179P ENDOTHELIN-1-INDUCED PROLIFERATION OF NEONATAL RAT CARDIAC FIBROBLASTS IS REDOX SENSITIVE

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Excessive proliferation of cardiac fibroblasts contributes to ventricular remodelling following ischaemia and reperfusion (Weber and Brilla, 1991). The mechanism of this reactive fibrosis is not known. Endothelin-1 (ET-1) is a candidate molecule since it is released upon reperfusion of ischaemic myocardium (Tsuji et al., 1991), and has a mitogenic effect on these cells under normoxic conditions (Piacentini et al., 1997). However, the effect of ET-1 on these cells under conditions of altered oxygen tension is not known. In this study, we examined the effects of hypoxia and reoxygenation, key components of ischaemia and reperfusion, on the ET-1-induced increases in DNA synthesis and cell proliferation using primary cultures of rat neonatal cardiac fibroblasts.

Primary cultures of neonatal rat cardiac fibroblasts were prepared by enzymatic dissociation of ventricular tissue from one-day old Sprague-Dawley rat pups. Fibroblasts were cultured on 24-well plates and maintained in media supplemented with 5 % serum until they had proliferated to ~ 50 % confluence. The cells were then mitogen-depleted for 48 h by incubation in serum-free media supplemented with bovine serum albumin (100 mg/ml), transferrin (1 mg/ml) and insulin (1 mg/ml). When necessary, cells were rendered hypoxic by incubation in a humidified chamber maintained at 37°C and gassed with a mixture of nitrogen (98 %), carbon dioxide (1 %) and oxygen (1%). After 24 h, cells were removed from the chamber, the media was replaced and the cells were allowed to recover in an incubator infused with ambient air supplemented with carbon dioxide (1%) for up to 36 h. At various time-points, cells were incubated with ET-1 (10nM) for 23 h, after which, [^{3}H]thymidine (10 μCi/ml) was added for a further 1 h incubation period. The incorporation of [^{3}H]thymidine into DNA, indicating the synthesis of DNA, was then measured. Three groups were examined. Group A consisted of cells subjected to 24 h hypoxia without a subsequent period of reoxygenation. In this group, ET-1 was added immediately after onset of hypoxia and cells were harvested whilst in the hypoxia chamber. In group B, ET-1 was added to media upon reoxygenation. In group C, ET-1 was added to media 8 h after onset of reoxygenation. All groups were compared to time-matched controls maintained under normoxic conditions. Comparisons between groups were made using a Student's t-test. All results are expressed as mean ± s.e.mean.

After 24 h hypoxia, ET-1-induced DNA synthesis was similar to that measured in normoxic cells (48 ± 7 vs. 40 ± 6 % over basal, n = 5), despite an 80 % decrease in basal DNA synthesis. In group B, basal DNA synthesis measured 24 h after onset of reoxygenation was similar to that measured in normoxic controls. However, ET-1-induced DNA synthesis was attenuated (2 ± 16 vs. 38 ± 16 % over basal, $P < 0.05$, n = 6). In group C, where addition of ET-1 was delayed until 8 h after initiation of reoxygenation, ET-1-induced DNA synthesis was higher than in normoxic controls (61 ± 20 vs. 37 ± 8 % $P < 0.05$, n = 8).

We conclude that the responsiveness of cardiac fibroblasts to ET-1 is redox-sensitive. It is maintained under reduced conditions and depressed when intracellular levels of reactive oxygen species would be expected to be high. This effect is reversible since the response to ET-1 recovers after prolonged reoxygenation.

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180P THE NITRIC OXIDE DONOR *S*-NITROSO-*N*-ACETYL PENICILLAMINE (SNAP) DOES NOT ALTER ENDOTHELIN-1-INDUCED CONSTRICTIONS IN HUMAN SMALL CELL ARTERIES

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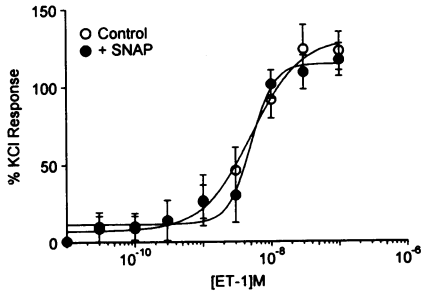
We have investigated the ability of endothelin-1 (ET-1) and nitric oxide (NO) to physiologically antagonise each other in human small coronary arteries. We have determined whether ET-1 can contract a preparation preincubated with a submaximal dose of the NO donor *S*-nitroso-*N*-acetyl penicillamine (SNAP) and conversely whether a submaximal concentration of ET-1 can be reversed by the addition of SNAP.

Small coronary arteries were obtained from 9 patients undergoing heart transplant operations and one cadaveric donor (mean age 42 ± 5 years). Epicardial arteries were dissected from the apices of the hearts, cleaned of surrounding connective tissue, and cut into 2mm rings. The segments were then mounted in a wire myograph for the measurement of isometric tension. Vessels were maintained at 37°C in an oxygenated Kreb's solution and were set to 90% of the internal diameter. Prior to constructing cumulative concentration-response curves to ET-1 and SNAP, preparations were stimulated with a potassium-rich (95mM) Kreb's solution three times.

Cumulative concentration-response curves were constructed to ET-1 (10pM - 100nM) in the absence (control) and presence of 100nM SNAP, which was pre-incubated with the tissue for 20 min. Results are expressed as a percentage of the average maximal potassium response for each ring ± SEM. In another set of experiments the ability of SNAP (10nM-30μM) to reverse a contraction induced by a sub-maximal (10nM) dose of ET-1 was investigated. Control rings of artery were contracted with 10nM ET-1 and the tension measured over the time course of the experiment. ET-1 dilutions were prepared in 0.1% acetic acid, SNAP dilutions were made in distilled water, kept on ice, in the dark and used within 3 hours.

The mean internal diameter was 652.5 ± 58.4μm and the mean contraction to 95mM potassium was 2.79 ± 0.46mN (20 observations from 10 hearts). ET-1 was found to potently constrict small coronary arteries, with a maximal response 124 ± 12% of the potassium response, and an EC_{50} value of 4.65nM (95% CI, 2.41 - 8.94nM; n = 5). In the presence of 100nM SNAP the maximal response was 117 ± 10%, with an EC_{50} value of 3.51nM (95% CI, 1.39 - 8.86nM; n = 5). EC_{50} values for the two curves were not significantly different (Mann- Whitney *U*-test, p = 0.83) and neither were the maximal responses (Student's *t*-test, p > 0.2).



SNAP (10nM-30μM) was unable to significantly reverse a contaction induced by 10nM ET-1 (Student's *t*-test p > 0.1 for all concentrations of SNAP; n = 5).

The results show that the NO-donor SNAP was unable to either prevent contractions to ET-1 or reverse an established ET-1 contraction.

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181P REDUCTION OF NITRERGIC NEURONAL VASODILATION IN RABBIT BASILAR ARTERIES BY INHIBITION OF SUPEROXIDE DISMUTASE

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Delayed cerebral vasospasm occurs in approximately one third of patients after intracranial bleeding. Two thirds of patients developing this syndrome die or suffer permanent neurological deficit as a result of ischaemic brain damage (Dorsch, 1995).

While the causes of vasospasm remain to be elucidated, there is considerable evidence to suggest that the generation of free radicals, and in particular superoxide, may be important. Superoxide can participate in a number of processes potentially leading to vasospasm, including inhibition of nitric oxide (NO)-induced vasodilation, endothelial damage and lipid peroxidation (Cook & Vollrath, 1995). Superoxide dismutase (SOD) has been shown to attenuate vasospasm in both animal and clinical studies (e.g., Shishido *et al.*,1994; Muizelaar *et al.*, 1993).

While the role of endothelium-dependent relaxation in vasospasm has been well studied, the role of neuronally released nitric oxide has received little attention. The aim of this initial study is to assess the role of superoxide in modulating nitrergic neuronal vasodilation in non-spastic cerebral vessels, by inhibiting endogenous CuZn-SOD using the copper-chelating agent diethyldithiocarbamic acid (DETCA).

Endothelium-denuded, isolated rabbit (New Zealand White, 1.9 – 3.5kg) basilar artery rings were set up for tension recording in a small-vessel myograph. Following treatment of rings with guanethidine (10μM), tone was raised by addition of histamine (10μM). Electrical field stimulation (EFS, 30-90 volts / 4ms pulse width / 2Hz / 5 seconds) of the intramural nerves was carried out via platinum electrodes above and below the ring.

EFS resulted in tetrodotoxin-sensitive relaxations of histamine induced tone. These relaxations were significantly inhibited by the presence of the nitric oxide synthase inhibitor L-NAME (300μM), from 38 ± 5 % to 16 ± 8 %, $P<0.01$, n=11. In separate rings, 100μM, but not 10μM, DETCA caused a significant reduction in EFS-induced relaxations, from 52 ± 6% to 9 ± 4%, $P<0.001$, n=9. Following washing, subsequent addition of exogenous CuZn-SOD (100 and 1000 units.ml.$^{-1}$), did not cause recovery of EFS-induced relaxations.

These results suggest that endogenous SOD is crucial in protecting neuronally released NO from destruction by superoxide. The relatively high molecular mass of SOD results in it having poor intracellular penetration. This may explain the failure of exogenously applied SOD to reverse the effect of DETCA.

It is concluded that increased superoxide production and /or a loss of antioxidant protection may be important factors in the pathophysiology of conditions characterised by maintained vasoconstriction, such as delayed cerebral vasospasm.

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182P LACK OF EFFECT OF INOSITOL 1,4,5 TRISPHOSPHATE ON Ca^{2+} UPTAKE INTO, AND Ca^{2+} RELEASE FROM, THE SARCOPLASMIC RETICULUM IN SAPONIN-SKINNED ISOLATED VENTRICULAR MUSCLES FROM RAT

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In smooth muscle inositol (1,4,5)-trisphosphate ($InsP_3$) plays a major role in releasing Ca^{2+} from the SR, by targeting an $InsP_3$ receptor. However in cardiac muscle, where Ca^{2+} -induced Ca^{2+} release (CICR) is the primary mechanism of Ca^{2+} release, the role of $InsP_3$ is unclear. $InsP_3$ could be important in the modulation of myocardial contractility by α-adrenergic stimulation, which has been shown to result in increased levels of diacylglycerol and $InsP_3$. The increase in myocardial contractile force during α-stimulation is due, at least in part, to an increase in Ca^{2+} release from the cardiac sarcoplasmic reticulum (SR). Therefore to investigate the possible role of $InsP_3$ in this positive inotropic effect, we examined the effect of $InsP_3$ on CICR and on the Ca^{2+} load of the cardiac SR.

Ventricular trabeculae (diameter 100-200 μm) were isolated from rats (Wistar or $LBNF_1$ strain, 200 – 250 g) and were skinned with saponin. We induced CICR by flash photolysis of nitr-5 in cytosol-like solutions (as described in Kentish & Xiang, 1997) and measured the resulting changes in [Ca^{2+}] using fluo-3. The Ca^{2+} transient (peak [Ca^{2+}] minus loading [Ca^{2+}]) in control solutions was 0.19 ± 0.06 μM (mean ± s.e.m., n = 5) and was not significantly affected by the presence of 10 μM $InsP_3$ (0.15 ± 0.03 μM; $P > 0.05$, paired t test). The amount of Ca^{2+} loaded into the SR under these conditions was studied using caffeine (10 mM) to release Ca^{2+} from the SR after a 3 min loading period, with BAPTA as the Ca^{2+} buffer. Under control conditions the caffeine-induced Ca^{2+} transient amounted to 0.64 ± 0.10 μM (n = 7) and it was not significantly different in the presence of 10 μM $InsP_3$ (0.59 ± 0.11 μM).

As $InsP_3$ can be converted to $InsP_4$ by the action of intracellular 3-kinases, we used a non-phosphorylatable analogue, 3-deoxy-3-fluoro-$InsP_3$ (3-F-$InsP_3$) and repeated the studies above. The Ca^{2+} transient resulting from CICR in the presence of 10 μM 3-F-$InsP_3$ (0.14 ± 0.03 μM, n=5) was not significantly different from the control (0.15 ± 0.03 μM). Similarly 3-F-$InsP_3$ did not alter the SR load, which was 0.43 ± 0.08 μM (n=4) under control conditions and 0.42 ± 0.10 μM in the presence of 10 μM 3-F $InsP_3$.

Richardson & Taylor (1993) reported that BAPTA and its derivatives (one of which is nitr-5) may inhibit the $InsP_3$ receptor. To see if this was responsible for the lack of effect of $InsP_3$ on the cardiac SR, we also carried out experiments using flash photolysis of nitrophenyl-EGTA to induce CICR, or using EGTA as a Ca^{2+} buffer in the SR load experiments. Once again we found no significant differences between the results in the absence and presence of 10 μM $InsP_3$.

Thus the observed potentiation of SR Ca^{2+} release during α-adrenergic stimulation of the myocardium is unlikely to be the result of a direct action of $InsP_3$ on the cardiac SR.

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183P THE EFFECTS OF α_1– AND β_2–ADRENOCEPTOR STIMULATION ON THE FREQUENCY FOR MAXIMUM POWER OUTPUT (f_{OPT}) OF RAT ISOLATED CARDIAC TRABECULAE UNDERGOING 'PHYSIOLOGICAL' CONTRACTIONS

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The work-loop technique (Josephson, 1985) investigates the mechanical performance of isolated muscles during cyclical changes in force and length which mimic the physiological contraction. We used this technique to examine the effects of α_1- and β-adrenoceptor stimulation on the power produced by isolated cardiac muscle at different frequencies.

Trabeculae (100-250 μm diameter) dissected from the right ventricles of rats (Wistar or $LBNF_1$ strain, 200 – 250 g) were bathed in modified Krebs-Henseleit solution (1 mM Ca^{2+}, 24°C) and were subjected to sinusoidal length changes (± 5% of muscle length) and stimulation at different cycle frequencies. A plot of force against length for a single cycle produced an anti-clockwise work-loop, the area of which represents the net work performed at that cycle frequency. Average power output was calculated as the product of net work and cycle frequency. The timing of the stimulus was adjusted to maximise net work and power output at each frequency. The power-frequency relationships derived during control conditions were compared to those established during stimulation of either α_1-adrenoceptors (with 100 μM phenylephrine + 10 μM propranolol) or β-adrenoceptors (with 5 μM isoprenaline), and to those produced following drug washout. Paired *t*-tests were used to compare the frequency for maximum power output (f_{opt}) under each condition (significance taken as $P < 0.05$).

