89P ARE B_1 BRADYKININ RECEPTORS INVOLVED IN THE ANTIARRHYTHMIC EFFECT OF BRADYKININ IN ANAESTHETISED DOGS?

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There is some evidence that in anaesthetised dogs bradykinin (25 ng kg^{-1} min^{-1}) infused intracoronarily prior to a 25 min occlusion of the left anterior descending coronary artery (LAD) markedly reduces the severity of myocardial ischaemia and suppresses the ischaemia-induced ventricular arrhythmias (Végh et al, 1991). The aim of the present study was to examine whether administration of Des-Arg9-Bradykinin (DBK), a selective B_1 bradykinin receptor agonist, could afford a similar antiarrhythmic protection.

Seventeen mongrel dogs were anaesthetised with the mixture of chloralose and urethane, thoracotomised and then subjected to a 25 min occlusion of the LAD. A small branch of the LAD was catheterised to infuse either saline (controls, n=10) or DBK in a dose of 25 ng kg^{-1} min^{-1} (n=7). The infusions were commenced 10 min prior to ischaemia and were maintained during ischaemia.

Compared to controls, administration of Des-Arg9-Bradykinin did not reduce the severity of ventricular arrhythmias. The number of ventricular premature beats (325±120 vs. 229±55 in controls), episodes of ventricular tachycardia (VT, 10.8±4.5 vs. 4.9±1.8 in controls), the incidence of VT (86% vs. 80% in controls), the incidence of ventricular fibrillation during occlusion (57% vs. 70% in controls) and survival from combined occlusion-reperfusion insult (14% vs. 20% in controls) were similar in both groups. These results might indicate that the cardioprotection afforded by bradykinin is not mediated through activation of B_1 bradykinin receptors.

Experiments with isolated left anterior descending coronary artery rings from intact animals support these results. Thus, bradykinin resulted in a dose-dependent vasorelaxation and reached a complete relaxation at a concentration of 1μM but Des-Arg9-Bradykinin relaxed the coronary artery rings with a maximum of 18% at a concentration of 10 mM which indicates the small density of B_1 bradykinin receptors in the left anterior descending coronary artery in dogs.

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90P BRADYKININ ELICITS DELAYED MYOCARDIAL PROTECTION IN RAT HEART VIA A NITRIC OXIDE-DEPENDENT MECHANISM

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The nonapeptide, bradykinin (BK) is an important trigger of classical preconditioning (Goto et *al.*, 1995) but its role in delayed preconditioning has not been characterised to date. Nitric oxide (NO) is known to play a role in triggering delayed preconditioning (Bolli et *al.*, 1998). The aims of this study were to investigate if exogenous BK could evoke delayed myocardial protection and to determine if any protection observed might be mediated by BK-induced NO generation.

Male Sprague Dawley rats (350-400g) received i.v bolus injections of BK (40μg/kg) or saline, via a tail vein preceded 15 minutes earlier by i.p injections of the NO synthase inhibitor nitro-L-arginine methyl ester (L-NAME, 10mg/kg) or saline.

Twenty four hours following these treatments, animals were anaesthetised with pentobarbitone sodium (50mg.kg^{-1}, i.p) and killed. The hearts were excised and Langendorff perfused with Krebs-Henseleit buffer (composition in mmol.l^{-1} NaCl 118, $NaHCO_3$ 25, glucose 11, KCl 4.7, $MgSO_4$ 1.2 KH_2PO_4 1.2, $CaCl_2$ 1.8). All hearts were stabilised for 20 minutes and subjected to 35 minutes of regional ischaemia by left coronary artery occlusion, followed by 120 minutes of reperfusion. Infarct sizes were measured using triphenyltetrazolium chloride staining, the risk zone being delineated with Evan's blue dye. The results obtained are highlighted in Table 1.

Table 1 Infarct Size

GROUP (n)	*RISK ZONE (cm^3)*	*INFARCT SIZE (%)*
Saline & Saline (9)	0.54±0.036	53.5±3.2
Saline & BK (8)	0.58±0.041	29.1±4.7*
L-NAME & BK (6)	0.60±0.040	52.3±5.0
L-NAME & Saline (7)	0.54±0.038	53.5±4.8

Infarct size is expressed as a percentage of the risk zone (%). Results are stated as mean±s.e.of mean of (n) experiments (one way ANOVA, followed by Fisher's protected least significant difference test) * = P<0.01 vs saline & saline

BK pretreatment caused a significant limitation of ischaemic injury 24 hours later. This delayed protection was completely abrogated by prior administration of a NO synthase inhibitor. Contractility expressed as rate pressure product (RPP) was similar in all groups. However, coronary flow rate (mls/min) was augmented in the BK treated group, an effect also abolished by prior administration of L-NAME.

These data are the first to show that BK can elicit a delayed preconditioning-like effect and that this action appears to be dependent on the early generation of NO. We speculate that NO may trigger protection via formation of peroxynitrite.

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91P THE ROLE OF K_{ATP} CHANNELS, ADENOSINE, NITRIC OXIDE AND PROSTAGLANDINS AS MEDIATORS OF REACTIVE HYPERAEMIA IN GUINEA-PIG HEARTS.

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Reactive hyperaemia (RH) is a transient vasodilation following a brief ischaemic period. Recent work suggests that ATP-dependent K^+ (K_{ATP}) channels may be important in mediating this response, however it is unclear whether mitochondrial K_{ATP} channels contribute to this process in the heart. To investigate this, we examined the involvement of K_{ATP} channels and the relative role of mitochondrial K_{ATP} channels and compared them to NO, adenosine, and prostaglandins as potential mediators of RH in the heart.

Male Dunkin-Hartley guinea-pigs (935 ±90 g) were sacrificed, the hearts were removed for perfusion according to the Langendorff technique, as previously described (Kingsbury, 2000). The RH response was assessed after global zero-flow ischaemia (5-120s) in the presence of the NO synthase inhibitor nitro-L-arginine methyl ester (L-NAME, 10^{-5}M, n=9), the adenosine A_1 antagonist 8-phenyltheophylline (8-PT, 10^{-6}M, n=10), the cyclooxygenase inhibitor indomethacin (10^{-5}M, n=12), the non-selective K_{ATP} inhibitor glibenclamide (10^{-6}M, n=12) and 5-hydroxy-decanoic acid (5-HD, 10^{-4}M, n=10) a selective mitochondrial K_{ATP} inhibitor. The specificity of glibenclamide and 5-HD in this protocol was confirmed by constructing dose-response curves to the non-selective K_{ATP} channel agonist pinacidil (38nmol-10µmol) and the mitochondrial selective K_{ATP} channel agonist diazoxide (42nmol - 2µmol). Values are expressed as mean ± SEmean, curves were fitted using non-linear regression analysis and Student's unpaired t-test enabled the comparison of groups; P<0.05 indicating statistical significance.

All hearts showed a reactive hyperaemic vasodilation in response to ischaemic challenges with a maximum control RH flow response (K_{max}) of 8.8 ± 0.1ml/min/g. L-NAME had relatively little effect on peak RH flow response although there was a decreased vasodilator response to short (5-20s) ischaemic challenges (P<0.05). Indomethacin had very little effect on the RH response with a tendency for an attenuated response to longer ischaemic challenges. In contrast 8-PT reduced the peak flow response independently of the duration of the ischaemic challenge with a 32% (P < 0.001) decrease in K_{max}. Glibenclamide had the largest effect of any of the antagonists examined on the peak RH flow, responses to ischaemic challenges of up to 40s were reduced by 87% (P < 0.01), with some residual vasodilator capacity to longer ischaemic challenges as demonstrated by the 49% reduction in response to 120s ischaemia. While 5-HD also inhibited the peak RH flow response with a 40% (P < 0.001) decrease in K_{max}, it was less potent than glibenclamide.

In conclusion, the RH response is multifactorial with roles for NO, adenosine and most importantly K_{ATP} channels. While glibenclamide was most effective in blocking the RH response, 5-HD also had a marked effect indicating that mitochondrial K_{ATP} channels were also involved but to a lesser extent than the sarcolemmal K_{ATP} channels. NO has a more minor role but is relatively more important in the response to shorter ischaemic challenges. Indomethacin was least effective indicating that cyclooxygenase products appear to have little, if any, role in RH in the guinea-pig coronary circulation.

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92P INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) EXPRESSION IN RAT AORTIC CULTURED SMOOTH MUSCLE CELLS TREATED WITH CAFFEIC ACID AND RELATED ANALOGUES

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Nordihydroguaiaretic acid (NDGA), an antioxidant and lipoxygenase enzyme inhibitor, has been shown to block lipopolysaccharide-(LPS)-induced expression of iNOS in rat aortic smooth muscle cells (SMC), suggesting a possible role for lipoxygenase products (e.g. leukotrienes) in this enzyme induction process (Zhou *et al.*, 1999). We therefore investigated if caffeic acid (3,4-dihydroxycinnamic acid), another lipoxygenase inhibitor (Koshihara *et al.*, 1984), might have similar effects to NDGA upon iNOS. Surprisingly, we have found instead that caffeic acid (CA) and chemically similar agents appear to *activate* iNOS expression by themselves in these cells.

SMC, from explants of adult male rat aortas, were grown at 37°C in Medium 199 containing 10% (v/v) foetal bovine serum and antibiotics in 5% CO_2 : 95% air. They were passaged by trypsinization into multi-well tissue culture trays to study and compare various drug treatments within each culture tray. CA and its analogues dissolved at 50mM in ethanol, were sub-diluted to 5µM with medium before addition to confluent cells. Medium for preparing LPS (from *E. coli*, serotype 055:B5) solutions and also for adding alone to "control" cells, contained the same final ethanol concentration. After 20h, nitrite concentration in medium from cells was measured by the Griess reaction to indicate cellular NO production. No drugs used altered the cell viability (MTT assay) or the absorbance produced by standard nitrite concentrations prepared in medium to calibrate the nitrite assay.

Nitrite concentration (4.2 ± 1.5 µM) in medium from cells treated with 1 µg/ml LPS was much higher than the low background levels in control cells (0.12 ± 0.08 µM). In these same cultures, treatment with LPS + 5 µM CA produced significantly greater nitrite (10.5 ± 1.3 µM) compared with cells treated with LPS alone (P < 0.05, Student-Newman-Keuls test). However, cells treated with CA alone also produced substantial nitrite (5.8 ± 1.6 µM; P <0.05 vs control cells). [Data above are means ± s.e.m. for n = 6 cultures from different rats]. Western blotting was carried out with a monoclonal iNOS antibody and protein extracts from drug-treated cells. iNOS was not detected in control cells, but an immunostained band was present with 1 µg/ml LPS-treated cells, and was of stronger intensity using cells treated with LPS + 5 µM CA (n=2). In three more cultures comparing control cells with CA-treated cells, iNOS was absent in controls but a clear band was seen with CA-treated cells.

Nitrite formation (to a similar extent as produced by CA) was also induced in SMC treated at 5 µM with some hydroxycinnamic (HC) acid isomers, *i.e.* 2-HC, 3-HC and 4-HC where the single hydroxyl group differs in position on the benzene ring. However, *trans*-cinnamic acid, an analogue of CA which lacks ring hydroxylation also induced nitrite formation. Here, experiments on 6 cultures detected zero nitrite in all control samples compared with 5.54 ± 0.36 µM in those treated with *trans*-cinnamic acid alone (P < 0.001).

While the mechanism(s) responsible for the apparent induction of iNOS expression by these agents remains to be investigated, it is of interest that CA and *trans*-cinnamic acid are natural constituents of various plants, and as such are absorbed as dietary factors by man (The Merck Index, 1989).

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93P DIFFERENTIAL EFFECTS OF VITAMIN C AND SUPEROXIDE DISMUTASE MIMETIC ON NO-MEDIATED VASODILATATION IN RABBIT AORTIC RINGS DURING OXIDANT STRESS

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Endothelium-dependent vasodilation is impaired in subjects with risk factors predisposing to atherosclerosis. A decrease in endothelium-derived nitric oxide (EDNO) bioavailability rather than decreased nitric oxide production may be the main deficit. Excess superoxide anion (O_2^-) inactivates NO and there is much interest in the potential use of antioxidants as therapeutic agents in conditions associated with oxidant stress. The aim of this work was to compare the ability of vitamin C and the cell permeable superoxide dismutase (SOD) mimetic manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP) to restore NO-mediated vasodilation in rabbit aortic rings during oxidant stress.

New Zealand white male rabbits (2 – 2.5 Kg) were killed by an overdose injection of sodium pentobarbitone via the lateral ear vein and exsanguinated. Descending thoracic aortas were removed and trimmed of adhering connective tissue and fat. Transverse 2 mm wide rings were cut and mounted in 3 ml organ baths containing oxygenated Krebs' solution at 37°C. Tissues were placed under 2g resting tension for 60 min and then re-tensioned to 2g for a further 30 min. Isometric measurements were recorded via force transducers (Grass FT03). Rings were constricted with noradrenaline to 80% maximum tension and then relaxed with acetylcholine (ACh, 10^{-8} - 10^{-5} mol/L) or the NO donor nitroprusside (NP, 10^{-6} - 10^{-4} mol/L).

Experiments were performed under basal conditions and after treatment with diethyldithiocarbamate (DETCA, 2 mmol/L), a cell permeable inhibitor of endogenous Cu/Zn SOD. Rings were pre-treated with DETCA for 15 minutes to increase intracellular oxidant stress. This was then followed by a washout period of 15 min with the addition of vitamin C, MnTMPyP or vehicle control. All experiments were performed using a minimum of 8 rings obtained from at least 4 animals. The sum of relaxations (SS, arbitrary units) to all doses of ACh or NP was used as a summary measure to quantify relaxation. Comparisons were made by Student's paired *t*-test (2-tailed).

Vitamin C (0.1-10 mmol/L) had no effect or inhibited relaxation to ACh. MnTMPyP (100 μmol/L) increased relaxation to ACh (SS: 22.7 ± 2.6 *vs.* 31.0 ± 2.6, means ± SE, $P<0.001$). DETCA inhibited relaxation to ACh (SS: 21.2 ± 3.0 *vs.* 3.9 ± 2.2, $P<0.001$) and this inhibition was abolished by MnTMPyP (100 μmol/L) but unaffected by vitamin C (3 mmol/L). Relaxation to NP was enhanced both by MnTMPyP (SS: 9.9 ± 1.9 *vs.* 29.5 ± 2.8, $P<0.001$) and by vitamin C (SS: 12.7 ± 2.6 *vs.* 36.1 ± 2.4, $P<0.001$). DETCA did not significantly influence relaxation to NP.

These results suggest that EDNO is more susceptible than NP derived NO to inactivation by intracellular O_2^- after inhibition of Cu/Zn SOD by DETCA, and that vitamin C is relatively ineffective at protecting EDNO from this stress. NO donated by NP may be more susceptible to extracellular inactivation and be protected from this by vitamin C. Alternatively vitamin C/MnTMPyP may potentiate the release of NO from NP.

94P MECHANISMS OF VASODILATION OF GREEN AND BLACK TEA EXTRACTS AND EPICATECHIN DERIVATIVES.

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Both green and black tea possess strong antioxidant activity and enhance plasma antioxidant activity (Yokozawa *et al.*, 1998). Vasodilator activity has been attributed to its epicatechin content, NO release and the inhibition of calcium influx (Huang *et al.*, 1998, 1999). Here we determine the effects and mechanisms of green and black tea extracts on vascular function *in vitro*. The role of the endothelium and the effect of the NO synthase inhibitor N^{ω}-nitro-L-arginine-methyl-ester (L-NAME, 10^{-4}M) was also examined. The vasodilator effect of 4 green tea epicatechin derivatives was also investigated, namely epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG).

Isolated aortic rings from male New Zealand rabbits (3.5 kg) were set-up at 37°C in organ baths (10 ml) filled with Krebs buffer solution and continuously bubbled with 16% O_2, 5% CO_2 and 79% N_2. Vessels were precontracted with phenyelphrine (PE, 10^{-7}M). Once a stable plateau had been reached, cumulative concentration response curves for the diluted tea extracts ($2x10^{-4}$ – 1.4x10 mg.ml^{-1}) and the epicatechin derivatives (10^{-8} – $3x10^{-4}$M) were obtained.

Table 1 demonstrates the ability of green and black tea extracts to induce significant vasorelaxation in isolated rabbit aortic rings *in vitro* Green and black tea extracts both evoked biphasic, concentration dependent response curves in the aortic rings. The initial phase ($2x10^{-4}$ – $1.4x10^{-2}$ mg.ml^{-1}) being abolished by the presence of L-NAME and the removal of the endothelium. The presence of L-NAME and endothelium removal reduced the second phase vasodilator response to high extract concentrations (Table 1). The 4 epicatechin derivatives all induced vasorelaxation to varying degrees, but only at high concentrations ($3x10^{-5}$ – 10^{-4}M). The vasorelaxation activity induced by the derivatives was: EGCG > EGC > ECG > EC (Table 1).

These results show that green and black tea extracts and green tea epicatechin derivatives may not only function as antioxidants and free radical scavengers in vitro, but may also play an important role as endothelium-dependent vasodilators.

Table 1. Relaxation values (± s.e.mean) to $1.4x10^{-1}$mg.ml^{-1} (sub-maximal) with green and black tea extracts and maximum relaxation values (± s.e.mean) obtained with green tea epicatechin derivatives in isolated rabbit aorta. n = number of animals.

	Sub-max % relax^n	n	*p*
GT (control)	41±8	7	
GT/-endothelium	7±4	4	ns
GT & L-NAME (10^4M)	5±10	6	*
BT (control)	36±5	6	
BT/-endothelium	10±2	6	*
BT & L-NAME (10^4M)	2±9	6	**
	Max % Relax^n	n	*p*
EC (control)	3±3	7	
ECG	16±9	6	ns
EGC	17±6	6	ns
EGCG	46±5	6	***

Statistical analysis was carried out using a one-way analysis of varience, followed by a Dunnet's Multiple comparisons test. ns = not significant, *P<0.05, **P<0.01, ***P<0.001 as compared to relevant control. EC: epicatechin; ECG: epicatechin gallate; EGC: epigallocatechin; EGCG: epigallocatechin gallate

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95P A COMPARISON OF THE RESPONSES OF EQUINE DIGITAL VEINS AND ARTERIES TO ENDOTHELIN-1

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Acute laminitis is a painful ischaemia and reperfusion injury of the equine digit. In experimental models of laminitis, selective venoconstriction has been implicated in its pathophysiology (Allen et al., 1990). Endothelin-1 (ET-1) is a potent vasoconstrictor of both arteries and veins which has been shown to raise pulmonary capillary pressure through selective venoconstriction (Rodman et al., 1992). The purpose of the present study was to compare the responses of equine digital arteries (EDA) and veins (EDV) to ET-1 and to examine the effect of the endothelium on these responses.

EDA and EDVs were obtained from mixed breed adult horses killed at an abattoir. Rings (4mm) were prepared for isometric tension recording as previously described (Bailey & Elliott, 1998). The endothelium was removed from some vessel segments by gentle rubbing. In the first experiment, EDA and EDV from the same horses (n=6) with intact endothelium (+e) were examined. In the second experiment, EDA or EDVs from the same horses (n=9) were examined with endothelium intact and removed (-e). All vessel were constricted initially with depolarising Krebs solution (DKS; 118 mM K^+). A second contraction to U44069 (9, 11-dideoxy-9α, 11α-epoxymethano-prostaglandin F2α; 30 nM) was obtained and their relaxant response to carbachol (1 μM) used to confirm the presence or absence of the endothelium. Cumulative concentration response curves (CRCs) to ET-1 (10^{-10} to 10^{-6} M) were then obtained with contractions being expressed as a percentage of the DKS response. CRCs were fitted using computerised non-linear curve fitting. EC_{50} and E_{max} values were compared for EDA (+e) and EDV (+e) using a paired Student's *t*-test. Similar comparisons were made for EDA(+e) vs. EDA(-e) and EDV(+e) vs. EDV(-e).

ET-1 proved to be a significantly more potent vasoconstrictor of EDV than EDA. There was no significant difference between the maximum response to ET-1 in the two vessel types. Removal of the endothelium had no significant effect on the responses of either EDA or EDV to ET-1. The data from these experiments are presented in Table 1.

Table 1

	EC_{50} (10^{-8} M) geometric mean (95% CL)		E_{max} (% DKS) Arithmetic mean ± sem	
Expt 1	EDA (+e)	EDV (+e)	EDA (+e)	EDV (+e)
	3.7 (2.4-4.9)	1.3 (0.8-1.9)*	124 ± 9.9	199 ± 41.3
Expt 2	+e	-e	+e	-e
EDA	3.0 (2.0-4.0)	4.7 (0.7-12.2)	110 ± 11.5	87.4 ± 12.7
EDV	1.2 (0.7-1.7)	0.8 (0.3-1.3)	188 ± 30.0	202 ± 20.6

* denotes P<0.05 vs. EDA(+e) using a paired Student's *t*-test.

ET-1 is a more potent vasoconstrictor of EDV than EDA and could, therefore, contribute to the selective venoconstriction which is proposed to occur during the developmental phases of laminitis. Mediators released by the endothelium do not appear to modulate the responses to ET-1 in either vessel type although further experiments using inhibitors of individual pathways are necessary to confirm this finding.

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96P RELAXATION OF THE PORCINE ISOLATED, PRE-CONTRACTED CORONARY ARTERY BY ATP AND ADP: MEDIATION VIA A_{2A} ADENOSINE RECEPTORS

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We have previously noted that ATP and ADP were able to elicit a concentration-dependent, endothelium-independent relaxation of the porcine isolated, pre-contracted coronary artery (Alexander *et al.*, 2000). Here we have tested the hypothesis that this relaxation is mediated through activation of A_{2A} adenosine receptors, reported to be expressed in this tissue (King *et al.*, 1990; Hasan *et al.*, 2000).

Segments of coronary artery (dissected from whole hearts rapidly transported in ice from the abattoir) from pigs (either sex) were mounted for isometric tension recordings, as previously described (Alexander *et al.*, 2000). Tissue viability was assessed by eliciting contractions in the presence of 60 mM KCl. The thromboxane A_2 analogue U46619 (11α,9α-epoxymethano-PGH_2) was utilised (up to 10 nM) to elicit a contraction of about 60 % of that to KCl. Thereafter, concentration-relaxation curves were constructed in the presence of increasing cumulative concentrations of the ligands. Where the effects of ZM241385 [4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5,}triazin-5-yl amino]ethyl)phenol, a high affinity, A_{2A}-selective antagonist (Poucher *et al.*, 1995)] were examined, an interval of at least 60 min (with multiple washouts) was allowed prior to repetition of the response curves in the presence of 10 nM or 100 nM ZM241385. Data reported are means ± s.e.m. of results from at least five separate preparations.

After contraction in the presence of U46619, tissues could be completely relaxed by ATP, ADP, adenosine or NECA (5'-*N*-ethylcarboxamidoadenosine, a stable adenosine analogue) in concentration-dependent manners (pD_2 values of 6.56 ± 0.24, 7.58 ± 0.23, 6.08 ± 0.15 and 6.99 ± 0.18, respectively). In the presence of ZM241385, concentration-response curves were right-shifted significantly (pD_2 values in the absence vs. presence of ZM241385, in all cases P<0.05, t-test). pK_i values could therefore be calculated and were 8.98 ± 0.27, 9.19 ± 0.40, 8.82 ± 0.38 and 8.78 ± 0.26 for ZM241385 against ATP, ADP, adenosine and NECA relaxations, respectively.

These pK_i values for ZM241385 are consistent with those previously reported for A_{2A} receptors in this tissue (Hasan *et al.*, 2000) and for the ligand at A_{2A} adenosine receptors in the pig brain (Alexander *et al.*, 1999). These results suggest, therefore, that relaxation of porcine isolated coronary artery by ATP and ADP is mediated via A_{2A} adenosine receptors. Whether there is direct activation of A_{2A} receptors by the nucleotides or rapid metabolism generates the active ligand (presumably adenosine) remains to be determined.

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97P EFFECT OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR AGONISTS ON CYTOKINE-STIMULATED ENDOTHELIN-1 PRODUCTION IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

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Peroxisome proliferator-activated receptors (PPARs) are a family of three nuclear hormone receptors, PPARα, -δ and -γ, that may have important immunomodulatory actions (see Bishop-Bailey, 2000). PPARα activators have been shown to inhibit thrombin-induced endothelin-1 (ET-1) production in human vascular endothelial cells (Delerive *et al.*, 1999). In this study we have investigated the effects of PPAR agonists on cytokine-stimulated ET-1 expression in human saphenous vein vascular smooth muscle cells (HVSMCs; Woods *et al.*, 1999).

Saphenous vein (SV) was obtained from patients undergoing coronary artery bypass graft surgery. Explants of HVSMCs were grown as previously described and were identified by α-actin staining (Woods *et al.*, 1999). HVSMCs were treated with a combination of TNF-α (10ng.ml^{-1}) and IFN-γ (1000U.ml^{-1}) for 24 h in the presence of either 15d-PGJ_2 (0.01-30μM), bezafibrate (0.1-300μM) or WY 14643 (0.1-300μM). The release of ET-1 was measured by specific sandwich ELISA (R&D Systems). Cell viability was assessed by the ability of cells to reduce MTT to formazan (Mosmann, 1983). Immunofluorescence of PPAR isoforms expressed in HVSMCs was determined using a previously described method (Bishop-Bailey *et al.*, 1999). Statistical analysis was carried out using Student's t-test.

15d-PGJ_2, a non specific PPAR agonist, and WY 14643, a PPARα agonist inhibited cytokine stimulated ET-1 release in a concentration dependent manner with M log IC_{50} values of –4.7 ±0.13 (n=4) and –3.7±0.06 (n=4), respectively. Bezafibrate a PPARα agonist, at the highest concentration used of 300μM inhibited cytokine stimulated ET-1 production by 53.3±5.6% ($p<0.01$; n=4). Neither 15d-PGJ_2, bezafibrate nor WY 14643 affected HVSMC viability. Immunofluoresecent staining of HVSMCs with subtype-specific antisera for the PPAR isotypes detected immunoreactivity for PPARα, -δ and -γ receptors. Pre-incubation of antibody with specific peptide antigen (1:50) or staining with secondary antibody alone showed no specific staining (n=3).

Our data indicate that PPARα, -γ and -δ receptor subtypes are expressed in HVSMCs and PPAR activators inhibit cytokine-stimulated ET-1 release. These findings indicate a novel role for PPARs in vascular smooth muscle function. Future work will elucidate the signaling pathway further.

This work was supported by grants from The British Heart Foundation (FS/95003, PG/99001).

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98P RECEPTOR-MEDIATED INTERNALISATION OF A FLUORESCEIN LABELLED ENDOTHELIN-1 IN A HUMAN ENDOTHELIAL CELL LINE

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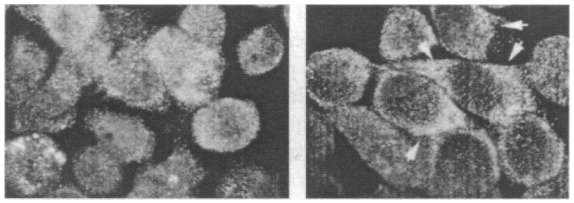
Increased circulating concentrations of endothelin-1 (ET-1) have been found in patients with a number of cardiovascular diseases (Levin, 1995). Although a causal role of the peptide has not been identified, there is evidence of prognostic value predicting clinical deterioration in patients with left ventricular hypertrophy and heart failure. We have recently observed increased expression of ppET-1 mRNA in animal models of both septic shock (Spiers *et al.*, 2000) and heart failure (Kelso *et al.*, 2000). The purpose of this study was to examine the fate of endothelin following binding to the receptor. We investigated the role of internalisation as a potential mechanism of clearance of the peptide in a cultured human endothelial cell line using fluorescein labelled ET-1 (FET-1).

ET-1 was labelled with fluorescein using a reactive dye pack and purified by thin layer chromatography (Panvera, USA). The integrity of the peptide and position of the label were confirmed by mass spectrometry. Biological activity of FET-1 was assessed using a rat aortic ring preparation. Thoracic aorta from female Wistar rats (225-275 g) were cut into rings, denuded of the endothelium, and mounted under 2g tension in organ baths (5 ml, 37°C, aerated with 95% O_2/ 5% CO_2) containing a modified Krebs buffer (KRB). Following stabilisation, the contractile response to increasing concentrations of ET-1 and FET-1 (0.1-3.1 nM) were established. Contractile response was expressed as a percentage of the maximal contractile response to 40 mM KCl. Human endothelial cells were plated onto 8 well TC chamber slides. Cells were washed with ice cold PBS and incubated at 4°C for 1 h in the presence of M199 supplemented with 50nM FET-1. Unbound FET-1 was removed by washing and the cells incubated for a further 1 h at 37°C to allow internalisation of the ligand-receptor complex. Cells were fixed, permeabilised and the fluorescent signal amplified (Alexa Fluo488 amplification kit; Molecular Probes) prior to mounting. The cells were visualised using a Leica DMIRB epifluorescence microscope and Improvision image analysis software.



Both ET-1 and FET-1 had similar logEC50 values (-8.8±0.29 vs –9.5±0.13) with respect to their contractile response. Under baseline conditions, the FET-1 was distributed uniformly on the surface of the cell membrane. Following incubation at 37°C for 1 hr, FET-1 was found to localise in the cytoplasmic/perinuclear region of the cell (arrows), depicting receptor-ligand internalisation.

In conclusion, internalisation may play a significant role in clearance of endothelin which would have implications for the clinical use of endothelin receptor antagonists as reduced clearance could lead to increased plasma levels of the peptide.

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This work was part funded by an equipment grant from the Health Research Board.

99P SYNTHESIS AND PRELIMINARY *IN VITRO* CHARACTERISATION OF A ^{18}F-LABELLED ANALOGUE OF SB209670, A PET RADIOLIGAND FOR THE ENDOTHELIN RECEPTOR

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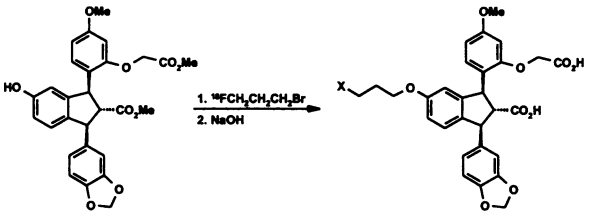
The very potent vasoconstrictor endothelin (ET) plays an important role in maintaining vascular tone by action on its two receptors ET_A and ET_B. Alteration in ET function has been suggested to play a role in a number of human vascular diseases (Miyauchi & Masaki, 1999). With positron emission tomography (PET) receptor-bound radioligands could be imaged and quantified *in vivo* with high sensitivity. The aim of this work was to develop a positron emitting radioligand as a means to study the significance of the ET receptor *in vivo* in normal and diseased tissue using PET.

Our target compound was SB209670 (Figure 1), an antagonist with high affinity for the ET_A and ET_B receptor (K_i = 0.43 nM and 14.7 nM, respectively) (Nambi *et al.*, 1994). The structure-activity evaluation of this compound indicated that the propyloxy moiety had little effect on the affinity for the receptor (Elliott *et al.*, 1994), suggesting that labelling with the positron emitting radionuclide ^{18}F in that position could be tolerated.

Figure 1. Structure of SB209670 (X=H) and synthesis of the fluorine-18 labelled analogue of SB209670 (X=^{18}F)



SB421672

The fluorine-18 labelled analogue of SB209670 was obtained by alkylation of SB421672 with 3-[^{18}F]fluoropropylbromide produced from [^{18}F]fluoride and 1,3-dibromopropane (Figure 1). [^{18}F]-SB209670 was synthesised from [^{18}F]fluoride in a total radiochemical yield of 17% in 156 min. The radiochemical purity of the isolated radioligand was 99%.

[^{18}F]-SB209670 was characterised using *in vitro* binding assays. Sections of human heart tissue were incubated at 23°C with either 1.4 nM [^{18}F]-SB209670 for increasing time periods (0-120 min, association experiment) or with increasing concentrations of [^{18}F]-SB209670 (3 pM - 10 nM, saturation experiment) for 1 hr. Non-specific binding was defined using 1 µM unlabelled ET-1.

Binding of [^{18}F]-SB209670 was time-dependant with an observed association rate constant (k_{obs}) of 0.182±0.032 min^{-1} at 23°C. Half time for association ($t_{1/2}$) was 3.8 min. [^{18}F]-SB209670 binds with subnanomolar affinity to the ET receptor with a Hill slope close to unity (Table 1).

