isoform of NOS were obtained from homogenates of rat (Male Wistar; 250-300g) brain as previously described (Mitchell et al 1991a). Endothelial NOS was obtained from homogenates of bovine aortic endothelial cells (BAE; Mitchell et al., 1991b). NOS activity was measured by the ability of homogenates (approximately 15 μg protein) to convert 3H-L-arginine to 3H-L-citrulline, in the presence of various co-factors (Mitchell et al., 1991a). The ability of homogenates to convert NAPNA to NO2Arg was determined under identical conditions as NOS measurements, spectrophotometrically by measuring the appearance of p-nitroaniline (absorbance 405 nm) in the homogenate supernatant (10,000 x g for 10 min), using authentic p-nitroaniline as standard (minimum detection= 3 μM; range 3-1000 μM; Southan et al., this meeting).

NAPNA and L-NMMA were equipotent inhibitors of endothelial NOS with IC_{50} values of 6 and 5 μM. However, NO2Arg was some 10 times more potent (approximate IC_{50} value of 0.6 μM; n=4-12 determinations from 4 separate enzyme preparations). NAPNA and NO2Arg were equipotent inhibitors of brain NOS activity (respective IC_{50} values, 1 and 0.8 μM. In contrast, L-NMMA was a weaker inhibitor of brain NOS (IC_{50} value of 45 μM; n=5-9 from 3 separate enzyme preparations). Homogenates of both rat brain and BAE were able to metabolize NAPNA to NO2Arg and p-nitroaniline. Brain homogenate (15 μg protein) converted 71±3% of NAPNA (100 μM) in 30 min (n=3), whereas BAE homogenate (15 μg protein) converted 16±2% of NAPNA (100 μM) in 30 min (n=3). These findings show that in crude preparations of NOS. NAPNA is a more potent inhibitor of the neuronal isoform present in rat brain than the isoform present in BAE. As rat brain preparations were able to metabolize NAPNA more efficiently than the endothelial preparations used in this study, it is conceivable that NAPNA must first be cleaved to NO2Arg in order to inhibit NOS activity. This hypothesis is supported by findings demonstrating that the ability of NAPNA to inhibit the cytokine-induced isoform of NOS requires hydrolysis to NO2Arg (Southan et al., this meeting). Clearly our finding that L-arginine analogues can afford selectivity, by virtue of the presence or absence of metabolizing enzymes, may form the theroretical basis for identifying tissue-selective NOS inhibitors.

This work was supported by a grant from GLAXO Research Ltd.

Babbedge, R.C., Moore, P.K., Gaffen, Z. and Hart, S.L., (1992). Br. J. Pharmacol.,1P, 107, 233.
Mitchell, J.A., Sheng, H., Forstermann, U. and Murad, F., (1991a). Br. J. Pharmacol., 104, 289-291.
Mitchell, J.A. Forstermann, U., Warner, T.D., Pollock, J.S., Schmidt, H.H.H.W., et al., (1991b). Biochem., Biophys. Res. Commun., 176, 1417-1423.
Thiemermann, C., Mustafa, M., Mester, P.A., Mitchell, J.A., Hecker, M. and Vane, J.R. (1991). Br. J. Pharmacol., 104, 31-38.

142P INDAZOLES: A NOVEL GROUP OF NITRIC OXIDE SYNTHASE INHIBITORS

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We have recently reported that 7-nitro indazole (7-NI) is a potent inhibitor of mouse and rat brain nitric oxide synthase (NOS) (Moore et al., 1993). Like L-N^G nitro arginine methyl ester (L-NAME; Moore et al., 1991), 7-NI exhibits anti-nociceptive activity in the mouse. However, unlike L-NAME, 7-NI does not increase mouse blood pressure suggesting that in vivo 7-NI does not influence endothelial NOS. We have now studied the effect of 7-NI administered i.p. in rats to inhibit NOS in different body regions and have assessed the ability of several other substituted indazole derivatives to inhibit cerebellar NOS.