α_1-Adrenoceptor stimulation increased power output and produced a small but significant increase in f_{opt}, from 3.1 ± 0.3 Hz to 3.5 ± 0.2 Hz (mean ± s.e.m., n = 6). Stimulation of the β-adrenoceptors produced a large potentiation of power output and significantly increased f_{opt} from 2.9 ± 0.3 Hz to 4.4 ± 0.2 Hz (n = 6). The effects of α_1- and β-stimulation on f_{opt} were not fully reversible: f_{opt} was 3.5 ± 0.2 Hz (n = 4) after phenylephrine washout and 3.5 ± 0.3 Hz (n = 4) after isoprenaline washout.

The β-mediated increase in f_{opt} was attributed largely to the reduction in twitch duration characteristic of β-stimulation, which allows the muscle to produce power effectively at higher frequencies. The increase in f_{opt} in response to α_1-stimulation was more surprising since α_1-stimulation is thought to prolong twitch duration (e.g. Terzic *et al.*, 1993). To address this issue we examined the effects of phenylephrine on twitch duration (assessed as the time to 90% relaxation, t_{90}) during isometric twitches at different frequencies. We observed that although α_1-stimulation significantly prolonged t_{90} at low stimulation frequencies (e.g. from 372 ± 41 ms to 402 ± 33 ms at 0.33 Hz, n = 7), at higher frequencies α_1-stimulation significantly reduced t_{90} (e.g. from 291 ± 15 ms to 270 ± 16 ms at 3 Hz, n = 5). The mechanisms underlying this frequency-dependent effect of α_1-stimulation remain unclear, but it may explain the α_1-mediated increase in f_{opt}.

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184P INVOLVEMENT OF α_1– AND α_2-ADRENOCEPTORS IN THE CONSTRICTION OF CAROTID ARTERIOVENOUS ANASTOMOSES (AVAs) IN ANAESTHETISED PIGS

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The present study set out to investigate the possible involvement of α_1- and α_2-adrenoceptors in the carotid vascular bed in pentobarbital anaesthetised, bilaterally vagosympathectomised female pigs (12-14 kg; n=28), using selective agonists (phenylephrine and BHT933) and antagonists (prazosin and rauwolscine) (Piascik *et al.*, 1996). Total carotid blood flow was measured with an electromagnetic flow meter, whereas its AVA and capillary fractions were calculated using the radioactive microsphere method (see Saxena, 1995). Phenylephrine (1-10 μg kg^{-1} min^{-1}) and BHT 933 (3-30 μg kg^{-1} min^{-1}) was infused (0.1 ml min^{-1}) for 10 min into the right carotid artery in animals pre-treated with or without prazosin (100 μg kg^{-1}, i.v.) or rauwolscine (300 μg kg^{-1}, i.v.), respectively (n=7 each). All data are expressed as mean±SEM. Baseline values (n=28) of heart rate (HR; beats min^{-1}), mean arterial blood pressure (MAP; mmHg) and total carotid, AVA and capillary conductances (dl min^{-1} $mmHg^{-1}$) were 100±2, 93±2, 124±6, 93±7 and 32±4, respectively. Prazosin and rauwolscine slightly decreased MAP (7±2 and 9±3%, respectively) without affecting carotid vascular conductance. Phenylephrine dose-dependently increased HR (by up to 29±6%). Prazosin did not affect HR changes, but unmasked a slight hypotensive response (-8±3%). BHT 933 did not affect HR or MAP. As shown in Figure 1, both phenylephrine and BHT 933 dose-dependently decreased total carotid conductance (maximum decreases: 74±4 and 59±4%, respectively), confined to the AVA part (maximum decreases: 92±3 and 84±1%, respectively). The capillary conductance was not affected. Prazosin and rauwolscine abolished, respectively, the phenylephrine- and BHT 933-induced total carotid and AVA constriction. In fact, phenylephrine slightly increased total carotid, AVA and capillary conductances after prazosin. In conclusion, the present results show that both α_1- and α_2-adrenoceptors mediate constriction of carotid AVAs in anaesthetised pigs. Since this *in vivo* model is predictive of anti-migraine activity (Saxena, 1995), α-adrenoceptor agonists at, particularly, the α_2-adrenoceptor (sub)types could provide new avenues for developing migraine abortive drugs.

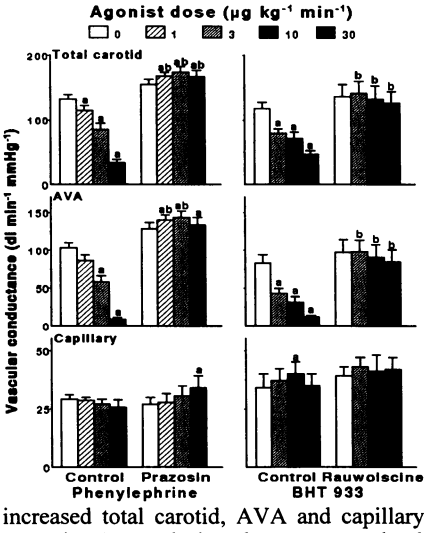

Fig. 1 *Carotid vascular effects of phenylephrine or BHT 933 in animals treated with or without prazosin (100 μg kg^{-1}, i.v.) or rauwolscine (300 μg kg^{-1}, i.v.), respectively (n=7 each). a, P<0.05 vs baseline; b, P<0.05 vs control.*

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185P EVALUATION OF α_1-ADRENOCEPTOR SUBTYPES IN THE RENAL VASCULATURE OF DIABETIC SPONTANEOUSLY HYPERTENSIVE RATS

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The renal sympathetic nerves play an important role in controlling kidney haemodynamic function. In previous studies (Sattar & Johns, 1994), we showed that adrenergic vasoconstriction in the kidney of normotensive, SHRSP, 2K1C and DOCA-salt hypertensive rats was primarily mediated by the post-junctional α1A-adrenoceptor subtype. Type I diabetes is associated with autonomic neuropathies which cause a defective autonomic control of major organs. This study aimed to examine the α_1-adrenoceptor subtype(s) involved in mediating adrenergically induced renal vasoconstriction in a rat model of diabetes and hypertension. The study was undertaken in spontaneously hypertensive male rats (220-280g) 7 days post-streptozotocin (50 mg/kg i.p.). After anaesthesia (pentobarbitone, 60 mg/kg i.p.), blood pressure was recorded from a carotid artery, the kidney exposed via the abdomen, renal blood flow (RBF) measured with electromagnetic flowmetry and an iliac artery cannulated for close renal arterial of infusion of drugs and saline. The renal nerves were identified and sectioned. The reductions in RBF to electrical renal nerve stimulation (RNS, at 1, 2, 4, 6, 8 and 10 Hz at 15 V and 2 ms), bolus doses of noradrenaline (NA, 25, 50, 100 and 200 ng/kg), phenylephrine (PHE, 0.25, 0.50, 1 and 2 μg/kg), and methoxamine (ME, 1, 2, 3 and 4 μg/kg) were determined before and after bolus doses of 5-methylurapidil (5-MeU) and chlorethylclonidine (CEC), 5 and 10 μg/kg plus 0.125 and 0.25 μg/kg/h respectively. Data, means ± s.e.m, were compared with a Student's 't' test and significance taken of the 5% level. In group A, BP was 154±3 mmHg, RBF 13.7ml/min/kg and blood glucose 247±18 M/dl and in group B, BP was 159±2 mmHg, RBF 13.9±0.7 ml/min/kg and blood glucose 238±25 M/dl.

The results obtained indicated that the renal vasoconstrictor responses in this model were attenuated by 5-MeU but not by CEC. These findings would suggest that α1A-adrenoceptor subtypes primarily mediate adrenergically induced constriction of the renal vasculature

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Table 1. Average % decrease in renal blood flow over RNS and agonist dose ranges (* = P<0.05).

GROUPS	TREATMENT	RNS	NA	PHE	ME
A	Control	31.43 ± 3.10	41.75 ± 4.05	41.32 ± 5.82	48.07 ± 4.89
(n = 8)	5 μg/kg 5-MeU	27.89 ± 2.60	43.07 ± 4.67	31.39 ± 4.35*	28.07 ± 4.35*
	10 μg/kg 5-MeU	31.26 ±3.15	43.14 ± 4.09	26.81 ± 4.46*	26.82 ± 3.62*
B	Control	31.44 ± 3.38	36.73 ± 4.23	33.58 ± 4.99	49.87 ± 4.70
(n = 8)	5 μg/kg CEC	32.65 ± 3.14	43.84 ± 4.27*	45.62 ± 5.45*	59.37 ± 5.40*
	10 μg/kg CEC	37.70 ± 3.50*	43.32 ± 4.29*	45.28 ± 4.43*	62.09 ± 5.39*

186P EFFECTS OF FR 167653 ON THE HAEMODYNAMIC RESPONSES TO LIPOPOLYSACCHARIDE INFUSION IN CONSCIOUS RATS

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One explanation for the lack of any consistent effects of anti-cytokine antibody treatment on the haemodynamic consequences of endotoxaemia (see Deitch, 1998 for review) is that the antibodies do not gain access to the local sites of synthesis and action of the endogenous cytokines. If so, a more successful approach may be to inhibit cytokine production. Yamamoto *et al.* (1996) recently described an inhibitor of TNF-α and IL-1β production (FR167653; 1-[7-(4-fluorophenyl)-1,2,3,4-tetrahydro-8-(4-pyridyl)pyrazolo [5,1-c][1,2,4]triazin-2-yl]-2-phenyl-ethane dione sulphate monohydrate), so we have assessed the effects of FR167653 on the regional haemodynamic responses to LPS infusion in conscious, male Long Evans rats (350-450 g). Animals were chronically instrumented with pulsed Doppler flow probes and intravascular catheters for recording regional haemodynamics. All surgery was performed under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p., supplemented as required), and experiments began 24 h after catheterization. FRI67653 (0.32 mg kg^{-1} h^{-1}; n = 8) or saline (0.4 ml h^{-1}; n = 8) were infused i.v. for 24 h, starting 1 h before co-infusion of LPS (*E.Coli* serotype 0127 B8, Sigma, 150 μg kg^{-1} h^{-1}). There were no differences between the 2 groups under resting conditions or just prior to infusion of LPS (Table 1). Some of the haemodynamic responses to LPS infusion are summarized in Table 2. Pretreatment with FR167653 prevented the LPS-induced early (1 h) and late (24 h) falls in MAP, without substantially influencing the regional vasodilatations. It is feasible that the beneficial effects of FR167653 were due to an improvement in cardiac output, possibly by inhibition TNF-α production in the heart.

These findings contrast with our previous studies, in which anti-cytokine antibody pretreatment failed to influence the hypotensive effects of LPS (Gardiner *et al.*, 1998).

Table 1. Heart rate (HR; beats min^{-1}), mean arterial pressure (MAP; mmHg) and renal (R), mesenteric (M) and hindquarters (H) vascular conductances (VC; (kHz mm Hg^{-1})10^3) under resting conditions, and just prior to LPS infusion in animals treated with saline (Sal; n = 8) or FR167653 (FR; n = 8). Values are mean ± s.e. mean.

	Rest		Pre-LPS	
	Sal	FR	Sal	FR
HR	336 ± 9	329 ± 5	327 ± 7	312 ± 6
MAP	103 ± 1	103 ± 2	102 ± 1	101 ± 2
RVC	80 ± 12	81 ± 4	85 ± 12	82 ± 5
MVC	66 ± 6	74 ± 5	66 ± 6	71 ± 4
HVC	43 ± 4	41 ± 2	42 ± 4	40 ± 2

Table 2. Cardiovascular changes during LPS infusion after pretreatment with Sal or FR. Abbreviations and units in Table 1.

		Time after onset of LPS infusion		
		1 h	8 h	23 h
HR	Sal	+17 ± 8*	+70 ± 10*	+79 ± 8*
	FR	+46 ± 8*†	+78 ± 14*	+58 ± 15*
MAP	Sal	-13 ± 2*	-3 ± 2	-9 ± 2*
	FR	-3 ± 1†	+2 ± 1	-1 ± 4†
RVC	Sal	+13 ± 6*	+41 ± 6*	+36 ± 7*
	FR	+13 ± 7*	+28 ± 6*	+31 ± 8*
MVC	Sal	+13 ± 4*	+9 ± 2*	-1 ± 2
	FR	+4 ± 4	0 ± 5	-1 ± 8
HVC	Sal	+3 ± 3	+7 ± 3*	+18 ± 3*
	FR	+3 ± 2	+5 ± 1*	+18 ± 4*

*P < 0.05 versus baseline (Friedman's test), †P < 0.05 versus Sal (Mann-Whitney U test).

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187P EFFECTS OF GLIBENCLAMIDE ON VASODILATATION DURING ENDOTOXAEMIA IN CONSCIOUS RATS

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In conscious rats, 5 h after the onset of continuous infusion of LPS in the presence of glibenclamide (GB), there is a substantial rise in mean arterial pressure accompanied by mesenteric and, particularly, hindquarters vasoconstriction (Gardiner *et al.*, 1997), but the relative contributions of inhibition of K_{ATP} channels or suppression of inducible nitric oxide synthase (iNOS) expression (Wu *et al.*, 1995) to these effects have not been delineated. In the present work we assessed the response to GB, 6 h after the onset of LPS infusion, i.e., when iNOS activity is maximal (Gardiner *et al.*, 1995), because GB does not influence iNOS activity (Wu *et al.*, 1995) and, hence, any effects of GB should be attributable to antagonism of K_{ATP} channels alone. Since the vasodilatation during LPS infusion is particularly marked when the vasoconstrictor actions of angiotensin II and endothelin are antagonised by losartan and SB 209670, respectively, (Gardiner *et al.*, 1996), we assessed the responses to GB under those conditions. Male, Long Evans rats were instrumented with pulsed Doppler probes and intravascular catheters (all surgery under sodium methohexitone anaesthesia 40-60 mg kg^{-1} i.p., supplemented as required) and were given i.v. losartan (10 mg kg^{-1}) and SB 209670 (300 μg kg^{-1}, 300 μg kg^{-1} h^{-1}) 1 h before the onset of continuous i.v. infusion of saline (Group A; n = 8) or LPS (*E.coli* serotype 0127 B8, Sigma; 150 μg kg^{-1} h^{-1}; Group B; n = 9). Six h later, GB was administered (20 mg kg^{-1} infused i.v. at 4 ml min^{-1} over 4 min) in 1% 2-hydroxypropyl-β-cyclodextrin/saline). Table 1 summarises some of the results. In the presence of losartan, SB 209670 and saline, GB caused a modest rise in blood pressure together with a small renal, and larger hindquarters, vasoconstriction. In the presence of losartan, SB 209670 and LPS, GB caused a pressor effect together with renal, mesenteric and hindquarters vasoconstrictions, and all these effects were greater ($P < 0.05$ Mann-Whitney U test) than in the absence of LPS. These results are consistent with activation of K_{ATP} channels contributing to the vasodilator effects of LPS infusion.