Table 1. Saturation data (mean±s.e.mean) for [^{18}F]-SB209670 - human heart tissue

n	K_D (nM)	B_{max} (fmol/mg protein)	nH
3	0.67±0.14	164.6±27.5	1.12±0.16

In conclusion, [^{18}F]-SB209670 has been synthesised in good radiochemical yields from [^{18}F]fluoride. The subnanomolar affinity for the ET receptor was retained indicating that [^{18}F]-SB209670 might prove to be a potential radioligand for PET.

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Support by grants from the BHF and MRC Technology Foresight.

100P DIFFERENCES IN POTENCY AND EFFICACY OF VASODILATORS *IN VITRO* ARE DEPENDENT ON THE CHARACTERISTICS OF THE APPLIED PRETENSION

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The relaxing properties of vasodilator drugs may depend on the characteristics of the contractile state of the vessel investigated.

In an *in vitro* set-up (isometric recording in organ baths containing physiological salt solution of 37°C) we used the male Wistar rat (240-260g) isolated thoracic aorta. We investigated the effect of different concentrations of α_1-adrenoceptor agonist phenylephrine (PhE) and the thromboxane A_2 agonist U46619 (U-46) on the vasodilator's concentration response efficacy (E_{max}) and potency (pD_2) of methacholine (MCh, endothelium-dependent), sodium nitroprusside (SNP, NO-donor) and forskolin (FSK, adenylate cyclase activator). To evaluate the influx of extra cellular Ca^{2+}, we performed an additional experiment, with the partial α_1-adrenoceptor agonist ST-587. This agonist is known to be dependent for about 84% on extracellular Ca^{2+} to cause a contraction in vascular tissue (Chiu *et al.*, 1986). Concentration response curves were constructed with nifedipine.

Data is presented as means ± S.E.M., except the values of the ST-587/nifedipine experiment, which are presented as mean ± SD, n = 6 in each group. All results presented are significant ($p < 0.05$) according to the Student t-test.

PhE was used in concentrations of 0.1, 0.3, 3µM which resulted in a contractile response of 4.8±1.8, 6.5 ± 1.5 and 7.8±2.8 mN, respectively. U46 was used in 0.18, 0.3, 1µM which resulted in a contractile response of 7.4 ± 2.6, 8.8±1.9, and 10.4∀2.8mN.

An increase of the PhE concentration from 0.1 to 3µM did not influence the relaxation response to FSK, while it reduced the pD_2 of SNP (8.6∀0.1 to 7.3∀0.1). Under these conditions only the E_{max} of MCh was reduced in the realaxation response (96.3%±4.3% to 43.3%±6.9%). Increasing the concentration of U-46 from 0.18 to 1µM affected only the relaxing efficacies of SNP (84%±4.4% to 17%±8.8%) and MCh (64.5%±12.3% to 0.0%±9.2%), while a shift of potency was observed for FSK (7.9∀0.2 to 7.1∀0.1). ST-587 used in the concentrations 1, 3, 10 and 30µM resulted in contractile responses of 6.4±0.6, 6.6∀1.2, 7.0∀1.1 and 7.3∀0.6 mN. These successive concentrations resulted in comparable pD_2 and E_{max} (7.9±0.5 and 79.4% ± 0.1) for the relaxation, concentration response curves of nifedipine.

From the presented data we conclude that, at least *in vitro* experiments, the efficacy and the potency of vasodilators depend on the characteristics of the applied precontraction. This has to be considered when comparing the effects of dilator drugs under unmatched conditions.

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101P VASODILATOR EFFECT OF ANGIOTENSIN II IN THE ISOLATED MESENTERIC VASCULAR BED OF THE RAT

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Angiotensin II (Ang II) the most important autacoid of the Renin-Angiotensin System, induces vasoconstriction, increases sympathetic discharge and activates the production of growth factors. The peripheral and central effects of Ang II are dependent on activation of at least two receptors subtypes: AT_1 and AT_2. The effect of Ang II on the vascular smooth muscle is usually constriction, however on the isolated mesenteric vascular bed (IMVB) of the rat this peptide does not induce its classical vasopresor effect. Therefore, we assessed the action of Ang II on the IMVB of the rat precontracted with norepinephrine in order to examine if a vasodilator effect could occur in this vascular bed.

The vasodilator effect of Ang II was studied in perfused IMVB of the rat, according to the method of MacGregor (1965). The IMVB was perfused at a constant rate with Krebs'buffer (4 mL/min), warmed to 37° C and gassed (95% O_2: 5% CO_2). Perfusion pressure, recorded via an arterial catheter, was raised by approximately 80 mm Hg by titration of norepinephrine (1 to 10 μM) added to the perfusate and the vasodilator effect of bolus injections of Ang II was studied before and during pre-treatment with losartan (30 μM), deoxycholic acid (1mg/mL, 5 min), L-Name (0.3 mM), TEA (1 mM), indomethacin (0.1 μM), captopril (0.1 μM), HOE 140 (0.01 μM) or PD 123319 (10 μM). The vasodilator effect of Ang II (0.1-100 nmol) was not altered by losartan, indomethacin (data not shown), captopril, HOE 140 or PD 123319. In contrast, the vasodilator effect of Ang II was significantly (P<0.05) reduced by L-Name and endothelial removal by deoxycholic acid (Figure).

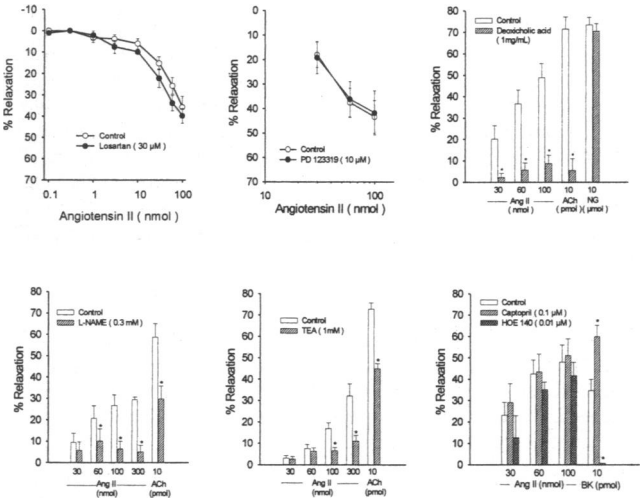

Figure 1. Vasodilator effect of Ang II, bradykinin (BK), acetylcholine (ACh) and nitroglycerin (NG) in the IMVB; n≥6 in each group.

These data indicate that in the IMVB of the rat Ang II induces an endothelium-dependent vasodilator effect that is not mediated by AT_1 or AT_2 receptors, prostaglandins or bradykinin release, but probably depends on NO and EDHF.

Reference: McGregor, D.D. (1965). *J. Physiol.* 177, 21-30.

102P 5-HYDROXYTRYPTAMINE RECEPTOR-MEDIATED VASOCONSTRICTION IS SELECTIVELY AUGMENTED IN PULMONARY RESISTANCE ARTERIES FROM FAWN HOODED RATS

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5-hydroxytryptamine (5HT) has a postulated role in pulmonary hypertension (PH). The Fawn hooded (FH) rat has an inherited susceptibility for the development of PH (Sato *et al.*, 1989). We have previously reported enhanced vasoconstriction to 5HT in perfused lungs of FH rats (Marriott *et al.*, 1991) and in pulmonary vessels from chronically hypoxic rats, in which responses to both $5HT_1$ and $5HT_2$ receptors stimulation are enhanced (MacLean *et al.*, 1996). In this study we have compared the vasoconstriction to 5HT in pulmonary and systemic resistance arteries of FH and 'normal' Wistar rats and examined the role of 5HT receptors by using 5HT, 5-carboxamidotryptamine (5CT, $5HT_1$ receptor agonist) and α-methyl-5HT ($5HT_2$ receptor agonist). U46619 (thromboxane A_2 receptor agonist) was tested as other vasoconstrictor.

Male FH and Wistar rats (10-12 wks) were sacrificed by an overdose of pentobarbitone sodium (*i.p.*). The pulmonary and mesenteric resistance arteries (100-200 μm) were dissected and mounted onto a wire myograph (Danish Myotech). The vessels were bathed in Krebs' solution bubbled with 21% O_2, /5% CO_2, balance N_2; 37°C. The vessels were subjected to tension to give transmural pressure equivalent to ~16 mmHg for pulmonary and ~100 mmHg for mesentery. The cumulative concentration response curves to 5HT, 5CT, α-methyl-5HT and U46619 were constructed. The maximum contraction (E_{max}) is expressed as % response of KCl (50 mM). Student's t-test was used to test the significance (P< 0.05, 4-8 rats per group, n= number of rings). Results are given as mean ± s.e.m.

The sensitivity (pEC_{50}) to, and the maximum response of, 5HT and α-methyl-5HT were significantly greater in pulmonary arteries of FH vs. Wistar rats (Table 1).

In addition, 5CT (0.1-30μM) produced greater contractions in pulmonary arteries of FH vs. Wistar rats (E_{max} to 30μM, in FH, 35 ± 6% vs. in Wistar, 13 ± 3 %, n=10; P<0.05).

In contrast, U46619 -mediated contractions in the pulmonary arteries of FH rats were similar in both strains (Table1).

Interestingly, in the mesenteric arteries, 5HT -evoked contractions in FH (pEC_{50} 6.98 ± 0.07, E_{max} = 154 ±8 %) vs. Wistar rats (pEC_{50} 6.66 ± 0.11, E_{max} = 153 ± 8 %, P> 0.05, n= 10) were not different.

In summary 5HT, α-methyl-5HT and 5CT-mediated vasoconstriction was augmented in the pulmonary arteries of FH compared to Wistar rats, whereas responses to U46619 were not altered. In contrast to the pulmonary arteries, 5HT-mediated contractions in the systemic resistance arteries were not altered in FH rats. These finding suggests that 5HT-induced vasoconstriction is selectively enhanced in the pulmonary resistance arteries of FH rats. $5HT_1$ and $5HT_2$ receptors appear to play an important role in the increased pulmonary vasoconstrictor sensitivity to 5HT in these rats.

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Sato, K., Webb, S., Tucker, A., *et al.* (1992). *Am. Rev. Respir. Dis.*, **145**, 793-797.

Table 1. Agonist-induced contractions in the pulmonary arteries.

Agonist	pEC_{50}		E_{max} (%)	
	Wistar	FH	Wistar	FH
5HT	5.66 ± 0.07 n=12	6.19 ± 0.07* n=18	60 ± 6 n=12	150±12* n=18
α methyl-5HT	5.93 ± 0.06 n=9	6.48 ± 0.07* n=10	25 ± 5 n=9	120±11* n=10
U46619	7.76 ± 0.09 n=7	8.07 ± 0.19 n=4 (NS)	145 ± 6 n=7	153±9 n=4(NS)

{*P< 0.01; NS, not significant; Wistar vs. FH pulmonary arteries}.

103P ANTAGONISM OF 5-HT-INDUCED CONTRACTION OF PORCINE ISOLATED CORONARY ARTERY BY SL 65.0472

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5-hydroxytryptamine (5-HT)-induced contractions of porcine isolated coronary arteries have been reported to be mediated by an atypical 5-HT_{2A} receptor or by a combination of 5-HT_{2A} and 5-HT_{1B}/5-HT_{1D} receptors depending on the experimental conditions (Cushing & Cohen, 1993; Hinton *et al.*, 1999). SL 65.0472 is a novel 5-HT receptor antagonist which has been shown to potently antagonise 5-HT_{1B}- and 5-HT_{2A}-receptor mediated vasoconstriction (O'Connor *et al.*, 2000). In this study we have investigated the antagonist properties of SL 65.0472 in porcine coronary arteries in the context of the 5-HT receptor subtypes involved.

Left anterior descending coronary arteries were obtained from Yucatan pigs post-mortem. Endothelium-denuded ring segments were mounted in a myograph for measuring changes in tension in Krebs solution at 37°C. Tissues were exposed to pargyline (500 µM) for 30 min and then washed. A single cumulative concentration response curve was performed to 5-HT in each tissue and responses expressed as a percentage of the response to 60 mM KCl. 5-HT contracted porcine coronary arteries with an EC_{50} of 0.52 ± 0.07 µM, E_{max} 56 ± 3% (n=14). In the presence of SL 65.0472 (0.1 – 10 nM, n=3-6) the concentration response curve to 5-HT was inhibited in non-competitive fashion with reductions in E_{max}. For example, following 1 nM SL 65.0472, 5-HT gave an EC_{50} of 4.7 ± 4 µM and an E_{max} of 19 ± 4%. The apparent pK_B value for SL 65.0472 was 9.4. By contrast, ketanserin (100 nM, n=3) displaced the 5-HT concentration response curve to the right in a parallel fashion without changing E_{max} (pK_B 9.1).

The affinity of SL 65.0472 for human recombinant 5-HT receptor subtypes determined according to previously described methods (Domenech *et al.*, 1997; Bonhaus *et al.*, 1995) was as follows. 5-HT_{2A} receptors IC_{50} 0.34 ± 0.04 nM (^{3}H-ketanserin binding in CHO-K1 cells, n=3), 5-HT_{1B} receptors 19 ± 4 nM (^{3}H-GR125743 binding in Hela cells, n=3), 5-HT_{1D} receptors IC_{50} 214 ± 46 nM (^{3}H-5-CT binding in CHO-K1 cells, n=3).

Hence, SL 65.0472 is a potent, non-competitive antagonist of 5-HT-induced contractions of porcine isolated coronary arteries. Its potency in this functional study would appear to be consistent with its 5-HT_{2A} receptor affinity. By contrast, in human coronary arteries, SL 65.0472 antagonises the effects of 5-HT with a pA_2 value of 8.8 (Galzin *et al.*, 2000) which is compatible with reports of a mixed 5-HT_{1B}/5-HT_{2A} receptor population in this tissue (Kaumann *et al.*, 1994).

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104P EFFECT OF TEMPERATURE ON 5-HYDROXYTRYPTAMINE-MEDIATED VASOCONSTRICTION OF EQUINE DIGITAL VEINS

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The circulation of the digit of the horse possesses numerous arterio-venous anastomoses probably reflecting its important role in thermoregulation (Molyneux et al., 1994). Ischaemia of the digit followed by reperfusion is thought to underlie the pathophysiology of the painful disease, laminitis. Cooling the feet has been advocated as a treatment for this condition yet cooling has also been shown to potentiate the effects of some vasoconstrictor agents (Harker et al., 1991). 5-hydroxytryptamine (5-HT) has been implicated as a possible trigger factor for equine laminitis (Bailey & Elliott, 1998a). The aim of this study was to determine the effect of temperature on responses of equine digital veins (EDV) to 5-HT-receptor mediated vasoconstriction.

EDVs were obtained from mixed breed adult horses killed (n=6 to 8) at an abattoir. Rings of EDV (4 mm) were cut, denuded of their endothelium and prepared for isometric tension recording as previously described (Bailey & Elliott 1998b). EDV were incubated either at room temperature (c22°C) or at 37°C and cumulative concentration response curves (CRCs) were obtained to 5-HT, sumatriptan (SUM) or α-methlyl 5-HT (α-Me) (all at 10^{-10} to 10^{-4} M). CRCs were fitted by computerised non-linear curve fitting and EC_{50} and E_{max} values compared at room temperature and 37°C for each agonist using a Student's *t*-test.

Vessels incubated at room temperature were significantly more sensitive to all three agonists tested with a 3.0, 3.7 and 2.2 fold decrease in EC_{50} values for 5-HT, SUM and α-Me respectively. The E_{max} value was significantly higher at room temperature in the case of SUM. There was no change in the efficacy of either 5-HT or α-methyl 5-HT. These data are presented in Table 1.

Table 1.

	EC_{50} (10^{-8} M) geometric mean (95% CL)		E_{max} (g/mg tissue) Arithmetic mean ± sem	
	37°C	Room temp	37°C	Room temp
5-HT	6.3 (4.9-8.5)	2.1 (1.6-2.7)[a]	0.53 ± 0.01	0.65 ± 0.02
SUM	12 (4.1- 35)	3.2 (1.3-7.7)[b]	0.15 ± 0.07	0.23 ± 0.03[b]
α-Me	6.0 (3.4-11)	2.7 (1.3-5.5)[a]	0.67 ± 0.03	0.64 ± 0.03

[a] denotes $P<0.05$ and [b] denotes $P<0.001$ vs. values obtained at 37°C using a Student's *t*-test.

These data demonstrate that cooling significantly increases vasoconstrictor response, particularly from stimulation of the 5-$HT_{1D/1B}$ receptor. This finding may be of importance in understanding the pathophysiology of equine laminitis.

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105P UPTAKE OF 5-HYDROXYTRYPTAMINE BY EQUINE DIGITAL VEIN ENDOTHELIAL CELLS: INHIBITION BY AMINES FOUND IN THE EQUINE CAECUM

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Acute laminitis is a painful ischaemia and reperfusion injury affecting the equine digit, which may be associated with the bacterial fermentation of carbohydrate in the hind gut: 5-hydroxytryptamine (5-HT) has been implicated as a possible trigger factor (Bailey & Elliott, 1998), and it is postulated that amines formed by gut bacteria may potentiate the effects of this vasoconstrictor by slowing its removal from the circulation. This study has examined the kinetics of the uptake of 5-HT by equine digital endothelial cells and the effects on this process of various monoamines found in the equine caecum.

Equine digital venous endothelial cells (EDVEC) were cultured from digits of adult mixed breed horses obtained at abattoir (Bailey & Cunningham, 1999). The cells were grown to confluency in Dulbecco's modified Eagle medium containing 10% foetal calf serum, 10% newborn calf serum and 100 IU/ml penicillin and streptomycin. Confluent monolayers in 24 well plates (2 $x10^5$ cells /well) were then incubated for a period of 30 min at 37°C in serum-free medium to which [^{3}H]5-HT (1-250 μM) was added. The cells were then washed five times with PBS, lysed with 0.15 M NaOH, and the activity of the cell contents quantified using a scintillation counter. Active uptake of 5-HT was expressed in pmoles /10^6 cells/ 30 min after subtracting blank values (wells with medium alone) and non-facilitated 5-HT diffusion (Pearson et al, 1977). In subsequent experiments, uptake of 5 μM [^{3}H]5-HT was measured over an incubation period of 60 min in the presence of one of the following amines: tyramine, tryptamine, phenylethylamine, isobutylamine, isoamylamine or spermine (1 μM – 1 mM). IC_{50} values and the amount of maximum inhibition were calculated for each amine (n=4 experiments).

EDVEC showed active uptake of [^{3}H]5-HT with a Km value of 41.6 ± 9.3 μM and a Vmax of 397.1 ± 27.7 pmoles /10^6 cells /30 min. The effects of the other amines on the uptake of 5-HT (5μM) are given in Table 1.

Table 1. Effects of amines on 5-HT uptake into EDVEC

amine	IC_{50} value geometric mean (95% CI)	Max inhibition (mean ±s.e.m.)
Tryptamine	$3.7 x10^{-6}$ M (3.1-4.3)	45.8 ± 4.9
Tyramine	$2.8 x10^{-5}$ M (1.8-4.4)	34.8 ± 3.3
Spermine	$6.4 x10^{-5}$ M (4.7-8.7)	41.1 ± 4.2
Phenylethylamine	$9.3 x10^{-5}$ M (4.4-19.4)	30.5 ± 4.5
Isoamylamine	ND	ND
Isobutylamine	ND	ND

ND not determined – no maximum reached at 1 mM concentration

These data suggest that endothelial uptake may play an important role in modulating the response of the digital circulation to 5-HT and that its inhibition by other monoamines may therefore potentiate the vasoconstriction caused by 5-HT.

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106P POST-JUNCTIONAL α_1- AND α_2-ADRENOCEPTOR-MEDIATED CONTRACTILE RESPONSES IN HUMAN RESISTANCE ARTERIES VARY WITH THE VASCULAR BED

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Nielson *et al.* (1990;1991) demonstrated the presence of post-junctional α_1- and α_2-adrenoceptors in human resistance arteries and also found that the α_2-mediated responses are predominant in resistance arteries. In this study α_1- and α_2-adrenoceptor-mediated responses in resistance arteries from three different anatomical regions - Inguinal subcutaneous (Ing), gluteal subcutaneous (Glt) and medialis/gastrocnemius skeletal muscle (SkM) - were compared using the agonists noradrenaline (NA, non-selective), phenylephrine (PE, α_1-adrenoceptor selective) and brimonidine (UK14304) (BR, α_2-adrenoceptor selective).

SkM-arteries (normalised diameter $L_{0.9}$=257±26 μm, n=9) were obtained from non-ischaemic areas of limbs amputated for critical limb ischaemia. Ing- ($L_{0.9}$=337±29 μm, n=8) and Glt-arteries ($L_{0.9}$=269±11 μm, n=5) were obtained from hernial operative biopsies and gluteal biopsies. Arterial segments were mounted on a small vessel wire myograph and normalised according to Mulvany & Halpern (1977). Cumulative concentration response curves to the agonists were obtained in a random order after activation with 123 mM KCl and 10 μM NA. Responses were expressed as effective resting transmural presure (ERTP) calculated according to the equation:

ERTP (kPa)=Wall tension/(Internal circumference/2π).

The ERTP obtained with 123 mM KCl in the arterial segments from all three vascular beds was similar. Maximum contractile responses and pEC_{50} values for the agonists are given in Table 1. In Ing- and SkM-arteries, pEC_{50} values for the three agonists were similar. Maximum responses to NA and PE were similar and greater than that produced by BR ($P<0.01$). In Glt-arteries pEC_{50} values for NA and BR were significantly higher than those of PE ($P<0.05$).

Table 1. Maximum contractile responses (ERTP, kPa) and pEC_{50} values of the agonists.

Tissue	NA		PE		BR	
	pEC_{50}	Mx Rsp	pEC_{50}	Mx Rsp	pEC_{50}	Mx Rsp
Ing	6.6±0.1	21±2	6.1±0.1	18±2	6.3±0.4	7±2
Glt	7.0±0.3	20±3	5.9±0.1	19±2	7.1±0.3	18±2
SkM	6.1±0.3	8±1	6.1±0.2	5±1	6.8±0.6	1±0.4

These results show that α_2-adrenoceptors contribute more than α_1- to NA-mediated responses in Glt-arteries but not the Ing- and SkM-arteries. The contribution of α_1- and α_2-adrenoceptors to the peripheral arterial resistance, thereby the mean arterial pressure, varies with the vascular beds from different anatomical regions.

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107P ELASTIC PROPERTIES OF THE AORTIC WALL IN α-1β-ADRENORECEPTOR KNOCKOUT MICE

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Studies evaluating the impact of changes in vascular smooth muscle tone on arterial wall elasticity have given contradictory results. To gain insight into this problem we evaluated aortic wall elasticity in alpha-1B-adrenoreceptor knockout mice ($\alpha_{1b}^{-/-}$), characterized by an hyporeactivity of smooth muscle cells to adrenergic α_1 vasoconstrictors (Cavalli et al., 1997), at baseline and following drug-induced hypertension with adrenergic or non adrenergic- vasoconstrictors.

Five mo-old C57BL/6J $\alpha_{1b}^{-/-}$ (n = 18) and control $\alpha_{1b}^{+/+}$ (n = 15) mice were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and cannulated with 3 polyethylene cannulas (intravascular portion 0.61/0.32 mm o.d./i.d., extravascular portion 0.96/0.58 mm): one into the descending thoracic aorta for measurement of mean aortic pressure (MAP) and heart rate, the second into the abdominal aorta for measurement of the thoraco-abdominal pulse wave velocity (PWV, according to the method previously used and extensively verified in rats, Niederhoffer et al., 1997) and the third into the abdominal vena cava for drug infusion. After a 20-minute's habituation period, baseline MAP, heart rate and PWV were determined, then mice received increasing doses of either phenylephrine, angiotensin II or vasopressin, in order to increase MAP from baseline to 170-180 mmHg. PWV was expressed as a function of MAP using an exponential regression ANOVA ($PWV = b.e^{a(MAP)}$). The slope of the exponential relationship (a) was used as a pressure-independent index of elasticity.

Results are expressed as means±s.e.m. and compared with an ANOVA associated to the Bonferroni test.

Under baseline conditions, MAP, heart rate and PWV were not different between $\alpha_{1b}^{-/-}$ mice (94±4 mmHg, 379±22 bpm, 347±15 cm/s) and $\alpha_{1b}^{+/+}$ (93±4 mmHg, 376±21 bpm, 361±23 cm/s). The dose of vasoconstrictor required to induce a similar increase in MAP in $\alpha_{1b}^{-/-}$ and $\alpha_{1b}^{+/+}$ mice was similar in the case of angiotensin II (28±8 and 28±2 μg/kg) and vasopressin (34±7 and 27±4 μg/kg), but the dose of phenylephrine doubled in $\alpha_{1b}^{-/-}$ (1220±379 μg/kg) compared to $\alpha_{1b}^{+/+}$ mice (659±308 μg/kg). Pressure-independent indices of aortic elasticity were similar in all groups of mice (Table 1).

Table 1: Slopes (10^{-3} cm/s.mmHg) of the exponential PWV-MAP curves following infusion of vasoconstrictors in $\alpha\alpha_{1b}^{-/-}$and $\alpha_{1b}^{+/+}$ mice (n = 5 to 6 per group).

	Phenylephrine	Angiotensin II	Vasopressin
$\alpha_{1b}^{-/-}$	7.3±0.6	8.6±0.5	7.0±0.8
$\alpha_{1b}^{+/+}$	7.6±0.6	7.0±0.7	5.7±0.4

$Alpha_{1b}^{-/-}$ mice showed hyporeactivity to phenylephrine, but no change in elastic properties of the aortic wall. Furthermore, the vasoconstrictors used had no specific impact on aortic wall elasticity. Given the proviso that drugs used may produce changes in aortic diameter (which was not measured), our results suggest that a change in smooth muscle cell tone is not an important determinant of elastic properties in the aortic wall.

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108P EFFECTS OF OXIDATIVE STRESS ON THE α1-ADRENOCEPTOR-INDUCED CONTRACTIONS IN THE RAT ISOLATED PORTAL VEIN.

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Oxidative stress has been shown to alter the function of autonomic receptors (Peters *et al*, 1998). In isolated rat left atria, exposed to electrically generated oxygen free radicals, we found an inversion of the α_1-adrenoceptor-induced positive inotropic effect. In the present study, we investigated whether this effect can be observed in another, non-cardiac tissue as well. The rat portal vein is a model to investigate drug effects on spontaneous and stimulated vascular myogenic activity.

Stimulation of α_1-adrenoceptors by full agonists has been shown to result in a typical biphasic response (Schwietert *et al*, 1991). In a low concentration range the agonist augments the amplitude of the spontaneous myogenic contractions whereas the baseline rises at higher concentrations.

Isolated portal veins from Male Wistar rats of 250-300 g were mounted in 5 ml organ baths. The isometric force of contraction was recorded under a constant pre-tension of 5 mN. The Tris buffered Krebs solution (37°C, pH 7.4) used contained 2.5 mM calcium for all experiments. Oxygen free radicals were generated by a constant current of 30 mA for 75 sec via electrodes at the bottom of the organ bath (Peters *et al.*, 1998). The spontaneous myogenic activity was recorded for half an hour before the electrolysis was applied. Cumulative concentration-response curves of the selective α_1-adrenoceptor agonists methoxamine (0.1 :M - 0.3 mM) and cirazoline (1 nM-30 :M) were constructed and the phasic and tonic part of the response analysed separately. At the end of each experiment, the preparations were exposed to an isotonic 60 mM KCl solution to test the maximal, depolarization-induced contraction. All results are given as mean values ± standard deviation of the mean of 4-6 experiments.

The electrolysis resulted in an immediate decrease of the spontaneous myogenic activity of 26.8 ± 9.1% (n=10). The potency ($-\log EC_{50}$, M) of methoxamine in inducing an increase in the phasic was unaltered, whereas for the tonic response it was reduced (Phasic: 5.7 ± 0.1, control n=6 vs. 5.4 ± 0.1, n=6 electrolysis; tonic 5.0 ± 0.04 control vs. 4.7 ± 0.07 electrolysis). However, the maximal effects were significantly reduced for both types of responses (Phasic: 5.22 ± 0.62 mN control *vs.* 2.46 ± 0.11 mN electrolysis; tonic: 5.2 ± 0.14 mN control *vs.* 2.44 ± 0.13 mN electrolysis). The same pattern holds true for cirazoline ($-\log EC_{50}$, M Phasic: 8.2 ± 0.1, control n=4 vs. 8.1 ± 0.06, n=4 electrolysis; tonic 6.3 ± 0.04 control vs. 5.0 ± 0.1 electrolysis; Emax Phasic: 3.80 ± 0.19 mN control *vs.* 2.98 ± 0.07 mN electrolysis; tonic: 4.08 ± 0.07 mN control *vs.* 2.63 ± 0.21 mN electrolysis) The KCl-induced contraction was not different in the two groups (7.63 ± 0.79 mN control n=10, 6.84 ± 0.38 mN electrolysis n=10).

From the presented data we conclude that oxygen free radicals, generated by electrolysis, impair the spontaneous myogenic activity as well as the reaction to α_1-adrenergic stimulation in the isolated rat portal vein. Although a quantitative difference could be observed, the exposure to oxygen free radicals did not change the quality of the response. A possible explanation of the discrepancy between the findings in cardiac and vascular tissue might be a difference in the susceptibility of the effector pathways of these two structures to oxygen free radicals.

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109P A PEPTIDE ACTIVATING PROTEASE ACTIVATED RECEPTOR -2 REDUCES THE VASCULAR RESPONSE TO PHENYLEPHRINE *IN VITRO* AND *IN VIVO*

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Protease activated receptor 2 (PAR-2) is a member of G protein coupled receptor activated by proteolytic cleavage (Dery *et al.*, 1998). *In vitro* activation of PAR-2 by synthetic peptides causes endothelium-dependent vasorelaxation. *In vivo*, on experimental animals, PAR-2 activation causes hypotension, only partially mediated by nitric oxide (Emilsson *et al.*, 1997). We have recently shown that hypotension induced by a PAR-2 activating peptide (PAR-2AP) is strongly increased in endotoxemic animals. This effect is paralleled by an increased expression of PAR-2 on vascular endothelium and smooth muscle (Cicala *et al.*, 1999), suggesting a role for the receptor in the development of endotoxic shock. The aim of this study was to evaluate the effect of PAR-2AP (SLIGRL) on the pressor response to phenylephrine (PE) both *in vivo* and *in vitro*.

Male Wistar rats (Charles River, 200-250g) were anaesthetised with urethane (sol 10% w/v; 10 ml/kg ip.), the right jugular vein was cannulated for drug administration and the left carotid artery was cannulated and connected to a pressure transducer for a continuous monitoring of blood pressure. Animals were pre-treated with the ganglion-blocking agent, chlorisondamine (2.5 mg/kg ip.). After blood pressure stabilisation, the pressor response to PE (10 µg/kg iv) was evaluated before and after 1 minute infusion of PAR-2AP (1mg/kg/min iv.).

In vitro experiments were performed on thoracic aorta excised from rats (200-250 g) sacrificed by exanguination. The tissue was cleaned from fat and cut in rings of 2-3 mm width, placed in an organ bath filled with oxygenated (95% O_2-5% CO_2) Krebs solution at 37°C and connected to an isometric transducer under resting tension of 0.5 g. After standardisation with PE (10^{-6} M), a stable contraction of the tissue was obtained with PE (10^{-6} M) and a cumulative concentration-response curve to Ach (10^{-8} -10^{-5} M) was performed to check the presence of an intact endothelium. A cumulative concentration response curve to PE (10^{-8}- 3 x 10^{-6} M) was then evaluated before and 15 minutes after incubation of the tissue with PAR-2AP (10^{-5} M). All results are expressed as mean ± s.e.m and analysed by using analysis of variance (ANOVA) followed by Bonferroni's test.

In vivo, administration of PE caused an increase in mean arterial blood pressure (MABP) of 62.00 ± 3.16 mmHg, that was significantly reduced to 14 .50 ± 2.36 mmHg after PAR-2AP infusion ($p<0.01$; n=4). Analysis of the concentration response curve to PE, obtained *in vitro*, showed that incubation of the tissue with PAR-2AP significantly reduced PE-induced contraction ($p<0.01$, two ways ANOVA; n = 4). In the presence of PAR-2AP, the EC50 of PE was increased from 4.8 ± 1.5 x 10^{-8} M to 1.1 ± 0.2 x 10^{-7} M ($p<0.05$), while Emax did not change. Our results show that PAR-2 activation reduces the vascular response to phenylephrine.