Cerebellum (CB), cerebral cortex (CX), olfactory bulb (OB), hippocampus (HC), adrenal gland (AD) and stomach (SF) were removed from Wistar rats (male, 200-280 g) 1 h after i.p. administration of 7-NI (10 mg kg^{-1}) or arachis oil (0.1 ml 100 g^{-1}) as control. All organs were homogenised (1:10 w/v) in 20 mM Tris buffer containing 2 mM EDTA (pH 7.4) and centrifuged (10,000 g, 15 min). Supernatant was passed over a 0.3 ml column of Dowex AG50WX-8 (Na^+ form) to remove endogenous L-arginine. Aliquots (25 ul) were incubated (15 min, 37°C) with [^{3}H] L-arginine (0.5 uCi, 120 nM), NADPH (0.5 mM), $CaCl_2$ (0.75 mM) and, where appropriate, 5 ul drug or vehicle. NOS activity was measured as [^{3}H] citrulline formation (Moore et al., 1993). Significant (all n=6, $P<0.05$) inhibition of NOS was observed in CB (31.1 + 3.2%), CX (38.0 ± 5.8%), HC (37.1 + 3.0%) and AD (23.9 ± 2.8%). OB and SF NOS activity was unaffected. In separate experiments cerebellar NOS prepared from untreated animals was inhibited by 7-NI (IC_{50}, 0.9 uM), 6-nitro indazole (6-NI; IC_{50}, 31.6 uM), 5-nitro indazole (5-NI; IC_{50}, 47.3 uM) and indazole (IC_{50}, 177.8 uM). 5- and 6-amino indazole and 3-indazolinone showed little NOS inhibitory activity (IC_{50}s >1 mM).

The present results confirm the ability of 7-NI to inhibit brain NOS in vitro and provide evidence for a similar effect in vivo. Both 5-NI and 6-NI as well as the parent compound, indazole, also inhibit cerebellar NOS in vitro although 7-NI exhibits greatest potency. In contrast, amination of the indazole ring at positions 5 or 6 effectively removes NOS inhibitory activity.

RCB is an SERC-CASE scholar with Wellcome Research Laboratories. PBW is supported by a scholarship from Glaxo Group Research. We thank the MRC for financial support.

Moore, P.K., Oluyomi, A.O., Babbedge, R.C. & Hart, S.L. (1991) Br. J. Pharmacol., 102, 198-202
Moore, P.K. Babbedge, R.C., Wallace, P., Gaffen, Z. & Hart, S.L. (1993) Br. J. Pharmacol., 108, 296-297

ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

1P **Li SW, Boughton-Smith NK, McKechnie K, Blackham A & Leff P** Inhibition by EP receptor agonists of formyl-methionyl-leucyl-phenylalanine (FMLP)-stimulated neutrophil superoxide production and relationship to increases in cAMP

2P **Mitchell JA, Akarasereenont P, Bishop-Bailey D, Wood EG, Thiemermann C & Vane JR** Aspirin is a more potent inhibitor of prostacyclin biosynthesis by endothelial cells than by LPS-treated J774 macrophages

3P **Cameron NE, Cotter MA, Dines KC & Maxfield EK** The effects of chronic flurbiprofen and L-N^G-nitro-arginine treatment on nerve function in non-diabetic and diabetic rats

4P **Mitchell JA, Swierkosz TA, Warner TD, Gross SS, Thiemermann C & Vane JR** Regulation of prostacyclin synthesis by the release of endogenous nitric oxide in response to bacterial lipopolysaccharide

5P **Ahluwalia A, Flower RJ & Perretti M** Dual action of prostaglandins (PGs) in the mouse skin oedema test

6P **Teixeira MM, Williams TG & Hellewell PG** Role of endogenous nitric oxide in leukocyte accumulation and oedema formation in guinea-pig skin

7P **Jamieson MJ, Ontiveros JA, Romano WF & Shepherd AMM** Insensitivity of mesenteric small veins to nitrovasodilators and endothelium-derived relaxing factor