Table 1. Cardiovascular variables in rats treated with losartan and SB209670 (at t = 0 min) followed by saline (Group A) or LPS (Group B) (at t = 60 min) and glibenclamide (at t = 420 min). Values are mean ± s.e. mean; MAP = mean arterial pressure (mm Hg); RVC, MVC, HVC = renal, mesenteric and hindquarters vascular conductance, respectively ([kHz mm Hg^{-1}]10^3). * $P < 0.05$ versus baseline; ° $P < 0.05$ versus 60 min value; † $P < 0.05$ versus 7 h value (Friedman's test).

		Time after treatment with losartan and SB209670			
		0	60	420	460 min
MAP	A	104 ± 1	87 ± 2*	85 ± 2*	95 ± 1*°†
	B	102 ± 1	83 ± 2*	55 ± 2*°	76 ± 3*°†
RVC	A	77 ± 7	110 ± 11*	109 ± 12*	98 ± 11*†
	B	73 ± 7	115 ± 10*	184 ± 18*°	157 ± 19*°†
MVC	A	80 ± 8	100 ± 11*	105 ± 10*	110 ± 8*
	B	67 ± 5	92 ± 9*	239 ± 14*°	193 ± 17*°†
HVC	A	44 ± 4	53 ± 5*	60 ± 6*	40 ± 5°†
	B	44 ± 3	57 ± 5*	86 ± 7*°	47 ± 3°†

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188P INTERINDIVIDUAL VARIABILITY IN BLOOD PRESSURES OF MALE, HETEROZYGOUS ((mRrn-2)27) TRANSGENIC RATS

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Over the last 5 years, we have been breeding the hypertensive transgenic ((m-Ren2)27) (abbreviated to TG) rat, originally described by Mullins *et al.* (1990). During that time we have, incidentally, noticed a relatively high degree of inter-individual variability in the resting blood pressures (BP) of adult, male heterozygous TG rats. As in most laboratories, our breeding schedule involves mating homozygous, male TG rats with normotensive Hannover Sprague Dawley (SD) female rats. For their survival, homozygous TG rats need maintenance antihypertensive treatment and, like Mullins *et al.* (1990), we have achieved this by putting captopril in their drinking water. However, during the mating period this results in the female SD rats being exposed to captopril. Whether or not *in utero* exposure to captopril influences the subsequent development of hypertension in TG rats is unknown. Therefore, in the present study, we have compared resting arterial BPs in age-matched (3-4 months old) male, heterozygous TG rats, bred under conditions in which captopril (50 mg l^{-1}) was present in the drinking water of the parents throughout the period of mating (3-5 days; Group A; n = 31), or when captopril was absent from the drinking water throughout the period of mating (Group B; n = 24).

Intra-arterial and intravenous catheters were implanted under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p. supplemented) and BPs were measured in conscious, unrestrained animals 24h later. Data were collected continuously over a 2 h period (between 07.00 and 09.00 h), using a custom-built haemodynamic processing system (Department of Instrument Services, University of Limburg, Maastricht, Netherlands) which sampled the signals and averaged data from 2 cardiac cycles every 2 s, then stored the information to disk every 10 s (as a mean of 5 measurements). Off-line, the data from each animal, collected over the entire 2 h period, were averaged.

In Group A, mean BP of individual animals ranged from 134 to 212 mm Hg and in Group B, the range was between 139 and 195 mm Hg. There were no differences between the resting BPs of the 2 groups (Table 1), or their variabilities. Moreover, interindividual differences were as great within, as between, litters.

Table 1. Resting BPs (mean ± s.d.) in conscious, male, heterozygous TG rats bred under conditions where captopril was present (Group A; n = 31) or absent (Group B; n = 24) during the period of mating.

	Group A	Group B
Systolic BP (mm Hg)	219 ± 15	219 ± 16
Diastolic BP (mm Hg)	138 ± 14	139 ± 13
Mean BP (mm Hg)	171 ± 15	173 ± 14

After the 2 h period of recording, a sub-group of animals (n = 13; 7 from Group A and 6 from Group B) were given the angiotensin (AT_1)-receptor antagonist, losartan (10 mg kg^{-1} i.v.) and BPs were recorded for a further 2 h starting 5 h later (i.e. between 14.00 and 16.00 h). The fall in mean BP following losartan (-33 ± 10 mm Hg; mean ± s.d.) was not significantly related to the starting BP value (r = 0.535) and not different in the animals from Group A (-28 ± 9 mm Hg) and Group B (-39 ± 9 mm Hg). Hence, one possible explanation for the variation in BP between heterozygous, male TG rats is variation in transgene expression influencing an hypertensinogenic factor other than angiotensin II.

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189P CONTRACTILE EFFECTS OF ENDOTHELIN-1 IN VENTRICULAR CARDIOMYOCYTES ISOLATED FROM NORMAL AND LIPOPOLYSACCHARIDE (LPS)-TREATED RATS

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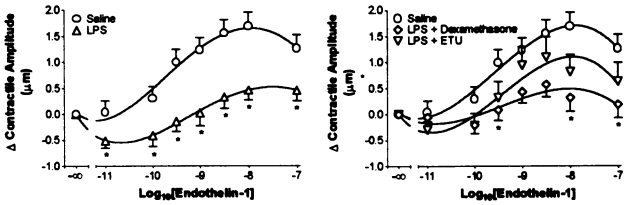
Endothelin-1 (ET-1) is a 21 amino-acid peptide with a potent inotropic effect in the heart. Elevated plasma levels of ET-1 are found in patients suffering from septic shock (Takakuwa *et al.*, 1994). Lipopolysaccharide is an integral part of the outer cell wall of gram negative bacteria, and is widely used to produce an experimental model of septic shock. The aim of the present study was to examine the contractile properties of ventricular cardiomyocytes isolated from normal and lipopolysaccharide treated rats in response to endothelin-1 and to assess the involvement of nitric oxide.

Wistar rats (275-325g, n=54) were injected with either saline or lipopolysaccharide (LPS; 5 mg kg^{-1}, i.p.; >8hr exposure) alone, or following pre-treatment with dexamethasone (5 mg kg^{-1}, i.p.). Ventricular cardiomyocytes were isolated by enzymatic dissociation (Kelso *et al.*, 1995). Cell contractions were recorded using a video edge detection system, and concentration-response relationships (cumulative) were established for ET-1 alone and in the presence of ethyl-isothiourea (ETU; 10 μM) in the LPS treated group. Data are expressed as mean ± s.e. mean. Data were analysed by one-way ANOVA with *post hoc* analysis (Bonferroni). A value of $P<0.05$ indicates statistical significance.

Resting cell length was similar for cardiomyocytes isolated from all groups compared to saline (114.6±1.8 μm) treated animals. Baseline contractile amplitude was reduced ($P<0.05$) in the LPS (7.3±0.2 μm), LPS+dexamethasone (6.7±0.3 μm) and LPS+ETU (6.9±0.3 μm) groups compared with saline (8.0±0.2 μm). The contractile response to ET-1 was attenuated in the LPS group compared to saline at all concentrations studied (Graph; $P<0.05$). Pre-treatment with dexamethasone prior to LPS administration did not prevent attenuation of the contractile response to ET-1 compared to saline. However, ET-1 in the presence ot ETU, in the LPS group, produced a concentration-dependent increase in contractile presponse similar to ET-1 alone in the saline group (Graph)



In summary, *in vivo* administration of LPS reduced the baseline contractile amplitude of isolated ventricular cardiomyocytes. Furthermore, the contractile response to ET-1 was attenuated following LPS administration, but was restored by co-perfusion with the non-selective nitric oxide synthase inhibitor, ethyl-isothiourea, but not by pre-treatment with dexamethasone, an agent which prevents the induction of iNOS.

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190P EFFECT OF BOSENTAN ON MYOGENIC TONE AND α-ADRENERGIC-DEPENDENT CONTRACTION OF RABBIT RESISTANCE ARTERIES

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Myogenic tone (MT), an increase in vascular tone in response to an increase in transmural pressure independent of neural or hormonal influences, is one of the fundamental mechanisms regulating blood perfusion in several vascular beds. Neuromediators, however, are also involved in the regulation of vascular tone. Both pressure- and agonist-induced contractions are modulated by the endothelium. The aim of this study was to investigate if endothelium-derived endothelin-1 (ET) and nitric oxide (NO) contributed similarely to the regulation of pressure- and agonist-induced smooth muscle contraction.

Rabbit mesenteric arteries (external diameter = 150-200μm) were isolated and cannulated, and changes in diameter were recorded as a function of perfusion pressure (PP, 10 - 160 mm Hg) or agonist addition at a PP of 60 mm Hg. All experiments were performed in the presence of indomethacin (10 μmol l^{-1}). At the conclusion of each experiment, passive [in Ca^{2+}-free solution containing sodium nitroprusside (1 μmol l^{-1}) and EGTA (1 mmol l^{-1})] and active (in the presence of 127 mmol l^{-1} KCl) diameters at a PP of 60 mm Hg or during each pressure step (10 - 160 mm Hg) were obtained. Contractile responses are expressed as changes (percentage) in diameter, normalized to the passive and minimum active diameter. In a series of experiments, the endothelium was removed by perfusion of the vessels with air. Experiments were performed after NO formation blockade with N^{ω}-nitro-L-arginine (L-NOARG, 0.1 mmol l^{-1}) or $ET_{A/B}$ receptor antagonism by bosentan (10 μmol l^{-1}). In another series of experiments, L-NOARG and bosentan were combined. Similar experiments were reproduced in the absence of endothelium. Results are given as mean±s.e.m. n represents the number of rabbits.

At a PP of 60 mm Hg, MT developed to represent 17±1% of the minimal diameter (n=23). The magnitude of the MT was increased by 140% ($P<0.05$) by inhibition of NO production with L-NOARG (n=20). Bosentan decreased ($P<0.05$) MT either alone (n=20) or in combination (n=21) with L-NOARG by ≈30%. Removal of the endothelium (n=25) decreased ($P<0.05$) MT (9±1%) to a similar level than in the presence of an intact endothelium and bosentan. Furthermore, neither L-NOARG nor bosentan affected the myogenic response of de-endothelialized vessels. Phenylephrine (PE), an α_1-adrenergic receptor (AR) agonist, induced contraction; the sensitivity to PE (pD_2, 6.2±0.2; n=11) was unaffected by bosentan alone (n=11) but increased ($P<0.05$, n=10) by L-NOARG (pD_2, 8.3±0.8). Addition of bosentan in the presence of L-NOARG (n=11) restored the sensitivity to its control value. Furthermore, endothelial denudation (n=13) decreased ($P<0.05$) PE sensitivity. Oxymetazoline (OXY), an α_2-AR agonist, induced contraction; the sensitivity to OXY (pD_2, 7.7±0.2; n=12) was unaffected by L-NOARG (n=10), bosentan, either alone (n=10) or combined with L-NOARG (n=10), and endothelial removal (n=12).

In conclusion, our results indicate that pressure-induced myogenic tone is independently regulated by endothelium-derived NO and ET since ET receptor antagonisms had a similar relaxant effect in the presence or in the absence of normal background NO. In contrast, α_1-AR stimulation-induced tone is potentiated by endothelium-derived ET in the absence of NO, whereas occupation of α_2-AR mediates a contraction that is unregulated by the endothelium. This highlights the complexity of endothelium-dependent regulation of vascular tone.

191P ENDOTHELIN-1-INDUCED SUPEROXIDE ANION PRODUCTION BY GUINEA-PIG CORONARY MICROVASCULAR ENDOTHELIAL CELLS: A ROLE IN LEFT VENTRICULAR HYPERTROPHY?

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Raised plasma levels of angiotensin II (Ang II) in left ventricular hypertrophy (LVH) are associated with increased NAD(P)H oxidase-mediated superoxide anion (O_2^-) production by coronary microvascular endothelial cells (CMVE) (Mosfer *et al.*, 1998). Ang II has also been shown to modulate the release of endothelin-1 (Et-1) from endothelial cells (Kubo *et al.*, 1998). In the present study we measured plasma levels of Et-1 (by radioimmunoassay, Penninsula Laboratories, UK) 6 weeks post-banding (band internal diameter 0.5mm) in a male Dunkin Hartley guinea pig (weight at banding 200-250g), supra-renal aortic-banded, pressure overload model of LVH (method as used by Linz & Scholkens (1992) for rats). Subsequently, CMVE (isolated from normal guinea pigs (350-400g) as described by Piper *et al.*, 1990 for rats) were cultured for 14 days (including one passage). Lucigenin-chemiluminescence was used to measure Et-1-induced changes in NADH/NADPH (both 1mM)-dependent O_2^- production in the membrane fraction of a CMVE lysate (method as used by Greindling *et al.*, 1990 for vascular smooth muscle cells). The integral for the first 10min of the reaction represents the total O_2^- produced over this time, and was normalised to lysate protein content. All data are expressed as mean ± standard error of the mean ($n \geq 8$), compared using one way analysis of variance followed by Student-Newman-Keuls test, and considered significant when $p<0.05$. Plasma Et-1 levels were unaltered in the sham-operated animals compared to normals (21.88±2.46 cf. 25.44±1.43 pg/ml respectively), but were significantly ($p<0.05$) elevated in the aortic banded animals (31.73±1.91 pg/ml). In control, CMVE NADH and NADPH oxidase activity was 1164.68±88.29 & 270.87±33.12 V.s/mg protein respectively. NADH oxidase activity was significantly ($p<0.05$) increased (1643.72±165.79 V.s/mg protein) by incubation with Et-1 (1nM for 9 hrs), but NADPH oxidase activity (309.98±40.17 V.s/mg protein) was unaltered. This Et-1 induced increase in NADH oxidase activity was significantly ($p<0.05$) inhibited (1226.26±75.70 V.s/mg protein) by the Et-1 antagonist BQ123 (1μM for 9 hours). These data demonstrate that the development of LVH in response to pressure overload is associated with increased plasma levels of Et-1, and that Et-1 may contribute to the overproduction of O_2^- associated with this condition.