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110P VASCULAR ACTIONS OF MDMA INVOLVE α-ADRENOCEPTORS AND 5-HT RECEPTORS

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We have investigated the effects of methylenedioxymethamphetamine (MDMA, 'ecstasy'), given intravenously, on diastolic blood pressure (DBP) in pithed and pentobarbitone anaesthetised rats, given that we have recently shown that MDMA has major actions as an agonist at α_2-adrenoceptors (Lavelle et al., 1999). An α_2-adrenoceptor agonist would be predicted to have central depressor and peripheral pressor actions by analogy to agonists such as clonidine.

Wistar rats (250-350g, male) were anaesthetised with pentobarbitone (Sagatal) (40 mg/kg, i.p., supplemented with 10 mg/kg, i.v. per hr) and respired with room air, or pithed under ether anaesthesia and respired with 100% O2. The jugular vein was cannulated for drug injection and the carotid artery for blood pressure recording. MDMA (5mg/kg) was injected following vehicle or test drug: the α_1-adrenoceptor antagonist prazosin, the α_2-adrenoceptor antagonist methoxyidazoxan (MI), the non-selective 5-HT antagonist methiothepin (MET), the 5-HT-2 antagonist ritanserin, the noradrenaline re-uptake blocker cocaine.

In pithed rats, pressor responses to MDMA were significantly reduced by prazosin (0.1mg/kg) or MET (0.1mg/kg), but not by ritanserin (1mg/kg). In anaesthetised rats, MDMA produced an initial pressor response of 34.4±2.6mmHg (mean±s.e.m.), followed by a sustained depressor response (maximum; -48.3±4.2mmHg, n=12). The combination of ritanserin and prazosin significantly reduced the initial pressor response to 18.2±3.6mmHg (P,0.05, analysis of Variance and Dunnett's test), although neither compound alone had any effect. The response to MDMA at 1 min was converted from a pressor response (14.3±3.4mmHg, n=12) to a depressor response by prazosin (0.1mg/kg) (-23.9±3.5mmHg, n=9). The maximum depressor response to MDMA was significantly reduced by MI (0.1mg/kg) (-32.6±4.0mmHg, n=7), and by cocaine (10 mg/kg) (-31.0±2.6mmHg, n=11).

It is concluded that the initial pressor response to MDMA (5mg/kg) involves α_1-adrenoceptors and 5-HT-2 receptors changing to a predominantly α_1-adrenoceptor response by 1 min, and the sustained depressor response to MDMA in the anaesthetised rat involves α_2-adrenoceptors.

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111P PERTUSSIS TOXIN INHIBITS THE DIURETIC AND NATRIURETIC EFFECTS OF SPHINGOSINE-1-PHOSPHATE AND SPHINGOSYLPHOSPHORYLCHOLINE IN ANAESTHETIZED RATS

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We have previously reported that i.v. infusion of the sphingolipids sphingosine-1-phosphate (SPP) and sphingosylphosphorylcholine (SPPC) increase diuresis and natriuresis in anaesthetized rats (Bischoff et al. 1999a, 1999b). Since SPP and SPPC can have intracellular and receptor-mediated effects and since the latter frequently are blocked by pertussis toxin (PTX) treatment (Meyer zu Heringdorf et al. 1997), we have investigated the effects of PTX treatment on diuresis and natriuresis induced by SPP (study I) and SPPC (study II) in anaesthetized rats.

Male Wistar rats (259-463 g) were anaesthetized with ketamine (100 mg kg^{-1}), and given PTX (10 μg kg^{-1}) or vehicle via the jugular vein (n = 6-7 per group). Three days later the rats were anaesthetized with thiobutarbitone (100 mg kg^{-1}) and surgically prepared as previously described (Bischoff et al.1996). Briefly, the femoral artery was catheterized for mean arterial pressure (MAP) measurements by a Statham transducer, the femoral vein was catheterized for volume substitution and sphingolipid infusions. Renal blood flow (RBF) was determined by a flow sensor placed on the right renal artery. Urine was collected from the cannulated ureters in 15 min collection periods. SPP and SPPC (30 μg kg^{-1} min^{-1} i.v. each) or vehicle were infused for 60 min. Statistical significance of PTX and sphingolipid effects was determined by two-tailed t-tests and two-way analysis of variance for overall treatment effects, respectively, with $P < 0.05$ considered significant. Data are mean ± s.e.m.

PTX-treatment significantly lowered basal urine flow rate in studies I and II (98 ± 21 vs. 180 ± 27 and 72 ± 8 vs. 165 ± 24 μl/15 min; n = 12-14 each, $p < 0.05$) and MAP in study I (85 ± 2 vs. 113 ± 2 mm Hg), while alterations of MAP in study II (90 ± 5 vs. 89 ± 7 mm Hg) and of RBF (7.1 ± 0.6 vs. 8.0 ± 0.4 6.3 ± 0.3 vs. 6.7 ± 0.5 ml min^{-1}) and Na^+ excretion (10 ± 4 vs. 17 ± 5 and 10 ± 2 vs.11 ± 3 μmol/15 min) in both studies did not reach statistical significance.

Infusion of SPP significantly but transiently lowered RBF by maximally 0.9 ± 0.3 ml min^{-1} in control but not in PTX-treated rats without significantly affecting MAP under either condition. Our new data show that SPP infusion significantly increased diuresis and natriuresis (e.g. by 217 ± 58 μl and 31 ± 10 μmol above basal, respectively, in the final 15 min collection period) in control rats but relative to vehicle did not significantly increase them in PTX-treated rats. Infusion of SPPC did not significantly affect RBF or MAP in control or PTX-treated rats. However, SPPC infusion significantly increased diuresis and natriuresis (e.g. by 138 ± 31 μl and 23 ± 8 μmol above basal, respectively, in the final 15 min collection period) in control rats but relative to vehicle did not significantly increase them in PTX-treated rats.

We conclude that SPP- and SPPC-induced diuresis and natriuresis in anaesthetized rats is PTX-sensitive and therefore likely to occur via receptors coupling to G-proteins of the $G_{i/o}$ type.

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112P VASORELAXATION TO 17 β-OESTRADIOL IN THE RAT ISOLATED MESENTERIC ARTERIAL BED

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The mechanisms of vasorelaxation to 17β-oestradiol are at present unclear. Stefano *et al.* (2000) recently demonstrated that 17β-oestradiol induces vasorelaxation via nitric oxide (NO). In contrast, Naderali *et al.* (1991) showed that relaxations to 17β-oestradiol were unaffected by NO synthase inhibitors. It has also been proposed that large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels are involved in the actions of 17β-oestradiol (White *et al.*, 1995). The aim of this study was to examine the roles of NO and K^+ channels in vasorelaxation to 17β-oestradiol in the rat mesenteric arterial bed.

Male Wistar rats (250-350g) were anaesthetized with sodium pentobarbitone (60mg kg^{-1}, i.p.) and exsanguinated. The mesenteric arterial bed was isolated (McCulloch *et al.*, 1997) and perfused with oxygenated Krebs-Henseleit solution. Following 30min equilibration, methoxamine was added to increase perfusion pressure (100-150mmHg). 17β-oestradiol was added cumulatively to the perfusion fluid (10pM-10μM). The vasorelaxant effects of 17β-oestradiol were assessed in the presence of 10μM indomethacin, and either 300μM N^G-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor or 300μM tetrabutlyammonium (TBA), a relatively selective inhibitor of K^+ channels. In other experiments, 60mM KCl was added to induce tone and to the investigate the effects of high extracellular K^+ on responses to 17β-oestradiol (McCulloch *et al.*, 1997). All data are expressed as mean ± s.e.mean. Values between two groups were compared by the Student's *t*-test for unpaired values.

In normal K^+ solution, 17β-oestradiol (10pM-10μM) caused concentration-related relaxations of methoxamine-induced tone (EC_{50} = 0.97±0.4 nM, maximal relaxation, R_{max} = 77.9±2.4%, n=12). L-NAME (300μM) had no effect on the potency or maximal response to 17β-oestradiol (EC_{50} = 1.1±0.6 nM, R_{max} =83.6±3.9%, n=7). In contrast, vasorelaxation to 17β-oestradiol (10pM-3μM) was abolished when tone was raised by 60mM KCl, except at 10μM 17β-oestradiol, when there was a relaxation of 59.9±14.5% (n=4). In the presence of TBA (300μM) vasorelaxation to 17β-oestradiol (100pM-3μM) was also abolished and only 10μM 17β-oestradiol induced a relaxation (60.3±5.6%; n=5).

This study has demonstrated that 17β-oestradiol causes potent vasorelaxations which are unlikely to be mediated via NO. High extracellular K^+ inhibited relaxations to 17β-oestradiol, which suggests that 17β-oestradiol induces vasorelaxation by increasing K^+ efflux. Consistent with this, the responses were also sensitive to TBA. It therefore seems likely that vasorelaxation to 17β-oestradiol involves K^+ channel activation. Whether this is endothelium-dependent (i.e. via endothelium-derived hyperpolarizing factor) or not is currently being investigated.

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113P VASORELAXATION TO CP55940 AND HU210 IN THE RAT ISOLATED MESENTERIC ARTERIAL BED IS NOT MEDIATED BY THE CB_1 CANNABINOID RECEPTOR

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The vasorelaxant effects of cannabinoid agonists are well recognised. However, the involvement of the CB_1 cannabinoid receptor in this vasorelaxation is disputed (Kunos *et al.*, 2000). We have now investigated the involvement of this receptor in the vasorelaxation to the cannabinoid agonists CP55940 ([α,2β-(R)-5α]-(-)-5-(1,1-dimethylheptyl) -2- [5-hydroxy-2- (3-hydroxypropyl) cyclohexyl]-phenol) and HU210 ((-)-11-hhydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl) in the rat isolated mesenteric arterial bed, using the CB_1 cannabinoid receptor antagonists SR141716A (*N*-piperindin-1-yl) -5- (4-cholorophenyl) -1- (2,4-dichlorophenyl) –4-methyl-1 *H*-pyrazole -3-carboxamide hydrochloride) and LY320135 ([6-methoxy-2-(4-methoxyphenyl) benzo[*b*] thien-3-yl] [4-cyanophenyl] methanone) (Pertwee, 1997).

Male Wistar rats (250-320g) were stunned and decapitated. The mesenteric arterial bed was cannulated via the superior mesenteric artery and perfused at 5ml min^{-1} with oxygenated Krebs-Henseleit solution (McCulloch & Randall, 1996). Perfusion pressure was monitored continuously by a pressure transducer placed close to the inflow cannula. Following 30min equilibration, perfusion pressure was increased by addition of methoxamine (5-20μM), and concentration-response curves were constructed to the cannabinoid agonists CP55940, HU210 and the stereoisomer of HU210, HU211 ((+)(3S,4S), 7-hydroxy-Δ^6-tetrahydrocannabinol-1,1-dimethylheptyl). The effects of the cannabinoid antagonists SR141716A (1,3 and 10μM) and LY320135 (2μM) on agonist concentration-response curves were also investigated.

Basal perfusion pressure was (20.8±4.2mmHg, mean±sem; n=35) and was increased by 69.2±9.4mmHg following the addition of 5-20μM methoxamine. CP55940 induced concentration-related reductions of established tone with a maximum relaxation (R_{max}) at HU210 also induced concentration-related vasorelaxation, maximum relaxation at 10μM being 66.0±10.9% (n=6). 1μM and 3μM SR141716A had no significant effect on maximum relaxation with the response to 10μM HU210 being 70.1±6.7% at 1μM (n=6) and 54.4±8.5% at 3μM SR141716A (n=3). At 10μM SR141716A, relaxation to HU210 was abolished, and resulted in constriction of 10.4±15.7% (n=5). HU211 induced concentration-dependent vasorelaxation, and at 10μM relaxation was 82.4±3.1% (n=3). This was not significantly different to vasorelaxation obtained in the presence of 10μM HU210. The presence of 1μM or 3μM SR141716A had no significant effect on vasorelaxation, with response to 10μM HU211 being 70.0±3.3% at 1μM (n=3) and 51.5±11.2% at 3μM SR141716A (n=5). 10μM SR141716A abolished vasorelaxation and resulted in vasoconstriction of 25.1±25.0% (n=3). 2μM LY320135 had no significant effect on relaxation to CP55940, maximum relaxation to 10μM being 84.6±2.8% (n=4). There was also no significant effect of 2μM LY320135 on vasorelaxation to HU210 (R_{max}=75.7±5.6% at 10μM HU210; n=5) or HU211 (R_{max}=74.7±5.3% at 10μM HU211; n=3).

The low potency of the agonists, the lack of stereoselectivity and the absence of antagonist effects at the relevant concentrations, indicate that the CB_1 cannabinoid receptor is not involved in vasorelaxation to the cannabinoid agonists CP55940 and HU210 in the rat isolated mesenteric arterial bed. The mechanisms of relaxation are under investigation.

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114P DISTRIBUTION OF THROMBOXANE RECEPTORS IN THE NORMAL HUMAN HEART AND ITS ALTERATION WITH DISEASE.

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The potent vasoconstrictor thromboxane A_2 (TXA_2) exerts a positive inotropic effect on the heart (Sakuma et al., 1989). Previous studies have shown that TXA_2 is synthesised from human cardiac atrial tissue (Mehta & Mehta., 1985). Animal studies have established the presence of specific thromboxane receptors (TP) in cardiac membrane preparations (Bowling *et al.*, 1994). Therefore, the aim of this study was to determine TP receptors in human heart tissue and see if receptor density is altered in cardiac diseases such as dilated cardiomyopathy (DCM), ischaemic heart disease (IHD) and primary pulmonary hypertension (PPH).

Following optimisation of binding conditions using the thromboxane receptor agonist, [^{125}I]-BOP (Morinelli *et al.*, 1989) in human vascular tissue (Katugampola & Davenport., 2000), 30 μm cryostat sections of human cardiac tissue were incubated with increasing concentrations (0.01-1.25 nM) of [^{125}I]-BOP. Non-specific binding was defined using 1 μM SQ29,548 (Monshizadegan *et al.*, 1992). Following a 30 min incubation sections were washed and the radioactivity retained was measured by γ-counting or the sections were apposed to film for 4 days for development. Developed images were quantified using computer assisted densitometry where receptor density was measured in amol/mm^2. Values of affinity (K_D) were compared using the Mann Whitney *U*-test, and receptor densities (B_{max}) were compared using unpaired Student's *t*-test with a significance value of $P<0.05$.

Saturation data are shown in Table 1. Autoradiography revealed the presence of TP receptors in the human inter-ventricular septum, with no significant difference in receptor density comparing DCM and IHD patients. TP receptors were also localised to human intramyocardial coronary arteries with no significant difference in receptor density between DCM and IHD.

Table 1. Ligand affinity and receptor density in human cardiac tissue. Values represent mean ± s.e.mean (n=4-5 individuals). $^+P<0.05$ compared to control of respective chamber. Hill slopes were close to unity.

Tissue	K_D (nM)	B_{max} (fmol mg^{-1} protein)
right atria-control	0.34 ± 0.10	33.9 ± 2.8
right atria-DCM	0.50 ± 0.03	59.9 ± 7.1$^+$
right atria-IHD	0.56 ± 0.16	32.3 ± 3.2
right ventricle-control	0.42 ± 0.08	37.9 ± 4.1
right ventricle-PPH	0.45 ± 0.12	66.6 ± 6.0$^+$
left atria-control	0.42 ± 0.09	35.8 ± 4.2
left ventricle-control	0.23 ± 0.10	28.4 ± 5.7
left ventricle-DCM	0.19 ± 0.03	25.5 ± 3.1
left ventricle-IHD	0.43 ± 0.15	36.1 ± 6.5

We report for the first time TP receptor density in human cardiac tissue and its alteration with disease. Surprisingly, we see an increase in TP receptor density in the right atria associated with DCM but not IHD. TP receptor density is significantly increased in the right ventricle of patients with PPH, where right ventricular hypertrophy and an increased TXA_2 production is apparent (Christman *et al.*, 1992). The functional importance of these alterations in TP receptor density in disease remains to be elucidated.

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115P CHARACTERISATION OF [^{125}I]-GHRELIN, THE ENDOGENOUS RADIOLABELLED LIGAND FOR THE GROWTH HORMONE SECRETAGOGUE ORPHAN RECEPTOR IN HUMAN CARDIOVASCULAR TISSUE.

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The 28 amino acid biologically active peptide, ghrelin (Kojima *et al.*, 1999) was recently identified as the endogenous ligand for the orphan G protein-coupled, growth hormone secretagogue receptor (GHS-R; Kojima *et al.*, 1999). Using synthetic growth hormone secretagogues like hexarelin, the distribution of GHS-R has been localised to human brain regions (Muccioli *et al.*, 1998) and to the rat heart (Bodart *et al.*, 1999). Therefore, the aim of this study was to characterise the radiolabelled endogenous ligand [^{125}I]-ghrelin, binding to GHS-R expressed in human cardiovascular tissue.

Human tissues were obtained with approval from the local ethics committee. Following optimisation of binding conditions, 30 μm cryostat sections of human tissue were pre-incubated in assay buffer containing 50 mM Tris, 10 mM EDTA, 10 mM EGTA and 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), pH 7.2 for 15 min. Following a 20 min incubation period the sections were rinsed in ice cold buffer (50 mM Tris) and radioactivity retained was measured by γ-counting or sections were apposed to film for 5 days for development. Saturation binding experiments were performed using increasing concentrations (0.01-1.25.nM) of [^{125}I]-ghrelin. Non-specific binding was determined using 1 μM hexarelin. Values of affinity (K_D) were compared using the Mann Whitney *U*-test, and receptor densities (B_{max}) were compared using unpaired Student's *t*-test with a significance value of $P<0.05$.

[^{125}I]-Ghrelin bound to sections of human left ventricle in a time dependent manner reaching equilibrium by 20 min with a half time for association ($t_{½}$) of 3 min. The binding was pH dependent with maximal binding being reached between pH 6.7 and pH 7.25.

Table 1. Ligand affinity, receptor density and Hill coefficient (n_H) for [^{125}I]-ghrelin binding to human cardiovascular tissue. Values represent mean ± s.e.mean, (n=3-4 individuals)

Tissue	K_D (nM)	B_{max} fmol mg^{-1} protein	n_H
left ventricle	0.35 ± 0.10	7.9 ± 2.8	0.87 ± 0.06
right atria	0.42 ± 0.13	7.6 ± 1.1	0.99 ± 0.01
coronary artery	0.22 ± 0.08	6.2 ± 2.1	0.96 ± 0.03
saphenous vein	0.29 ± 0.10	7.2 ± 2.7	1.04 ± 0.05
aorta	0.43 ± 0.19	17.5 ± 2.5	1.06 ± 0.05

[^{125}I]-Ghrelin bound with sub-nanomolar affinity to human cardiovascular tissue with a comparable receptor density (Table 1). Cardiac peptides such as endothelin-1, angiotensin II, adrenomedullin and newly identified peptide ligands such as apelin and TIP39 did not compete for the [^{125}I]-ghrelin binding site. Autoradiographical visualisation revealed the presence of [^{125}I]-ghrelin binding sites in atherosclerotic coronary arteries as well as saphenous vein grafts, on both the media as well as the proliferated intimal smooth muscle layers.

We have demonstrated for the first time the binding of [^{125}I]-ghrelin, the radiolabelled endogenous ligand for the GHS-R in the human cardiovascular system, suggesting this ligand to be a useful tool for further characterisation of GHS-R in other human tissues.

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116P PROSTAGLANDIN E2 PREVENTS CLOFILIUM-INDUCED TORSADE DE POINTES

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Severe arrhythmias, especially torsade de pointes (TdP) can be evoked by drugs that prolong repolarisation e.g. clofilium (Farkas & Coker, 2000). Prostaglandin E_2 (PGE_2) can open ATP dependent K^+ channels (Bouchard *et al.*, 1994), and thus shorten repolarisation and in theory could prevent TdP. On the other hand PGE_2 can inhibit delayed rectifier K^+ current (Ren *et al.*, 1996), and thus prolong repolarisation which could worsen arrhythmias. The present study was designed to examine whether PGE_2 reduces or exacerbates clofilium-induced TdP.

Male NZW rabbits (2.6-3.1kg) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.) and artificially ventilated with room air via a tracheal cannula. The chest was opened, and ECGs (leads I, II and III), arterial blood pressure and left ventricular epicardial monophasic action potential were recorded. Rabbits were assigned randomly to one of four groups (n=10) to receive vehicle (1% v/v ethanol in saline) or PGE_2 (0.28, 0.84 or 2.8 nmol kg^{-1} min^{-1}) and clofilium. Each animal received three infusion rates of clofilium (20, 60 and 200 nmol kg^{-1} min^{-1}, i.v.) with each infusion administered for 19 min. PGE_2 was given simultanously with clofilium but to the left ventricle via a carotid cannula. Five min prior to each drug infusion, phenylephrine infusion was started and given i.v. for 24 min at increasing rates (75, 150, 225 and 300 nmol kg^{-1} min^{-1} for 15, 3, 3 and 3 min, respectively). All three 24 min dosing cycles were followed by a 10 min drug free interval.

The highest rate of PGE_2 infusion decreased the incidence of clofilium-induced TdP. This dose of PGE_2 had no effect on heart rate but lowered blood pressure and shortened QT intervals before clofilium could exert its maximal pro-arrhythmic effect at the end of the 2nd dosing cycle (Table 1).

Table 1. The effect of PGE_2 (nmol kg^{-1} min^{-1}) on the incidence of TdP at any time, and on mean ± s.e. mean values for mean arterial blood pressure (MBP) heart rate (HR) and QT intervals measured 10 min after commencing the 2nd drug infusion.

	Control	PGE_2 0.28	PGE_2 0.84	PGE_2 2.8
TdP (%)	50	20	20	0[#]
MBP (mmHg)	86±5	72±4	70±5*	59±5*
HR (beats min^{-1})	186±10	188±6	188±7	200±10
QT (ms)	287±14	274±6	271±6	245±10*

[#]$P<0.05$ compared to control, Fisher's exact test. *$P<0.05$ compared to control, Kruskal-Wallis test.

These data indicate that PGE_2 prevents clofilium-induced TdP probably by interfering with the ability of clofilium to prolong repolarisation, or by reducing blood pressure. These results suggest that some pathological conditions, in which endogenous PGE_2 concentrations are increased (e.g. fever or inflammation) might reduce drug-induced TdP.

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117P CARDIAC GENE EXPRESSION ANALYSIS OF THE LEFT VENTRICLE AND ATRIA IN ANTHRACYCLIN-INDUCED CARDIOMYOPATHY IN RABBITS

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Changes in ventricular performance and myocardial structure are key elements of cardiomyopathy and congestive heart failure. The purpose of this study was to (i) assess the expression of foetal isoforms, (ii) investigate expression of ET-1 and its receptor subtypes, and (iii) examine expression of genes involved in altered geometry of the myocardium, including collagen-1 (COL-1), matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), in an experimental model of cardiomyopathy using the anthracycline, epirubicin.

New Zealand White rabbits (2-2.5kg) were treated with twice weekly injections of epirubicin (1 mg kg^{-1}) or saline (n=21) for a period of 10-15 weeks (20-22 mg kg^{-1}) followed by a washout period of 2 weeks. Cardiac geometry was assessed by echocardiography at weekly intervals. Plasma levels of ANP were measured by radioimmuno-assay following extraction using C_{18} Bond Elut columns. Total cellular RNA was isolated from the left ventricle and atria by an acid guanidinium thiocyanate-phenol-chloroform extraction. First stand cDNA was synthesised by reverse transcription, and amplified by polymerase chain reaction (PCR) using gene specific primers. PCR products were separated by electrophoresis, quantified by densitometry and normalised relative to an internal standard (GAPDH). Data are expressed as mean ± s.e.m. and analysed by one-way ANOVA with *post hoc* analysis (Bonferroni). A value of $P<0.05$ indicates statistical significance

Two treatment groups were identified as non-dilated (n=8) and dilated (n=6) based upon echocardiographic evidence. Plasma levels of atrial natriuretic peptide (ANP) were elevated ($P<0.05$) in both non-dilated (5.17± 0.34 ng ml^{-1}) and dilated (5.43±0.45 ng ml^{-1}) compared to control (2.09±0.10 ng ml^{-1}) groups. Re-expression of the foetal isoform genes, skeletal-α actin (SkαA) and ANP, were evident in left ventricle from both non-dilated and dilated myocardium, whereas expression of SkαA and myosin heavy chain-β (MHC-β) were up-regulated in atria from only dilated hearts. Expression of the ET-1 gene was up-regulated in dilated cardiomyopathy, although there was no evidence for altered expression of the ET_A and ET_B receptor subtypes. COL-1, MMP-9 and TIMP-1 mRNA were up-regulated in left ventricle from both non-dilated and dilated myocardium, but only atria from dilated myocardium.

	LEFT VENTRICLE		ATRIA	
	Non-dilated	Dilated	Non-dilated	Dilated
SkαA	31%*	38%*	⇔	80%§
ANP	3.6 fold§	4.3 fold*	⇔	⇔
MHC-β	⇔	⇔	⇔	2.1 fold§
ET-1	⇔	2.8 fold*	⇔	4.3 fold§
ET_A	⇔	⇔	⇔	⇔
ET_B	⇔	⇔	⇔	⇔
MMP-9	3.7 fold§	3.1 fold§	⇔	30%*
TIMP-1	33%§	37%§	⇔	41%§
COL-1	4.2 fold*	4.1 fold§	⇔	48%*

**P<0.05 & §P<0.001 vs expression in normal cardiomyocytes*

In conclusion, (1) Alterations in mRNA levels of foetal isoform genes is compatible with an advanced cardiomyopathy following chronic administration of epirubicin. (2) Expression of ET-1 is up-regulated in dilated cardiomyopathy. (3) Increased expression of collagen-1, MMP-9 and TIMP-1 mRNA reflects (i) significant gross remodelling of the myocardium during dilated cardiomyopathy, and (ii) specific remodelling of the left ventricles in non-dilated cardiomyopathy.

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118P BILIVERDIN, THE PRODUCT OF HEME OXYGENASE METABOLISM ATTENUATES SIN-1 INDUCED APOPTOSIS IN WKY 3M-22 RAT AORTIC SMOOTH MUSCLE CELLS.

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Heme oxygenase (HO) metabolises heme to biliverdin with the concomitant production of carbon monoxide (Maines et.al., 1982). Biliverdin is an antioxidant that has been shown to reduce the cytotoxicity of nitric oxide (NO) donors in primary cultures of rat aortic smooth muscle cells (RASMCs) (Hamilton & Warner, 1999). 3 morpholinosydnonimine (SIN-1) is an NO donor that also produces equimolar amounts of superoxide anion (O_2^-). Together NO and O_2^- can generate peroxynitrite ($ONOO^-$), a highly reactive and cytotoxic free radical species, that is an extremely potent inducer of apoptosis. Apoptosis in vascular smooth muscle cells may play an important role in the stability of atherosclerotic lesions. Here we establish whether biliverdin prevents SIN-1 induced smooth muscle cell death by arresting the process of apoptosis.

RASMCs (WKY 3M-22; Gordon *et al.*, 1986; a gift from Dr. David Han, University of Washington, Seattle) were grown to confluence in 96-well plates. Medium was then replaced with fresh serum-free medium, with or without 30 μM biliverdin and the cells left for 2 h before incubation with SIN-1 (0.03-3 mM) for a further 16 h. The medium was then removed and its content of lactate dehydrogenase (LDH) determined (Roche Cytotoxicity Detection Kit). In a separate series of experiments, to determine the time course of action of SIN-1 and assess the induction of apoptosis, WKY 3M-22 cells were grown to ≈ 50% confluence on glass cover slips and then exposed for 0, 0.5, 1, 2, 4, 8 and 16 h to 3 mM SIN-1. LDH release was determined as above, and the cells fixed and stained with Hoechst 33258 dye. The slips were then viewed under a fluorescent microscope and apoptotic cells distinguished by their characteristic morphology (nuclear condensation and presence of apoptotic bodies). The % apoptotic cells in 3 fields (x40 magnification) per data point were counted.

SIN-1 produced a concentration-dependent increase in cell death, with 3mM causing 67±11 % cytotoxicity (n=5) (100 % cytotoxicity = LDH released by 1% Triton-X 100). Pre-treatment of cells with 30 μM biliverdin significantly ($p<0.001$, 2 way ANOVA plus Bonferroni's test) reduced cell death; e.g. 3 mM SIN-1 caused 1±1 % cytotoxicity (n=5). The cytotoxic effects of 3 mM SIN-1 increased in a time-dependent manner, e.g. 15±6 % at 4 h and 57±5 % at 16 h (both n=3). Apoptosis was detectable approx. 2 h before LDH release, and increased time-dependently reaching 83±12 % at 8 h (n=3) (<10 % of cells remained on the cover slip at 8 h; by 16 h the number of adherent cells left was too low to permit analysis). 30 μM biliverdin markedly reduced the rate of apoptosis such that at 8 h it was only 4±1 % (n=3; $p<0.001$, 2 way ANOVA plus Bonferroni's test).

In conclusion, biliverdin inhibits apoptotic cell death induced by SIN-1. Hence, biliverdin production by HO could reduce the rate of apoptosis of smooth muscle cells under the influence of endogenous NO and oxidative stress. HO would, therefore, aid plaque stability in atherosclerotic lesions, where NO production and oxidative stress are elevated and rates of apoptotic cell death are greater than normal.

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119P EFFECTS OF TYRPHOSTIN AG126 ON THE MULTIPLE ORGAN FAILURE CAUSED BY SEVERE HAEMORRHAGE AND RESUSCITATION IN THE ANAESTHETISED RAT

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Phosphorylation of proteins on tyrosine residues by protein tyrosine kinases plays an important role in the regulation of cell proliferation, cell differentiation and signalling processes in cells of the immune system. Receptor tyrosine kinases participate in transmembrane signalling, whereas the intracellular tyrosine kinases take part in the signal transduction to the nucleus. Enhanced activity of tyrosine kinases has been implicated in the pathophysiology of many diseases associated with local or systemic inflammation including sepsis and septic shock (Levetzki & Gazit, 1995). The tyrosine kinase inhibitor, tyrphostin AG126, attenuates the circulatory failure and organ injury caused by endotoxin in the rat (Ruetten & Thiemermann, 1997). Here we investigate the effects of tyrphostin AG126 on the organ injury and dysfunction (kidney and liver) associated with haemorrhagic shock (HS) in the anaesthetised rat.

Thirty-three male Wistar rats were anaesthetised with thiopentone sodium (120 mg·kg^{-1} i.p.). The carotid artery was cannulated to measure mean arterial blood pressure (MAP) and heart rate (HR), the jugular vein for the intravenous administration of drugs, and the femoral artery for blood withdrawal. After a 15 min equilibration period, animals were pre-treated with tyrphostin AG126 (5 mg·kg^{-1}) or its vehicle (50% v·v^{-1} DMSO). Thirty min later, rats were subjected to haemorrhage (to lower MAP to 45 mmHg for 90 min) and resuscitation with the shed blood. Four hours after the onset of resuscitation, blood was taken for measurement of biochemical markers of organ injury. HS produced rises in the serum levels of (i) urea and creatinine (renal dysfunction), (ii) aspartate aminotransferase (AST), and alanine aminotransferase (ALT), (liver injury), and (iii) creatine kinase (CK) (neuromuscular injury) (n=9).

Administration (30 min prior to haemorrhage) of tyrphostin AG126 (5 mg·kg^{-1} i.p, n=9) reduced the serum levels of urea, AST, and ALT, but did not affect the rise in the serum levels of creatinine and CK associated with HS (see Table 1).

Table 1: *p<0.05 when compared with HS by ANOVA followed by Dunnett's). All data are expressed as mean±s.e.mean. Statistical differences between groups were analysed by ANOVA followed by a Bonferroni's test (p<0.05).

Biochemical Parameter	Sham-DMSO (n=8)	Sham-Tyr (n=7)	HS-DMSO (n=9)	HS-Tyr (n=9)
Urea (mmol·L^{-1})	8 ± 0.8*	8 ± 1.0*	18 ± 1.1	14 ± 1.0*
Creatinine (μmol·L^{-1})	52 ± 5*	56 + 3*	108 ± 17	85 ± 6
AST (iu·L^{-1})	263 ± 55*	229 ± 62*	1070 ± 276	298 ± 59*
ALT (iu·L^{-1})	136 ± 15*	134 ± 26*	721 ± 200	222 ± 41*
CK (iu·L^{-1})	687 ± 89*	546 ± 67*	2495 ± 716	1281 ± 380

Thus, the tyrosine kinase inhibitor tyrphostin AG126 attenuates the liver injury caused by severe haemorrhage and resuscitation.