8P **Rowe ID, Haynes WG, Williams BC & Webb DJ** CHAPS attenuates both endothelium-dependent dilatation and response to vasoconstrictors in the perfused superior mesenteric arterial bed in the rat

9P **Southan GJ, Gross SS & Vane JR** Amides and esters of N^w-nitro-L-arginine (e.g. L-NAME) must be hydrolyzed to become active NO synthase inhibitors

10P **Brave SR, Gibson A & Tucker JF** The inhibitory effects of hydroquinone on nitric oxide-induced relaxation of the mouse anococcygeus are prevented by native thiols

11P **Szabó Cs, Mitchell JA, Gross SS, Thiemermann C & Vane JR** An antagonist of platelet-activating factor (WEB 2086) inhibits the induction of nitric oxide synthase by bacterial lipopolysaccharide

12P **Gross SS & Vane JR** Inhibitors of GTP cyclohydrolase cause superinduction of nitric oxide synthase by immunostimulants in aortic smooth muscle cells

13P **Swierkosz TA, Mitchell JA, Bishop-Bailey D & Vane JR** *In vivo* increase of prostaglandin H2 synthase (cyclo-oxygenase) and nitric oxide synthase activity by bacterial lipopolysaccharide

14P **Mohammed SP, Adcock JJ & Coleman RA** Effect of the prostanoid EP_2-receptor agonist, AH13205, on airway rapidly-adapting stretch receptors in the cat

15P **Whelan CJ, Vardey CJ, Coleman RA & Johnson M** Evidence that inhibition of histamine-induced plasma protein extravasation by salmeterol in guinea-pig lung is not subject to tachyphylaxis

16P **Milne AAY & Piper PJ** Different effects of two anti-CD18 antibodies on antigen-induced airway hyper-responsiveness and cell accumulation in guinea-pigs

17P **Borsodi A, Ozdemirler G, Nevin S, Kabasakal L, Otvos F & Toth G** Binding characteristics of the delta antagonist (1,5'-^{3}H)naltrindole in rat brain membranes

18P **Cheronis JC, Goodfellow VS, Loy SD, Marathe M, Elder M, Hanson WL & Whalley ET** *In vitro* and *in vivo* activity of a peripherally acting μ opioid receptor agonist/BK_2 receptor antagonist heterodimer

19P **Whalley ET, Loy SD, Cheronis JC, Blodgett JK & Allen LG** Novel peptide heterodimers with actions at BK_2 and either μ opioid, NK_1 or NK_2 receptors: *in vitro* studies

20P **Rane A, Zhurong L, Henderson C & Wolf CR** Selective down-regulation of hepatic cytochrome P450 isozymes by morphine - a neuroendocrine mechanism?

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The proceedings of the British Pharmacological Society are published as a supplement to the *British Journal of Pharmacology*.

The *British Journal of Pharmacology* is published monthly by the Scientific & Medical Division, Macmillan Press Ltd.

All business correspondence and reprint requests should be addressed to the Scientific & Medical Division, Macmillan Press Ltd., Houndmills, Basingstoke, Hampshire RG21 2XS, UK. Telephone: (0256) 29242; Fax: (0256) 810526.

Annual subscription prices for 1992 EEC £412, elsewhere £445/US$825 (sterling rate is definitive). Orders must be accompanied by remittance. Cheques should be made payable to Macmillan Press, and sent to: Macmillan Press Ltd., Subscription Department, Brunel Road, Houndmills, Basingstoke, Hampshire RG21 2XS, UK.

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Second Class postage paid at Rahway NJ, US Mailing Agent: Mercury Airfreight International Ltd, Inc., 2323 Randolph Avenue, Avenel, New Jersey, NJ 07001, USA.

British Journal of Pharmacology

Proceedings Supplement

Proceedings of the British Pharmacological Society Meeting

University of Aberdeen
Joint Meeting with British Opioid Colloquium

14th–16th April, 1993

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