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192P ANGIOTENSIN-INDUCED ENDOTHELIN EXPRESSION IN ISOLATED RAT AORTA, FUNCTIONAL CONSEQUENCE AND INHIBITION BY CALCIUM ANTAGONIST

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Angiotensin II (AII) appears to play a major role in tissue remodeling occurring in hypertension (Kim et al., 1996). At least part of this action may result from activation of the expression of endothelin-1 (ET1) gene (Lüscher & Lerman, 1998). It has been reported that AII potentiates noradrenaline-evoked contraction in perfused mesenteric bed by a mechanism involving ET1 overexpression (Dohi et al. 1992). The purpose of the present experiments was to examine if those AII effects could be reproduced in isolated rat aorta and if they could be altered by a calcium antagonist. We have used lacidipine, a long-acting calcium channel blocker, which prevents tissue remodeling and ET-1 overexpression in salt-loaded SHR-SP (Kyselovic et al., 1998).

Aortae were isolated from SHR 12 week old and were bathed at 37° in phosphate-free Krebs solution containing LNNA (10^{-4} M) and indomethacin (10^{-5} M). Dose-effect curves to noradrenaline were performed by measuring the contractile response of rings, before and after 4 hours incubation with or without AII (10^{-7} M). Paired control and treated preparations were isolated from a same animal. Because initial dose-effect curves were superimposed, comparisons were made on EC_{50} obtained after 4 hours incubation. In parallel experiments, total RNA was extracted from pools of 3 aortae and subjected to Northern blot analysis. Prepro-ET1 mRNA levels were expressed relative to GAPDH mRNA (Kyselovic et al.,1998)

Noradrenaline EC_{50} values (nM) were equal to 7.9 ± 1.30 in controls (n=12) and to 4.0 ± 0.61 in aortae (n=12) exposed to AII ($P<0.05$). This twofold shift to the left was not observed when AII was added in the presence of the $ET_{A/B}$ receptor antagonist bosentan (10^{-5} M). When preparations were exposed to lacidipine 10^{-8} M, EC_{50} values were respectively equal to 46.0 ± 7.48 (n=7) and to 39.9 ± 5.41 (n=7), without and with AII. This difference was not significant.

In four experiments, comparing controls and AII treated vessels, Northern blot analysis of mRNA showed a 25 ± 3% AII-dependent increase in the abundance of prepro-ET1 mRNA. This AII-evoked increase was blocked when AII was added in the presence of lacidipine 10^{-8} M ($P<0.05$).

Those observations show that lacidipine prevented both the bosentan-sensitive potentiation of the noradrenaline response evoked by AII in rat aorta and the AII-related increase in prepro-ET1 mRNA abundance in the vessel wall. The former action may be related to the interaction of lacidipine with L-type Ca Channels, the activity of which is increased by ET-1 (Godfraind et al. 1989). The mechanism of the latter action needs still to be investigated.

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193P INHIBITION OF BIG ENDOTHELIN-1 (BIG ET-1) RESPONSES IN ENDOTHELIUM-DENUDED HUMAN CORONARY ARTERY BY THE SELECTIVE ENDOTHELIN-CONVERTING ENZYME-1 (ECE-1) INHIBITOR PD159790

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Increased production of the potent vasoconstrictor peptide endothelin-1 (ET-1) may contribute to the development of coronary artery disease. Isoforms of the endothelin-converting enzyme (ECE), which catalyses the synthesis of ET-1 from its precursor big ET-1, have been localised to human coronary artery endothelial cells (Russell, *et al.*, 1998). ECE activity persists in isolated blood vessels following endothelium removal. We have reported that this non-endothelial ECE activity is up-regulated in human coronary artery disease (Maguire & Davenport, 1998). PD159790 is a newly described ECE-1 inhibitor (Ahn *et al.*, 1998) which, unlike phosphoramidon, has no inhibitory effect against neutral endopeptidase 24.11. We have therefore investigated the ability of this compound to block big ET-1 responses in endothelium-denuded human coronary artery *in vitro*.

Coronary arteries were obtained from 8 patients (6 male, 2 female; 24-50 years) undergoing cardiac transplantation. Rings of human coronary artery were denuded of their endothelium and set up for isometric tension recordings in 5ml baths containing oxygenated Krebs solution (37°C). Cumulative concentration-response curves were constructed to big ET-1 (10^{-9}-$7x10^{-7}$M) in the absence and presence of PD159790 (10-100μM) which was added 30 minutes earlier. Experiments were terminated by the addition of 50mM KCl to determine the maximum contractile response for each preparation and big ET-1 responses were expressed as a percentage of this.

PD159790 shifted concentration-response curves to big ET-1 to the right in a concentration-dependent manner (Figure 1). There was no effect of PD159790 on responses to ET-1 (data not shown).

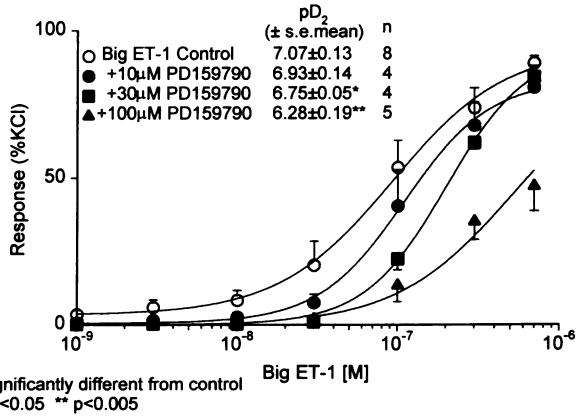

Figure 1. Inhibition of Big ET-1 by PD159790

PD159790 is one of the first inhibitors developed which is highly selective for ECE-1. We have demonstrated that this compound will block big ET-1 responses in endothelium-denuded human coronary artery implying the presence of an ECE-1 like enzyme on human vascular smooth muscle cells. Inhibitors of this ECE, which is up-regulated in human coronary artery disease, may therefore have novel therapeutic potential in man.

We thank the Consultant and theatre staff of Papworth Hospital. Supported by grants from the British Heart Foundation.

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194P EFFECT OF MAST CELL DEGRANULATION ON A_3-RECEPTOR-MEDIATED PROTECTION FROM HYPOXIA-INDUCED MYOCARDIAL STUNNING

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An A_3-adenosine receptor agonist, IB-MECA (N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide), protects against hypoxia-induced myocardial stunning (Gardner & Broadley, 1997). Activation of the A_3-adenosine receptor causes release of mast cell mediators (Hannon *et al*, 1995) and partial depletion of mast cell mediators by A_3-receptor activation may protect the heart from damage by a full-scale release of mediators during hypoxia or ischaemia (Linden, 1994). This study examined the effect of mast cell degranulation on hypoxia-induced myocardial stunning in guinea-pig isolated left atria and papillary muscles and whether A_3-receptor activation was still cardioprotective.

Left atria and left ventricular papillary muscles from male Dunkin-Hartley guinea-pigs (250-300g) were set up in Krebs-bicarbonate solution at 37±0.5°C gassed with 5% CO_2 in O_2. Tissues were paced at 2Hz with threshold voltage + 50%. Resting tensions of 0.5-1.0g were applied and isometric tension recorded. Differences between means±SEM ($n \geq 4$) were taken to be significant if $p<0.05$ from a paired or unpaired t-test.

After normoxic equilibration (pO_2 560-620 mmHg), hypoxia (pO_2 50-60 mmHg) was induced by switching from gassing with 5% CO_2 in oxygen to 5% CO_2 in nitrogen. Hypoxia was maintained for 30 minutes before reoxygenation 60 min with 5% CO_2 in oxygen. Tissues were paced throughout. Hypoxia caused a fall in developed tension to 13.1±1.8 and 13.2±2.6% of the pre-hypoxic level in left atria and papillary muscles, respectively. On reoxygenation, the atrial developed tension returned to 79.5±5.1, 68.1±1.5 and 75.5±3.8 at 5, 10 and 60 min after reoxgenation, respectively (i.e. biphasic recovery). Papillary muscles recovered to 52.6±7.6, 54.2±6.0 and 71.2±4.8%, respectively. These were significantly below the pre-hypoxia resting levels, indicating myocardial stunning.

IB-MECA ($3x10^{-8}$M) added at reoxygenation, significantly improved recovery of atria and papillary muscles from hypoxia. The developed tensions at 5 and 60 min post-reoxygenation were 91.7±5.0 and 93.7±2.4% in atria and 80.6±2.6 and 95.6±7.0% in papillary muscles.

The mast cell degranulator, compound 48/80 (0.1mg/ml), added to the bath for 5min after a 10min normoxic equilibration caused positive inotropy in both left atria (135.5±18.1% increase in developed tension, n=8) and papillary muscles (177.9±31.4%). After washout, compound 48/80 (0.1mg/ml) was added again and the positive inotropy was significantly (paired t-test) reduced in both left atria (47.9±8.3%) and papillary muscles (33.1± 7.9%). After 5 minutes this was also washed from the bath. Compound 48/80 did not significantly affect the recovery from hypoxia in either left atria or papillary muscles compared to controls. The developed tensions at 5, 10 and 60 min post-reoxgenation were 78.6±5.7, 65.6±7.9 and 82.4±5.9% in left atria and 57.5±4.7, 66.0±2.2 and 74.4±5.0% in papillary muscles. Compound 48/80 did not significantly affect the improvement in recovery from hypoxia seen with IB-MECA. The developed tensions at 5, 10 and 60 min were 101.4±7.0, 81.4±3.9 and 103.5±6.3% in atria and 76.3±7.4, 82.1±8.3 and 94.6±3.2% in papillary muscles, compared to the compound 48/80 control values.

It therefore appears that mast cell degranulation products are not involved in hypoxia-induced myocardial stunning and do not mediate the cardioprotective effects of the A_3-receptor agonist, IB-MECA.

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195P CHARACTERISATION OF RECEPTORS MEDIATING VASOMOTOR EFFECTS OF ADENOSINE ANALOGUES IN HAMSTER CHEEK POUCH

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The hamster cheek pouch preparation was used to investigate the vasomotor effects of adenosine receptor agonists and antagonists. Male golden (Syrian) hamsters were anaesthetised with sodium pentobarbitone (50mg kg^{-1} i.v., maintained with 15mg kg^{-1} i.v.). The cheek pouch was prepared as described by Hall & Brain (1994). All drugs were applied topically to the cheek pouch by superfusion and concentration-response curves were obtained cumulatively. Vasomotor effects of adenosine agonists on arterioles (20-40μM) were expressed as % change in basal vessel diameter.

Very low doses (3pM-1nM) of NECA and low doses (1-10nM) of CPA, IBMECA, 2CADO and adenosine constricted the arterioles. CGS21680 was never observed to induce vasoconstriction. Higher agonist concentrations (10nM-1μM) induced vasodilatation and the agonist potency order (with EC_{20} values, n = 4-11) for the dilatation was NECA (0.003μM) > CPA (0.1μM) = 2CADO (0.1μM) = CGS21680 (0.1μM) > IBMECA (0.2μM) > adenosine (2.5μM). EC_{20} values were calculated because, within the concentration range tested, adenosine only achieved a 20% change in basal diameter and CGS21680 plateaued at 25% dilatation. The dilator response to CPA was variable, with some tissues constricting to CPA even at higher concentrations. The EC_{20} value was calculated from the tissues that constricted at lower doses of CPA and dilated to higher concentrations of the agonist.

8-SPT (50μM, n=4) abolished, or markedly reduced, constrictor responses to NECA and 2CADO but appeared to have no effect on constrictions to CPA. 8-SPT (50μM) also antagonized the vasodilatation to NECA with an apparent pK_B of 5.3. 8-SPT (50μM) also appeared to antagonize the dilator response to higher concentrations of CPA in those tissues that dilated to this agonist. 8-SPT (50μM, n = 4) however did not antagonize dilator responses to either 2CADO or IBMECA, and in fact responses to IBMECA appeared to be potentiated in the presence of 8-SPT.

Vasoconstrictor responses to NECA were blocked by DPCPX (1nM, n = 3) but dilator responses to NECA were unaffected suggesting that A_1 mediated responses do not compromise the dilator responses measured. In the presence of DPCPX (1nM), ZM241385 (10nM) antagonized vasodilator responses to NECA giving an apparent pK_B of 8.8 (n = 3).

Overall these results suggest that NECA dilates hamster cheek pouch arterioles via an A_{2A} adenosine receptor. 8-SPT blocks constrictions to NECA and 2CADO and DPCPX (1nM) also inhibits constrictions to NECA, which suggests that vasoconstriction induced by these agonists may by via A_1 adenosine receptors. 8-SPT (50μM) however did not antagonize dilatations to 2CADO and IBMECA which suggests that they may be acting via an antagonist-resistant site as seen in other larger blood vessels such as the hamster aorta (Prentice & Hourani, This Meeting).

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196P DIFFERENT EFFECTS OF ADENINE NUCLEOTIDE ANALOGUES ON THE RESPONSES MEDIATED BY ADENOSINE 5'DIPHOSPHATE (ADP) RECEPTORS ON HUMAN PLATELETS

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Adenosine 5'-diphosphate (ADP) induces human blood platelets to change shape and aggregate, and it has been suggested that these two responses are mediated by separate ADP receptors. The structure-activity relationships for a number of analogues of adenine nucleotides in causing aggregation and shape change were measured and compared. ADP and its analogues, 2-methylthio-ADP, α,β-methylene-ADP, Sp-ADPαS and ADPβS were used as agonists and the $p[A]_{50}$ values were calculated. ATP (100μM) and its analogues, Ap_5A (100μM), α,β-methylene-ATP (100μM), and 2-methylthio-ATP (0.1-100μM) and UTP (100μM) were used as antagonists, and the apparent pK_B values were calculated from the shifts in the concentration-response curves.

The $p[A]_{50}$ value of ADP for aggregation was 6.0 and the $p[A]_{50}$ value of ADP for shape change was 6.1. In general, the structure-activity relationships for both responses were similar, but three compounds did show differences (Table 1). Sp-ADPαS and ADPβS were much more potent agonists relative to ADP for shape change than for aggregation. For shape change ADPβS ($p[A]_{50}$=5.2) was 6-fold less potent than ADP, whereas it was only weakly active in causing aggregation, achieving at 100μM only 20% of the maximum aggregation induced by ADP. Sp-ADPαS also showed much higher potency as an agonist for shape change than for aggregation, and in fact acted as an antagonist to ADP for aggregation. In addition, 2-methylthio-ATP was a better antagonist of aggregation than of shape change. For aggregation, the pK_B value for 2-methylthio-ATP (1μM) was 7.0, and 10 or 100μM 2-methylthio-ATP almost abolished responses to ADP (100μM). For shape change however, 2-methylthio-ATP was inactive as an antagonist at 1 and 10μM, and at 100μM a pK_B value of 5.2 was obtained. These results support the existence of more than one type of ADP receptor or pathway mediating these two responses in human platelets.