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120P EFFECTS OF L-N-(L-IMINOETHYL)LYSINE (L-NIL) ON THE CIRCULATORY FAILURE AND MULTIPLE ORGAN INJURY IN HAEMORRHAGIC SHOCK IN THE ANAESTHETISED RAT

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An enhanced formation of nitric oxide (NO) by the inducible isoform of NO synthase (iNOS) contributes to the circulatory failure in endotoxic and haemorrhagic shock (Thiemermann *et al.*, 1993). Selective inhibition of iNOS activity with either L-N^6-(l-iminoethyl)lysine (L-NIL) or 1400W attenuates the circulatory failure, but not the multiple organ injury and dysfunction caused by endotoxin in the rat (Wray *et al.*, 1998). Here we investigate the effects of L-NIL on the delayed circulatory failure and the organ injury and dysfunction (kidney and liver) associated with haemorrhagic shock (HS) in the anaesthetised rat.

Thirty-seven male Wistar rats were anaesthetised with thiopentone sodium (120 mg·kg^{-1} i.p.). The carotid artery was cannulated to measure mean arterial blood pressure (MAP) and heart rate (HR), the jugular vein for the intravenous administration of drugs, and the femoral artery for blood withdrawal. After a 30 min stabilisation period, rats were subjected to haemorrhage to lower MAP to 45 mmHg for 90 min. Animals were then treated with either L-NIL (3 mg·kg^{-1} i.v. followed by 3 mg·kg^{-1}·h^{-1} until the end of the experiment) or its vehicle (saline, 1 ml·kg^{-1} i.v. followed by 1 ml·kg^{-1}·h^{-1}) and subsequently resuscitated with the shed blood. Four hours after the onset of resuscitation, blood was taken for measurement of biochemical markers of organ injury.

In animals treated with vehicle for L-NIL, HS caused a delayed fall in MAP (circulatory failure) (n=12, p<0.05). In addition, HS caused significant rises in the serum levels of (i) urea and creatinine (renal dysfunction), (ii) aspartate aminotransferase (AST), and alanine aminotransferase (ALT), (liver injury), and (iii) creatine kinase (CK) (neuromuscular injury) (n=12) (Table 1).

The iNOS inhibitor L-NIL significantly attenuated the delayed fall in MAP as well as the rise in the serum levels of ALT (n=10, p<0.05). In contrast, L-NIL did not significantly reduce the rise in the serum levels of urea, creatinine, AST and CK associated with HS (see Table 1).

Table 1: *p<0.05 when compared with HS by ANOVA followed by Dunnett's). All data are expressed as mean±s.e.mean. Statistical differences between groups were analysed by ANOVA followed by a Bonferroni's test (p<0.05).

Biochemical Parameter	Sham-Saline (n=8)	Sham-L-NIL (n=7)	HS-Saline (n=9)	HS-L-NIL (n=9)
Urea (mmol·L^{-1})	7 ± 0.6*	6 ± 0.6*	15 ± 0.8	13 ± 1.0
Creatinine (μmol·L^{-1})	29 ± 5*	32 + 5*	76 ± 10	64 ± 11
AST (iu·L^{-1})	226 ± 21*	234 ± 38*	726 ± 176	565 ± 170
ALT (iu·L^{-1})	99 ± 7*	234 ± 38*	476 ± 150	170 ± 16*
CK (iu·L^{-1})	477 ± 78*	476 ± 55*	1910 ± 396	1404 ± 417
MAP 4h after Resuscitation. (mm Hg)	107 ± 3*	119 ± 5*	64 ± 7	90 ± 7*

Thus, the selective iNOS inhibitor L-NIL attenuates the circulatory failure and the liver injury associated with severe haemorrhage and resuscitation.

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Wray, G. *et al.*, (1998). *Shock*, **9,** 329-335.

121P EFFECTS OF 5-AMINOISOQUINOLINONE ON REGIONAL MYOCARDIAL ISCHAEMIA AND REPERFUSION IN THE ANAESTHETISED RAT

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Poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme, which is activated by single strand breaks in DNA. Inhibitors of PARP activity reduce myocardial infarct size (Thiemermann *et al.*, 1997). In 1991, Suto and colleagues reported that 5-aminoisoquinolinone (5-AIQ) is a water-soluble inhibitor of PARP activity in a cell free preparation (enzyme from calf thymus) (Suto *et al.*, 1991). We have recently reported a novel route for the synthesis of 5-AIQ and shown that this compound is a potent inhibitor of PARP activity in human cardiac myoblasts (McDonald *et al.*, 2000).

Exposure of H9c2 cells to hydrogen peroxide (Bowes *et al.*, 1998) (H_2O_2, 1 mM for 30 min, n=9) caused a significant increase in PARP activity. Pre-treatment of these cells with 5-AIQ (3 μM to 1 mM, 10 min prior to H_2O_2) caused a concentration-dependent inhibition of PARP activity (IC_{50}: ~ 4.5 μM, n=6).

Forty-five male Wistar rats (220-280 g) were anaesthetised with thiopentone sodium (120 $mg \cdot kg^{-1}$ i.p.). All animals were tracheotomised and ventilated (inspiratory oxygen concentration: 30%; tidal volume: 8-10 $ml \cdot kg^{-1}$; respiration rate: 70 $strokes \cdot min^{-1}$). The carotid artery was cannulated to measure mean arterial blood pressure (MAP), the jugular vein for the administration of drugs. The chest was opened (left sided thoracotomy) and a 6-0 silk thread placed around the left anterior descending coronary artery (LAD). The animals were allowed to recover for 30 min and subsequently the LAD was occluded for 25 min and then reperfused for 2 h. The PARP-inhibitor 5-AIQ or its vehicle (saline, 1 $ml \cdot kg^{-1}$) was given 1 min prior to the onset of reperfusion (high dose: 0.3 $mg \cdot kg^{-1}$ i.v. bolus plus 0.3 $mg \cdot kg^{-1} \cdot h^{-1}$ throughout the reperfusion period, n=6; medium dose: 0.1 $mg \cdot kg^{-1}$ i.v. plus 0.1 $mg \cdot kg^{-1} \cdot h^{-1}$, n=9; low dose: 0.03 $mg \cdot kg^{-1}$ i.v. plus 0.03 $mg \cdot kg^{-1} \cdot h^{-1}$, n=11). At the end of the experiment, the LAD was re-occluded, and 1 ml of Evans Blue dye (2% w/v) was injected into the jugular vein to determine the non-perfused (area at risk, AR). Infarct size was determined by incubation of small pieces of the AR with nitro-blue tetrazolium (NBT, 0.5 $mg \cdot ml^{-1}$ at 37°C for 40 min). All data are expressed as mean±s.e.mean. Statistical differences between groups were analysed by ANOVA followed by a Bonferroni's test ($p<0.05$).

5-AIQ caused a dose-related reduction in infarct size from 50±3% (vehicle-control, n=12) to 25±2% (high dose, $p<0.05$) and 31±6% (medium dose) of the AR ($p<0.05$). The low dose did not have a significant effect on infarct size (40±4%, $p>0.05$). The AR was similar in all of the groups studied (control: 47±3%, high dose: 49±1%, medium dose: 56±2%, low dose: 50±3%; $p>0.05$). There were no significant differences for MAP or heart rate in any of the experimental groups studied.

Thus, 5-AIQ (i) is a potent, water-soluble inhibitor of PARP activity in rat cardiac myoblasts *in vitro* and (ii) reduces the infarct size caused by regional myocardial ischaemia and reperfusion in the anaesthetised rat. This finding supports the view that inhibitors of PARP activity may be useful in conditions associated with ischaemia-reperfusion of the heart and other organs (Thiemermann *et al.*, 1997; McDonald *et al.*, 2000).

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McDonald, M.C. *et al.*, (2000). *Br. J. Pharmacol.*, **130,** 843-850.
Bowes, J. *et al.*, (1998). *Cardiovasc. Res.*, **41,** 126-134.

122P EFFECTS OF 15-DEOXY-D12,14-PROSTAGLANDIN J2 ON THE INFARCT SIZE CAUSED BY MYOCARDIAL ISCHAEMIA AND REPERFUSION IN THE RAT

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The peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors. The endogenous prostaglandin D_2 metabolite 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) binds to PPAR-γ, modulates the transcription of target genes and has been suggested to serve as endogenous PPAR-γ ligand (Ricote *et al.*, 1998). Activators of PPAR-γ including 15d-PGJ_2 attenuate the expression of the adhesion molecules intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) caused by tumour-necrosis factor-α in endothelial cells (Pasceri *et al.*, 2000). Not all of the effects of 15d-PGJ_2, however, are due to the activation of PPAR-γ. For instance, the inhibition by 15d-PGJ_2 of the expression of inducible nitric oxide synthase (iNOS) in activated microglia is independent of the activation of PPAR-γ (Petrova *et al.*, 1999). This study investigates the effects of 15d-PGJ_2 on the infarct size caused by regional myocardial ischaemia and reperfusion in the anaesthetised rat.

Twenty-six male Wistar rats (230-290 g) were anaesthetised with thiopentone sodium (120 $mg \cdot kg^{-1}$ i.p.). All animals were tracheotomised and ventilated (inspiratory oxygen concentration: 30%; tidal volume: 8-10 $ml \cdot kg^{-1}$; respiration rate: 70 $strokes \cdot min^{-1}$). The carotid artery was cannulated to measure mean arterial blood pressure (MAP), the jugular vein for the administration of drugs. The chest was opened (left sided thoracotomy) and a 6-0 silk thread placed around the left anterior descending coronary artery (LAD). The animals were allowed to recover for 30 min. The PPAR-γ ligand 15d-PGJ_2 or its vehicle (10% $v \cdot v^{-1}$ dimethyl sulphoxide, DMSO, 1 $ml \cdot kg^{-1}$) was then administered as an i.v. bolus injection (low dose: 100 $\mu g \cdot kg^{-1}$, n=6; high dose: 300 $\mu g \cdot kg^{-1}$, n=7). Thirty min later, the LAD was occluded for 25 min and then reperfused for 2 h. At the end of the experiment, the LAD was re-occluded, and 1 ml of Evans Blue dye (2% $w \cdot v^{-1}$) was injected into the jugular vein to determine the non-perfused (area at risk, AR). Infarct size (IS) was determined by incubation of small pieces of the AR with nitro-blue tetrazolium (NBT, 0.5 $mg \cdot ml^{-1}$ at 37°C for 40 min). All data are expressed as mean±s.e.mean. Statistical differences between groups were analysed by ANOVA followed by a Bonferroni's test ($p<0.05$).

When compared to rats, which had been subjected to thoracotomy, but not LAD-occlusion (sham-operation, n=7, IS: 1±1%,), occlusion and reperfusion of the LAD of vehicle-treated rats resulted in an infarct size of 46±6% (n=6) of the AR. The high dose of the PPAR-γ ligand 15d-PGJ_2 caused a reduction in infarct size from 46±6% (vehicle-control) to 7±1% ($p<0.05$). In contrast, the low dose did not have a significant effect on infarct size (42±2%, $p>0.05$). The AR was similar in all of the groups studied (sham: 51±2%, control: 53±3%, high dose: 49±2%, low dose: 45±2%; $p>0.05$). There were no significant differences for MAP or heart rate in any of the experimental groups studied.

Thus, the endogenous PPAR-γ ligand 15d-PGJ_2 causes a pronounced reduction in IS caused by regional myocardial ischaemia and reperfusion in the anaesthetised rat. The mechanism of the cardioprotective effect of 15d-PGJ_2 warrants further investigation.

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Petrova T.V. *et al.*, (1999). *Proc. Natl. Acad. Sci. U.S.A.* **96,** 4668-4673.
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123P THE POLY(ADP-RIBOSE) POLYMERASE INHIBITOR 5-AMINOISOQUINOLINE REDUCES OXIDATIVE STRESS-MEDIATED RENAL DYSFUNCTION IN THE RAT *IN VIVO* AND *IN VITRO*

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Generation of reactive oxygen species (ROS) has been implicated in the pathogenesis of renal ischaemia-reperfusion (I/R) injury (Paller *et al.*, 1984). ROS produce DNA strand breaks which leads to the activation of the DNA-repair enzyme poly(ADP-ribose) polymerase (PARP) (Schraufstatter *et al.*, 1986). During oxidant stress, excessive PARP activation results in depletion of its substrate NAD and subsequently of ATP, leading to cellular dysfunction and cell death (Schraufstatter *et al.*, 1986). PARP inhibitors can provide beneficial effects against I/R and oxidative stress-mediated renal injury (Chatterjee *et al.*, 1999, 2000). However, current PARS inhibitors are either weak inhibitors and/or need to be dissolved in dimethyl sulphoxide, which exerts non-specific effects. The aims of this study were to investigate the effects of the novel, water-soluble PARP inhibitor 5-aminoisoquinolinone (5-AIQ) on (i) the renal dysfunction mediated by I/R of rat kidneys *in vivo* and (ii) the cell injury and death of rat renal proximal tubular (PT) cells exposed to oxidative stress (hydrogen peroxide, H_2O_2).

For *in vivo* studies, 26 male Wistar rats (240-280 g) were anaesthetised with sodium thiopentone (120 mg kg^{-1} i.p.). After performing a midline laparotomy, rats were divided into 3 groups; (i) 'Shams', in which rats were maintained under anaesthesia for the duration of the experiment, (ii) 'I/R only', in which rats underwent bilateral clamping of the renal pedicles for 45 min followed by reperfusion for 6 h and (iii) 'I/R + 5-AIQ', in which rats underwent I/R, but were administered an i.v. bolus of 5-AIQ (0.3 mg kg^{-1} in saline) 5 min prior to beginning reperfusion followed by an infusion of 5-AIQ (0.3 mg kg^{-1} h^{-1} in saline) throughout reperfusion. At the end of the experiment, plasma samples from each rat were collected and levels of urea and creatinine were measured (Vetlab Services, Sussex).

For *in vitro* studies, PT cells were isolated from the kidney cortex of 6 male Wistar rats (250-300 g) using collagenase digestion, differential sieving and Percoll density centrifugation. PT cells were cultured on 24 well plates in Minimum Essential Medium (MEM) containing 10% (v v^{-1}) fetal calf serum. Once confluent, cultures were divided into three groups; (i) PT cell incubated with MEM only ('Untreated'), (ii) PT cells treated with 1 mM H_2O_2 for 3 hours ('H_2O_2 only') or (iii) PT cells treated with 1 mM H_2O_2 and 1 mM 5-AIQ ('5-AIQ + H_2O_2'). Cellular injury and death were assessed spectrophotometrically by measurement of the mitochondrial-dependent conversion of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into formazan or by the measurement of intracellular lactate dehydrogenase (LDH) released into the incubation medium, respectively.

Renal I/R produced significant increases in plasma urea and creatinine concentrations, which were significantly reduced by infusion of 5-AIQ (Table 1). Incubation of rat PT cell cultures with 1 mM H_2O_2 for 3 hours significantly inhibited mitochondrial respiration and increased LDH release, respectively (Table 2). Incubation with 5-AIQ significantly reduced the H_2O_2-mediated inhibition of mitochondrial respiration and increase in LDH release (Table 2).

Thus, I/R of rat kidneys produces significant renal dysfunction, which can be significantly reduced by administration of 5-AIQ. Furthermore, 5-AIQ reduces H_2O_2 (and therefore ROS)-mediated cellular injury and death in primary cultures of rat PT cells. We therefore propose that PARP inhibitors may be beneficial in renal disorders mediated by oxidative stress.

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Paller, M.S., Hoidal, J.R. & Ferris, T.F. (1984) *J. Clin. Invest.* **74**, 1156-1164.
Schraufstatter, I.U., Hyslop, P.A., Hinshaw, D.B. *et al.*, (1986) *J. Clin. Invest.* **77**,1312-1320.

Table 1 (*in vivo*)	N	Plasma Urea (mmol L^{-1})	Plasma Creatinine (μmol L^{-1})
Sham	10	6 ± 1	33 ± 4
I/R only	10	23 ± 1*	172 ± 4*
I/R + 5-AIQ	6	19 ± 1*+	130 ±11*+

Table 2 (*in vitro*)	N	Mitochondrial respiration (% control)	LDH release (% Triton X-100 control)
Untreated	6	100	24 ± 5
H_2O_2 only	6	20 ± 2*	70 ± 2*
5-AIQ + H_2O_2	6	47 ± 2*+	50 ± 3*+

Table 1: Effect of I/R and 5-AIQ biochemical indicators of renal dysfunction (*P<0.05 vs. Sham, +P <0.05 vs. I/R only). Table 2: Effect of 5-AIQ on H_2O_2-mediated inhibition of mitochondrial respiration and LDH release, respectively (*P <0.05 vs. Untreated, +P <0.05 vs. Control). Data are expressed as mean ± s.e. mean of N rats, analysed using one-way ANOVA followed by the Bonferroni's post significance test. A P value of less than 0.05 was considered to indicate significance.

124P THE R(+) ENANTIOMER OF AMLODIPINE HAS AN ENDOTHELIAL DEPENDENT VASODILATORY EFFECT ON CANINE CORONARY ARTERY

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Amlodipine is a potent calcium channel blocker, of the dihyropyridine class, with a racemic mixture currently used for the treatment of hypertension and angina. Previous studies have shown that the amlodipine racemate releases nitric oxide (NO) from canine coronary microvessels (Zhang & Hintze, 1998). As the calcium channel blocking activity of amlodipine predominantly resides with the S(-) enantiomer of amlodipine, the aim of this study was to determine whether the R(+) enantiomer contributes to the vasodilation of dog coronary artery induced by the racemate.

Canine coronary artery rings with or without endothelium, were mounted in organ baths in Krebs Solution at 37°C, under a resting tension of 5g. The tissues were contracted with either $PGF_{2\alpha}$ ($3x10^{-6}$M) or KCl (30mM) and cumulative concentration response curves to the amlodipine racemate, the R(+) and S(-) enantiomers were carried out over a concentration range of $1x10^{-7}$M - $3x10^{-5}$M, using a contact time of 2 minutes per concentration. Subsequently experiments using the racemate and the R(+) enantiomer were repeated following pre-incubation with the NO synthase inhibitor L-NAME ($3x\ 10^{-5}$M). The peak height of the $PGF_{2\alpha}$ and KCl-induced contractions was measured, together with the contraction height following each concentration of compound. The % relaxation was calculated and the results analysed by REML (residual maximum likelihood).

The R(+) enantiomer induced significant rapid relaxation at concentrations of $1x10^{-5}$M and above, in both KCl and $PGF_{2\alpha}$ pre-contracted tissues, which was significantly attenuated by endothelium removal or pre-treatment with L-NAME (Table 1). The effects of racemic amlodipine were also reduced by L-NAME, but endothelium removal only inhibited effects on $PGF_{2\alpha}$ contracted tissues. Why there was a difference between the effect of the racemate on KCl and $PGF_{2\alpha}$ contracted tissues on endothelium removal is unclear at the moment. The R(+) enantiomer at $3x10^{-6}$M had no effect on tissues contracted with either KCl or $PGF_{2\alpha}$.

Table 1: Effect of racemate & R(+) enantiomer on coronary artery

Coronary artery rings	% relaxation KCl contracted tissues		% relaxation $PGF_{2\alpha}$ contracted tissues	
	R(+) ($3x10^{-5}$M)	racemate ($3x10^{-5}$M)	R(+) ($3x10^{-5}$M)	racemate ($3x10^{-5}$M)
+endothelium	40.2±4.4	87.7±2.2	92.4±4.9	100.0±0.0
-endothelium	9.0±4.6†	88.6±4.2	30.9±4.4†	60.8±9.4†
+L-NAME	17.1±3.2†	50.0±3.9†	17.4±4.8†	38.5±5.0†

†p<0.001 *vs* tissues +endothelium n=6-16, mean ± s.e.mean

This study has demonstrated that at concentrations of $1x10^{-5}$M and above, the R(+) enantiomer of amlodipine induces an endothelial dependent relaxation of canine isolated coronary artery which is partly mediated by NO. The endothelial and NO dependent relaxation induced by the high concentrations of the racemate used in this study, may be partly attributable to the R(+) enantiomer and independent of any calcium channel blocking activity.

Zhang and Hintze (1998) *Circulation* 97 (6) 576-580

125P HAEMODYNAMIC EFFECTS OF A NOVEL HYPOTENSIVE VASOPRESSIN PEPTIDE.

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An antihypertensive component to arginine vasopressin (AVP) has been described (Balakrishnan & McNeill, 1996). The recent development of a novel vasopressin analogue, $d(CH_2)_5$[D-Tyr(Et)^2, Arg^3,Val^4 Arg^7,Eda^9]AVP (HYPO-AVP) that lowers BP (Manning *et al.*, 1999) might help to explain the mechanism of this postulated antihypertensive effect of AVP. The BP lowering properties of this peptide were not mediated by known classical AVP or oxytocin receptors and appeared independent of peripheral autonomic, bradykinin, prostaglandin, and nitric oxide systems (Manning *et al.*, 1999). However, it is not known if the BP lowering effects are due to decreases in total peripheral resistance (TPR) or to decreases in cardiac output (CO). The aim of this study was to address this issue.

Under anaesthesia (isoflurane by inhalation), male Sprague-Dawley rats (310 – 490g) were instrumented with aortic ultrasonic flowprobes for the recording of CO and with femoral artery telemetry probes for the recording of BP. After recovery from surgery, the changes in BP, CO, and total peripheral conductance (TPC=CO/BP) to I.V. infusions of HYPO-AVP (one dose per day) were recorded in conscious unrestrained rats. TPC is the reciprocal of TPR. Procedures were in accordance with the Canada Council on Animal Care. HYPO-AVP was kindly supplied by M. Manning, Med. Coll. of Ohio, Toledo, Ohio.

Following a 2 h pre-infusion control period, infusions of 0.6, 1.0, 2.0 and 4.0 µg/kg/min of HYPO-AVP for 30 min evoked dose-related decreases in BP (Table 1).

Table 1: Control values and maximum changes from these values by 30 min I.V. infusions of HYPO-AVP. *P < .05 compared to pre-infusion control values (ANOVA).

Dose of Hypo-AVP	BP mm Hg	CO ml/min/kg	TPC ml/min/kg/mmHg
control; n=9	112±3	183±11	1.64±.10
0.6µg/kg/min	- 7±1*	+10±4	+.20±.05*
control; n=9	107±4	184±8	1.72±.04
1.0µg/kg/min	-21±5*	-49±14*	-.18±.10*
control; n=7	106±2	197±14	1.85±.11
2.0µg/kg/min	-51±5*	-115±14*	-.39±.15*
control; n=6	106±2	189±15	1.80±.15
4.0µg/kg/min	-54±6*	-150±12*	-.96±.14*

The fall in BP evoked by the lowest dose of 0.6 µg/kg/min was associated with a small increase in TPC. However, the falls in BP evoked by the three higher doses were associated with dose-related decreases in CO. In fact, TPC decreased at these higher doses indicating peripheral vasoconstriction.

The results suggest that the BP lowering effects of HYPO-AVP are due primarily to changes in CO, not TPC. The antihypertensive effect of AVP was also due to a decrease in CO (Balakrishnan & McNeill, 1996). Thus, the antihypertensive effect of AVP and the BP lowering effects of HYPO-AVP share similar hemodynamic profiles.

Balakrishnan, S. and McNeill, J.R. (1996) Am.J.Physiol., **271**, H1728-H1733.
Manning *et al.* (1999) J. Peptide Sci. 5:472-490.

126P THE ISOLATED PIGLET PURKINJE FIBRES PREPARATION AS A SUITABLE MODEL TO PREDICT DRUG-INDUCED VENTRICULAR ARRHYTHMIAS

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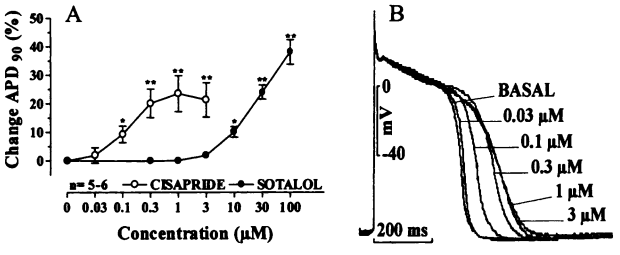
The CPMP (Committee for Proprietary Medicinal Products) points to consider document (986/96; 1997) has highlighted the need to address the proarrhythmic potential of new drugs in safety pharmacology, recommending a greater focus on QT-interval "in vivo" and measurements of cardiac action potential using intracellular recordings "in vitro", specially with non-cardiovascular drugs. The aim of this study was to characterise piglet Purkinje fibres as a suitable preparation to determine the arrhythmogenic proclivity by investigating the electrophysiological effects of cisapride (a gastrointestinal prokinetic) and (±)-sotalol (an antiarrhythmic) with reported proarrhythmogenic effects (Carlsson, 1997; Carmeliet, 1984).

Piglets of either sex (4 – 6 kg) were stunned in a CO_2 chamber, bled and their hearts were quickly removed from the chest and placed in iced Krebs' physiological salt solution, equilibrated with a mixture of 95 % O_2 - 5 % CO_2. Free running strands of Purkinje fibres were carefully dissected from right and left ventricles and placed in an organ bath containing Krebs' solution at 36.5 ± 0.5°C. Each preparation was stimulated at a supramaximal voltage with a cycle length of 1000 ms (1 Hz) and 1 msec duration. After a 60-minute stabilisation period, increasing concentrations of drug (0.03-100 µM) were superfused at a constant rate of 7 ml/min. Following a 30-minute incubation period, the diastolic membrane potential (DMP), action potential amplitude (APA), action potential duration (APD) and maximum velocity of the action potential upstroke (V_{max}) were recorded. Data were analysed by Student's t-test for paired data. Values in Figure 1A are mean ± s.e. mean.

The results obtained showed that cisapride and (±)-sotalol increased the APD at 90 % repolarisation, in a concentration-dependent manner, reaching significance ($p<0.05$) starting at 0.1 µM for cisapride and 10 µM for (±)-sotalol (Figure 1A). No effect on DMP, APA and V_{max} was observed at any of the concentrations tested. Figure 1B shows the lengthening of the action potential when cisapride concentration was increased.

FIGURE 1



Each point represent mean ± s.e.mean
* p<0.05, ** p<0.01 vs. basal (Student's t-test for paired data).

These results support the usefulness of piglet cardiac Purkinje fibres as a suitable model for predicting the potential of drug-induced arrhythmogenic effects.

Carlsson, L., et al., (1997), JPET. **282**, 220-227
Carmeliet, E., (1984), JPET. **232**, 817-825

127P SELECTIVITY OF CALCIUM CHANNEL BLOCKERS ON T- AND L-TYPE CALCIUM CURRENTS IN GUINEA-PIG VENTRICULAR MYOCYTES

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Both L- (CaL) and T-type (CaT) calcium channels are present in the heart (McDonald *et al.*, 1994). They have different distribution, functional role, and electrophysiological and pharmacological properties. CaL in cardiac myocytes are blocked by classical calcium channel blockers (CCB), which are classified in different chemical classes: phenylalkylamines, dihydropyridines and benzothiazepines. T-type channels are thought to be insensitive to these drugs and to be selectively blocked by the tetralol derivative mibefradil, recently withdrawn from the market due to multiple metabolic interactions.

Due to their different voltage-dependence, CaT or CaL blockade by CCB is usually studied by applying steps from more (-90 mV) or less negative (-50 mV) holding potentials (HP), respectively. Thus it is practically impossible to extrapolate from the data obtained by using voltage clamp protocols the relative selectivity of CCB under physiological conditions. Thus, we aimed to compare the CaT/CaL blocking selectivity (T/L) of several CCB by evaluating their effects on both components evoked in the same cell.

A voltage-clamp protocol mimicking a normal action potential was used: HP of -90 mV, 200 ms depolarising steps to -50/+50 mV. A concentration of CCB, reported to block at least 50% of CaL evoked from a HP in the range of -50 to -30 mV, was used: 1 μM for amlodipine (AML), lacidipine (LAC) and lercanidipine (LER); 10 μM for diltiazem (DIL); 1 μM for verapamil (VER). Mibefradil (MIB, 3 μM) was used as representative CaT blocker. All drugs except MIB were dissolved in DMSO (0.1%). Methods for cell isolation and patch-clamp recordings were as described previously (Cerbai *et al.*, 97; Koidl *et al.*, 1997). Guinea-pig ventricular myocytes having both CaT and CaL were superfused with a Na^+ and K^+ free solution pre-warmed to 35°C, to abolish overlapping currents. Data are expressed as mean±s.e.m. Statistics was performed by means of ANOVA followed by the Student-Neuman-Keuls test. Probability of less than 0.05 was considered significant.

Using the described voltage protocol, all CCB blocked less than 20% CaL, with the exception of LAC which reduced CaL by 61.3% of control. Surprisingly, all CCB blocked a significant amount of CaT, varying from 0.8% (DIL) to 28% (MIB). We calculated for each cell the ratio T/L between CaT and CaL blockade. As expected, MIB showed the highest T selectivity (1.3±0.1, n=5); LAC and DIL resulted to be L selective (T/L= 0.4±0.1, n=3 and 0.8±0.1, n=8, $p<0.05$ versus MIB). VER (0.99±0.05, n=8) and AML (0.92±0.1, n=5) were not selective, while LER showed a slight T selectivity (1.1±0.05, n=6). Thus, CCB can be differentiated also on the basis of their T/L selectivity.

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128P CALLIPELTIN A: CARDIAC EFFECTS AND INHIBITION OF Na/Ca EXCHANGER

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It has been recently reported (Tsunoo & Kamijo, 1999) that cyclic depsipeptides produced by microorganisms induce positive inotropic and negative chronotropic effects in rat heart. Callipeltin A is a cyclic depsipeptide recently isolated from the New Caledonian Lithistida sponge *Callipelta sp.*, (Zampella et al., 1996). In the present study we have investigated the effect of Callipeltin A in cardiac preparations.

Force of contraction was measured in isolated guinea-pig left atria electrically driven at 1Hz (Cargnelli et al., 1994). Na/Ca exchange activity measured as Na_i-dependent ^{45}Ca uptake, Na,K ATPase and Ca ATPase activities, and Ca binding were determined in bovine cardiac sarcolemmal vesicles as reported previously (Luciani et al., 1995). Type III phosphodiesterase activity of bovine heart was measured as reported by Floreani et al. (1997). The pCa-tension relationship of skinned papillary bundles of guinea-pig heart was measured as described by Danieli-Betto et al. (1990). All data are means ± SEM (n=4).

In guinea pig left atria Callipeltin A (2 μM) increases the force of contraction from 100 ± 25 mg to 250 ± 60 mg accompanied by a rise in the resting tension (50 ± 15 mg). In cardiac sarcolemmal vesicles Callipeltin A (0.5 - 4 μM) inhibits the Na/Ca exchange activity with a IC_{50} of 0.89 ± 0.15 μM and a maximal inhibition of 95% at 3 μM. On the contrary, Callipeltin A at a concentration that inhibits the Na/Ca exchanger, does not inhibit Ca-binding and other enzymatic activities (Table I) and does not show Ca-ionophore action (no ^{45} Ca uptake in the absence of Na gradient). 4 μM Callipeltin A does not change the pCa-tension relationship of skinned papillary bundles: pCa50 6.09 ± 0.05 and 6.12 ± 0 in control and treated fibers, respectively; Hill coefficient 3.28 ± 0.35 and 3.35 ± 0.33 in control and treated fibers, respectively.

Table I. Specificity of Callipeltin A (2 μM)

	Control	Treated	% change
Na/Ca exchange (nmol Ca/mg prot/10 s)	4.23 ± 0.15	0.72 ± 0.12	-83
Ca binding (nmol Ca /mg prot/10min)	2.23 ± 0.17	1.9 ± 0.1	-15
Na, K ATPase (nmol ATP/mg prot /min)	400 ± 73	533 ± 65	+33
Ca ATPase (nmol ATP/mg prot /min)	120 ± 50	107 ± 38	-11
Phosphodiesterase III (nmol cAMP/mg prot /min)	0.44 ± 0.02	0.41 ± 0.02	-7

The data presented show that Callipeltin A induces a positive inotropic effect and is a potent and selective inhibitor of the Na/Ca exchanger. It is proposed that the positive inotropic effect of Callipeltin A on guinea-pig atria is due to the inhibition of the Na/Ca exchanger.