Table 1. Effects of analogues of adenine nucleotides on human platelet responses. Each value is the mean and s.e.mean of at least 3 determinations.

Compound	Platelet Responses	
	Aggregation	Shape change
Agonists ($p[A]_{50}$)		
ADP	6.0±0.1	6.1±0.2
2-methylthio-ADP	7.0±0.4	7.2±0.2
α,β-methylene-ADP	< 4 [1]	< 4 [1]
ADPβS	< 4 [1]	5.2±0.1
Sp-ADPαS	< 4 [1]	5.9±0.2
Antagonists (apparent pK_B)		
Sp-ADPαS	5.4±0.1	-----
ATP	4.9±0.1	4.6±0.1
Ap_5A	4.8±0.2	4.6±0.1
α,β-methylene-ATP	3.5±0.1 [2]	3.7±0.1 [2]
UTP	3.4±0.4 [2]	3.3±0.2 [2]
2-methylthio-ATP	7.0±0.2	5.2±0.2

[1] very weak agonist - response at 100μM less than 30% of the response to ADP.

[2] very weak antagonist - concentration ratio at 100μM less than 2.

197P CHARACTERISATION OF ADENOSINE RECEPTORS MEDIATING RELAXATION IN HAMSTER ISOLATED AORTA

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The aim of this study was to characterise the adenosine receptor(s) mediating relaxations in the hamster isolated aorta, to complement work *in vivo* in the hamster cheek pouch (Nicholls, Hourani & Hall, This Meeting). Cumulative relaxant curves were obtained in rings contracted with 1μM phenylephrine. Where possible $p[A_{50}]$ values were estimated by logistic curve fitting. For curves which did not reach a plateau, pIC_x values were estimated by regression of the linear portion of the curve, where x approximates to half of the maximum % relaxation of the phenylephrine contraction attained for each agonist.

Adenosine relaxed the aorta but relaxations were not blocked by 8-sulphophenyltheophylline (8-SPT, 50μM, pIC_{20} control = 3.74±0.38; + 8-SPT = 3.78±0.13, n=4&6). However, in the presence of the uptake inhibitor nitrobenzylthioinosine (NBTI,1μM), curves to adenosine were left-shifted ($p[A_{50}]$ + NBTI = 6.14±0.04) and an apparent pK_B for 8-SPT of 5.79±0.05 could be obtained (n=4&6). NECA (5'-N-ethyl-carboxamidoadenosine) also relaxed the aorta but curves were biphasic. The first phase of the curve was blocked by 8-SPT (pA_2 = 5.75±0.14, n=4) and the A_{2A} selective antagonist ZM 241385 (4-(2-[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-a] [1,3,5] triazin-5-ylamino] ethyl)-phenol, pK_B = 9.17±0.10, n=3&4). Likewise, the A_{2A} selective agonist CGS 21680 (2-p-[(2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamido-adenosine) relaxed the tissues but curves were biphasic and the first phase was again blocked by ZM 241385 ($p[A_{50}]$ control = 6.42±0.39; + 10nM ZM 241385 = 5.33±0.24, apparent pK_B = 9.06±0.34, n=5&6). The potency of CGS 21680 and the affinity estimates obtained for ZM 241385 are consistent with the presence of A_{2A} (Poucher *et al.*, 1995). However relaxations to R-PIA (N^6-R-phenylisopropyl-adenosine), CPA (N^6-cyclopentyladenosine), 2-CADO (2-chloroadenosine) and IB-MECA (N^6-(3-iodobenzyl)adenosine-5'-N-methyluron-amide) were not blocked by 8-SPT (50μM, n=3-7). The agonist potency order for the 8-SPT-resistant responses including NECA (in the presence of 1μM ZM 241385) is as follows: IB-MECA (pIC_{50}=4.93±0.14) > 2-CADO (pIC_{50}=4.65±0.10) ≥ R-PIA (pIC_{50}=4.40±0.13) > CPA (pIC_{30}=4.05±0.28) = NECA (pIC_{30}=3.95±0.25) ≥ adenosine (pIC_{20}=3.74±0.38). This potency order suggests involvement of A_3 receptors, however responses to IB-MECA were not blocked by the A_3 receptor antagonist MRS1191 (3-ethyl 5-benzyl2-methyl6-phenyl-4-phenylethynyl-1,4-dihydropyridine -3,5-dicarboxylate, 1μM; Jacobson et al., 1997, n=4&5).

Overall, the data suggest the presence of A_{2A} receptors activated by NECA and adenosine (in the presence of uptake blockade) and another as yet undefined site resistant to blockade by 8-SPT, which is activated by adenosine (in the absence of uptake inhibitor), CPA, R-PIA, 2-CADO, IB-MECA and high concentrations of NECA.

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198P TRANSFORMING GROWTH FACTOR β1 LIMITS INFARCT SIZE WHEN ADMINISTERED PRIOR TO REPERFUSION IN AN ISOLATED RAT HEART MODEL

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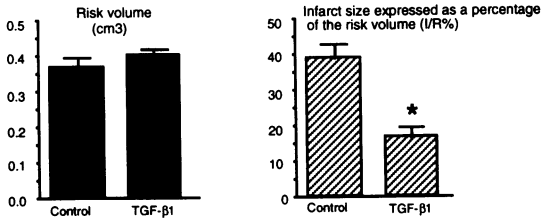
There is evidence that various endogenous growth factors may be cardioprotective in the setting of myocardial ischaemia-reperfusion injury. Transforming growth factor-β1 (TGF-β1) has been reported to limit myocardial ischaemia-reperfusion injury (Lefer *et al.*, 1990) but the mechanism of protection is unknown. Previously, we reported that TGF-β1 limited infarct size in the rat isolated heart when administered prior to the onset of myocardial ischaemia (Mocanu et al., 1998). This protective action appeared to be mediated by p38 MAP kinase signalling. In the present study we have examined the ability of TGF-β1 administration to limit reperfusion injury.

Male Sprague-Dawley rats (300-350 g) were deeply anaesthetised with pentobarbitone sodium (50 mg/kg i.p.). The hearts were excised and Langendorff perfused with Krebs-Henseleit buffer at constant pressure (80 mm Hg). All hearts were subjected to 35 min left coronary artery occlusion followed by 120 min reperfusion. The risk zone size was delineated using fluorescent microspheres and infarcted tissue was determined with triphenyltetrazolium staining. Computerised planimetry was used to quantitate these volumes and the percentage infarction within the risk zone (I/R %) was calculated.

Hearts were randomised into 2 experimental groups. Group 1 (n=8) was control hearts which underwent coronary occlusion but received no drug intervention. Group 2 (n=8) hearts were perfused with TFG-β1 0.2 ng/ml for 35 min beginning 5 min before reperfusion.

There were no significant differences between groups concerning heart rate, left ventricular developed pressure and coronary flow throughout the time course of the experiments.



Results are expressed as mean ± s.e.mean.
* = $p < 0.01$ (Student's unpaired t test)

Administration of TGF-β1 immediately prior to reperfusion significantly limited infarct size. This beneficial effect is independent of blood-borne, humoral or neuronal interventions, suggesting a direct cytoprotective action on myocardium. We speculate that the ability of TGF-β1 to limit infarct size may be associated with attenuation of apoptosis during early reperfusion.

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199P MECHANISMS UNDERLYING LEUCOCYTE ADHESION FOLLOWING VASCULAR INJURY AND THE RELEVANCE OF LEUKOCYTES IN NEOINTIMA FORMATION IN A RABBIT MODEL OF RESTENOSIS

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Previous work in our laboratory has demonstrated that leukocyte adhesion to balloon injured rabbit subclavian arteries is enhanced 24 and 48 hours after injury (Kennedy *et al*, 1997) and that this enhanced adhesion may be related to increased expression of adhesion molecules on the injured vessel (Kennedy *et al*, 1998). Vascular injury generates a range of cytokines (Ross, 1993) which may result in the upregulation of the adhesion molecules. Leukocytes could then adhere and migrate into the vessel wall where they may contribute to neointima formation. The aim of the current study was to determine the effect of the cytokines TNFα and IL-1β on leukocyte adhesion and to assess the effect of leukocyte depletion on neointima formation in balloon injured rabbit left subclavian artery.

Rabbit isolated subclavian artery rings were incubated with either TNFα or IL-1β (0.01-10ng/ml) for 4 hours. After washing, each artery was opened longitudinally and incubated with 5μl of ^{51}Cr-labelled leukocytes for 30 minutes at 37°C, washed and gamma counted. Leukocyte adherence was calculated as % of total leukocytes added. This experiment was repeated using a fixed concentration of 1ng/ml but different times of incubation (0.25-4 hours). Groups were compared using one-way ANOVA followed by Dunnett's Multiple Range Test. To evaluate the importance of leukocytes in neointima formation, angioplasty of the left subclavian artery was performed under halothane/nitrous oxide anaesthesia (Hadoke *et al*, 1995) in leukopenic rabbits. Leukopenia was induced by mustine hydrochloride (1.7mg/kg) i.v. 4 days before angioplasty. Vessel areas (mm^2) were assessed by planimetry 28 days after angioplasty. Groups were compared using one-way ANOVA followed by a Tukey Test.

Pre-treatment of isolated rings of rabbit subclavian artery *in vitro* with either TNFα or IL-1β enhanced the adhesion of leukocytes to the artery in both a concentration and time dependent manner. For TNFα, significance was reached at 10ng/ml (% leukocyte adhesion was 47±4 in treated artery vs. 21±5 in the control artery, n=6; $p<0.01$) and at 0.5 hours (% leukocyte adhesion was 28±3 in treated artery vs. 18±3 in the control artery, n=6; $p<0.05$). For IL-1β, significance was reached at 1ng/ml (% leukocyte adhesion was 38±4 in treated artery vs. 21±5 in the control artery, n=6; $p<0.05$) and at 0.5 hours (% leukocyte adhesion was 29±2 in treated artery vs. 18±3 in the control artery, n=6; $p<0.05$).

The *in vivo* data demonstrated that leukopenia decreases neointima formation in injured arteries (neointimal area was 1144±411mm^2 in mustine-treated arteries, n=5, vs. 4800±388mm^2 in control arteries, n=6; $p<0.001$). No difference was seen in the areas of adventitia or media between control and mustine-treated arteries.

In conclusion, TNFα and IL-1β are capable of enhancing leukocyte adhesion to isolated blood vessels. This effect may be important in the events that occur after vascular injury. The reduction in neointima in leukopenic rabbits suggests a role for leukocytes in restenosis.

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200P CASPASE INHIBITORS IMPROVE POST-ISCHAEMIC FUNCTIONAL RECOVERY IN RAT ISOLATED WORKING HEARTS

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Apoptosis has been demonstrated to contribute to myocardial cell death as a result of reperfusion injury (Fliss & Gattinger, 1996). However, the underlying signalling events are largely unknown. In this study, we assessed the effects of specific inhibitors of interleukin-1β-converting enzyme-like proteases (caspase 1), Ac-Tyr-Val-Ala-Asp-CHO (Ac-YVAD-CHO), and CPP32-like proteases (caspase 3), Ac-Asp-Glu-Val-Asp-CHO (Ac-DEVD-CHO), on postischaemic functional recovery after low flow ischaemia and reperfusion in isolated rat working hearts.

Male Wistar-Kyoto rats were anaesthetised with pentobarbital (60 $mg \cdot kg^{-1}$ i.p.) and ventilated with room air via a PE tube inserted into the trachea. After the chests were opened, hearts were quickly removed and attached by the aorta to a perfusion apparatus (Hugo Sachs Elektronik, Germany). Hearts were temporarily retrogradely perfused with oxygenised Krebs-Henseleit solution. The pulmonary artery was cannulated for determination of coronary flow (CF) and the left atrium for anterograde perfusion. After an equilibration period of 15 min, the retrograde perfusion of the hearts was switched to an anterograde perfusion (working heart mode) using a preload of 11 mmHg and an afterload pressure of 51 mmHg. Hearts were paced at a frequency of 5 Hz. Global low flow ischaemia was induced by reducing CF to 10%, maintained for 30 min, followed by 30 min of reperfusion. Hearts were either perfused with Ac-YVAD-CHO (1 μM; n=8), Ac-DEVD-CHO (1 μM; n=10) or their vehicle (DMSO 0.01%; n=15), starting 15 min prior to ischaemia and continued throughout the experiment. Left ventricular developing pressure (LVDP), dP/dt_{max} (maximal rate of pressure increase), aortic flow (AF), coronary flow (CF), cardiac output (CO) and external heart power (EHP) were determined in the working heart mode after 15 min of equilibration and at the end of the experiment. From these data the recovery percentage were calculated. Data are expressed as mean±s.e.m. *$P<0.05$ vs. vehicle, unpaired Student's t test.

Administration of Ac-YVAD-CHO, Ac-DEVD-CHO or the vehicle had no effect on baseline values. In the vehicle-treated hearts, the recovery percentages for LVDP (38±10%), dP/dt_{max} (40±11%), AF (21±8%), CF (30±9%), CO (30±9%) and EHP (21±8%) after 30 min of ischaemia and reperfusion were significantly depressed. In contrast, treatment of hearts with Ac-YVAD-CHO or Ac-DEVD-CHO significantly increased the recovery for LVDP (87±4% and 86±4%), dP/dt_{max} (89±4% and 88±2%), AF (53±5% and 62±6%), CF (75±4% and 81±3%), CO (60±4% and 67±5%) and EHP (55±7% and 64±6%) (all $p<0.05$), respectively.

These results demonstrate that Ac-YVAD-CHO or Ac-DEVD-CHO significantly improve functional recovery after low flow ischaemia and reperfusion in isolated rat working hearts. Thus, inhibition of caspase-1 and/or caspase-3 activity might be useful in myocardial ischaemia and reperfusion injury.

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201P INSULIN MODIFIES MYOCYTE APOPTOSIS AND REDUCES MYOCARDIAL INFARCT SIZE WHEN ADMINISTERED AT REPERFUSION: A NOVEL MECHANISM OF PROTECTION

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Reperfusion of ischaemic myocardium is essential for tissue salvage, but can paradoxically contribute to cell death (reperfusion injury). Previous attempts to reduce reperfusion injury by administration of pharmacological protective agents at the onset of reperfusion have provided conflicting results. Recently, it has been demonstrated that administration of a "metabolic cocktail" comprising glucose, insulin and potassium (GIK) during the post-ischaemic reperfusion period can be cardioprotective in *in vivo* rat hearts (Jonassen *et al.*, 1996). Although the majority of cell death during myocardial ischaemia-reperfusion results from cellular necrosis, we would hypothesis that programmed cell death (apoptosis) may in part contribute to overall myocyte death during the reperfusion period. An anti-apoptotic effect of insulin has been recently reported in cultured neurones. In this study, we investigated the potential cardioprotective role of insulin given at the moment of reperfusion in isolated rat hearts. We also describe experiments in isolated rat cardiomyocytes, investigating the possible contribution of the anti-apoptotic effect of insulin to its cardioprotective action.

Hearts from male Wistar rats, anaesthetised with Sodium Pentobarbital (50 mg/kg i.p.), were retrogradely perfused with Krebs-Heinseleit buffer on a Langendorff apparatus. Following 20 min of stabilisation, all hearts underwent 35 min regional ischaemia followed by 120 min reperfusion. Infarct size (I) was determined using tetrazolium staining, the risk zone (R) with fluorescent microspheres and the % I/R ratio was calculated. Insulin (0.3 mU/ml) was added to the perfusate at the start of reperfusion. The effect of tyrosine kinase (TK) and phosphatidyl-inositol-3-kinase (PI3-kinase) blockade was assessed using Lavendustin A (Lav) (0.1μM) and Wortmannin (Wort) (1μM), respectively. These inhibitors were infused for 30 min (from 30 min of ischaemia until 25 min of reperfusion). In a separate group of experiments the anti-apoptotic effects of insulin, when added at the moment of reoxygenation, was evaluated using cultured neonatal rat myocytes exposed to 6 h hypoxia followed by 2 h reoxygenation using the dUPT nick-end labelling assay (TUNEL) for DNA fragmentation.

Table 1. Infarct size and apoptotic data.

	Isolated Hearts		Isolated myocytes
GROUP	n	% I/R	% apoptotic cells (TUNEL)
Control	7	46.2±2.5	49.2±3.1
Ins at reperf	9	23.3±2.8*	28.5±1.8*
Lav alone	6	50.3±3.1	
Ins + Lav	6	50.3±6.5	
Wort alone	5	51.1±5.5	
Ins + Wort	6	50.9±4.6	

Mean ± s.e.m. * $P < 0.05$ vs control group; (1-way ANOVA)
Ins at reperf=insulin given at reperfusion. Ins=insulin.

Reperfusion treatment with insulin resulted in a significant reduction in infarct size compared to controls. The protective effect of insulin during reperfusion was abolished by either Lav or Wort, implying a role for both TK and PI3-kinase in mediating this protection. The percentage of apoptotic cells was also significantly reduced in the insulin group compared to untreated cells. These data strongly suggest that the cardioprotective effect of insulin during reperfusion may be due to attenuation of ischaemia//reperfusion-induced apoptosis, and may be mediated via a tyrosine kinase and PI3-kinase activated signalling pathway. In addition we speculate that insulin, as a growth factor, may protect the myocardium by attenuation of apoptosis during early reperfusion.

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202P ACTIONS OF ARACHIDONIC ACID ON CONTRACTION AND SPONTANEOUS ACTIVITY IN GUINEA-PIG ISOLATED VENTRICULAR MYOCYTES

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Recent evidence suggests that arachidonic acid (AA), and/or its metabolites may influence cardiac excitation-contraction coupling (Hoffmann et al. 1995). The aim of the present study was to investigate actions of AA on contraction and spontaneous activity in guinea-pig isolated ventricular myocytes.

Myocytes were isolated from guiea-pig ventricle and superfused with a balanced salt solution containing 2.5 mM calcium. Contractions were monitored using an edge-detection system applied to the video image of cells viewed via a microscope. L-type calcium currents (ICa_L) were activated by depolarisations from -40mV to 0mV for 200ms (switched voltage clamp). Calcium transients were constructed from the magnitude of calcium-activated tail currents following interruption of action potentials by voltage clamp to -70mV (Terrar & White 1989). In one series of experiments, spontaneous activity was induced by application of 40 nM isoprenaline. Spontaneous activity was quantified as the number of spontaneous action potentials and spontaneous depolarizations. Statistical significance was assessed using Student's paired t test.

Exposure of myocytes to 10 μM AA (10 min) reduced contraction amplitude by 40 ± 4% (n=6). This was associated with a reduction in action potential duration: after 10 min exposure to 10 μM AA, APD_{20} was 88 ± 2% and APD_{90} was 90±2% of that before AA was applied. Exposure of the myoytes to 10 μM AA also caused a reduction of ICa_L by 39 ± 6% (n=6). In addition, the amplitude of calcium transients was reduced by 38±4% (n=8) in the presence of 10 μM AA. The effects of AA were not prevented by exposure of the myocytes to the cyclo-oxygenase inhibitor trihydroindomethicin (10 μM) before exposure to 10 μM AA (reduction of contraction by 42 ± 4% (n=6; $P<0.05$). Reduction of contraction was also not prevented by 10 μM 5,8,11,14-eicosatetraynoic acid, an inhibitor of AA metabolising enzymes (reduction of contraction by 46 ± 3% with 10 μM AA in the presence of the inhibitor). In another series of experiments, spontaneous activity was provoked by exposure of the myocytes to 40 nM isoprenaline. This spontaneous activity was consistently abolished within 10 min following exposure to 10 μM AA (6 of 6 showed the suppression of activity).

In conclusion, the observed negative inotropic actions and suppression of spontaneous activity by AA appears to be associated with a reduction in the cytosolic calcium transient amplitude, occurring in part through a decrease in calcium entry through L-type calcium channels. However additional actions of AA on sarcoplasmic reticulum stores cannot be ruled out, and may also contribute.

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203P EFFECTS OF HALOTHANE ON CALCIUM TRANSIENTS AND CONTRACTIONS IN GUINEA-PIG AND RAT ISOLATED VENTRICULAR MYOCYTES

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The aim of this study is to investigate inotropic effects of the inhalational anaesthetic halothane at concentrations in the clinical range. Previous work has shown that halothane reduces calcium currents and interferes with net uptake and release of calcium from intracellular stores (Terrar & Victory 1987; Ramsay & Terrar, 1991). In the present experiments calcium transients were measured using the fluorescent probes indo-1 and fluo-3.

Single myocytes were isolated from guinea-pig and rat ventricle and superfused with a balanced salt solution containing 2.5 mM calcium (pH 7.4, 36 C). Cells were stimulated to contract by field stimuli (2 to 5 ms duration, 1 Hz) applied via platinum electrodes at the edges of the flow chamber. Contraction was measured using an edge-detection system applied to the video image of cells viewed via a microscope. In experiments where calcium transients were to be recorded, myocytes were loaded with either indo-1 or fluo-3 using their AM esters. In the case of indo-1, excitation was at 340 nm, and emission was recorded at 410 and 490 nm (the 410/490 ratio was taken as a measure of cytsolic calcium). For fluo-3, excitation was at 490 nm and emission was at wavelengths longer than 525 nm. The fluorescence signals were recorded using an intensified CCD camera (Photonic Sciences). Halothane at a concentration of 1 mM was dissolved in 0.1% DMSO and applied to the cells using a rapid switch system. Student's t test (paired unless otherwise stated) was used to evaluate statistical significance.

In guinea pig cells, halothane caused an initial increase in contraction (similar to that reported for rat cells by Robinson et al 1993), and this was followed by reduction of contraction to 40 ± 5% (n=6 cells, P<0.001). The effects were reversible (showing recovery to 84 ± 1% after 60 s wash in halothane-free solution). In rat myocytes under the same conditions, 1 mM halothane reduced contraction to 13 ± 3% (P<0.05) of that before halothane. Again the effects were reversible (recovery to 93 ± 3% on washout). The decrease in contraction with halothane appeared to be larger in rat than in guinea-pig myocytes (P<0.05, two sample t test). Halothane (1 mM) decreased the peak amplitude of calcium transients measured with indo-1 (410/490 ratio) by 40 ± 4% (n=6 cells; P<0.001) in guinea-pig myocytes. When fluo-3 was used to measure calcium transients, halothane (1 mM) reduced the calcium fluorescence signal in guinea-pig myocytes by 50 ± 6% (n=7 cells; P<0.05). The reduction of fluorescent signals corresponding to the calcium transients appeared to be significantly larger when fluo-3 was used rather than indo-1 (P<0.05, two sample t test); non-linearities in the relationship between cytosolic calcium and fluorescence, together with differences between the dyes in their affinity for calcium, must be taken into account when evaluating these observation. Reductions in fluorescence signals for both dyes were reversible on washout of halothane (recovery after 30s wash to 85 ± 8% in the case of indo-1, and to 85 ± 5% in the case of fluo-3).

The observations provide further support for the hypothesis that the reduction in contraction caused by clinical levels of halothane depends at least in part on reductions of cytosolic calcium transients. Additional effects of halothane on myofilament sensitivity to calcium cannot be ruled out and may also contribute.

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204P REGULATION OF CYCLIC ADP RIBOSE (cADPR) PRODUCTION BY PROTEIN KINASE A IN CARDIAC VENTRICULAR MICROSOSMES PREPARED FROM GUINEA-PIG HEART

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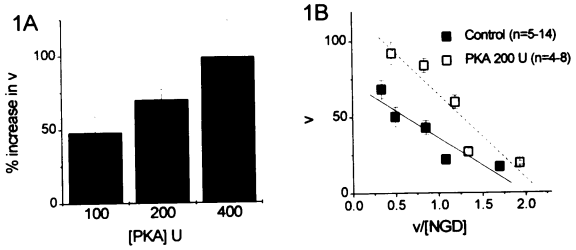
Cyclic ADPR is a metabolite of NAD which has been shown to mediate Ca^{2+} mobilisation in various cellular systems including the heart (Lee, et al., 1994; Iino et al., 1997). The possible regulation of cADPR production in the heart by protein kinase A (PKA) was investigated in microsomes using the NAD analogue, NGD which is converted by ribosyl cyclase to the fluorescent product cGDP ribose.

Microsomes were prepared from the hearts from guinea-pigs by a method based on that described by Sitsapesan and Williams (1990) and were diluted to a concentration of 2-5% in a high K^+ solution supplemented with phosphocreatine (20 mM), creatine phosphokinase (20 U), Na Azide (1 mM), ATP (1 mM) and the protease inhibitors leupeptin, aprotinin and soybean trypsin inhibitor. Experiments were carried out at 36 °C and NGD conversion into the fluorescent product cGDPR was monitored using a PTI fluorimeter (excitation at 300 nm and emission measured at 410 nm) with PTI Felix software and measured in arbitrary units. Data are mean ± s.e.mean and Student's t test (paired data) was used for statistical analysis with P<0.05 taken to indicate significance.

Addition of NGD (10-200 μM) to the cuvette containing the microsomes consistently caused an increase in fluorescence which gradually reached a steady level. The initial rate of production (v) of cGDPR was determined by fitting a line to the increase in fluorescence during the first few minutes of production of cGDPR. Following the addition of 400 units (U) of the catalytic subunit of PKA (cs-PKA), the initial rate of NGD (200 μM) conversion to cGDPR was increased by 99 ± 25 % (p<0.05; n=5). The effect of cs-PKA was concentration dependent over the range tested (Figure 1A) and also dependent on the substrate concentration with the greatest effect at the highest concentration of NGD. A plot of v/[NGD] against v (Eadie-Hofstee plot), in which the slope is $-K_m$ and the y intercept V_{max} revealed that cs-PKA increased the K_m from 36 to 55 μM and increased the V_{max} from 72 to 120 (arbitrary units) n=4-14).



These data are consistent with a role for the activation of the protein kinase A signal transduction pathway in elevating the level of endogenous cADPR in the heart.

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This work was supported by the British Heart Foundation.

205P EFFECTS OF AVP AND ANTAGONISTS ON CORONARY AND MESENTERIC ARTERIES IN INFARCTED RATS

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Arginine-vasopressin (AVP) may play a role in the pathogenesis of heart failure (HF) by increasing peripheral resistance via V_{1a} receptors and fluid retention via V_2 receptors. Since specific non-peptide V_{1a} and V_2 receptor antagonists have become available recently, this offers new possibilities to study the effects of both aspects of AVP. In this study we examined the role of V_{1a} and V_2 receptors in coronary arteries (CA) and small mesenteric arteries (SMA) in sham and HF rats. HF was induced by myocardial infarction (MI) through coronary artery ligation.

At 1 day and 3 weeks after MI, CA responses to AVP were studied in isolated perfused hearts. Dose-response curves were obtained by bolus injections of AVP (100 µl; 10^{-10}-10^{-5} M), in the presence or absence of the V_{1a} receptor antagonist SR-49059 (10^{-8} M) or the V_2 receptor antagonist OPC-31260 (10^{-6} M) (Fig.1).

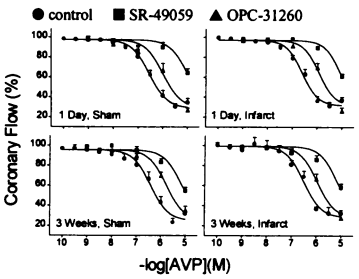

Fig.1. Decrease on rat coronary artery flow by AVP.

In sham hearts, coronary flow (CF) was maximally decreased by 70 ± 3% from baseline value (12.1 ± 1.1 ml/min) at 1 day after surgery (pEC_{50}: 6.55 ± 0.09), and 73 ± 2% from baseline value (12.0 ± 1.4 ml/min) at 3 weeks after surgery (pEC_{50}: 6.41 ± 0.14). The curves were similar in MI rats (pEC_{50}: 6.61 ± 0.08 and 6.53 ± 0.04, respectively). SR-49059 caused a significant rightward shift ($p<0.01$) in all groups with an apparent pA_2 of 9.25. OPC-31260 caused less rightward shift (only significant in infarct hearts at 1 day and sham hearts at 3 weeks) with an apparent pA_2 of 6.43. At 3 weeks after MI, SMA were studied in a Mulvany myograph. AVP dose-response curves (10^{-10}-10^{-6} M) were made in the presence or absence of SR-49059 (10^{-8} M) or OPC-31260 (10^{-6} M) and presented as percentage of the maximal contraction to 125 mM KCl (Fig.2).

The SMA of both MI and sham rats showed a comparable response to 125 mM KCl of respectively 6.3 ± 0.3 mN and 7.5 ± 0.6 mN. SMA of sham and MI rats showed a similar maximal contraction of respectively 287 ± 16% (pEC50: 9.55 ± 0.07) and 266 ± 18% (pEC50: 9.22 ± 0.07). Both SR-49059 and OPC-31260 caused a significant rightward shift ($p<0.01$) with a apparent pA_2 of 9.65 and 6.73 respectively. Although E_{max} appears to be lower in the presence of both antagonists than the control E_{max}, the changes were not statistically significant ($p>0.05$).