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129P CO-ADMINISTRATION OF SPERMINE AND NEWBORN CALF SERUM TO RATS ELEVATES BLOOD PRESSURE *IN VIVO*

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A strong correlation exists between hypertension and polyamine content in cardiovascular tissues of spontaneously hypertensive rats (Ibrahim et al., 1996) and in hypertension induced by angiotensin II (Ibrahim et al., 1995). However, it is not clear whether accumulation of polyamines contribute to hypertension. We have therefore examined the effects of spermine on rat blood pressure *in vivo*. We have also examined whether spermine acts directly or through aldehyde derivatives generated following its metabolism by amine oxidases present in newborn calf serum.

Male Wistar rats (270 – 370 g, Charles River, Italy) were treated daily with normal saline (NS; 0.9 %, w/v; 2 ml/rat), newborn calf serum (NCS; 1.5 ml/rat), spermine (SPR; 20 mg/kg), or NCS (1.5 ml/rat) plus SPR (20 mg/kg) for four weeks. Mean blood pressure (MBP) was measured daily using a tail cuff. Diastolic and systolic blood pressure were determined by carotid cannulation in rats sacrificed 4 weeks after treatment. Serum nitrite was determined by the Griess assay in samples collected immediately after sacrifice.

NCS plus SPR caused a significant increase in MBP, elevating the latter from 130±6 mmHg to 160±4.0 mmHg (n=10, $P<0.01$) by week four. Comparing NS group to NCS plus SPR both diastolic and systolic blood pressure were both elevated, increasing from 67.8±8.2 to 103.6±4.07 ($p<0.005$) and from 105±10 to and 136.2±3.2 ($p<0.05$) respectively. In addition, accumulated plasma nitrite was inhibited by approximately 50%. There was also a statistically significant correlation ($P<0.05$) between plasma nitrite levels and MBP. Neither the administration of NS nor NCS or SPR caused a significant change in MBP, diastolic and systolic blood pressure or plasma nitrite levels.

In conclusion, our results demonstrate that the polyamine spermine, in the presence of NCS, elevates blood pressure *in vivo* and significantly attenuates plasma nitrite levels presumably by inhibiting the constitutive NO pathway. These findings may have important implications in pathologies such as hypertension and pre-eclampsia, where elevated levels of polyamine have been documented.

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130P EFFECTS OF E-4031, CISAPRIDE, TERFENADINE AND TERODILINE ON CARDIAC REPOLARISATION IN CANINE PURKINJE FIBRE AND HERG CHANNELS EXPRESSED IN HEK293 CELLS.

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In response to the emphasis placed on *in vitro* assays to define a novel compound's potential to prolong the electro-cardiogram QT interval in man we report the results of testing E-4031, cisapride, terfenadine and terodiline in 2 such assays: the dog isolated Purkinje fibre and inhibition of the HERG (Human Ether-a-gogo Related Gene) potassium current expressed in HEK293 cells.

Intracellular recordings were made from Purkinje fibres, of male beagle dogs, as previously described (Gwilt et al., 1991). HERG current recorded from HEK293 cells using standard whole-cell patch clamp techniques (Hamill et al., 1981), was activated by applying "action potential-like" voltage pulses.

E-4031 and cisapride prolonged the dog Purkinje fibre action potential duration at 90% repolarisation (APD90; Table). Neither terfenadine nor terodiline had any effect on APD90 compared to vehicle controls. All 4 compounds blocked HERG current in a concentration dependent manner (Table 1).

The free plasma concentrations for E-4031, cisapride, terfenadine and terodiline associated with around 10% prolongation of QT in man are approximately 5, 16, 10 and 100nM, respectively (Honig et al., 1993, Thomas et al., 1995). At these concentrations all 4 compounds inhibit HERG current by 10-20%. E-4031, also prolongs Purkinje fibre APD90 to a similar extent and at similar concentrations to those inhibiting HERG. The other agents tested which may have effects at additional cardiac ion channels produced smaller or negligible APD90 prolongation possibly as a result of those mixed channel-blocking properties.

Table 1.

Drug	Concentration (nM)	ΔAPD90 % (mean±SEM (n=5))	HERG (IC50 nM)
E-4031	20	29.3 ± 5.7*	17
	200	94.6 ± 20.7*	
	2000	115.6 ± 23.6*	
Cisapride	3	3.0 ± 1.4	35
	30	8.5 ± 1.3	
	300	37.0 ± 4.7*	
Terfenadine	20	1.9 ± 1.4	50
	200	3.1 ± 2.1	
	2000	5.8 ± 2.2	
Terodiline	10	0.2 ± 0.9	364
	100	0.5 ± 1.8	
	1000	1.1 ± 1.9	

*P<0.01 vs time-matched vehicles (data not shown) using Kruskall-Wallis.

In conclusion concentrations of E-4031, cisapride, terfenadine and terodiline associated with QT prolongation in man inhibit HERG current by around 20%. This assay provides a sensitive measure of a compound's potential to prolong the QT-interval by blockade of the delayed rectifier current associated with HERG. Subsequent testing in Purkinje fibre reveals which compounds appear to have mixed ion channel effects.

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131P EFFECTS OF E-4031, CISAPRIDE, TERFENADINE AND TERODILINE ON MONOPHASIC ACTION POTENTIAL DURATION (MAPD) AND INCIDENCE OF TORSADE DE POINTES (TdP) IN THE DOG.

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E-4031, cisapride, terfenadine and terodiline affect cardiac ionic currents and cardiac repolarisation. Here we examine their effects on in vivo cardiac electrophysiology in normal, and arrhythmogenicity in chronically AV-blocked isoflurane-anaesthetised male beagle dogs, respectively.

MAPD was recorded from the right ventricle during pacing at 150bpm (n=4-6 per group). TdP was recorded from the electro-cardiograms of dogs previously prepared by ablating the atrio-ventricular node and pacing at 40bpm via a pacemaker. Compounds were given intravenously in a loading bolus and maintenance infusion to achieve steady-state levels.

All four compounds prolonged MAPD in a dose-dependent manner and were associated with an incidence of TdP (Table 1). The free (drug not bound to plasma protein) concentrations associated with a 10% prolongation of MAPD were 0.3, 8, 2.8 and 140 nM for E-4031, cisapride, terfenadine and terodiline, respectively. These are comparable to the free levels associated with prolongation of the electrocardiogram QT interval in man and block of HERG (Human Ether-a-gogo Related Gene) potassium currents in vitro (Leishman et al., 2000). Maximum prolongation of MAPD appeared lower for terfenadine than E-4031, which may be related to the mixed channel effects noted in Purkinje fibre. TdP was associated with the plasma levels required to prolong MAPD in the normal anaesthetised dog by 8-17%. Interestingly in the case of cisapride and terfenadine some dogs showed no evidence of TdP despite even higher levels of drug which were associated with marked prolongation of MAPD in the anaesthetised dog.

In conclusion concentrations of E-4031, cisapride, terfenadine and terodiline associated with around 10% QT prolongation in man prolong MAPD in the anaesthetised dog to a similar extent. Furthermore, in the AV-blocked dog these concentrations are associated with TdP. Since these active concentrations are comparable to the potency and rank order of the compounds for inhibiting HERG current, the *in vitro* HERG assay may be useful in predicting *in vivo* effects on QT.

Table 1.

Drug	Dose ($\mu gkg^{-1} + \mu gkg^{-1}min^{-1}$)	ΔMAPD (%, mean ± SEM)	TdP (incidence)
E-4031	8 + 0.05	8.4 ± 1.8	1/5
	32 + 0.19	16.2 ± 2.3 *	4/4
	128 + 0.77	26.0 ± 4.0*	NT
	512 + 3.07	25.6 ± 4.2*	NT
Cisapride	40 + 0.21	4.5 ± 0.5	0/5
	160 + 0.85	10.4 ± 1.3 *	1/5
	640 + 3.41	17.0 ± 2.3 *	3/4
	2560 + 13.65	24.8 ± 2.3*	0/1
Terfenadine	40 + 1.07	5.2 ± 2.0	0/5
	160 + 4.27	7.1 ± 1.4	0/5
	640 + 17.07	13.2 ± 3.4	1/5
	2560 + 68.27	13.8 ± 3.3 *	0/4
Terodiline	40 + 0.23	1.9 ± 0.9	0/5
	160 + 0.91	5.2 ± 1.1	0/5
	640 + 3.63	7.1 ± 1.4	0/5
	2560 + 14.61	12.9 ± 1.0 *	2/5

P<0.05 compared to vehicle controls (data not shown; ANOVA)

Leishman, D.J., Helliwell, R., Wakerell, J & Wallis, R.M. (2000). *This Meeting*

132P DIVERGENT REGULATIONS OF MMP-2 AND MMP-9 IN ADULT RAT CARDIAC FIBROBLASTS: MECHANISM OF proMMP-2 ACTIVATION

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The migration and proliferation of cardiac fibroblasts in the areas of myocyte loss following ischaemia and reperfusion leads to fibrosis and ventricular remodelling (Weber & Brilla, 1991). Basement membrane degradation is the prerequisite for initiating the cell migration and the activation of matrix metalloproteinase-2 (MMP-2) is essential for both these processes (Morgunova *et al.*, 1999). MMP-2 is secreted as a zymogen (proMMP-2): in a number of different cell systems its activation is thought to take place primarily on the cell surface and to involve membrane-type 1 matrix metalloproteinase (MT1-MMP) and tissue inhibitor 2 of matrix metalloproteinase (TIMP-2) (Nagase & Woessner, 1999). In this study, we sought to examine the effects of endothelin-1 (ET-1), angiotensin II (Ang II), phorbol 12-myristate 13-acetate (PMA) and Concanavalin A (Con A) on the release and activation of MMP-2 from cardiac fibroblasts. Primary cultures of adult rat cardiac fibroblasts were prepared by enzymatic dissociation of left ventricular tissue from male Sprague-Dawley rats (250 - 300 g). Once confluent, cells were re-plated onto 6-well plates or 100 mm dishes and maintained in MEM containing new-born calf serum (10 %) until confluent. The medium was changed to MEM plus transferrin (1 mg/ml), insulin (1 mg/ml) and bovine serum albumin (100 mg/ml) for 48 h before onset of experimental protocols. Cells were treated with ET-1 (10^{-7} M), Ang II (10^{-6} M), PMA (1 – 200 nM), and Con A (1 - 50μg/ml) for 24 hrs. Media samples were then collected and whole cell lysates were prepared. MMP activity was assessed using gelatin-zymography followed by densitometric analysis of the lytic bands.

The expressions of MT1-MMP and TIMP-2 in the media and whole cell lysates were detected by Western blotting. Where appropriate, results are expressed as mean ± s.e.m. Each experiment was repeated at least three times using independent cell populations. We found that proMMP-2 was the only gelatinase constitutively secreted by cardiac fibroblasts (n = 20). In contrast to previous studies (Guarda *et al.*, 1993), neither levels nor activation of MMP-2 were modulated by either Ang II or ET-1 (Figure 1). PMA (100 nM) induced secretion of proMMP-9 and increased secretion of proMMP-2 by 40 ± 6 % (n = 4, P< 0.05). Treatment with Con A induced activation of MMP-2 and this coincided with expression of MT1-MMP and TIMP2 in whole cell lysates. These findings suggest that in common with other cell types, activation of MMP-2 secreted from cardiac fibroblasts requires the presence of MT1-MMP and TIMP-2 at the cell membrane.

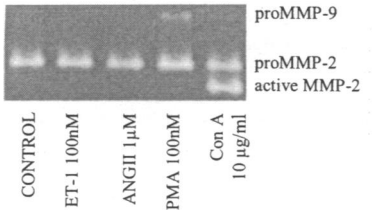

Figure 1. Zymographic analysis of media from fibroblasts treated as indicated.

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133P PREVENTION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA, ECSTASY) INDUCED NEURODEGENERATION BY THE CLOMETHIAZOLE ANALOGUE AR-A008055

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It was recently shown that both (R)-(+)-AR-A008055 and (S)-(-)-AR-A008055 protected against ischaemic damage to gerbil hippocampus (Nelson et al., 2000a). It was also demonstrated that (S)-(-)-AR-A enhanced $GABA_A$ receptor function in an analogous manner to clomethiazole (CMZ), involving the direct opening of the chloride channel of the receptor ionophore (Nelson et al., 2000a; Green et al., 2000), while (R)-(+)-AR-A enhanced GABA function through an unrelated mechanism. CMZ also protects against the neurotoxic degeneration to 5-HT nerve endings that follows 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) by a mechanism independent of its effect on body temperature (Colado et al., 1998), unlike pentobarbitone which protects by preventing MDMA induced hyperthermia (Colado et al., 1999). We have now investigated the effect of the AR-A008055 enantiomers on MDMA-induced damage to cerebral 5-HT nerve endings.

Male DA rats (150-170g) were injected with a neurotoxic dose of MDMA (15 mg kg^{-1}i.p.). Groups were injected with saline (controls), (R)-(+)-AR-A or (S)-(-)-AR-A (100 mg kg^{-1}i.p.) 5 min prior to and 55 min after the MDMA injection. Rectal temperature was measured at this time. Seven days later the concentration of 5-HT was measured by hplc in hippocampus, striatum and cortex. In a further experiment, rats given MDMA plus the AR-A enantiomer were kept warmed with a homeothermic blanket so that their rectal temperature was similar to animals given only MDMA. All neurochemical data were analysed by 1 way ANOVA followed by Newman-Keuls.

Rats injected with MDMA displayed a hyperthermic response of approx. 1.5 °C for several hours, an effect abolished by both (R)-(+)- and (S)-(-)-AR-A. 7 days later the MDMA-treated rats had a major decrease in the 5-HT content of the hippocampus (47±3% of control, n=5-6; $p<0.01$) and the other 2 regions. Neither enantiomer altered basal 5-HT concentrations, but both attenuated the MDMA-induced decrease [(R)-(+)-AR-A: 68± 3%; (S)-(-)-AR-A: 67±6% of control values; both different from control $p< 0.01$]. When rats given MDMA+ enantiomer were warmed and their rectal temperature kept similar to rats given only MDMA, no protection was seen in hippocampus following (R)-(+)-AR-A (5-HT content: 51± 9% of control values, while (S)-(-)-AR-A was still neuroprotective (61±3% of control; different from MDMA group $p<0.01$).

These data demonstrate that (S)-(-)-AR-A008055, like CMZ, protects against MDMA-induced neurodegeneration by a mechanism independent of its effect on MDMA-induced hyperthermia. As both compounds interact with the $GABA_A$ receptor in the same unique way, this mechanism may account for the protective effects seen here. Pentobarbitone and (R)-(+)-AR-A008055, both enhance GABA function through other mechanisms (Nelson et al., 2000a; 2000b), and only protect because they prevent MDMA-induced hyperthermia.

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134P THE NOVEL DOPAMINE D2 RECEPTOR PARTIAL AGONIST, SLV-308, REVERSES MOTOR DISABILITY IN MPTP-LESIONED COMMON MARMOSETS (*CALLITHRIX JACCHUS*).

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SLV-308, a benzoxalozone, is a dopamine D2 'like' receptor partial agonist with 5-HT1A receptor agonist activity that has therapeutic potential in the treatment of psychoses (Feenstra *et al*, 2000; Ichikawa & Meltzer, 2000). However, partial D2 receptor agonists may also have efficacy in treating Parkinson's disease (PD; Brucke et al, 1988). L-DOPA is the most commonly used treatment for PD, although its beneficial actions are complicated by psychosis. A partial dopamine receptor agonist with antipsychotic activity may be of potential benefit in treating the disease. For this reason, we have investigated the effects of SLV-308 on motor deficits in the MPTP-treated common marmoset.

Adult common marmosets (*Callithrix Jaccus*; n=2 male & n=2 female: 304-433g), previously treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce stable motor deficits (Pearce *et al*, 1998), were treated with domperidone (2mg/kg po) 40 minutes prior to SLV-308 (0.003-0.3mg/kg ip in 0.9% sterile saline) administration. A modified latin square design was employed with one week between treatments. Locomotor activity was measured in automated photocell cages for 8 hours following SLV-308. During this period, disability was assessed 30 minute intervals by a trained observer, blinded to the treatment (Pearce *et al*, 1998).

SLV-308 produced a long-lasting (8h), dose-dependent increase in locomotor activity and decrease in disability scores compared to vehicle (Table 1). The threshold dose for inducing motor activity was SLV-308 0.03mg/kg, and the maximum effect was observed at 0.3 mg/kg.

treatment	locomotor counts	disability score
vehicle	2037 ± 752	120 ± 13
0.003mg/kg	5341 ± 1503	112 ± 10
0.01mg/kg	8387 ± 2261	52 ± 8*
0.03mg/kg	10883 ± 2450*	52 ± 4*
0.1mg/kg	14575 ± 2914*	42 ± 6*
0.3mg/kg	14757 ± 4387*	42 ± 4*

Table 1: Cumulative locomotor counts and disability scores following SLV-308. Data are expressed as the mean ± s.e.m. (n=4) * $p<0.05$ vs. vehicle (Friedman followed by Wilcoxon-Signed Ranks Test).

These data show that SLV-308 has potent antiparkinsonian activity, reversing both akinesia and disability. As a partial agonist, SLV-308 presumably has full D2 receptor activity in the denervated striatum. However, its partial D2 'like' receptor efficacy in the mesolimbic and mesocortical systems may contribute to its anti-psychotic actions. In conclusion, SLV-308 may be a useful alternative adjunct for late stage L-DOPA treatment of PD patients.

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135P THE EFFECTS OF ORG 25935 ON THE EXTRACELLULAR LEVELS OF GLYCINE IN BRAIN REGIONS OF FREELY MOVING RATS

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The uptake of glycine into presynaptic nerve terminals or the neighbouring fine glial processes constitutes an efficient mechanism by which its postsynaptic action can be regulated. This process is carried out by two different glycine transporters, GlyT-1 and GlyT-2. It has been demonstrated that the GlyT-1 is present throughout the central nervous system (e.g. Kim et al., 1994) whereas the GlyT-2 mRNA is dominantly located in the spinal cord, brainstem and cerebellum (Jursky & Nelson, 1995; Zafra *et al.*, 1995). Previous reports have demonstrated that inhibition of glycine re-uptake by a selective GlyT-1 inhibitor Org 24598 (Walker et al., 1999), causes significant increases in the extracellular glycine levels in various brain regions of freely moving rats (Ge et al., 1999). Glycine re-uptake inhibitors may enhance glutamatergic function or normalise the glutamatergic hypofunction in schizophrenia via increases in glycine levels in the CNS. In the present study, we have investigated the effect of a chemically unrelated, selective and potent glycine re-uptake inhibitor, Org 25935 (cis-N-methyl-N-(6-methoxy-1-phenyl-1,2,3,4-tetrahydronaphthalen-2-ylmethyl)amino methylcarboxylic acid hydrochloride), on the extracellular levels of glycine in frontal cortex, hippocampus and striatum of freely moving rats using microdialysis.

Male rats (Wistar, 250 - 300 g, Harlan) were anaesthetised before 15 mm long guide cannulae were stereotaxically inserted and allowed at least a week for recovery. A custom-built microdialysis probe with 4 mm microdialysis membrane (molecular weight cut off 5,000) was gently inserted into the hippocampus (final microdialysis probe tip location, mm, A -4.5, V -7.9, L -4.9, relative to Bregma), frontal cortex (mm, A +3.5, L -1.5, V -5.5, relative to Bregma) or striatum (mm, A +0.5, L -2.5, V +6.8, relative to Bregma; Paxinos & Watson, 1986) and was perfused with an artificial cerebrospinal fluid (aCSF) at 2 µl/min. Dialysate glycine levels were quantified by HPLC coupled with fluorescence detection.

Systemic administration of Org 25935 (3, 6 and 10 mg/kg, i.p.) maximally increased the glycine levels by 35, 80 and 130 % ($n=3-5$, $p<0.01$ - 0.5; ANOVA Dunnett's t-test), above the basal levels, in rat striatum, whilst Org 25224, the optical isomer of Org 25935 failed to modify the glycine levels. In frontal cortex and hippocampus, Org 25935 (6 mg/kg, i.p.) also induced significant increases in glycine levels by 75 and 70 % ($n=3-5$, $p<0.01$), respectively, above the basal levels. Sub-chronic administration (3 mg/kg, p.o. daily for 2, 3 and 4 days) elevated striatal glycine by 35-40 % ($n=4-6$, $p<0.05$; ANOVA Dunnett's t-test) above basal levels on each of the days.

The present studies demonstrated that systemic administration of Org 25935 dose-dependently increased the extracellular glycine levels in striatum of freely moving rats. Org 25935 also caused significant elevation in glycine levels in rat frontal cortex and hippocampus with no significant regional differences. Radio-labelled uptake studies have indicated that Org 25935 possesses high affinity for GlyT-1, suggesting that Org 25935-induced elevation in glycine levels is directly related to inhibition of this transporter.

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136P DETERMINATION OF THE EXTRACELLULAR GLYCINE CONCENTRATION IN THE RAT SPINAL CORD *IN VIVO*

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Binding of glycine at the strychnine-insensitive site is known to modulate opening of the NMDA receptor channel. It has remained unclear, however, whether this binding site is saturated *in vivo*. Although the concentration of glycine in cerebrospinal fluid (CSF) is sufficient to support saturation, several studies have reported potentiation of NMDA receptor-mediated neuronal activity in the spinal cord by agonists of this site in vivo (*e.g.* Budai *et al.*, 1992). This may be due to lower glycine levels in the extracellular fluid (ECF) which, rather than the CSF, is in closest contact with the synapse. We have, therefore, determined the glycine concentration ([glycine]) of the ECF in the rat spinal cord dorsal horn using the zero net flux method of quantitative microdialysis (Lönnroth *et al.*, 1987). This is compared to the glycine content of CSF from the same rat.

Methodology for spinal cord microdialysis was adapted from Gerin & Privat (1996). Male Wistar rats (250-230g; Charles River) were used and anaesthetised throughout under halothane/N_2O). The microdialysis probe was introduced through a burr hole in the dorsal surface of vertebra Th13 to place a 1.5 mm length of dialysis membrane unilaterally into the dorsal horn of the L3/L4 lumbar region of the spinal cord. The probe was perfused at a rate of 2 $\mu l.min^{-1}$ with an artificial extracellular fluid solution (aECF). Following a 20 min recovery period, perfusion was switched between aECF containing glycine at nominal concentrations of 0, 4, 8, 12 and 25 µM. Four dialysate samples (10 µl) were collected at each perfusate [glycine], the order of which was randomised between rats. Perfusate and equilibrated dialysate [glycine] (mean last two samples at any given perfusate [glycine]) was determined by HPLC coupled to fluorescence detection. CSF was collected from the lumbar cord prior to microdialysis probe insertion. Samples were filtered to 5000 MW (Ultrafree®- MC, Millipore, UK) and the glycine content determined by HPLC with fluorescence detection.

ECF [glycine] was determined to be 2.59 ± 0.31 µM (mean ± s.e. mean, n = 5). Correlation coefficients ® for linear regression were >0.9 in all experiments. CSF [glycine] was 6.37 ± 1.11 µM (mean ± s.e. mean, n = 5). There was no correlation between ECF and CSF concentrations (r = 0.15).

The CSF [glycine] reported here is in agreement with previous studies (*e.g.* Budai *et al.*, 1992). The ECF [glycine], though 2 – 3 fold lower than in the CSF, would still support saturation of the strychnine-insensitive site *in vivo*. However, the presence of specific uptake mechanisms for lgycine in the CNS may allow lower intrasynaptic glycine levels. The lack of correlation between ECF and CSF [glycine] would indicate that the ECF level is regulated independently of the CSF concentration.

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137P PRE-TREATMENT WITH IMIDAZOLINE$_2$ BINDING SITE COMPOUNDS INCREASES THE BINDING OF [^{3}H]2BFI IN RAT BRAIN MEMBRANES

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It has been previously reported (Steinberg *et al.*, 1999) that the monoamine oxidase (MAO) inhibitor tranylcypromine (TCP) is capable of potentiating the binding of [^{3}H]2BFI (2-(2-benzofuranyl)-2-imidazoline) in human brain and platelets. This potentiation of binding has not been seen in other species and appears to involve only MAO B, but does not seem to relate directly to the inhibition, by TCP, of this enzyme. Here we report a potentiation of [^{3}H]2BFI binding in rat brain membranes caused by pre-incubation with the imidazoline$_2$ (I_2) biding site ligands 2BFI and BU224 (2-(4,5-dihydroimidazol-2-yl)quinoline).

Whole brain membranes (male, Wistar, ~250g) were prepared as previously described (Lione *et al.*, 1998). Aliquots, 2.7ml containing ~3.3mg protein were incubated (20min at 37°C) with either 2BFI or BU224 (10μM, 300μl) or a buffer control (50mM ortho phosphate, 1mM $MgCl_2$, pH 7.4, 300μl). Similarly 2.7ml aliquots containing ~12mg protein were incubated with 2BFI (35μM, 300μl) or a buffer control (300μl). At the end of the incubation the sample was diluted with 25ml ice cold buffer vortexed and centrifuged to precipitate the membrane (32,000g for 20min). Any remaining compound was then removed by repeated washing in excess buffer. Buffer, 30ml, was added, the precipitate re-suspended and centrifuged (32,000g for 20min), this was repeated four times. The resulting membrane precipitate was then tested for its ability to bind specifically the selective I_2 ligand [^{3}H]2BFI either at a single concentration (5nM) or over a range of concentrations (0.005-10nM) as described elsewhere (Lione *et al.*, 1998). These data were analysed using the nonlinear regression and statistical analysis supplied with GraphPad Prism version 3.00 for Windows (San Diego California USA).

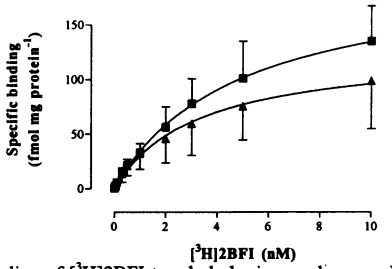

Figure 1. Binding of [^{3}H]2BFI to whole brain membranes. Pre-treatment of membranes with either buffer controls (▲) or 2BFI 35μM (■). Each curve represents the means of five experiments performed in triplicate, vertical bars represent the S.D.

Pre-incubation with 10μM BU224 or 2BFI resulted in a significant increase in the specific binding of 5nM [^{3}H]2BFI, 114.3±17.5 and 123.6±29.8 fmol mg of protein^{-1} respectively, when compared with the control, 69.0±9.3 fmol mg of protein^{-1}, (P<0.01, ANOVA with Dunnetts post test) Figure 1 shows the saturation binding of [^{3}H]2BFI to the rat brain membranes pre-treated with 35μM 2BFI. Again there is a significant increase in the B_{MAX} of the treated samples, 199.2± 45.8 fmol mg of protein^{-1}, compared with the controls, 130.7±46.3 fmol mg of protein^{-1}, (P<0.05 student t-test, two tailed). This increase in binding is most easily explained by the hypothesis that the BU224 or 2BFI are making available a further population of sites that then remain accessible after removal of the I_2 ligands. Whether this is the case or if this potentiation is similar to that seen by others (Steinberg *et al.*, 1999) requires confirmation.

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138P EFFECT OF BU99006, AN IRREVERSIBLE IMIDAZOLINE$_2$ BINDING SITE LIGAND, IN THE DISTRIBUTION OF [^{3}H]2BFI AND OF [^{3}H]RX821002 BINDING TO RAT BRAIN SECTIONS

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BU99006 (5-isothiocyanato-2-benzofuranyl-2-imidazoline) is a selective, irreversible ligand for I_2-binding sites (BS) *in vitro* (Coates *et al.*, 2000). Irreversible inactivation of I_2-BS *in vivo* has also been demonstrated in rat whole brain membranes following BU99006 pre-treatment (Tyacke *et al.*, 2000). In the present study, we have used receptor autoradiography to determine the effect of BU99006 pre-treatment on the distribution and density of both I_2-BS and α_2-adrenoceptors (AR) in rat brain sections.

Male Wistar rats (270-310g) were anaesthetised with sodium pentobarbital (60 mg kg^{-1}, i.p.) and treated with BU99006, (15 mg kg^{-1}, i.v.) or vehicle. The animals remained anaesthetised and 30 mins post injection were perfused, trans-cardiac to remove any un-reacted BU99006 and blood from the brains. The brains were removed and immediately frozen in isopentane on dry ice and stored at -70°C. Brains were sectioned (12μm) and binding with [^{3}H]2-BFI (1nM) ± BU224 (10μM) or [^{3}H]RX821002 (1nM) ± rauwolscine (10μM) performed according to the methods described by Lione *et al.*, (1998) or Hudson *et al.*, (1992) respectively. Autoradiograms were analysed using computer-assisted densitometry (MCID 5) and comparison between BU99006-treated and vehicle controls made using Student's unpaired t-test for each brain area analysed.

Results for specific [^{3}H]2-BFI binding showed a reduction across all brain areas in animals treated with BU99006 compared with control animals (figure 1). A residual level of binding was observed across all brain areas analysed in the BU99006-treated group. However, the binding density was at the limits of detection of the apparatus (~5 fmol mg tissue^{-1}). Binding to α_2-AR was not affected by pre-treatment with BU99006 with similar binding densities observed in brain areas associated with α_2-adrenoceptor expression. Mean section density for [^{3}H]RX821002 binding to controls (35.7 ± 3.3 fmol mg tissue^{-1}) and BU99006-treated (37.2 ± 2.5 fmol mg tissue^{-1}) did not differ significantly.

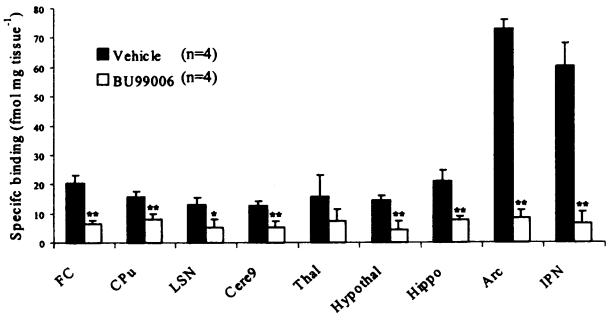

Figure 1: Specific [^{3}H]2-BFI binding to specific brain areas in rat brain sections. Results shown as Mean ± SD, *P<0.05, **P<0.01

These data show BU99006 is a selective irreversible ligand for I_2-BS *in vivo*. Furthermore, the autoradiographical study confirms a reduction in I_2-BS in all brain areas investigated including discrete regions associated with high levels of I_2-BS. In conclusion, BU99006 selectively inactivates I_2-BS in all brain areas investigated in rat brain confirming its value as a research tool for further studies into this binding site.

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139P AMPA RECEPTOR-MEDIATED EXCITOTOXIC CELL DEATH IN RAT CULTURED OLIGODENDROCYTE PROGENITOR CELLS

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A large body of evidence suggest neurones are vulnerable to glutamate receptor-mediated toxic insults (excitotoxicity). However, recent data suggest that oligodendrocytes (macroglial cells responsible for CNS axonal myelination) may also be vulnerable to excitotoxic insults (Matute, 1998; McDonald *et al.* 1998). The present study examined the ability of selective AMPA and kainate receptor ligands to influence oligodendrocyte progenitor cell (OPC) viability.

Mixed glial cell cultures were prepared from Wistar rat cerebral cortices (2-3 day old, mixed sex) and cultured for 9-11 DIV in DMEM/10% fetal calf serum medium. Loosely attached contaminating microglia were removed by pre-shaking the culture flasks (1h, 200rpm). Consequently, a purified OPC cell suspension was obtained (18h, 200rpm) and seeded (25,000 cells well^{-1}) onto poly-D-lysine-coated 24 well plates. Purified OPCs were maintained for 3 to 5 days in chemically-defined (Bottenstein-Sato N2) medium, supplemented with basic Fibroblast Growth Factor (10ng ml^{-1}) and Platelet-derived Growth Factor-AA (10ng ml^{-1}). OPC identity was confirmed via indirect immunofluorescence using a monoclonal anti-A_2B_5 antibody. Assessment of OPC viability was performed 24 hours following initial drug application via fluorescein diacetate and propidium iodide fluorescence microscopy (20x objective) (Jones & Sneft, 1985). Data are presented as percentage control cell viability (± s.e.m.). Statistical analyses were performed using the Mann-Whitney *U*-test.

Both kainate (pEC_{50}=3.9 ± 0.3, E_{max}=54.5 ± 7.1%, n=5) and (S)-5-iodowillardiine (pEC_{50} = 4.9 ± 0.2, E_{max}= 31.6 ± 3.9%, n=4) induced OPC death. When tested alone, 1mM L-glutamate elicited limited OPC death (91.9 ± 3.4%, n=4, $p<0.05$), whereas 100μM (S)-AMPA was ineffective (n=4). However, in the presence of 100μM cyclothiazide (a selective AMPA receptor desensitisation inhibitor), both 100μM (S)-AMPA and 1mM L-glutamate elicited cell death (65.2±9.3%, n=4, $p<0.05$ and 57.8 ± 6.4%, n=4, $p<0.05$, respectively). When tested alone, 100μM cyclothiazide failed to influence OPC viability (n=4).