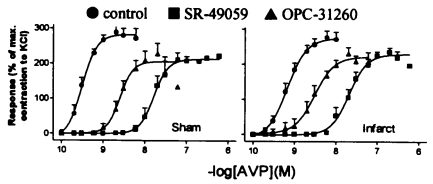

Fig.2. Constriction of rat small mesenteric artery by AVP.

These data indicate that AVP induced similar vasoconstrictor responses in sham and MI rats, independent of the vascular bed that is studied. Since these effects were antagonised by SR-49059, the V_{1a} receptor mediates AVP-induced vasoconstriction. The V_2 receptor antagonist OPC-31260 also displays antagonism on the constrictor response. However, the shape of the dose-effect curves and the parallel shift may suggest that the antagonism by OPC-31260 reflects its affinity for the V_{1a} receptor, rather than V_2 receptor involvement.

206P THE STABLE NITROXIDE RADICAL TEMPOL PROTECTS HUMAN ENDOTHELIAL CELLS AGAINST THE CELLULAR INJURY CAUSED BY HYDROGEN PEROXIDE

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Reactive oxygen species (ROS) including superoxide anions, hydroxyl radicals and hydrogen peroxide contribute to ischaemia-reperfusion injury, septic shock or atherosclerosis. The pathophysiology of endothelial cell injury is a prominent feature of all of these conditions. Tempol (4-hydroxy-tempo) is a water-soluble analogue of the spin label tempo (2,2,6,6-tetramethylpiperidine-N-oxyl), which is widely employed in electron spin resonance spectroscopy. Tempol is a stable piperidine nitroxide (stable free radical) of low molecular weight, which permeates biological membranes and scavenges superoxide anions *in vitro* (Laight *et al.*, 1997). We have discovered that tempol reduces the infarct size caused by regional myocardial ischaemia and reperfusion in the isolated perfused heart of the rat (McDonald *et al.*, 1998) and attenuates the renal and liver injury caused by endotoxin in the rat (Leach *et al.*, 1998). In this study we investigated the effect of tempol on cellular injury in cultures of a human endothelial cell line (EA.hy 926) exposed to oxidant stress in the form of hydrogen peroxide, and compare the effects of tempol with those obtained with other agents well known to reduce the generation or effects of ROS (1,3-dimethyl-2-thiourea, catalase and deferoxamine mesylate).

Human endothelial cells (passages 40 to 49) were seeded onto 96-well plates and cultured to confluence in Dulbecco's Modified Eagle Medium (DMEM) containing glutamine (2 mM) and 10% foetal calf serum (FCS). Cell injury (i.e. reduction in mitochondrial respiration) was assessed spectrophotometrically by measurement of the mitochondrial-dependent conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into formazan. To evaluate the degree of cell injury induced by H_2O_2, the cells were exposed to various concentrations of H_2O_2 (0.03, 0.1, 0.3, 1, 3, 10 mM, n=4). In the subsequent intervention studies, the cells were challenged with a submaximal concentration of H_2O_2 (3 mM) for 4 h. To elucidate the effect of tempol, cells were preincubated (10 min prior to H_2O_2) in media (1% FCS) in the absence or presence of increasing concentrations of tempol (0.03-30 mM), 1,3-dimethyl-2-thiourea (DMT, 0.03-30 mM), deferoxamine mesylate (0.03-30 mM) or catalase (0.03-30 U/ml). Controls (without exposure to H_2O_2) were performed for all groups (n=4). Statistical differences between groups were analysed by ANOVA followed by a Bonferroni's test ($p<0.05$).

Table 1: Effect of increasing concentrations of catalase, DMT, deferoxamine and tempol on H_2O_2-mediated inhibition of mitochondrial respiration (mean ± s.e.m. of % control) (*$p<0.05$ vs. H_2O_2 only, n=4).

	catalase	DMT	deferoxamine	tempol
0	23±3	10±5	25±4	20±1
0.03	18±2	6±2	24±3	25±3
0.1	21±2	10±4	37±5	26±3
0.3	25±5	10±3	68±3*	34±5
1	43±14	26±5	80±5*	61±5*
3	81±5*	78±8*	71±13*	58±10*
10	94±2*	95±11*	toxic	toxic
30	104±5*	toxic	toxic	toxic

Exposure of EA.hy 926 cells to H_2O_2 for 4 h caused a significant reduction in mitochondrial respiration. The presence of 1,3-dimethyl-2-thiourea, catalase or deferoxamine mesylate caused an attenuation of the cytotoxic effects of H_2O_2 (see Table 1). Tempol (1 or 3 mM) attenuated the H_2O_2-mediated inhibition of mitochondrial respiration in a dose-related fashion. Higher concentrations of DMT, deferoxamine or tempol were toxic to the EA.hy 926 cells. These experiments demonstrate that the stable free radical tempol can protect human endothelial cells against cell injury caused by hydrogen peroxide.

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207P THE MULTIPLE ORGAN DYSFUNCTION CAUSED BY LIPOTEICHOIC ACID AND PEPTIDOGLYCAN IS ATTENUATED BY THE STABLE NITROXIDE RADICAL TEMPOL IN THE RAT

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Endotoxaemia, sepsis and septic shock are associated with the generation of reactive oxygen species (ROS) including superoxide anions and hydroxyl radicals (Vespasiano *et al.*, 1993). Tempol (4-hydroxy-2,2,6,6-tetra-methylpiperidine-N-oxyl) is a stable piperidine nitroxide (stable free radical) of low molecular weight, which permeates biological membranes and scavenges ROS including superoxide anions (Laight *et al.*, 1997). Recently we reported that the spin trap agent tempol attenuated the circulatory failure and multiple organ injury/dysfunction (MODS) caused by endotoxin (Leach *et al.*, 1998). In this study we investigate the effects of tempol in a model of gram-positive septic shock in the rat.

Male Wistar rats (240-300 g, n=34) were anaesthetised with sodium thiopentone (120 $mg \cdot kg^{-1}$, i.p.). The trachea was cannulated to facilitate respiration. Catheters were inserted in the left carotid artery for the measurement of mean arterial blood pressure (MAP) and heart rate (bpm), and the jugular vein for drug administration. At completion of the surgical procedure, animals were allowed to equilibrate for 15 min after which they received a bolus of tempol (100 $mg \cdot kg^{-1}$ i.v. over 5 min) or its vehicle (saline, 2 $ml \cdot kg^{-1}$ i.v.). After 15 min two cell wall fragments of the pathogenic gram-positive bacterium *Staphylococcus aureus* were administered to induce a gram-positive septic shock (Kengatharan *et al.*, 1998): lipoteichoic acid (LTA, 3 $mg \cdot kg^{-1}$ i.v.) was given followed by peptidoglycan (PepG, 10 $mg \cdot kg^{-1}$ i.v.) over 15 min. After this, an infusion of tempol (30 $mg \cdot kg^{-1} \cdot h^{-1}$ i.v.) or saline (1.5 $ml \cdot kg^{-1} \cdot h^{-1}$ i.v.) was maintained during the experiments. All values are expressed as mean ± s.e.mean.

Gram-positive shock for 6 h resulted in significant rises in the serum levels of urea and creatinine (creat) (indicators of renal dysfunction/failure), aspartate aminotransferase (AST, a non-specific marker for hepatic injury) and alanine aminotransferase (ALT, an indicator of liver injury). Tempol caused a transient fall in MAP and rise in heart rate (HR, not shown), and significantly attenuated the renal dysfunction as well as the liver injury/dysfunction caused by LTA/PepG (Table 1). In rats not receiving LTA/PepG (sham + tempol, n=6), tempol did not affect any of the parameters measured (data not shown).

Table 1: Effects of tempol after 6 h on MAP (mmHg), HR (bpm) and organ injury/dysfunction (urea: $mmol \cdot L^{-1}$, creat: $\mu mo \cdot L^{-1}$, AST, ALT: $i.u. \cdot L^{-1}$) caused by LTA/PepG (*: $p<0.05$ vs. sham + saline, #: $p<0.05$ vs. shock + saline. ANOVA followed by Bonferroni's test).

	MAP	HR	urea	creat	AST	ALT
sham + saline (n=6)	119±5	365±15	5±1	35±3	176±19	100±9
shock + saline (n=12)	95±5 *	384±13	11±2 *	55±5 *	458±28 *	217±35 *
shock + tempol (n=10)	86±1 *	411±8	9±1	39±1 #	302±17 *#	125±10 #

Thus, pretreatment of rats with the superoxide anion scavenger tempol attenuates the renal and liver dysfunction and/or injury caused by gram-positive septic shock in the rat. These results support the view that an enhanced formation of reactive radical species contributes to the MODS associated with gram-positive sepsis.

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208P NEITHER HEAT STRESS NOR ISCHAEMIC PRECONDITIONING ALTER MYOCARDIAL mRNA EXPRESSION OF THE ATP-SENSITIVE POTASSIUM SUBUNIT Kir 6.2

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K_{ATP} channels are known to be involved in cardioprotection afforded by heat stress (HS; Pell *et al*, 1997) and ischaemic preconditioning (PC; Gross & Auchampach, 1992). Severe ischaemia in the rat increases mRNA expression of the K_{ATP} channel subunits Kir6.1 and Kir6.2 in the heart (Akao *et al*, 1997). The effects of HS and PC on mRNA expression of the Kir6.2 subunit were, therefore, investigated in the rabbit. Three time points were chosen to assess Kir6.2 mRNA: (i) during the acute phase of protection (1 h), (ii) between the two phases (3 h) and (iii) during the delayed phase of protection (24 h).

Pentobarbitone anaesthetised male New Zealand White rabbits (2.2-3.5 kg) were entered into either a HS or PC protocol. *HS*. Rabbits underwent HS for 15 min at a core temperature of 42±0.2°C; sham controls were anaesthetised only. Animals in both groups were recovered for either 1, 3 or 24 h. Hearts were then harvested, under anaesthetic overdose, and left ventricular (LV) tissue rapidly frozen (n=4 for all groups except 1 h following sham HS for which n=2). *PC*. Rabbits underwent a midline sternotomy and pericardiotomy. PC was performed using 4 cycles of 5 min left coronary artery occlusion each separated by 10 min reperfusion. Hearts were harvested as above at 1, 3 or 24 h after PC (n=3 for each group). Sham controls were anaesthetised only; two animals were recovered for 1 h and three animals for 24 h. RNA was prepared from heat stressed, preconditioned and control LV tissue. Kir6.2 mRNA expression was assessed by Northern blot analysis using ^{32}P-labelled rabbit Kir6.2 cDNA. Equal loading was confirmed using ^{32}P-labelled rat glycerol-3-phosphate dehydrogenase (GAPDH).

Kir6.2 mRNA expression was not regulated at 1, 3 or 24 h following HS or PC. Representative Northern blots are shown in Figures 1. Whilst these results suggest that there is no change in the gene expression pattern of the gene encoding Kir6.2 in association with cardioprotection, translational and post-translational modifications which alter the functional properties of the K_{ATP} channel cannot be excluded.

T.J. Pell holds a Glaxo Wellcome studentship.

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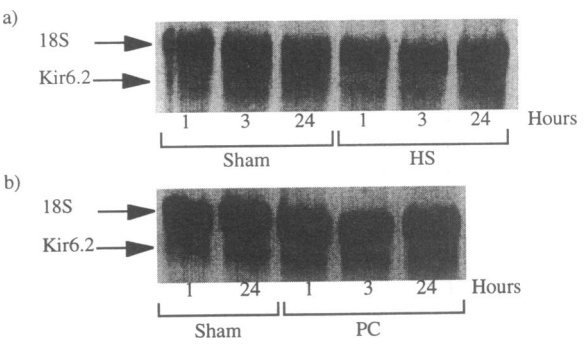

Figure 1: Kir6.2 mRNA expression at 1, 3 and 24 h follwing HS (panel a) and PC (panel b). Representative Northern blot.

209P MODIFICATION OF RESPONSES OF THE RAT HEART TO BENZODIAEPINE AGONISTS AND INVERSE AGONISTS BY PK 11195 AND FLUMAZENIL

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Cardiac effects of diazepam are modified by PK 11195 [1-(2-chlorophenyl-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide] and flumazenil (Zeegers et al., 1998). Cardiac actions of zolpidem, a partial benzodiazepine agonist, were compared to those of Ro 19-4603, a partial inverse agonist of the central-type receptor.

Hearts of female rats (160 g), anaesthetized, heparinized, aortae cannulated, were excised and perfused with Tyrode's solution (37° C, pH 7.0), gassed with 5% CO_2 in O_2. Coronary flow rate was measured by collecting the perfusate in a tumbling vessel; the tumbling time was measured and the flow rate expressed as ml/min. Inotropy (mm Hg) was recorded, using a latex balloon filled with water and inserted into the left ventricle. The heart was exposed to 22.0 to 650 µg zolpidem, or 3.9 to 51.5 µg Ro 19-4603, in the absence or presence of PK 11195 or flumazenil (10^{-8} to 10^{-5} M) (n = 9). Changes in coronary flow rate or inotropy at exposure were expressed as average percentage rate or force of control value, measured immediately before exposure ± SEM. Statistics were performed using analysis of variance followed by Bonferroni statistics.

Coronary flow rate was dose-dependently decreased by zolpidem (up to maximal -71.0% ± 9.1%), whereas Ro 19-4603 induced dose-dependent increase of flow rate (Emax 71.3% ± 4.1) after an initial decrease at the lowest dose. PK 11195, 10^{-5} M, antagonized the zolpidem-induced response at 650 µg; and flumazenil, 10^{-8} M, potentiated the response at 125 up to 167 µg zolpidem. In the presence of PK 11195 the Ro 19-4603-induced increase of perfusion rate was decreased concentration-dependently (at 10^{-5} M Emax 19.5% ± 0.7), whereas only 10^{-5} M flumazenil reduced the response (Emax 22.9% ± 0.8). *Inotropy* was increased dose-dependently by zolpidem (Emax 180% ± 5.1), Ro 19-4603 induced a biphasic response (Emax of the positive inotropic response: 128% ± 8.0). At lower concentrations the response to zolpidem was intensified (at 10^{-7} M, Emax: 283% ± 8.6), then reversed and antagonized by PK 11195 (at 10^{-5} M, Emax: 54.1% ± 0.8). Flumazenil potentiated the response (at 10^{-5} M, Emax 358% ± 12.6). The negative inotropy was antagonized significantly by 10^{-5} M PK 11195 and enforced by 10^{-6} M flumazenil. Both antagonists significantly reduced the positive inotropy (Emax at 10^{-5} M 46.1% ± 2.5, and 40.1% ± 2.7 respectively.).