Both 300μM kainate and 100μM (S)-AMPA/100μM cyclothiazide-mediated OPC death were abolished by the selective non-competitive AMPA receptor antagonist, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzo-diazepine hydrochloride (GYKI 52466, 50μM). Additionally, the putative AMPA receptor antagonist Evans Blue (5μM), reduced 300 μM kainate-mediated cell death (75.3±5.6%, n=4, $p<0.05$).

These data suggest that OPCs are susceptible to AMPA receptor-mediated excitotoxic insults. Therefore, AMPA receptor antagonists may be useful in limiting white matter degeneration during excitotoxic CNS insults.

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140P PRESYNAPTIC ADENOSINE RECEPTORS MODULATE [^{3}H]D-ASPARTATE RELEASE IN RAT CORTICAL NERVE ENDINGS

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The presence of release-regulating presynaptic adenosine receptors in rat cerebral cortex was investigated by monitoring the release of endogenous glutamate or of previously accumulated [^{3}H]D-aspartate ([^{3}H]D-ASP) from isolated nerve endings in superfusion.

Synaptosomes were prelabeled with [^{3}H]D-ASP and stimulated with 15 mM KCl using normal medium (with Ca^{2+} 1.2 mM) or Ca^{2+}-free medium. The [^{3}H]D-ASP overflow, in normal medium was 1.80±0.07 % (n=15) and in Ca^{2+}-free medium was 0.58 ± 0.08 % (n=7) indicating that 67.52% of the overflow was Ca^{2+}-dependent. The K^+-evoked endogenous glutamate release was also studied to evaluate the Ca^{2+} dependency. The K^+-evoked release in normal medium was 1.8 ± 0.1 ng/mg protein and 0.6±0.05ng/mg protein in a Ca^{2+}-free medium (67.72% of the release was therefore Ca^{2+}-dependent).

The effect of 2-*p*-(carboxyethyl)phenethylamino-5'-N-ethylcarbox amidoadenosine (CGS21680), an adenosine$_{2A}$ (A_{2A}) receptor agonist was studied on both basal and K^+-evoked (15 mM) release of [^{3}H]D-ASP. CGS21680 at 0.1 and 0.01 nM did not produce any effect on basal release (B_2/B_1 ratio of controls = 0.992 ± 0.001, n = 5; B_2/B_1 ratio in presence of CGS21680 0.1 nM = 0.994± 0.024, n=5; B_2/B_1 ratio in presence of CGS21680 0.01 nM = 1.029 ± 0.036, n = 5) but significantly increased theK^+-evoked overflow ($p < 0.01$) with an $EC_{50} \simeq 1.0$ pM, the maximal effect being 26.23% at 0.01 nM concentration. This potentiating effect was not present when Ca^{2+} was omitted from the superfusion medium (the K^+-evoked percent of overflow was in the control 0.58 ± 0.08% and in presence of CGS21680 0.64 ± 0.1%). The potentiating effect of CGS21680 was antagonized by 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine (SCH58261) with a $K_i \simeq 5$ nM. This latter compound did not produce *per se* any effect on both basal and K^+-evoked [^{3}H]D-ASP overflow. Adenosine (1-1000 μM) produced an inhibitory effect (20.59 ± 2.62%, maximal effect; $p < 0.01$) on [^{3}H]D-ASP overflow which was completely counteracted by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1 μM). When used at lower concentration, 0.1 nM to 0.1 μM, adenosine did not modify the K^+-evoked release even in presence of SCH58261 (0.1 μM), DPCPX (1 μM) or both. However adenosine was able to antagonize the potentiating effect of CGS21680 (0.01 nM) with an IC_{50} = 0.1 nM. The A_1 receptor agonist CCPA also produced an inhibitory effect on [^{3}H]D-ASP overflow when used at 0.1μM (27.60%; $p<0.01$) and this effect was counteracted by DPCPX(1μM) but not by SCH58261(0.1μM).

In conclusion, our data indicate the existence of two different subtypes of adenosine receptors on cortical glutamatergic nerve endings mediating inhibition (A_1) or potentiation (A_{2A}) of neurotransmitter release respectively.

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141P INVESTIGATION OF mGlu RECEPTORS IN PROTOPLASMIC AND FIBROUS ASTROCYTES: IMPLICATIONS FOR RECEPTOR CROSS-TALK.

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The aim of this study was to investigate the effects of activation of metabotropic glutamate (mGlu) receptors, and their possible interactions with other glial neurotransmitter systems, on cAMP and inositol phosphates (IPx) accumulation in primary cultures of protoplasmic and fibrous astrocytes.

Cortical astrocytes were obtained from 1 day old male Wistar rats and maintained for at least 15 days in DMEM/F12 medium, as described by McCarthy and De Vellis (1980). Fibrous astrocytes were produced *via* activation of protoplasmic cells by switching cell cultures to DMEM medium, supplemented with growth factors as described by Miller *et al.*, (1993) with slight modification. Evaluation of the influences of agonists on cAMP and IPx accumulation was according to Brown *et al.*, (1972) and Miller *et al.*, (1993). Statistical analysis was done employing two way ANOVA.

Glutamate (Glu) and 1S,3R-ACPD (100μM) treatment failed to increase IPx synthesis in protoplasmic cells, while the response to noradrenaline (NA,100μM) was 310 ± 56% of basal. After astrocyte activation, responses to 100μM of Glu, ACPD, NA, Carbachol (CCh) and Histamine, but not 5-HT were significantly (n=4, $p<0.0001$) enhanced. In cAMP experiments, notable (n=4, $p<0.05$) inhibition of cAMP formation by 100 μM of DCG-IV, Clonidine, CCh, 5-HT and Baclofen was observed in both cell types. However, the response to DCG-IV was selectively increased (n=4, $p<0.01$) by astrocyte activation (EC_{50} of 660 ± 25 nM in protoplasmic and 250 ± 26 nM in fibrous cells). Preincubation of fibrous astrocytes with 100 μM DCG-4 and Clonidine or 10 μM AMPA and kainate did not alter production of IPx induced by 100μM (S)-3,5-DHPG (n=6). When fibrous astrocytes were treated with DHPG (100μM), prior to addition of Gi-coupled receptor agonists listed above, inhibition of cAMP synthesis was completely abolished (n=4, $p<0.01$). This effect of DHPG on cAMP inhibition mediated by DCG-IV and Clonidine was abolished (n=3, $p<0.01$) by pre-incubation with 10 μM of the PKC inhibitor Bisindolylmaleimide (GF 109203X).

In contrast to previous investigations, induction rather than enhancement of response to Glu, ACPD, CCh and Histamine took place upon astrocyte activation, while the response to NA was enhanced. The selective increase in the response to DCG-IV in fibrous cells is consistent with the molecular biological data obtained by Minoshima and Nakanishi (1999). While the DHPG response was not altered by DCG-IV and Clonidine, the effect of Gi-coupled receptors agonists was abolished by DHPG pretreatment. This interaction is dependent on PKC activation mediated *via* group I mGlu receptors.

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McCarthy and De Vellis. (1980). *J. Cell Biol.* **85,** 890-902.
Miller *et al.*, (1993). *Brain Res.* **618,** 175-178.
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142P EFFECT OF METABOTROPIC GLUTAMATE RECEPTORS ON ASTROCYTE PROLIFERATION: INVOLVEMENT OF PKC AND P;D

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Expression of metabotropic glutamate (mGlu) receptors in astrocytes, mainly mGlu5 and mGlu3, is well documented (Pearce *et al.*, 1986). However, their functional roles have not yet been established. Astrocytes differ from their neuronal counterparts in many ways; for example, they may undergo robust proliferation in response to external signals. It was previously reported that mGlu receptors mediate astrocyte proliferation without affecting the overall cell viability (Condorelli *et al.*, 1989; Ciccarelli *et al.*, 1997). However, the intracellular signalling pathway remained to be studied. We report here, that activation of mGlu5 and mGlu3 receptors influences cortical astrocyte proliferation via PKC and PLD.

Cerebral cortical astrocytes were prepared from Wistar rat pups using methods similar to those previously described (McCarthy and DeVellis, 1980). Cells were seeded onto flasks and cultured in modified DMEM/F12 medium. Between 12-14 days, cells were trypsinised and plated onto glass cover slips or 48-well plates. Astrocytic identity (>95%) was confirmed by immunoreactivity for glial fibrillary acidic protein (GFAP). For proliferation studies, agonists and antagonists were added in serum free medium. After 24 hours, cells were incubated with tetrazolium reagent (CellTiter, Promega) for a further 3 hours and absorbance recorded at 490 nm. The group I mGlu receptor agonist, (S)-3,5-dihydroxyphenylglycine (DHPG, 100 μM) increased cortical astrocyte proliferation in the presence and absence of serum. Further studies showed that DHPG and phorbol 12-myristate 13-acetate (PMA) increased proliferation in a dose-dependent manner (EC_{50}s of 14.6 ± 1.2 μM and 480.7 ± 1.6 nM respectively; n=4). A selective mGlu5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) was able to inhibit DHPG (100 μM) induced astrocyte proliferation in a concentration-dependent manner (IC_{50}= 14.24 ± 1.4 μM; n=5). A selective PKC inhibitor, bisindolylmaleimide (GF109203x) also inhibited the proliferative actions of both PMA (100nM; n=4) and DHPG (100 μM) with IC_{50}s of 14.24 ± 1.4 μM and 794.7 ± 2.6 μM respectively. Finally, varying concentrations of butanol, known to inhibit phosphatidic acid formation by PLD, also inhibited the effects of DHPG (100 μM; $P<0.001$; n=6). In contrast, activation of group II mGlu receptors by 2,3-dicarboxycyclopropylglycine (DCG-IV; n=3) inhibited basal astrocyte proliferation. This was inhibited by the group II selective antagonist α-amino-α-[(1S,2S)-2-carboxycyclo-propyl]-9H-xanthine-9-propionic acid (LY341495; 1 μM; n=3).

We conclude that mGlu5 receptors mediate cortical astrocyte proliferation *via* PKC and PLD, whereas mGlu3 receptor activation inhibits proliferation *via* an as yet unknown mechanism.

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Ciccarelli *et al.* (1997). *Glia* **21,** 390-398.
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143P NALOXONE BENZOYLHYDRAZONE IS A PARTIAL AGONIST AT HUMAN NOCICEPTIN RECEPTORS EXPRESSED IN CHINESE HAMSTER OVARY CELLS (CHO_{NCR}): STUDIES WITH GTPγ^{35}S.

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Naloxone benzoylhydrazone (NalBzOH) is a κ-opioid agonist, a μ-opioid antagonist and has variable activity at the nociceptin receptor (NCR) (Calo et al., 2000). We have previously shown that for cAMP inhibition, NalBzOH acts as a partial agonist (E_{max} 44%) compared to nociceptin (NC, 100%) in CHO_{NCR} cells (Nicol et al, 1999). In this study we have further examined the action of NalBzOH using a GTPγ^{35}S binding assay.

Studies were performed with 20μg membranes prepared from CHO_{NCR} cells in 50mM Tris, 0.2mM EGTA, 100mM NaCl, 1mM Mg^{2+}, 0.15mM bacitracin, 1mg. ml^{-1} BSA, 100μM GDP, pH7.4 and ~150pM GTPγ^{35}S. NalBzOH and NC (with peptidase inhibitors) were added in various combinations. Incubations were for 1h at 30°C. Non-specific binding was defined in the presence of 10μM GTPγS. Data are mean±s.e.mean (n≥3).

At 100μM GDP, NC stimulated GTPγ^{35}S binding with a pEC_{50} value of 8.53±0.02. NalBzOH was ineffective. At 10μM, NalBzOH produced a parallel rightward shift in the concentration response curve to NC yielding a pK_B of 6.93±0.03. In a previous study Berger et al (2000) reported that partial agonism at the NCR is clearly observed at lower (5μM) GDP and suggested that a coupling reserve may exist. Studies with NC and NalBzOH were therefore repeated at this lower GDP concentration. Net binding of GTPγ^{35}S increased ~4.3fold and the pEC_{50} of NC increased (9.29±0.02). Moreover, partial agonist activity of NalBzOH was again evident (12.8% relative to NC; pEC_{50} 7.00±0.10), Figure 1.

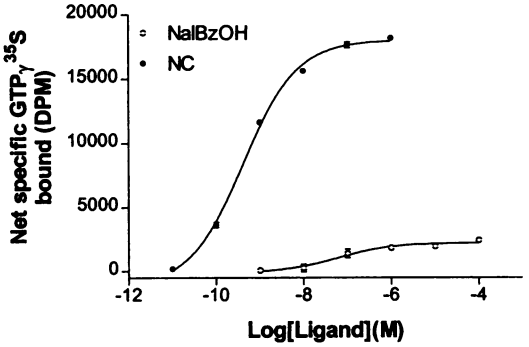

Figure 1. Effect of NC and NalBzOH on GTPγ^{35}S binding at 5μM GDP. Unstimulated GTPγ^{35}S binding has been subtracted to yield net specific binding.

These data are in agreement with the study of Berger et al., (2000) which showed clear partial agonist activity of the nociceptin receptor ligands [F/G]NC(1-13)NH_2 and Ac-RYYRIK-NH_2 at 5μM GDP and essentially no efficacy at 100μM GDP. Consistent with this report our data may also suggest the presence of a coupling reserve in this experimental system (CHO_{NCR}).

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144P A COMPARATIVE STUDY OF THE PHARMACOLOGICAL PROPERTIES OF HOMO-OLIGOMERIC AND HETERO-OLIGOMERIC HUMAN RECOMBINANT 5-HYDROXYTRYTAMINE TYPE-3 (5-HT_3) RECEPTORS

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Recombinant 5-HT_3 receptors assembled as homo-oligomers (5-HT_{3A}) and hetero-oligomers (5-HT_{3A} and 5-HT_{3B}) differ markedly in their biophysical properties (Davies *et al.*, 1999). This study examines whether the 5-HT_{3B} subunit also affects the pharmacological profile of the receptor.

Radioligand binding assays using the selective antagonist [^{3}H]granisetron were performed on membrane homogenates of HEK 293 cells transiently transfected with cDNAs encoding human 5-HT_{3A} and 5-HT_{3B} receptor subunits, either individually, or in combination. The pharmacological profiles of homo- and hetero-oligomeric receptors were determined in saturation and competition binding assays (Hope *et al.*, 1996). Functional characterization of the receptor was performed using voltage-clamp recordings upon *Xenopus laevis* oocytes injected intra-nuclearly with the appropriate cDNAs (Mair *et al.*, 1998). Data are reported as the mean ± s.e.m. Statistical significance ($P<0.05$) was assessed by two-tailed t-test.

The K_d of [^{3}H]granisetron at homo- and hetero-oligomeric receptors was not significantly different (1.00 ± 0.1 nM and 1.05 ± 0.3 nM respectively; n = 3). Six other structurally distinct antagonists, in competition with [^{3}H]granisetron, did not distinguish between the receptor isoforms (Figure 1). By contrast, five agonist ligands consistently exhibited a lower apparent affinity for the hetero-oligomeric receptor (Figure 1).

Against current responses to 5-HT applied to oocytes at EC_{50}, the open channel blocker diltiazem (Gunthorpe & Lummis, 1999) selectively blocked the homo-oligomeric (pIC_{50} = 5.21 ± 0.03) *versus* hetero-oligomeric (pIC_{50} = 4.52 ± 0.09) receptor.

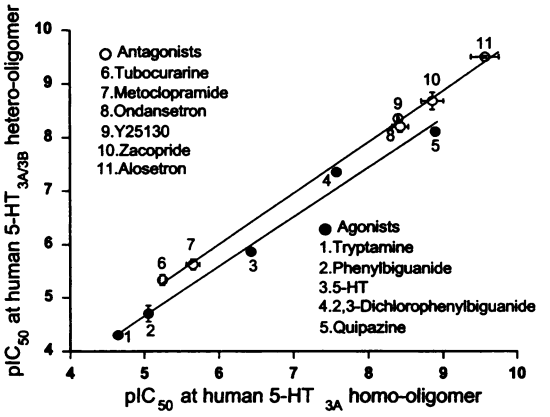

Figure 1. Binding parameters (pIC_{50}) for agonist and antagonist ligands at homo- and hetero-oligomeric 5-HT_3 receptors. Data points are mean ± s.e.m of 3-6 independent experiments.

In summary, the 5-HT_{3B} subunit influences 5-HT_3 receptor pharmacology in a subtle manner.

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145P SB-272183, A SELECTIVE 5-HT_{1A}, 5-HT_{1B} AND 5-HT_{1D} RECEPTOR ANTAGONIST.

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Reports in the literature demonstrate that 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors function as 5-HT autoreceptors, modulating 5-HT release and, in the case of $5HT_{1A}$ receptors, cell firing (Davidson & Stamford, 1995). Blockade of serotonergic autoreceptors are postulated to increase 5-HT transmission which, in turn, may lead to antidepressant activity. In this study we report the *in vitro* profile of SB-272183 (5-Chloro-2,3-dihydro-6-[4-methylpiperazin-1-yl]-1-[4-[pyridin-4-yl]napth-1ylaminocarbonyl]-1H-indole) a novel selective 5-$HT_{1A/1B/1D}$ autoreceptor antagonist.

SB-272183 displayed pK_i values of 8.0±0.1 (n=12), 8.1±0.1 (n=12) and 8.7±0.1 (n=12) at human recombinant 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors respectively. This compound has 30 fold selectivity over 5-HT_7 and β_2 receptors but > 100 fold selectivity at all other 5-HT receptors tested. Affinity at rat and guinea-pig receptors was generally lower (see Watson *et al., 2000* & Selkirk *et al., 1998* for methods): 5-$HT_{1B/1D}$ affinity was 7.8±0.1 (n=3) and 7.7±0.1 (n=3) at rat and guinea-pig striatum respectively, while 5-HT_{1A} affinity was 7.7±0.1 (n=3) at both rat and guinea-pig cortex.

[^{35}S]-GTPγS binding studies (see Watson *et al.*, 2000 for method) demonstrated that SB-272183 was a partial agonist, with intrinsic activities of 0.4±0.1 (n=3), 0.4±0.0 (n=10) and 0.8±0.1 (n=3) and pEC_{50} values of 7.7±0.0 (n=3), 7.7±0.1 (n=10) and 8.2±0.1 (n=3) at human recombinant 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors respectively. However, competitive antagonism of the 5-HT response was evident at 5-HT_{1A} and 5-HT_{1B} receptors, generating pA_2 values of 8.2±0.2 (n=3) and 8.5±0.1 (n=3) respectively.

SB-272183 (10 nM – 1 μM) produced a concentration dependent increase in stimulated [^{3}H]5-HT release (see Roberts *et al.*, 1996 for method) from rat and guinea-pig cortical slices. The maximum potentiation of release was 225±21 (n=7) and 181±22 (n=5) % of control for guinea-pig and rat respectively. In addition, 1 μM SB-272183 produced a complete reversal of a 5-HT (30 nM)-induced inhibition of release in rat cortex. Therefore, SB-272183 acts as a 5-HT autoreceptor antagonist at both rat and guinea-pig native tissue.

Measurement of cell firing (see Corradetti *et al.*, 1996 for method) or 5-HT efflux (see Roberts *et al.*, 2000 for method) from rat dorsal raphe nucleus confirmed that SB-272183 (1 μM) lacked intrinsic activity at cell body 5-HT_{1A} or 5-$HT_{1A/1B/1D}$ receptors respectively. However, 1 μM SB-272183 produced a complete reversal of a 8-OH-DPAT (10 nM)-induced inhibition of cell firing and sumatriptan (100 nM)-induced inhibition of 5-HT efflux

In summary, SB-272183 is a mixed 5-HT_{1A}, 5-HT_{1B} and 5-HT_1 receptor ligand with selectivity over other receptors. In recombinan human receptor systems this compound displays partial agonism at al three receptors. In contrast, SB-272183 displays antagonism at nativ rat and guinea-pig 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors. In addition data presented at this meeting (Watson *et al.*) show that SB-272183 i an antagonist at human native 5-HT_{1A} receptors.

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146P REGULATION OF CAMKII BY THE MAPK PATHWAY IN LONG-TERM POTENTIATION

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Mitogen-activated protein kinase (MAPK) has long been known to affect neuronal protein expression by regulating gene transcription, thereby influencing long-term cellular processes such as differentiation. However, recent work in synaptic plasticity has shown that MAPK may be involved in a relatively rapid, synapse-specific phenomenon: the induction of long-term potentiation (LTP). LTP is a use-dependent, persistent increase in synaptic efficiency that is widely studied as model system for cellular memory. We used a protocol to produce LTP that may be particularly relevant to normal patterns of activity at the hippocampal CA3-CA1 synapse in animals during learning. Hippocampal slices (400 μm thick) were prepared from male Sprague-Dawley rats (125-150 g), and excitatory postsynaptic potentials (EPSPs) were monitored by field recording in stratum radiatum of area CA1. LTP was induced by theta-pulse stimulation (TPS, 150 pulses at 10 Hz) of the Schaffer collaterals in area CA3 paired with activation of ß-adrenergic receptors (1 μM isoproterenol for 10 min) as described by Thomas and coworkers (1996). The resulting LTP was MAPK-dependent, since it was prevented by preincubation with 50 μM PD98059, an inhibitor of the MAPK kinase MEK. Immunohistochemical visualization using a phospho-specific antibody raised for phospho-MAPK showed that this form of stimulation caused an increase in phospho-MAPK throughout the dendritic tree of CA1, as well as in the cell bodies including the nuclei. Phospho-MAPK positive neurons in CA1 increased from 13.3±3.0 (n=6) in sham controls to 86.8±11.9 (n=7) at 2 min after the stimulation ($p<0.01$), whereas MAPK phosphorylation in areas CA2-CA4 was not affected by the stimulation. Western immunoblots established that the increase in MAPK phosphorylation was restricted to the p42 isoform of the enzyme, peaking at approximately 2 min after stimulation (174.2±46.1% above controls, n=8, $p<0.05$) and returning to baseline level within 60 min. The stimulation protocol also affected CaMKII processing in area CA1 by two temporally-distinct mechanisms: a transient rise in the fraction of CaMKII that was phosphorylated (99.3±19.6% above controls at 15 min, n=13, $p<0.05$), and a delayed increase in the total amount of CaMKII (46.6±15.6%, above controls, n=10 $p<0.05$), which required protein translation. Together, these phenomena maintained the amount of phospho-CaMKII at elevated levels for at least one hour following LTP induction (43.7±15.1%, n=5; sham, -1.8±12%, n=5, $p<0.05$). The increase in fractional CaMKII followed a time course similar to that of p42-MAPK. Double-label confocal microscopy, using phospho-specific antibodies for MAPK and CaMKII, showed phospho-CaMKII and phospho-MAPK to be colocalized in the dendrites and cell bodies of CA1 pyramidal neurons. The inhibition of MEK by PD98059 prevented both the early phosphorylation of CaMKII (+25±19.5%, n = 4; not statistically different from sham controls measuring +14. ±17.8%, n=11), and the delayed increase in CaMKII levels (+0.54±5.07%, n = 5). We propose that the MAPK pathway may serve as a temporal bridge in LTP, enabling a rapid increase in synaptic efficiency that is followed by a prolonged, protein synthesis-dependent phase.

Thomas et al., Neuron, 17, 475-482, 1996

147P ELECTROPHYSIOLOGICAL AND ELECTROENCEPHALOGRAPHICAL CHARACTERISATION IN RATS INDICATES CX516 (Ampalex) TO BE AN ANXIOLYTIC Ampakine®

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Allosteric modulation of AMPA receptor deactivation and/or desensitisation are associated with differing pharmacological effects in electrophysiological and behavioural paradigms (Yamada, 1998). In the present study we have examined both electrophysiological and electroencephalographical (EEG) effects of the Ampakine® CX516, which has been shown to positively modulate the AMPA receptor by largely affecting deactivation kinetics (Arai and Lynch, 1998).

Electrophysiological analysis involved whole-cell patch clamp recording from cultured rat hippocampal neurones and extracellular field recordings from rat (4-6 weeks) hippocampal brain slices. In the former assay, CX516 (1-100 μM) increased both the peak amplitude and the steady-state inward current response (n=4/4) induced by a 1s application of glutamate (0.5 mM). In hippocampal slices, CX516 (100μM) increased the amplitude of field EPSPs without increasing half-width.

Male, adult, Sprague-Dawley rats (n=27), weighing between 440 and 660g were anaesthetised with Sagatal (60 mg/kg, i.p.) and provided with electrodes for recording the cortical electroencephalogram and nuchal electromyogram. This allowed automated classification of sleep-wake states and pharmaco-EEG analysis (Ruigt *et al.*, 1989). A 15-hour baseline registration period was preceded by a vehicle injection (5% mulgofen, i.p.) and followed the next day by a 15-hour experimental registration period preceded by administration of either vehicle or CX516 (10, 30 or 100 mg/kg, i.p.). Percentages of time in active and passive wakefulness, pre-REM, REM, light, and deep sleep were calculated for each bin of 30 minutes and compared to vehicle values (Mann-Whitney test, $p<0.05$). The changes in sleep-wake organisation were subsequently analysed using a discriminant analysis in which the effects of CX516 were tested against a database of known effects by reference drugs of different therapeutic classes (i.e., anti-depressants, anti-psychotics, anxiolytics, hypnotics, sedatives, and anti-convulsives). CX516 decreased REM sleep and deep sleep at 30 mg/kg during the first 2 hours post-administration, while at 100 mg/kg REM, intermediate stage, light, and deep sleep were decreased during the first 4 hours post-injection. Passive waking increased during the first 5 hours. Analyses revealed that CX516 at 10 mg/kg exerted effects, which resembled placebo with the highest probability (39%). However, at 30 mg/kg and at 100 mg/kg the effects of CX516 most reliably resembled the effects of anxiolytic class of compounds (41% and 37%, respectively).

The present study suggests that CX516 enhances AMPA receptor activity and changes sleep-wake organisation in a way best comparable to anxiolytic drugs.

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148P THE EFFECT OF CGRP AND $CGRP_{8-37}$ ON SYNAPTIC TRANSMISSION IN PURKINJE CELLS IN THE CEREBELLUM

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The levels and distributions of calcitonin gene-related peptide (CGRP) change throughout development within the cerebellar cortex yet the density of receptor expression in the molecular layer is maintained (Goodman & Iversen, 1986; Morara *et al.*, 1998). To date, the role that CGRP plays within the cerebellum has not been established. We have used electrophysiological techniques to assess the effects that CGRP and the antagonist $CGRP_{8-37}$ have on synaptic transmission between parallel fibres and Purkinje cells and on spontaneous synaptic events.

14 – 21 day old male Wistar rats were decapitated under halothane anaesthesia and the cerebellum removed. 200μm thick sagittal slices were obtained and whole cell patch-clamp recordings were made at room temperature from Purkinje cells whilst stimulating parallel fibres at 0.2Hz. 10 minute exposure to CGRP depressed the amplitude of the EPSCs elicited from Purkinje cells in a concentration-dependent manner. 50nM CGRP resulted in a significant and prolonged decrease in the amplitude of EPSCs within 25 minutes compared to control recordings (control 99.1 ± 4.9%, n = 7; CGRP 60.5 ± 9.7%, n = 7, $P<0.05$ Kruskal-Wallis). Synaptic depression was accompanied by an increase in the ratio of pairs of pulses applied at 50ms intervals. The effect of 50nM CGRP was not significantly blocked by the receptor antagonist $CGRP_{8-37}$ over a range of concentrations from 1μM to 30nM when applied for 5 minutes before and during the application of CGRP. At a concentration of 10nM, applications of $CGRP_{8-37}$ alone also significantly decreased EPSC amplitudes to 45.8 ± 10.3%, of baseline levels (n = 6; $P<0.01$, Kruskal-Wallis) and increased the paired pulse ratio. A similar level of depression occurred when 50nM CGRP was applied in the presence of 10nM $CGRP_{8-37}$ (47.1 ± 9.7%, n = 6; $P>0.05$). Since the effect of CGRP on the paired pulse ratio suggested a presynaptic site of action, we investigated the effect of CGRP and $CGRP_{8-37}$ on miniature EPSCs (mEPSCs) recorded from Purkinje cells in a raised calcium extracellular solution containing 400nM TTX. Both 10nM $CGRP_{8-37}$ and 50nM CGRP significantly decreased the frequencies of mEPSCs within 25 minutes of application compared to control recordings (control 98.7 ± 13.1%, n = 6, 10nM $CGRP_{8-37}$ 80.4 ± 13.1%, n = 6, 50nM CGRP 62.4 ± 7.4%, n = 5, $P<0.01$ vs. control, Kruskal-Wallis), but did not alter the amplitude of the mEPSCs.

In conclusion applications of CGRP and $CGRP_{8-37}$ to cerebellar slices depressed synaptic transmission between parallel fibres and Purkinje cells. The site of action is likely to be presynaptic. The mechanism of action is being investigated.

This work was supported by the Wellcome Trust.

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149P A COMPARISON OF THE ANTAGONIST ACTIONS OF [^{11,18}Ser]CGRP$_{8-37}$ WITH CGRP$_{8-37}$ ON THE CGRP RECEPTORS EXPRESSED ON L6, SK-N-MC AND COL 29 CELLS.

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The CGRP antagonist CGRP$_{8-37}$ binds with high affinity to CGRP$_1$ receptors (found on rat L6 and human SK-N-MC cells) and with lower affinity to CGRP$_2$ receptors (found on human Col 29 cells). The N-terminus of CGRP$_{8-37}$ forms an amphipathic α-helix essential for high affinity binding; replacement of arginines at positions 11 and 18 by alanines reduces the potency of the antagonist at least 100-fold on L6 and SK-N-MC cells and to unknown extent on Col 29 cells (Howitt & Poyner, 1997; Poyner *et al.*, 1998). It is unclear whether arginines 11 and 18 make specific contacts with the receptor (or residues elsewhere on the peptide) or whether they simply interact with aqueous solvent (perhaps correctly orientating the α-helix on the receptor). We have investigated this with [^{11,18}Ser]CGRP$_{8-37}$. This is amphipathic but specific residue-residue interactions are unlikely to be preserved.

L6, SK-N-MC and Col 29 cells were grown to confluence in 24 well plates at 37°C in a humidified atmosphere of 95% air/5% CO_2, as described previously (Poyner *et al.*, 1998) and these conditions were maintained whilst examining the effects of the drugs. Cells were pre incubated with Krebs solution containing isobutyl methyl xanthine for 30 min before addition of any drug. Concentration-response curves to CGRP were constructed in the presence or absence of CGRP$_{8-37}$ (100nM L6, SK-N-MC cells; 3μM Col 29 cells) and 10μM [^{11,18}Ser] CGRP$_{8-37}$, measuring cyclic AMP accumulation. Cells were pre-incubated with antagonists for 5 min before addition of CGRP for a further 5 min. Incubations were terminated by boiling. Cyclic AMP was extracted and assayed by radio-receptor assay as described previously (Poyner *et al.*, 1998). Apparent pKb values were calculated for the antagonists from each experiment as log(dose ratio-1)- log[antagonist].

CGRP stimulated cyclic AMP production in a concentration dependent manner (pD_2 values of 9.51±0.08 (L6 cells), 8.48±0.10 (Col 29 cells), 8.88±0.09 (SK-N-MC cells), n=6 in all cases). Antagonists shifted the curves to the right without depression of maximum (range 99%-104% of the maximum seen with CGRP alone). The apparent pKb values for CGRP$_{8-37}$ and [^{11,18}Ser]CGRP$_{8-37}$ were respectively as follows: L6, 7.88±0.02 and 5.91±0.04; SK-N-MC, 7.97±0.04 5.58±0.11; Col 29, 6.57±0.12 and 5.70±0.11 (n=3, all values). For each cell line, the pKb value for [^{11,18}Ser]CGRP$_{8-37}$ was significantly less than that for CGRP$_{8-37}$ ($P<0.05$, Student's unpaired *t*).

Replacement of arginines at positions 11 and 18 by serines reduces affinity as much as replacement by alanines (Poyner *et al.*, 1998). This suggests that the arginines are not simply involved in maintaining hydrophilicity but make specific residue-residue contacts. CGRP$_2$-like receptors are less sensitive to this effect than CGRP$_1$-like receptors.

We thank Dr. J. Longmore and colleagues at Merck Sharpe and Dohme, Harlow for the gift of [^{11,18}Ser]CGRP$_{8-37}$ and the Wellcome Trust for financial support.

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150P SOME EFFECTS OF MEMBRANE CHOLESTEROL ON AMPA RECEPTORS IN ACUTELY DISSOCIATED HIPPOCAMPAL NEURONES

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The cholesterol content of the plasma membrane of cells is an important determinant of membrane fluidity but it is also clear that the cholesterol interacts with specific recognition sites on some membrane proteins (Yeagle, 1993). Both phenomena have been reported to affect the properties of neuronal oxytocin and cholecystokinin receptors (Gimpl *et al.*, 1997) and also the $GABA_A$ receptor (Sooksawate & Simmonds, 1998, 2001). We now report preliminary results from an analogous investigation of AMPA receptors.

Experiments were performed on acutely dissociated hippocampal neurones from young Wistar rats. Whole cell current responses to local applications of AMPA were recorded from patch electrodes using a similar methodology to that described for the GABA experiments (Sooksawate & Simmonds, 1998). Pre-incubation of the neurones with a complex of 0.15 mM cholesterol and 1.5 mM methyl-β-cyclodextrin for 20 min at 30°C enriched neuronal cholesterol to 210 ± 3.2% of control whereas pre-incubation with 5 mM methyl-β-cyclodextrin alone depleted the cholesterol to 70 ± 1.8% control. Averaged log AMPA - current response curves from cholesterol-depleted, control and cholesterol-enriched neurones are shown in Fig. 1. The respective EC_{50} values (mean ± s.e.m.) derived from the curve fitting procedure (GraphPad Prism) were 9.52 ± 0.76 μM (n=2), 28.0 ± 2.02 μM (n=4) and 56.8 ± 15.6 μM (n=3). These values differ significantly from each other (ANOVA $P< 0.05$).

The reduction in AMPA potency with cholesterol enrichment is analogous to the reduction in GABA potency on $GABA_A$ receptors in similarly enriched neurones, which was suggested to be due to reduced membrane fluidity (Sooksawate & Simmonds, 2001). In contrast, the increase in AMPA potency with cholesterol depletion is opposite to the effects on $GABA_A$ receptors, which showed a reduction in potency with depletion as well as enrichment, probably involving different mechanisms. The results obtained with AMPA are compatible with a single mechanism of effect of cholesterol which remains to be identified.

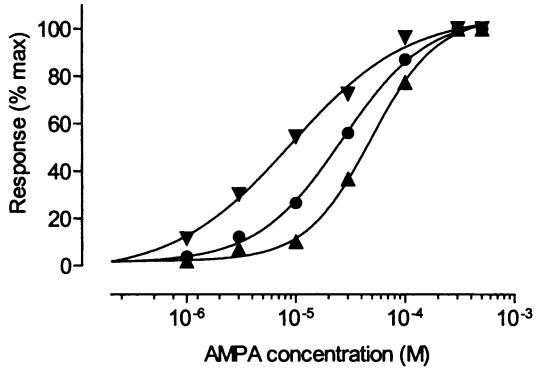

Fig. 1. Whole cell current responses to AMPA recorded from dissociated hippocampal neurones clamped at -40 mV. Averaged data are shown from 4 control neurones (●), 2 cholesterol-depleted neurones (▼) and 3 cholesterol-enriched neurones (▲).

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151P COLORECTAL DISTENSION-EVOKED iNOS, NITROTYROSINE AND C-FOS EXPRESSION IN THE ANAESTHETISED RAT IS INHIBITED BY THE SELECTIVE iNOS INHIBITOR GW274150

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Repeated colorectal distension (CRD) evokes an inflammatory response in the colon, and Fos-like immunoreactivity (Fos-LI) in discrete areas of the rat spinal cord associated with nociceptive input (Traub et al, 1992). The inducible enzyme iNOS may be expressed under such conditions, and has been implicated in nociceptive mechanisms. We report the effect of the selective iNOS inhibitor GW274150 ((S)-2-amino-7-acetamidino-5-thioheptanoic acid) on CRD evoked iNOS expression and nitrotyrosine immunolabelling in the rat colon, DRG and spinal cord, as well as on numbers of Fos-LI nuclei in the rat spinal cord. GW274150 has been identified as a potent and selective iNOS inhibitor (IC_{50} 2.19μm) with selectivity over both human eNOS (x248) and human nNOS (x79) (Clayton et al, 2000).

Male Wistar rats (320-460g) were anaesthetised with urethane ($1.5gkg^{-1}$ i.p.) and a colorectal balloon was inflated to 80mmHg for 30s, every 2min, for 120min (c-fos study) or for 2, 4, or 6 hours (iNOS study). GW274150 ($15mg.kg^{-1}$ i.v.) or saline were administered 10min before CRD onset. In sham distended animals, the balloon was inserted, but not inflated. For the iNOS study, tissue from the colon (2-4cm from anus), L5-L6 DRG and L5-S2 spinal cord was removed into 4% formaldehyde overnight, and 5μm sections processed for visualisation of iNOS and nitrotyrosine (NT) expression. 40μm sections of L6-S1 spinal cord were processed for visualisation of Fos-LI using standard immuncytochemical techniques. Statistical significance (c-fos study) was assessed using one way ANOVA and Dunnett's test ($P<0.05$ significant).

There was increased expression of iNOS in all tissues following 2, 4 and 6 hours CRD. NT expression was also seen following 2 hours CRD. There was no iNOS or NT expression in sham distended animals (n=3-4). Pretreatment with GW274150 ($15mgkg^{-1}$ i.v.) had no effect on iNOS

Repeated CRD significantly increased numbers of total bilateral Fos-LI nuclei in the spinal cord to 2914±312 vs 926±114 in sham distended animals (total in 12 sections, L6-S1; $P<0.05$; n=3-4), with 821±61 in laminae I-II, 966±108 in lateral V-VI and 907±202 in medial V-X. Pretreatment with GW274150 ($15mgkg^{-1}$ i.v.) significantly reduced total Fos-LI nuclei to 1949±87 ($P<0.05$; n=4), with 31%, 36% and 43% reductions in laminae I-II, lateral V-VI and medial V-X respectively ($P<0.05$).

Tissue	iNOS expression:			% control response +GW274150 (2h)	
	2h	4h	6h	iNOS	NT
colon (mean)	72±42	169±15	111±26	96%	27%*
DRG (% area)	65±10	65±2	67±3	100%	110%
Spinal cord (%area)	13±5	14±2	12±3	54%	140%

Table 1. n=3-4 throughout. *denotes significant inhibition ($P<0.05$)

In conclusion, visceral stimulation using repeated noxious CRD results in significant iNOS expression in colon, DRG and spinal cord neurones. Such expression may contribute to visceral nociceptive mechansims, since the iNOS inhibitor GW274150 significantly inhibits CRD-evoked c-fos expression in the spinal cord, in laminae associated with nociceptive processing. Inhibition of iNOS may therefore provide a novel approach to the treatment of visceral pain.

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152P THE N^3-SUBSTITUTED WILLARDIINE ANALOGUE (S)-3-(4-CARBOXYBENZYL)WILLARDIINE SELECTIVELY ANTAGONISES AMPA OVER KAINATE RECEPTORS ON NEONATAL RAT SPINAL MOTONEURONES

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There are few antagonists that show selectivity for AMPA over kainate receptors (Bleakman & Lodge, 1998). The natural product willardiine is a selective agonist for AMPA over kainate receptors (Wong *et al.*, 1994). The aim of the current study is to convert the AMPA receptor selective agonist activity of (S)-willardiine into that of an antagonist by incorporating an N^3-substituent. The activity of one such compound, (S)-3-(4-carboxybenzyl)willardiine (3-CBW), as a selective antagonist of AMPA receptor-mediated responses on neonatal rat spinal motoneurones is described.

Recordings were made from ventral roots of hemisected spinal cords from 2-5 day old Wistar rats of either sex bathed in medium containing tetrodotoxin (0.1 μM) to allow measurement of depolarisations evoked by exogenously applied agonists (1 min applications) (Evans *et al.*, 1982). Non-cumulative concentration-response curves (CRCs) were constructed to the AMPA receptor agonist (S)-5-fluorowillardiine (Wong *et al.*, 1994) in the absence and presence of 3-CBW (50 μM; 30 min pre-incubation). Selectivity studies were also carried out by adding approximately equi-effective doses of the non-NMDA receptor agonists (S)-AMPA, kainate, (S)-5-iodowillardiine and ATPA in the absence and presence of either 3-CBW (100 μM) or the selective non-competitive AMPA receptor antagonist GYKI 53655 (50 μM) (for a review of the activity of these compounds see Bleakman & Lodge, 1998).

A further study measured the ability of 3-CBW to block the fast component of the dorsal root evoked ventral root potential (fDR-VRP) which is thought to be mediated by AMPA/kainate receptors (Long *et al.*, 1990). Recordings were taken from the ventral root following stimulation of the corresponding dorsal root (16x threshold V, 2 pulses min^{-1}). CRCs were constructed for 3-CBW (5 min applications), in the presence of 2 mM $MgSO_4$ / 50 μM (R)-AP5 (30 min pre-incubation) to block NMDA receptors.

A parallel shift in the CRC to (S)-5-fluorowillardiine was observed in the presence of 50 μM 3-CBW giving an apparent K_D value of 21 ± 3 μM (n=3; mean ± s.e.m.). In addition, 3-CBW was found to reduce the fDR-VRP with an IC_{50} value of 10.3 ± 2.4 μM (n=3). 3-CBW (100 μM) was shown to block AMPA-evoked depolarisations by 93 ± 4 % whilst those evoked by kainate were reduced by only 56 ± 5 %, thus demonstrating some selectivity for AMPA over kainate receptors. Responses to ATPA and (S)-5-iodowillardiine were reduced by 87 ± 6 and 95 ± 4 %, respectively (all determinations n=4). In a similar manner, GYKI 53655 (50 μM) blocked AMPA responses by 98 ± 2 % and responses to kainate, ATPA and (S)-5-iodowillardiine by 33 ± 19, 52 ± 13 and 77 ± 10 %, respectively (all determinations n=3).

Residual responses to kainate, ATPA and (S)-5-iodowillardiine were observed in the presence of GYKI 53655 (50 μM) whereas the AMPA response was almost completely abolished. Therefore, it is likely that a component of the responses to these agonists is mediated by kainate receptors.

By increasing the inter-acidic group chain length of willardiine its agonist action at AMPA receptors can be converted into that of an antagonist. As 3-CBW shows selectivity for AMPA over kainate receptors it is likely to be a useful tool for isolating kainate receptor-from AMPA receptor-mediated responses on motoneurones.

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153P ARE GLUCOSE-RECEPTIVE NEURONES IN LATERAL HYPOTHALAMUS MODULATED BY OREXIN-A? A COMBINED ELECTROPHYSIOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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The lateral hypothalamus (LH) is crucially involved in feeding behaviour but the neuronal pathways involved are uncertain. Orexin-A and -B are expressed exclusively in the LH, and stimulate feeding acutely (Williams *et al.*, 2000). Glucose-sensitive neurones, which make up ~40% of LH neurones (Fukuda *et al.*, 1984) are thought to be important in the regulation of energy balance (Bergen *et al.*, 1996). We have investigated the structural and functional links between these two neuronal populations, using a combination of intracellular recording and immunohistochemical techniques.

Rats (2-3 weeks old, Wistar, either sex) were anaesthetised with sodium pentobarbital (40 mg/kg.i.p.) and decapitated. Transverse hypothalamic slices (200-400 μm) were placed in a recording chamber perfused with gassed modified artificial cerebral spinal fluid (ACSF) at room temperature. Intracellular recordings were made from neurones situated in the LH, and their glucose responsiveness determined during exposure to normal (10 mM), low (3 mM) and high (15 mM) glucose levels in the ACSF. Neurobiotin 2% was included in the electrode filling solution to enable visualization of impaled neurones.

Application of orexin-A (1 μM) induced depolarisation in both glucose-sensitive and glucose-insensitive neurones. However, in glucose-insensitive neurones, orexin-A produced relatively short-lived and modest depolarisations (2.9±0.5 mV; n=13). By contrast, orexin-A elicited prolonged and significantly greater depolarisation (13.7±1.9 mV, n=8; P<0.001 *vs* insensitive cells, Student *t*-test), in glucose-sensitive neurones.

Together with depolarisation, orexin-A (1 μM) induced spontaneous discharges in silent cells and increased the firing rate in active cells. When the membrane potential was clamped to the pre-drug level, orexin-A (1 μM) augmented input resistance (31.2±4.3%; n=4, P<0.05), increased the number of evoked action potentials elicited by the depolarising step at a fixed membrane potential (from 2.2±0.4 to 4.2±0.7; n=5, P<0.01), induced burst-like firing resulting from postinhibitory rebound when hyperpolarising currents were injected (n=6), and reduced the threshold current required to evoke an action potential (from 0.11±0.02 nA to 0.07±0.01 nA; P <0.01; n=8), in glucose-sensitive neurones.

Cells studied electrophysiologically were injected with neurobiotin and later detected with streptavidin-FITC. Orexin-A containing neurones were visualised using a rhodamine-tagged secondary antibody and a polyclonal primary antibody raised against full-length orexin-A. Dual fluorescent studies were performed using confocal microscopy on a Zeiss LSM510, taking overlapping confocal slices and creating 3-D renders of the stack. None of the cells (n=28) shown to be glucose-sensitive stained positive for orexin-A, but some processes of orexin-A cells were closely associated with glucose-sensitive neurones.

Orexin neurones may release the peptide, which then evokes depolarisations in glucose-sensitive cells. We suggest that orexin-A may regulate glucose-sensitive neurones in the LH, and that this interaction may contribute to the regulation of feeding behaviour.

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154P EFFECT OF ADENOSINE A_{2A} RECEPTOR ANTAGONISM ON THE HISTOLOGICAL DAMAGE, NEUROLOGICAL DEFICIT AND STRIATAL ADENOSINE AND AMINO ACID OUTFLOW FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT

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Evidence exists that adenosine A_{2A} receptor antagonists administered systemically or injected locally in the hippocampus are neuroprotective in "in vivo" models of brain ischemia or kainate-induced excitotoxicity. However, the neuroprotective action mechanism of adenosine A_{2A} antagonist has to be clarified.

The aims of the study were: 1) to investigate the protective effect of the selective A_{2A} receptor antagonist SCH 58261 (7-(2-phenyl-ethyl)-5 amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4 triazolo [1,5-*c*]pyrimidine) in a rat model of permanent focal ischemia; 2) to relate it to modifications of excitatory aminoacid outflow. A vertical microdialysis probe was placed in the right striatum of male Wistar rats (270-290 g) anesthetized with choral hydrate. Animal body core temperature was maintained at a constant 37°C with a recirculating pad and K module. After 24 h, permanent medial cerebral artery occlusion (MCA-O) was induced by the intraluminal suture technique in 5% halothane anesthetized rats (Zea Longa *et al* 1989). Adenosine and amino acid concentrations in the dialysates, collected during 4 h after inducing ischemia in awake freely moving rats, were determined by HPLC coupled with a fluorometric detector (Melani *et al.* 1999).

The neurological deficit, evaluated according to Garcia *et al* (1995), and histopathological outcome were assessed 24 h after MCA-O. MCA-O induced damage (calculated in relation to the volume of ipsilateral hemisphere; mean±SEM; n=13) of 17.3±1.2% in the striatum and 37.8±2.0% in the cortex. After SCH 58261 (n=8), administered at a dose of 0.01 mg/Kg i.p. five min after inducing the focal ischemia, the damage was 15.7±2.0% in the striatum and 29.3±3.6% in the cortex (two-tailed t test: p<0.04 in the cortex). The outflow of all transmitters, evaluated in eight rats, significantly increased after MCA-O. Maximal increases were 25-fold for adenosine, 2.5-fold for glutamate, 2.3-fold for aspartate, 140-fold for GABA and 7-fold for taurine. The mean±SEM (calculated as percent of pre-ischaemic values) of transmitter outflow during 4 h after MCA-O was 970±5% for adenosine, 152±30% for glutamate, 156±12% for aspartate, 7,33 8±2,760% for GABA and 520 ± 230% for taurine. SCH 58261 (n=8) reduced the outflow to 416±88% for adenosine, 100±12% for glutamate, 105±16% for aspartate, 737±333% for GABA and 354±69% for taurine.

The reductions of adenosine, glutamate, aspartate and GABA were statistically significant (one-way ANOVA: p<0.05). SCH 58261, on the other hand, failed to modify the motor-sensory functions: the neurological scores were (mean±SEM) 9.5±0.6 in control rats (n=13) and 9.1±1.2 in SCH 58261 treated rats (n=8). It is concluded that the A_{2A} antagonist SCH 58261 is protective against the cortical histopathologic outcome and increased transmitter outflows in the present model of focal ischemia.

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155P ACTIVATION OF NMDA RECEPTORS SUPPRESSES PRESYNAPTIC RESPONSES TO ADENOSINE

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We have previously reported that the inhibitory effects of adenosine on glutamate release in the hippocampal CA1 region were dependent on the presence of magnesium (Bartrup & Stone, 1990). The loss of adenosine responses in magnesium-free medium was prevented by antagonists acting at N-methyl-D-aspartate (NMDA) receptors, suggesting that the interaction involved the activation of NMDA receptors. We have now examined the effects of adenosine and NMDA itself using the paired-pulse paradigm, which provides a more accurate indication of presynaptic events than the study of single population spikes and postsynaptic potentials.

Hippocampal slices 450μM thick were prepared from adult male Wistar rats (150-200g) as described previously (Nikbakht & Stone, 2000). Briefly, slices were prepared in artificial cerebrospinal fluid (acsf) of composition (mM): NaCl 115; KCl 2; KH_2PO_4 2.2; $CaCl_2$ 2.5; $MgSO_4$ 1.2; $NaHCO_3$ 25, D-glucose 10, gassed with 5%CO_2 in oxygen. Slices were maintained for at least 1h at room temperature in a water-saturated atmosphere of 5%CO_2 in oxygen before being transferred to the recording chamber and superfused with acsf at 30°C. Paired-pulse stimuli were applied to the Schaffer collateral fibres using interstimulus intervals of 10,20 and 50ms, with population spikes being recorded in the stratum pyramidale by a glass microelectrode containing 1M NaCl (~5MΩ).

At 6 or 10μM, NMDA itself produced a depression of population spikes, and 4μM was selected for subsequent experiments. A concentration of 10μM adenosine was selected as it depressed spike size by 70.6% ± 5.0 (n=10), allowing increases or decreases of response to be detected. When NMDA at 4μM was superfused simultaneously, adenosine decreased the spike size by only 45.0% ± 4.8 (n=4; $p<0.05$). The inclusion of 2-amino-5-phosphono-pentanoic acid (2AP5; 50μM) prevented the suppression of the adenosine response.

At 10μM, adenosine suppressed paired-pulse inhibition at an interpulse interval of 10ms, and enhanced facilitation at 20 and 50ms. NMDA had no effect itself, but significantly reduced the effect of adenosine (43% ± 5.2 at 10ms, $p<0.05$; 48% ± 4.4 at 20ms, $p<0.05$; 60.0% ± 3.2 at 50 ms, $p<0.01$; all n=5). The effect of NMDA was prevented by 2AP5. The suppressant effect of NMDA was still seen in slices superfused with bicuculline methobromide, 30μM. Superfusing slices with magnesium-free medium also prevented the response to adenosine by a mechanism which could be antagonised by 2AP5.

These results show directly the ability of NMDA to suppress the presynaptic inhibitory effect of adenosine, and raise the possibility that such an interaction could be involved in the physiological regulation of synaptic transmission.

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156P EVIDENCE THAT β-MSH IS THE ENDOGENOUS ANTI-OBESITY LIGAND ACTING AT HYPOTHALAMIC MELANOCORTIN-4 RECEPTORS

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Recent evidence suggests a role for the pro-opiomelanocortin-derived melanocortins in the regulation of energy balance. These act through melanocortin-4 receptors (MC4-R) in the hypothalamus to reduce feeding and increase thermogenesis (Huszar *et al.*, 1997). α-MSH is generally assumed to be the endogenous agonist for MC4-R, but there is no conclusive evidence to support this hypothesis; rather there is emerging evidence to suggest the contrary. Of the endogenous melanocortin peptides, β-MSH has the highest affinity for human MC4-R (Schioth *et al.*, 1996), and is also reported to inhibit feeding (Abbott *et al.*, 2000). β-MSH is therefore a strong candidate as the endogenous MC4-R agonist, but this hypothesis has previously received little investigation. In this study, we utilised various complementary approaches to elucidate this potential role, including (i) classical binding studies using cloned receptors obtained as a kind gift from Dr. V. Chhajlani (AstraZeneca, Sweden) and hypothalamic tissue from male Wistar rats (250g); (ii) immunohistochemical determination of peptide distribution, with a highly specific antiserum, using the method of Coons, (1958) to identify whether it is present in the appropriate appetite-regulating brain regions; and (iii) [^{35}S] GTPγS autoradiography to identify functional activation of receptors through coupling with intracellular signal transduction systems (G-proteins; Sim *et al.*, 1995). β-MSH bound with a high affinity at both human and rat MC4-R (K_i = 11.1 ± 0.1 nM and K_i = 5.0 ± 0.4 nM, respectively), greater than the affinity for α-MSH (K_i (human) = 356 ± 16 nM and K_i (rat) = 22.5 ± 2.3 nM; both $p<0.005$ vs β-MSH). Moreover, using agonist-stimulated [^{35}S] GTPγS binding we found that specific hypothamic nuclei of male Wistar rats (250g) showed significantly increased activity upon application of β-MSH. 5 μM β-MSH significantly increased [^{35}S] GTPγS binding over basal conditions, in the hypothalamic ventromedial (60±7%; $p<0.005$; Student's t-test; n=7 per group), dorsomedial (46±4%; $p<0.005$), paraventricular (51±3%; $p<0.005$) and arcuate nuclei (58±6%; $p<0.005$). These regions of the brain are considered particularly important in the regulation of feeding behaviour and also express high levels of MC4-R (Harrold *et al.*, 1999). Finally, the anatomy of this binding corresponded closely to β-MSH fibre terminal fields, as determined by immuno-histochemistry. In conclusion, we suggest that the activity of hypothalamic MC4-R may be governed by availability of β-MSH rather than α-MSH, and that this is the endogenous MC4-R ligand which reduces food intake and decreases body weight.

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157P ROLE OF INTRACELLULAR REGULATORY PATHWAYS UPON UPTAKE AND HANDLING OF L-DOPA BY CAPILLARY CEREBRAL ENDOTHELIAL CELLS, ASTROCYTES AND NEURONAL CELLS

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The present study examined the nature of the inward L-DOPA transporter in rat capillary cerebral endothelial (REB 4 cells; Gomes & Soares-da-Silva, 1999), type 1 astrocytes (DI TNC1 cells; Radany *et al.*, 1992), and Neuro 2A neuroblastoma cells (Calderon *et al.*, 1999), and whether protein kinases differentially modulate the activities of these transporters. Cells were grown at 37° C in a humidified atmosphere (5% CO_2) on 2 cm^2 plastic culture clusters in Minimum Essential Medium adjusted to contain 1.5 g/L sodium bicarbonate and 1.0 mM sodium pyruvate supplemented with 10% fetal bovine serum and 100 U ml^{-1} penicillin G, 0.25 μg ml^{-1} amphotericin B and 100 μg ml^{-1} streptomycin. After 6 days, the cells formed a monolayer and each 2 cm^2 culture well contained about 100 μg of cell protein; 24 h before the experiments the cell culture medium was changed to a serum free medium. In uptake studies, cells were preincubated (30 min) with Hanks' medium with added tolcapone (1 μM) and benserazide (10 μM). L-DOPA was assayed by h.p.l.c. with electrochemical detection. L-DOPA was applied from the apical cell side at non-saturating (2.5 μM) and saturating (up to 1000 μM) concentrations for 6 min. Results are arithmetic means with s.e.mean, n=4-6. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. A P value less than 0.05 was assumed to denote a significant difference.

In all three types of cells the accumulation of L-DOPA was saturable at 250 μM, being the inward transfer of L-DOPA largely promoted through the 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BHC)-sensitive and sodium-independent L-type amino acid transporter. Only in DI TNC1 cells was the effect of manoeuvres that increase intracellular cAMP levels (forskolin, 100 μM; isobutylmethylxanthine, 1 mM) accompanied by increases in L-DOPA uptake (82±14% and 92±30% increase, respectively). Again, only in DI TNC1 cells was the effect of the guanylyl cyclase inhibitor LY 83583 (30 μM) accompanied by 65±10% increase in L-DOPA accumulation, whereas the nitric oxide donor sodium nitroprusside produced a 25±6% decrease in L-DOPA accumulation. The activators of protein kinase C and inhibitors of protein tyrosine kinase failed to affect the accumulation of L-DOPA. In all three types of cells, the Ca^{2+}/calmodulin inhibitors calmidazolium and trifluoperazine inhibited L-DOPA uptake in non-competitive manner. Thapsigargin (1 μM) and A23187 (1 μM) failed to alter L-DOPA accumulation in RBE 4 and Neuro 2A cells, but markedly increased L-DOPA uptake in DI TNC1 cells (thapsigargin, 132±14% increase; A23187, 41±10% increase). Forskolin produced a marked increase in cAMP levels in all three types of cells (% increase: RBE, 589±81 ;DI TNC1, 1549±160; Neuro 2A, 355±35), this being significantly ($P<0.05$) reduced by thapsigargin, but not by A23187, which suggests that Ca^{2+}-dependent effects on L-DOPA accumulation were independent of changes in cAMP levels. It is concluded that L-DOPA in RBE 4, DI TNC1 and Neuro 2A cells is transported through the L-type amino acid transporter and appears to be under the control of Ca^{2+}/calmodulin mediated pathways. DI TNC1 cells, however, are endowed with other processes that appear to regulate the accumulation of L-DOPA, responding positively to increases in intracellular Ca^{2+} and cAMP and decreases in cGMP.

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158P DOPAMINE D-1, D-2 AND D-3 RECEPTOR BINDING DENSITY WAS NOT ALTERED IN TETRAHYDROBIOPTERIN-DEFICIENT hph-1 MICE

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The hph-1 mouse has defective tetrahydrobiopterin (BH4) biosynthesis and it is an animal model of L-dopa-responsive dystonia in man (Segawa et al., 1971; McDonald et al., 1988). The brain concentration of BH4 is reduced by approximately 50% and tyrosine hydroxylase activity is decreased and monoamine (dopamine) metabolism is also impaired, as a consequence (Brand et al., 1995; Hyland et al., 1996). We previously reported that gene expression of preprotachykinin (PPT), but not preproenkephalin (PPE-A), was increased in the striatum of hph-1 mouse, suggesting that alterations in the striatal direct output pathway was linked to the onset of dystonia. This was suprising since PPT is normally under excitatory dopamine control and should decrease if dopamine deficiency occurs (Zeng et al., 1997). We now report on the effect of BH4 deficiency on dopamine D-1, D-2 and D-3 receptor expression in the striatum and nucleus accumbens of hph-1 and normal control C57BL mice using quantitative autoradiography.

Seven hph-1 and eight normal C57BL mice (30 days of age, weight 10-12 g) were killed by cervical dislocation. The brains were removed and flash frozen. For assessment of D-1, D-2 and D-3 receptor density, coronal sections (20 μm) through the striatum (AP 0.2 mm from bregma) and nucleus accumbens (AP 2.2 mm from bregma) were incubated with [^{3}H]-SCH23390 (0.5 nM, for D-1), [^{3}H]-spiperone (0.5 nM, For D-2) and [^{3}H]-7-OH-DPAT (0.5nM, for D-3) respectively. Non-specific binding was defined with 10 μM cis-flupenthixol for D-1, with 10 μM sulpiride for D-2 and with 10 μM dopamine for D-3 respectively. Quantitative evaluation of autoradiograms was undertaken by computerised densitometry and results (mean±sem) were analysed by paired Student's *t*-test.

The intensity of D-1, D-2 and D-3 receptor binding in the nucleus accumbens of hph-1 mice was not significantly different from that of normal C57BL mice (Table 1). Similarly, D-1, D-2 and D-3 receptor binding density in the striatum of hph-1 mice was not altered compared to normal C57BL mice.

Table 1: Density of dopamine D-1, D-2 and D-3 receptors in the striatum and nucleus accumbens of hph-1 and C57BL mice (fmol/mg). Data is expressed as mean ±sem (7-8 animals/group). NA, nucleus accumbens; STR, striatum.

Strain		NA	STR
hph-1 mice	D-1	13.7±2.5	13.8±1.9
C57BL mice		12.4±2.3	14.6±2.6
hph-1 mice	D-2	0.74±0.2	1.1±0.2
C57BL mice		0.71±0.1	1.0±0.1
hph-1 mice	D-3	0.55±0.1	0.3±0.1
C57BL mice		0.52±0.1	0.2±0.1

The present study shows that in both striatum and nucleus accumbens, D-1, D-2 and D-3 receptor binding density was not altered in hph-1 mice despite decreased dopamine turnover (Hyland et al., 1996). Consequently, the increase in PPT-mRNA in the striatal direct output pathway may not reflect decreased dopamine activity but deficiencies in other BH4 needed enzyme, such as nitric oxide synthase (NOS).

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159P PREFERENTIAL COUPLING OF D_{2S} DOPAMINE RECEPTORS TO αo G PROTEINS OVER αi2 G PROTEINS

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The interaction of receptor and G protein is a critical event in the mechanism of agonist action at G protein coupled receptors. The D_2 dopamine receptor has been shown to interact with different Gαi/o proteins to influence different signaling events (Neve & Neve, 1997), but a clear definition of its selectivity for different G proteins has not yet emerged.

Human dopamine D_{2S} receptors were epitope tagged at the N-terminus with a tag derived from gp120 by PCR, subcloned into the baculovirus expression vector pBlueBac4.5 and recombined into Bac-N-Blue baculovirus DNA. Baculoviruses encoding the G protein subunits αi2, αo, β1, γ2 were donated by T Kozasa (University of Texas, Dallas). Receptor and G protein subunits were expressed in *Spodoptera Frugiperda* (*Sf*9) cells at different multiplicities of infection (Table 1). Cell membranes were prepared 48 hours after infection. Receptor levels were determined by saturation analysis with [^{3}H]spiperone as described by Gardner *et al.*, (1996). The number of G proteins was estimated by western densitometry. The affinity of dopamine for the receptors was determined by competition against [^{3}H]spiperone (0.25nM, 3hrs, 25°C). Dopamine potency for stimulating [^{35}S]GTPγS binding was determined using a variation of the method described by Gardner *et al.*, (1996) (100μl final volume, 5μg membranes, 200pM [^{35}S]GTPγS). Saturation analysis of [^{3}H]spiperone binding to epitope tagged D_{2S} receptors gave a K_d of 90pM (pK_d 10.05±0.12, mean±s.e.m. n=4). This was unaffected by the co-expression of G protein subunits. Receptor levels in the two preparations used for co-expression studies were comparable and the level of αo was not significantly different to the level of αi2 ($p>0.05$ Student's *t* test) (Table 1).

Competition experiments with dopamine in the absence of sodium ions and GTP were best described by a two site binding model with high and low affinity states (K_h, K_l) (Table 2). The percentage of receptors exhibiting high affinity for dopamine was increased by the co-expression of αo subunits ($p<0.05$, Student's *t* test). An increase was also apparent in membranes co-expressing αi2 but this was not statistically significant ($p>0.05$, Student's *t* test). Competition experiments were best fitted by a one site binding model when GTP and sodium ions were included in the buffer. The low affinity state for dopamine (K_l) was similar to the affinity measured in the presence of sodium ions and GTP (K_{iGTP}) (Table 2).

Dopamine was unable to stimulate [^{35}S]GTPγS binding in membranes expressing only receptor and β1γ2 subunits, whereas clear stimulation was observed when α subunits were also expressed. Dopamine potency at stimulating [^{35}S]GTPγS binding was higher in the αo preparation relative to the αi2 preparation ($p<0.05$, Student's *t* test) (Table 3), indicating that D_{2S} receptors activated by dopamine display a preference for coupling to the αo G protein over the αi2 G protein.

Table 1. D_{2S} receptor and G protein levels as measured by [^{3}H]spiperone saturation binding and western densitometry respectively (n=3)

Multiplicity of infection (R:α:β:γ)	D_{2S}β1γ2 (2:0:4:4)	D_{2S}αoβ1γ2 (2:4:3:3)	D_{2S}αi2β1γ2 (2:4:4:4)
Bmax D_{2S} (pmol/mg)	1.38±0.09	1.20±0.05	1.18±0.02
α (pmol/mg)	-	209±70	275±67

Table 2. Binding of dopamine to D_{2S} receptors and D_{2S} receptors co-expressed with αo or αi2 G protein subunits (n=3)

Multiplicity of infection (R:α:β:γ)	D_{2S}β1γ2 (2:0:4:4)	D_{2S}αoβ1γ2 (2:4:3:3)	D_{2S}αi2β1γ2 (2:4:4:4)
pK_h±s.e.m (K_h nM)	7.42±0.18 (38)	7.42±0.27 (38)	7.39±0.24 (41)
pK_l± s.e.m (K_l nM)	4.83±0.07 (14800)	4.91±0.21 (12300)	4.93±0.08 (11700)
%Rh	22±3	45±4	33±5
pK_{iGTP}± s.e.m (K_{iGTP} nM)	4.88±0.06 (13200)	4.93±0.10 (11700)	4.89±0.02 (12900)

Table 3. Potency of dopamine at stimulating [^{35}S]GTPγS binding at D_{2S} receptors & D_{2S} receptors co-expressed with αo or αi2 G protein subunits.

Multiplicity of infection (R:α:β:γ)	D_{2S}β1γ2 (2:0:4:4)	D_{2S}αoβ1γ2 (2:4:3:3)	D_{2S}αi2β1γ2 (2:4:4:4)
pEC_{50}± s.e.m (n=4) (EC_{50} nM)	-	6.89±0.18 (129)	6.07±0.06 (851)

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160P THE A_{2A} ADENOSINE RECEPTOR ANTAGONISTS ARE EFFECTIVE IN MODELS OF PARKINSON'S DISEASE THROUGH THE MODULATION OF BOTH D2 AND D1 DOPAMINERGIC PATHWAYS

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Disinhibition of striatal A_{2A}/D_2 output neurons, resulting in excessive GABAergic drive of the external segment of the globus pallidus, is one of the principal mechanisms whereby the generation of motor deficits in Parkinsonian patients occurs. The A_{2A} receptor antagonists are remarkably effective in different models of Parkinson's disease (PD) (Ongini & Fredholm, 1996). However, whether their effects are entirely attributable to the A_{2A}/D_2 antagonistic interaction or they partially reflect a modulatory activity of this class of drugs on A_1/D_1 containing neurons has not been fully established (Fuxe et al., 1998). Moreover, to our knowledge, nothing has been reported as to their duration of action *in vivo*. The first part of this study was aimed at defining the *in vivo* activity of two highly selective A_{2A} receptor antagonists, SCH 58261 (SCH) and KW 6002 (KW) at reverting the hypokinetic state elicited by either D_2 or D_1 receptor blockade using haloperidol or SCH 23390, respectively. All data presented are averaged from at least three different experiments made of 6-8 animals for each group. Moreover, to reveal possible species differences, all tests were performed in adult male Sprague-Dawley rats and CD1 mice. In the second part, the unilateral 6-hydroxydopamine (6-OHDA) lesion model was used to assess their time-response profile. Data were analysed using Dunnett's t-test.

Regardless of the species used, both drugs reduced haloperidol- or SCH 23390-induced catalepsy in a dose-dependent fashion. More specifically, in rats the systemic administration of a fully effective dose (10 mg/kg) of either SCH or KW inhibited by 45±11% or 75±2% the cataleptic response elicited by haloperidol and by 40±10% or 50±12% that elicited by SCH 23390 when compared to vehicle-treated animals. Similar effects were observed in mice (SCH, 60±8% and 80±11% inhibition in haloperidol and SCH 23390, respectively; KW, 75±4% and 80±13% inhibition in haloperidol and SCH 23390, respectively). In addition, we determined the duration of action of SCH and KW in rats unilaterally lesioned using 6-OHDA (8 μg/4 μl/4 min). Regardless of the dose tested, neither SCH nor KW elicited any effects *per se*, however they both produced a long-lasting potentiation of the circling behaviour induced by a subthreshold dose of L-DOPA (4 mg/kg, ip). SCH (10 mg/kg, ip) elicited a significant effect as compared to vehicle (45±20 turns/2hrs, n=10), at 1h (880±164 turns/2hrs, n=10) and 3 hrs post dosing (300±80 turns/2hrs, n=6), but it returned to basal levels at 6hrs (238±100 turns/2hrs, n=6). Very similar effects were recorded following the administration of an equipotent dose of KW.

Consistent with previous observations, A_{2A} receptor antagonists have proven to be effective in different animal models of PD and have a long-lasting action. Moreover, our data support the notion that the *in vivo* effects of A_{2A} blockers are, at least partially, mediated *via* modulation of the A_1/D_1 striatal output pathway. Altogether these findings indicate that A_{2A} antagonists represent a valuable non-dopaminergic therapeutic approach for the treatment of PD.

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Ongini & Fredholm, (1996) Trends Pharmacol. Sci., *17*, 364-372

161P EFFECT OF CYTOCHROME P4502E1 INHIBITION ON FREE RADICAL FORMATION AND DOPAMINE EFFLUX IN THE RAT STRIATUM: A MICRODIALYSIS STUDY

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Susceptibility to develop Parkinson's disease (PD) has been linked to abnormalities of cytochrome P450 (CYP) function (Jenner *et al.*, 1992). Multiple P450 enzymes are expressed in the brain (Kohler *et al.*, 1988) but the relationship of these to PD is unclear. In particular CYP2E1 is localised in the substantia nigra, co-localising with tyrosine hydroxylase (Reidl *et al.*, 1998) but its functional role is unknown. However, it may contribute to the oxidative stress thought to underlie PD (Jenner, 1998). This study investigates the role of CYP2E1 in free radical formation and dopamine (DA) efflux in the striatum *in vivo* using diallyl sulphide (DAS) to inhibit 2E1 activity.

Male Wistar rats (250-350g) were implanted with dialysis probes into the right striatum using standard stereotaxic techniques under halothane anaesthesia. Twenty hours following implantation, the probes were perfused with artificial extracellular fluid (aECF/2µl/min). Hydroxyl radicals were measured by the inclusion of sodium salicylate (5mM) in the aECF, forming 2,3 dihydrobenzoic acid (2,3-DHBA) and 2,5-DHBA. After an equilibration period, basal dialysates were collected every 10min over a 2h period before administration of either vehicle or DAS (100 or 200mg/kg i.p.) and for a further 2h thereafter. Levels of DA, 3,4 dihydroxyphenylacetic acid (DOPAC), 2,3-DHBA and 2,5-DHBA were determined by HPLC with electrochemical detection. Data (expressed as mean ± s.e.m) were analysed by one-way ANOVA followed by a *post hoc* Dunnett's test.

DAS (100mg/kg or 200mg/kg) had no effects on levels of striatal 2,3-DHBA ,2,5-DHBA, DA efflux (Figure 1) or DOPAC formation when compared to vehicle controls ($p = or > 0.05$).

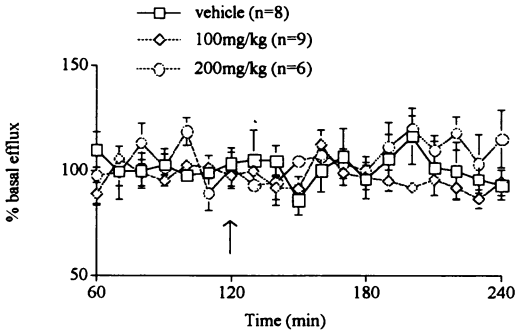

Figure 1 The effect of diallyl sulphide on striatal DA efflux. ↑ indicates i.p. injection of vehicle or drug

The data suggest that inhibition of CYP2E1 does not alter DA efflux in the normal rat striatum and does not contribute to hydroxyl radical formation. However, whether CYP2E1 is important in the parkinsonian substantia nigra rather than striatum is not known and requires further investigation

RK is supported by the Parkinson's Disease Society

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162P EFFECT OF A SELECTIVE CANNABINOID CB_1 RECEPTOR AGONIST ON SPINAL NOCICEPTIVE TRANSMISSION IN CARRAGEENAN INFLAMED ANAESTHETISED RATS

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Recent interest has focused on the analgesic potential of cannabinoids for the treatment of sustained pain states including inflammatory pain. A selective CB_1 agonist arachidonyl-2-chloroethylamide (ACEA), a derivative of anandamide, has been synthesised (Hillard *et al.*, 1999). Here electrophysiological methods were employed to investigate the effects of spinal administration of ACEA on noxious and innocuous evoked neuronal responses in carrageenan inflamed anaesthetised rats.

Extracellular recordings of convergent dorsal horn neurones were made in anaesthetised (1% halothane in 66% N_2O/33% O_2) male Sprague Dawley rats (Drew *et al.*, 2000). Neuronal responses to transcutaneous electrical stimulation (3x C-fibre threshold, train of 16 stimuli at 0.5Hz) of the receptive field were recorded, post-stimulus histograms were constructed. Responses were quantified on the basis of post-stimulus latencies: Aβ-fibre 0-20ms; Aδ-fibre 20-90ms; C-fibre 90-300ms and post-discharge 300-800ms. λ-carrageenan (100µl, 2% in saline) was injected into the plantar surface of a hindpaw 3 hours before the ACEA (0.5-500ng/50µl) dose-response data was obtained. Control values were taken and 50µl of ACEA (0.5-500ng) was applied to the spinal cord (n=9). In a separate group of rats with carrageenan inflammation, spinal pre-administration of SR141716A (0.01µg/50µl) (CB_1 receptor antagonist) was given prior to spinal ACEA (500ng/50µl, n=7). Data are presented as mean maximal effects. Mean area under the curve (AUC) of % of control response versus the time since drug administration (0-60mins) was calculated to compare the effect of ACEA in the presence and absence of SR141716A. Statistical analysis was performed using Mann-Whitney t-test, repeated measures ANOVA and Dunnett's *post hoc* test.

Mean depth of neurones studied was 874±43µm (mean ± s.e.m; n=16) and the C-fibre threshold and latency of responses were 1.7±0.1mA and 149±11ms, respectively. Control mean Aβ-, Aδ-, C-fibre and post-discharge responses of the neurones were 82± 10, 92± 10, 454± 51, 326± 36 action potentials respectively.

ACEA (0.5, 5, 50, and 500ng/50µl) reduced Aδ-fibre evoked responses (83± 4, 76± 6, 71± 6, 73± 5% of control, $p < 0.05$) and non-potentiated component of the C-fibre evoked responses (74± 9, 68± 11, 69± 11 and 60± 8%, $p<0.05$ of control) of spinal neurones. Spinal ACEA reduced post-discharge responses of dorsal horn neurones (84± 10, 67± 8, 67± 11, 58± 5% of control $p<0.01$). Aβ-fibre evoked responses of spinal neurones were not altered by spinal ACEA.

Pre-administration of SR141716A reduced effects of ACEA on Aδ-fibre evoked responses (AUC: 2703± 139 and 3847± 90 in absence and presence of SR141716A respectively, $p<0.01$) and post-discharge responses (AUC: 2423± 236 and 3754± 128 in absence and presence of SR141716A respectively, $p<0.01$) of neurones.

Effects of ACEA reported here are comparable to those previously described with the non-selective cannabinoid agonist HU210. This study demonstrates the contribution of spinal cannabinoid CB_1 receptors to the antinociceptive effects of cannabinoid agonists in a model of inflammatory pain.

Drew, L.J *et al.*, (2000) *Euro. J. Neurosci.*, **12**, 2079-2086.

Hillard, C.J. *et al.*, (1999) *JPET.*, **289**, 1427-1433.

163P CANNABINOID RECEPTOR ACTIVATION REDUCES CAPSAICIN EVOKED INCREASES IN $[Ca^{2+}]_i$ IN ADULT DORSAL ROOT GANGLIA CELLS

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Activation of spinal cannabinoid (CB) receptors inhibits C-fibre driven neuronal responses *in vivo* (Drew et al.,2000). This has prompted the present investigation of the effects of the cannabinoid receptor agonist HU210 on capsaicin evoked Ca^{2+} responses in dorsal root ganglion (DRG) cells in primary culture.

DRG were isolated from adult Wistar rats and neurones cultured as described by Lindsay (1988). Cells were grown on 13mm glass cover slips for 24 hours prior to incubation with Fura 2-AM (5μM, 30 min, 37°C). The mean diameter of the cells sampled was 24.3 +/-0.8 μm. Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in individual neurones in fields of 30-40 cells were calculated as the ratios of peak fluorescence intensities (measured at 500 nm) at excitation wavelengths of 340 and 380 nm respectively. DRG neurones were superfused with different concentrations of the vanilloid receptor agonist capsaicin, alone or in combination with the cannabinoid receptor agonist HU210 (1μM, 60 seconds) in the presence or absence of the cannabinoid CB_1 receptor antagonist SR141716A (1μM) with 45 minute wash-out periods between drug applications. Data are expressed as mean +/- SEM. Statistical analysis was performed using one way ANOVA or Mann Whitney test.

Ratio values in untreated cells were 1.3 ± 0.03 (n=29). Capsaicin evoked increases in $[Ca^{2+}]$i over the concentration range 10nM-1μM, with a peak increase of 1.9±0.1 fold basal at 1μM (n = 29, Fig 1A). 45% of cells studied responded to 1μM capsaicin. There was little desensitisation of capsaicin responses. Exposure of cells twice to 100nM capsaicin with a 45 min inter-treatment interval; second response 85± 6% of the first response (n = 26). SR141716A (1μM) had no effect on capsaicin (100nM) evoked responses (92 ± 2% of control capsaicin response). In the presence of 1μM HU210, the capsaicin (100nM) evoked response was significantly reduced (45 ± 5% of control capsaicin response, p <0.001, n=58); this reduction was partly blocked (70 ± 4 % of control response) by the co-application of 1μM SR141716A (Fig1B).

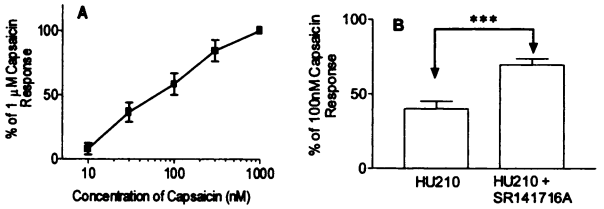

Figure 1. A. Capsaicin evoked increases in $[Ca^{2+}]_i$ in DRG cells ($p<0.0001$, one way ANOVA). Data are presented as percentages of the response to 1μM capsaicin. B. CB_1 receptor-dependent reduction in capsaicin response. DRG cells were incubated in the presence of HU210 (1μM) or HU210 plus SR141716A (1μM) prior to stimulation with capsaicin (100nm). (Mann Whitney test)

These data show that activation of cannabinoid CB_1 receptors reduce capsaicin evoked responses, probably mediated by VR_1 receptors, in DRG cells. This study demonstrates functional inhibitory effects of CB receptors at this level, which may contribute to antinociceptive effects of cannabinoids.

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Lindsay R.M. (1988) J.Neurosci., **8**, 2394-2405.

164P ANALGESIC EFFECT OF BISPHOSPHONATES IN MICE

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Bisphosphonates are analogues of inorganic pyrophosphate and are inhibitors of bone resorption. Many derivatives have been developed for the treatment of enhanced bone resorption; several reports reveal that treatment with bisphosphonates is able to reduce the pain associated with different painful diseases.

The aim of the present work is to study the analgesic effect, after intravenous (i.v.) and intracerebroventricular (i.c.v.) administration, of several different bisphosphonates, using the tail-flick Test in mice, in comparison with the analgesic effect of morphine and of acetylsalicylic acid.

Male mice (strain CD_1), weighing 20 to 25 g, were used in all experiments. Antinociception was assessed by the tail-flick method of D'Amour as modified by Dewey (1970). At least 10 animals per group were used and for each animal we calculated the difference between post-dose and pre-dose latency time (Δ Time) *.

The following drugs were administered into the tail lateral vein 30 minutes before the test: clodronate 5, 10, 30, 100 mg/kg, alendronate and etidronate 1, 3, 10, 30, 60 mg/kg, pamidronate 0.31, 0.62, 1.25. 2.50 mg/kg. Acetyl salicylic acid, given at the doses of 10, 30 and 60 mg/kg, was used in order to have a positive control of the antinociception. To estimate the central action of the drugs we evaluated the antinociceptive effect produced by i.c.v. injection of clodronate, etidronate, pamidronate, at the doses between 0.3 to 0.75 μg/mouse, in comparison with morphine. Conscious mice, under a light ethyl ether anaesthesia, were treated according to Haley (1957).

In the tail-flick test, after i.v. administration, clodronate induced a dose-dependent antinociceptive effect that was statistically significant ($p<0.05$) for all the doses used. The mean values of differences between post-dose and pre-dose latency time, in seconds, were 4.50 ± 1.60, 4.73 ± 0.61, 5.52 ± 1.28 and 6.71 ± 1.12 for the doses of 5, 10, 30 and 100 mg/kg respectively. Alendronate and etidronate induced a modest and not statistically significant increase of the tail-flick latency time at the doses of 1, 3, 10, 30 mg/kg i.v.. Only with the high dose of 60 mg/kg the antinociceptive effect of alendronate (Δ time 4.40 ± 0.97 seconds) and etidronate (Δ time 4.11 ± 0.25 seconds) was pronounced and statistically significant. Pamidronate produced a significant dose-dependent increase ($p<0.05$) of the Δ time (2.98 ± 0.29, 3.43 ± 0.40, 4.61 ± 0.49) with the 3 highest doses administered. Acetyl salicylic acid induced a dose-dependent antinociceptive effect that was statistically significant for all the 3 doses used. (Δ time 3.99 ± 0.69, 6.07 ± 1.24, 8.25 ± 1.28).

After i.c.v. administration, morphine produced a dose-dependent anti-nococeptive effect with an ED_{50} of 0.11 μg/mouse.

Clodronate and pamidronate showed a potent dose-dependent effect for all the doses tested ($p<0.05$), except for the lowest dose of clodronate, with an ED_{50} of 0.55 and 0.39 μg/mouse respectively. Etidronate did not produce any statistically significant antinociceptive effect.

In conclusion there is evidence that bisphosphonates, particularly clodronate and pamidronate, have a pharmacological role in modulation of antinociception under acute conditions not related to osteolysis or acute inflammatory bone process.

* Statistics, Kruskal – Walles one-way analisis of variance followed by the distribution-free multiple comparison was used

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165P DETECTION OF SOMATOSTATIN AND SOMATOSTATIN RECEPTOR TYPES IN THE RAT TRIGEMINAL GANGLION

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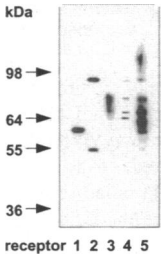
Migraine headache is associated with activation of trigeminal neurones which relay information to the brain. It is believed that the modulation of nociceptive transmission by the neuropeptide, somatostatin (SRIF), results in analgesic effects. Hence, clinical studies using octreotide, a long-acting analogue of somatostatin, have proved effective in the treatment of acromegaly, leading to the alleviation of all symptoms of the disorder including headache and joint pain. Putative target sites for octreotide have been shown to exist in the trigeminal system and periaqueductal gray (reviewed in Selmer *et al.*, 2000). Hence, we have used semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) along with a combination of Western blotting, immunohisto and -cytochemical techniques to identify the presence of specific SRIF receptor types in the trigeminal ganglion at both the mRNA transcript and protein level.

Whole trigeminal ganglia were isolated from both female adult Wistar rats (150-250g) and 3-day-old Wistar neonates (mixed sex). Rat trigeminal ganglion neuronal cultures were prepared as previously described (Eckert *et al.*, 1997) and maintained *in vitro* for 5 days in the presence of NGF (50 ng ml^{-1}) and cytosine-β-D-arabinofuranoside (20 μM). The SV total RNA isolation system (Clontech) was used to extract RNA. RT-PCR was carried out using sequence specific primers for sst_{1-5} receptors and SRIF (Clontech). Semi-quantification of PCR reactions was achieved by normalisation to the house-keeping gene, GAPDH. Immunohisto, -cytochemistry and Western blotting was carried out as previously described (Schindler *et al.*, 1997) using selective polyclonal antibodies raised to sst_{1-5} receptors and SRIF (Dako). Control experiments involved the omission of primary antibody and the specificity of the polyclonal antibodies have been previously demonstrated by Western blotting (Cole *et al.*, 2000).

Single significant bands of anticipated size were observed for all SRIF receptors (sst_1-sst_5) following RT-PCR using both adult and neonatal trigeminal material. The data suggest that expression of the sst_2 receptor type maybe developmentally regulated with apparently higher levels of this receptor present in adult trigeminal neuronal tissue when compared to those observed in neonatal samples.

Figure 1



Western blotting data, using total cell lysates, derived from both dissociated adult and neonatal trigeminal ganglion neurones are in good agreement with the RT-PCR data (Figure 1, adult trigeminal material). Whilst the predicted molecular weight for SRIF receptors derived from primary structure analyses is approximately 45kDa, our data indicate the presence of higher molecular weight species for all SRIF receptor types. This may be a result of retardation of covalently modified receptors via glycosylation, addition of phosphate moieties or receptor dimerisation.

Localisation studies on both adult and neonatal trigeminal ganglion slices and dissociated neurones, employing fluorescence and DAB detection systems, showed the presence of immunoreactivity corresponding to somatostatin and all of its receptors in a number of neurones, showing both cytoplasmic and membrane-bound staining. All signals were absent in control experiments.

Our data indicate the presence of somatostatin and all five somatostatin receptors in both neonatal and adult trigeminal ganglia. Whilst the functional activities of these receptors in this tissue remain to be determined, it is tempting to speculate that somatostatin may play a neuromodulatory role in the trigeminal system.

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Schindler, M. *et al*, (1997), *Neuroscience* 76, 225-240
Selmer, I-S *et al*, (2000), *Regul. Pept.* 90, 1-18

166P IDENTIFICATION OF THE H^+ ION MODULATORY SITE ON A $GABA_A$ RECEPTOR

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$GABA_A$ receptors are responsible for the majority of neuronal inhibition within the central nervous system (CNS). They are composed from several subunit classes including: α, β, γ, δ, ϵ, π, θ and ρ.

Changes in extracellular pH can accompany neurodegeneration, CNS pathologies and even synaptic transmission (Chesler and Kaila, 1992). These changes in extracellular pH may regulate $GABA_A$ receptor function in a manner dependent upon the subunit composition (Krishek *et al.*, 1996). The pH titration curve profile for α1βi $GABA_A$ receptors (where i = 1 or 2) revealed a potentiation of the GABA-activated current in acidic pH.

Using the redox reagents DL-dithiolthreitol (DTT) and 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) and diethylpyrocarbonate (DEPC) both cysteine and histidine residues were investigated respectively to determine if they had a role in H^+ ion modulation. Using α1βi expressing HEK cells, whole-cell patch clamp techniques were used to measure changes in GABA-activated currents.

Altering the pH of the external solution from pH 7.4 to pH 5.4 increased the maximum of the GABA concentration-response curves by 2.5 - 3 fold (measured at 10 μM GABA) for α1βi $GABA_A$ receptors, with minimal change in the EC_{50} values: α1β1 at pH 7.4 : 4.3±0.5μM, pH 5.4: 3.5 ±0.5μM; α1β2 pH 7.4 : 2.7 ±0.1μM, pH 5.4: 2.27 ±0.02μM (n =3 -13). The redox reagents had no significant influence on the pH modulation, suggesting that cysteine residues are not involved in H^+ modulation.

Application of DEPC (1mM) inhibited the GABA concentration-response curves for α1βi $GABA_A$ receptors in a non-competitive manner at pH 7.4 and pH 5.4 (EC_{50} s after DEPC : α1β1 pH 7.4 : 5.1 ± 1.4μM, pH 5.4 : 17.1 ± 4.4μM; α1β2 pH 7.4 : 2.7 ± 0.9μM, pH 5.4 : 13.8 ± 0.9μM; n =3-8). DEPC also abolished the potentiation of the GABA-activated response at pH 5.4 for α1βi receptors. These results suggest that it is one or more histidine residues that are responsible for pH modulation.

Previous studies with zinc have indicated competitive inhibition between Zn^{2+} and H^+ ions, suggesting that both ions are acting on the same site (Krishek *et al.*, 1998). H267 on the β-subunit does influence zinc inhibition (Wooltorton *et al.*, 1997). When the mutation H267A was introduced into the β-subunit ($\alpha1\beta i^{H267A}$) the pH potentiation was abolished. EC_{50} values for the mutants were as follows, $\alpha1\beta1^{H267A}$, pH 7.4 : 3.5 ±0.6μM, pH 5.4: 14.0 ± 2.7μM, and $\alpha1\beta2^{H267A}$ pH 7.4: 3.5 ±0.4μM, pH 5.4: 7.2 ± 2.0μM (n =3-9). The maximum GABA responses for $\alpha1\beta i^{H267A}$ at pH 7.4 and pH 5.4 were similar to that of the controls at pH 7.4.

In conclusion we suggest that the histidine residue at position 267 on the β-subunit of the α1βi $GABA_A$ receptors is responsible for the potentiation of the GABA-activated current in acidic pH.

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167P REGULATION OF CAPSAICIN-EVOKED RELEASE OF CGRP FROM RAT ISOLATED DURA MATER *IN VITRO*.

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The release of calcitonin gene related peptide (CGRP) from the peripheral terminals of the first division of the trigeminal nerve has been implicated as one of the underlying mechanisms involved in migraine pathogenesis (Goadsby et al.,1988). In this study we have investigated the effect of a number of drugs including the anti-migraine drug sumatriptan on capsaicin-evoked release of CGRP from the rat isolated dura mater *in vitro*.

Male Wistar rats (225g–260g) were killed by CO_2 inhalation followed by decapitation. The skull was divided longitudinally and the cerebral hemispheres and brain stem carefully removed as previously described (Ebersberger et al.,1999). The hemisected skulls with adherent dura mater were placed in a heated jacket and the skull cavities filled with 400µl pre-warmed (37°C) Krebs solution containing (mM) NaCl (118), $NaHCO_3$ (25), KCl (4.7), $MgSO_4.7H_2O$ (0.6), KH_2PO_4 (1.2), D-glucose (11.1), $CaCl_2.6H_2O$ (1.3), gassed with 95% O_2/5% CO_2. One of the cavities acted as a control to monitor spontaneous changes in capsaicin (one minute contact) evoked release of CGRP from 2 successive challenges given one hour apart. Samples were collected every 5min after an initial wash period of 30min. Drugs were administered to the test chamber 10 minutes prior to the second capsaicin challenge. In some studies $CaCl_2$ was removed from the bathing solution 30 min before the second capsaicin challenge. The concentration (pg/ml) of CGRP in the bathing medium was assayed using a CGRP enzyme linked immunoassay (SPBio). Evoked release was measured as the sum of the CGRP concentration found in the bathing medium during the capsaicin exposure and subsequent 5min sample, corrected for basal values prior to capsaicin challenge. All values shown are mean ± se mean from n separate experiments.

The basal concentration of CGRP in the bathing medium prior to the first capsaicin challenge was 17 ± 1 pg /ml (n=38). Capsaicin-evoked release of CGRP was significantly attenuated by prior exposure of the dura mater to capsazepine or removal of $CaCl_2$ from the external bathing medium (Table 1). Capsaicin-evoked release was not modified by the selective adenosine A1 agonist, GR 79236 (Gurden et al.,1993), or the somatostatin sst_2 agonist, octreotide. Sumatriptan also did not modify capsaicin evoked release of CGRP.

Table 1: Capsaicin (100nM) evoked release of CGRP from rat isolated dura mater in the presence (challenge 2) and absence (challenge 1) of various drug treatments. Controls show CGRP levels following 2 successive challenges in the absence of treatment.

	Increase in Concentration CGRP (pg/ml)		
Treatment	**Challenge 1**	**Challenge2**	**n**
Control	65±7	41±5	19
Capsazepine (3µM)	57±3	1±2*	4
Ca^{2+} removal	39±9	10±1*	4
Sumatriptan (1µM)	43±14	54±17	4
GR79236 (1µM)	84±31	74±19	4
Octreotide (1µM)	51±13	40±17	3

*Significantly different $p<0.05$ from Control Challenge 2 (Students unpaired t-test).

In conclusion capsaicin evoked release of CGRP in rat isolated dura mater is mediated via activation of vanilloid VR1 receptors and is dependent upon extracellular calcium entry. However surprisingly, this release could not be modified by a number of agonists including the antimigraine drug, sumatriptan.

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168P *EX VIVO* MEASUREMENT OF *c-fos* mRNA USING TaqMan® QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR).

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The immediate early gene *c-fos* is a widely used molecular marker of neuronal activation following painful stimuli (Bullitt, 1990). *c-fos* expression has previously been assessed using techniques of *in situ* hybridisation and immunocytochemistry (ICC); however, quantification can be difficult and time-consuming. TaqMan® qPCR is a high throughput integrated system for the quantitative detection of nucleic acids. We have utilised qPCR methodology for the quantitative measurement of *c-fos* mRNA *ex vivo* following systemic application of capsaicin.

Male Sprague Dawley rats (200-400g) were anaesthetised with urethane (1.25 g kg^{-1} i.p.), and capsaicin (0.3–10.0 µmol kg^{-1}) or vehicle was administered via the left jugular vein (1 ml kg^{-1}). Respiration was maintained artificially (10 ml kg^{-1}, 70 strokes min^{-1}). Rats were killed by exsanguination 45 mins post capsaicin, and brainstems rapidly dissected. Tissue samples (+1 mm to –5 mm obex) were snap frozen on dry ice and stored at -70°C. MK-801 (1 mg kg^{-1}) was administered i.v. 5 minutes prior to capsaicin (0.3 µmol kg^{-1}).

RNA was isolated (Promega SV Total RNA Isolation System), and complimentary DNA (cDNA) generated by reverse transcription. qPCR amplification of the cDNA was carried out using TaqMan® (ABI PRISM® 7700 Sequence Detection System). The threshold cycle (C_T) indicates the cycle number at which the amount of amplified target reaches a fixed detection threshold. Changes in *c-fos* expression were quantified with reference to the endogenously expressed 'housekeeper' gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as follows: C_T FOS – C_T GAPDH = ΔC_T; $\Delta C_{T\,test}$ - $\Delta C_{T\,control}$ = $\Delta\Delta C_T$. Results are expressed as fold change over control ($2^{-\Delta\Delta CT}$). Upper and lower confidence intervals were calculated using $2^{-\Delta\Delta CT \pm stdev}$, and statistical analysis performed against ΔC_T values using one way ANOVA and Dunnett's post hoc test ($P<0.05$ significant).

For ICC experiments, rats were perfuse-fixed with 4% paraformaldehyde 2 hours post capsaicin (0.3-10.0 µmol kg^{-1}), and brainstems removed +1mm to –5mm obex). 14µm sections were cut using a cryostat and slides processed according to the ABC method of Hsu *et al.*, 1981. Results are expressed as number of Fos-like immunoreactive cells (Fos-LI) per section ± s.e.mean. Statistical significance was tested as described above.

Dose response curves to i.v. capsaicin were generated using Taqman qPCR and ICC methods. A significant increase in *c-fos* mRNA expression over vehicle treated animals was seen at all doses tested using qPCR ($P<0.01$; n=4-6). The lowest dose of 0.3 µmol kg^{-1} produced a 6.0 fold elevation in *c-fos* mRNA (3.7-9.5), which increased to a maximum of 13.8 fold at 3.0 µmolkg^{-1} (9.7-19.7). 10 µmol kg^{-1} showed no greater response. In comparison, in the tissue processed using ICC (n=4), the minimum dose of capsaicin required to achieve a significant response over vehicle treated animals (0±0.1 cells) was 1.0 µmol kg^{-1} (179±32 cells; $P<0.01$). 0.3 µmolkg^{-1} produced a non-significant increase (44±25 cells). The greatest response was seen at the dose of 10 µmolkg^{-1} (347±32 cells; $P<0.01$).

Subdivision of the brainstem revealed a greater expression of *c-fos* mRNA in the region of -1mm to -3mm obex. Here, 0.3µmol kg^{-1} capsaicin produced a 9.3 fold increase over control (6.0–14.6; n=10), which was significantly reduced by MK-801 (1 mg kg^{-1}) to 1.7 fold (0.9-3.4; n=10; $P<0.01$), suggesting an involvement of NMDA receptors in this effect.

Using capsaicin as a stimulus to induce *c-fos* expression in the brainstem, we have shown that TaqMan® qPCR provides a sensitive, alternate method for the *ex vivo* quantitative measurement of CNS activation, and that this method is suited to protocols appropriate for pharmacological studies.

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