In conclusion, the benzodiazepine agonist and the inverse agonist exert opposite actions on both coronary flow rate and inotropy. Antagonists interact with these changes in a different manner. This latter observation suggests that ambiguous (multiple receptor) mechanisms are involved in cardiac effects of benzodiazepines.

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210P CERAMIDE RELAXES RAT MESENTERIC MICROVESSELS

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Ceramide is an intermediary product of the sphingolipid metabolism. It has recently been shown that ceramide can cause relaxation of isolated rat aorta (Johns *et al.* 1997). Since aorta contributes little to the regulation of peripheral resistance, we have investigated possible effects of ceramide on the tone of isolated rat mesenteric microvessels.

Mesenteric microvessels (200-300 µm diameter) were prepared from adult male Wistar rats and mounted in a Mulvany-Halpern myograph for measurement of isometric force of contraction as previously described (Chen *et al.* 1996). Microvessels were precontracted by addition of 100 µM methoxamine, which increased force of contraction by approximately 14 mN. Thereafter, ceramide was added and force of contraction was monitored. C2-*erythro*-ceramide and microvessels with functionally intact endothelium (assessed as maintained relaxation response to 100 µM carbachol) were used throughout unless otherwise indicated. Data are mean ± s.e. mean of n experiments and expressed as % of force 5 min prior to ceramide addition.

Addition of 100 µM ceramide caused a slowly developing relaxation of mesenteric microvessels which reached maximum values after 9-10 min and partially abated thereafter, yielding a biphasic time course. The maximum relaxation reached by 10, 30 and 100 µM ceramide was 15 ± 4% (n = 15), 20 ± 5% (n = 15) and 65 ± 5% (n = 16), respectively. The relaxation was stereospecific since 100 µM C2-*threo*-ceramide caused significantly less relaxation (43 ± 11%; n = 8; $P < 0.05$ vs. C2-*erythro*-ceramide in a one-way analysis of variance). C8-ceramide (100 µM) caused only very little if any relaxation of 100 µM methoxamine-contracted microvessels (12 ± 11%, n = 10). In contrast, the structurally related sphingosylphosphorylcholine (100 µM) did not reduce but rather enhanced the 100 µM methoxamine-induced contraction by an additional 16 ± 1% (n = 8). Sphingosylphosphorylcholine even caused microvessel contraction in the absence of methoxamine (Czyborra *et al.* 1999)

Mechanical removal of the endothelium by introduction of a hair into the vessel lumen abolished the relaxant effect of 0.1 mM carbachol but had only little effect on the relaxant effect of 100 µM ceramide (with endothelium 52 ± 8%; n = 15; without endothelium 44 ± 7%; n = 13; not significantly different).

We conclude that ceramide can cause concentration-dependent relaxation of rat mesenteric microvessels. This relaxation has pharmacological specificity involving predominantly short-chain ceramides and occurs largely independent of an intact endothelium. We speculate that abatement of relaxation at later time points may involve vasocontracting metabolites of ceramide.

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211P RAT MICROVESSEL CONTRACTION BY SPHINGOLIPIDS: STRUCTURE-ACTIVITY RELATIONSHIP AND ROLE OF ENDOTHELIAL MEDIATORS

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We have previously reported that sphingosine-1-phosphate causes a concentration-dependent contraction of rat mesenteric and intrarenal microvessels *in vitro* and reduce mesenteric and renal blood flow *in vivo* (Bischoff *et al.* 1998). We have now investigated these effects in more detail by testing several analogues of sphingosine-1-phosphate, i.e., sphingosylphosphorylcholine, D-*erythro*-sphingosine, psychosine and glucopsychosine, and studied the roles of extracellular Ca^{2+} and endothelium-derived mediators.

In vitro experiments on mesenteric and intrarenal microvessels (200-300 µm diameter) from adult male Wistar rats were performed in a Mulvany-Halpern myograph as previously described (Chen et al. 1996). Data are mean ± s.e. mean of n experiments and are expressed as % of the maximal noradrenaline effect in each vessel, which was approximately 18 and 6 mN for mesenteric and renal microvessels, respectively.

All five sphingolipids caused concentration-dependent microvessel contraction *in vitro*. At a bath concentration of 100 µM, the contraction in mesenteric and renal vessels was 50 ± 11% and 22 ± 7% for sphingosine-1-phosphate, 48 ± 5% and 54 ± 8% for sphingosylphosphorylcholine, 26 ± 10% and 25 ± 7% for D-*erythro*-sphingosine, 17 ± 5% and 21 ± 5% for psychosine, and 6 ± 2% and 19 ± 5% for glucopsychosine (n = 8-10 each), respectively.

The contractile response to sphingosylphosphorylcholine in mesenteric vessels was partially inhibited at each agonist concentration by 300 nM of the Ca^{2+} entry blocker nitrendipine and even more by complete removal of extracellular Ca^{2+} (no nominal Ca^{2+} plus 0.5 mM EGTA). Thus, contraction at 100 µM sphingosylphosphorylcholine was 40 ± 6% under control conditions, 29 ± 6% in the presence of nitrendipine, and 6 ± 1% in the absence of extracellular Ca^{2+} (n = 11-13 each; $P < 0.001$ in a two-way analysis of variance).

To test a possible contribution of endothelium-derived mediators, we investigated the effect of 1 mM N^G-nitro-L-arginine and 10 µM indomethacin on contraction of mesenteric microvessels by 0.1-100 µM sphingosylphosphorylcholine. For example, contraction by 30 µM sphingosylphosphorylcholine was 95 ± 3% under control conditions, 95 ± 7% in the presence of N^G-nitro-L-arginine, 71 ± 6% in the presence of indomethacin, and 68 ± 8% in the combined presence of N^G-nitro-L-arginine and indomethacin (n = 8-9; not significantly different in a one-way analysis of variance).

We conclude that sphingolipid-induced contraction of rat microvessels has a specific pharmacological profile. It depends on the presence of extracellular Ca^{2+} but is largely independent of endothelium-derived mediators.

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212P EFFECTS OF PROSTANOIDS ON INOSITOL PHOSPHATE FORMATION AND PROTEIN SYNTHESIS IN RAT NEONATAL VENTRICULAR CARDIOMYOCYTES

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We have recently shown (Pönicke et al., 1998) that in rat neonatal ventricular cardiomyocytes stimulation of $G_{q/11}$-coupled receptors such as α_1-adrenergic- or ET_A-receptors causes increases in inositol phosphate (IP) formation and in rate of protein synthesis. The thromboxane A_2 (TXA_2)-mimetic U 46619 also increased IP-formation and rate of protein synthesis; however, its potency was low (pEC_{50}-values of 5.5 to 6.0); in addition, the selective TP-receptor antagonist SQ 29548 was only a weak inhibitor of both responses. We, therefore, wondered whether another prostanoid (PG)-receptor might be involved in IP-formation and rate of protein synthesis. To test this hypothesis we assessed, in neonatal rat cardiomyocytes, the effects of several prostanoids (PGD_2, $-E_1$, $-E_2$, $-F_{2\alpha}$, Carbocyclin as well as U46619) on IP-formation and rate of protein synthesis.

Isolation of neonatal cardiomyocytes, IP-formation (determined as accumulation of total [^{3}H]-IP's in [^{3}H]-myo-inositol prelabelled cells during a 60 min incubation at 37°C in HANKS buffered saline solution that contained 10 mM LiCl) and rate of protein synthesis (determined as [^{3}H]-phenylalanine incorporation during a 24 hours incubation) were performed as recently described (Pönicke et al., 1997). All data are means ± SEM of n experiments.

Among the PG's tested $PGF_{2\alpha}$, $-D_2$, $-E_2$, and U46619 concentration-dependently increased IP-formation and rate of protein synthesis; PGE_1 and Carbocyclin exerted effects only in very high concentrations (>1µM); pEC_{50}- and E_{max}-values are given in the table.

	Protein-Synthesis		IP-Formation	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}
$PGF_{2\alpha}$	8.3±0.2	71±12	7.2±0.3	189±39
PGD_2	6.9±0.1	80± 5	5.7±0.1	166±14
PGE_2	6.2±0.2	72±10	5.1±0.1	193±18
U 46619	6.0±0.2	33± 5	5.5±0.1	128±35
Carbocyclin	n.d	n.d.	n.d.	n.d.
PGE_1	n.d	n.d.	n.d.	n.d.

n=5-8, E_{max} = maximal increase above basal in %
n.d. = no maximal response could be determined

The fact, that in rat ventricular neonatal cardiomyocytes PG's stimulate IP-formation and rate of protein synthesis with an order of potency $PGF_{2\alpha} > PGD_2 > PGE_2 =$ U46619 > Carbocyclin = PGE_1 is in favour of the idea that the receptor involved is a FP-receptor (Coleman et al., 1994).

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213P EFFECT OF MIBEFRADIL AND VERAPAMIL ON THE TACHYCARDIC RESPONSE TO NORADRENALINE AND ELECTRICAL STIMULATION IN THE PITHED RAT

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It was recently shown by Göthert and Molderings that the T-type calcium channel prevalent blocker mibefradil (MIB) blocks the ω-conotoxin GVIA-sensitive sympathetic noradrenaline release in human cardiac tissue at micromolar range. This is most probably due to the N-type calcium channel blocking properties of the drug (Bezprozvanny & Tsien, 1995). It was the aim of our study to investigate a possible effect of mibefradil on the cardiac noradrenaline release in the pithed rat preparation. To compare the effects with direct adrenoceptor stimulation we applied noradrenaline in a separate group of animals. Furthermore, electrical stimulation of the preganglionic cardioaccelerator nerves as well as the noradrenaline dose-response curves were performed with the L-type calcium channel blocker verapamil (VER) as well.

During anesthesia male normotensive Wistar rats of 250-300g received a tracheal cannula and one group of animals were pithed and immediately thereafter subjected to artificial respiration with room air (60 strokes/mm, 10 ml/kg) using a positive pressure pump. The right jugular vein was catheterized and heparin (1 000 IU/kg) was injected via this route. A cannula was placed in the ipsilateral common carotid artery and arterial blood pressure was measured via a pressure transducer connected to a MacLab data acquisition system. The animals were pretreated with tubocurarine (1 mg/kg, i.v.) and atropine (1 mg/kg s.c.) and bilateral vagotomy was performed in the cervical region. The pithing rod was coated with enamel, except for a segment of 1 cm length, 7cm distally from the tip for a maximal stimulation of the cardiac sympathetic nerves (C7-T1). After an equilibration period of 5 min, the animals received saline (1 ml/kg) (controls), MIB or VER either 3 or 10 μmol/kg intra arterially. After an incubation period of 15 min, in the pithed group the preganglionic cardioaccelerator nerves were electrically stimulated between the pithing rod and the dorsally-located indifferent electrode using monophasic rectangular pulses of 2 ms duration and supramaximal voltage (50V). Trains of pulses of 25s duration were applied and the increase in cardiac frequency (beats/min) was measured. Between the periods of stimulation, sufficient time was allowed to ascertain complete return of the cardiac frequency to pre-stimulation value. The second group received cumulatively noradrenaline 0.1-1000 nmol/kg in a total volume of 0.5ml/kg intravenously.

The maximum increase in heart rate (bpm) in response to electrical nerve stimulation was 96±7 (control, n=6), 71±8* (MIB 3μmol/kg, n=6), 57±6* (MIB 10μmol/kg, n=5), 94±8 (VER 3μmol/kg, n=4) and 46±7* (VER 10μmol/kg, n=5). The maximum increase in heart rate (bpm) in response to noradrenaline was 96±4 (control, n=6), 87±20 (MIB 3 μmol/kg, n=6), 42±9* (MIB 10 μmol/kg, n=5), 73±5* (VER 3μmol/kg, n=5) and 40±7* (VER 10 μmol/kg, n=6). Under control conditions and in the presence of 3 μmol/kg MIB and VER the maximum effect of noradrenaline was reached at 0.1 μmol/kg whereas in the presence of 10 μmol MIB and VER it was reached at a dose of 1 μmol/kg.

* denotes P-value <0.05 when compared to the respective control.

MIB at a dose of 3 μmol/kg was significantly more effective in reducing the tachycardic response to electrical stimulation when compared to externally applied noradrenaline. For verapamil the opposite holds true. These differences were not observed with doses of 10 μmol/kg MIB and VER.

We conclude that the reduction of the noradrenaline release from cardiac sympathetic neurons by mibefradil is responsible for the observed phenomenon which would be in line with the findings on N-type calcium channel blocking properties of this compound. However, in our model this particular effect is only observable at a moderate dose of MIB, whereas the effect of higher doses might be mediated mainly by L-type calcium channel blockade.

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214P DIFFERENTIAL VASORELAXANT EFFECTS OF ROSIGLITAZONE, ITS METABOLITES AND TROGLITAZONE

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Thiazolidinedione (TZD) PPARγ (peroxisome proliferator activated receptor γ) agonists are new treatments for Type 2 diabetes that enhance glucose metabolism by increasing insulin sensitivity in target tissues. At high doses these agents lower blood pressure in animals (Buchanan *et al*, 1995), possibly by reducing peripheral resistance via blockade of calcium currents in vascular smooth muscle cells (Zhang *et al*, 1994), and in clinical studies the TZD troglitazone (TRO) can cause oedema (Kelley & Killian, 1998). Rosiglitazone (RSG) is a potent aminopyridyl TZD currently in Phase III clinical studies. It is metabolised to 6 desmethylated or hydroxylated phase I metabolites (Bolton *et al*, 1996). We have compared the actions of RSG and its metabolites on vascular relaxation and calcium channels with those of TRO.

The order of potency to relax phenylephrine (1μM) pre-contracted adult female Sprague-Dawley rat isolated aorta was (IC_{50} values [95% confidence limits] in parentheses): TRO (59 μM [44.7-78.5]) > RSG (130 μM [119-140]) > SB-244675 (380 μM [331-434]) ≥ SB-275286 (390 μM [344-432]) ≥ SB-237216 (410 μM [353-472]) ≥ SB-243914 (500 μM [431-580]) > SB-280789 (650 μM [585-722]). SB-271258 achieved only 15% relaxation at the highest concentration studied (1mM). The calcium channel binding properties of the compounds were determined in adult male Sprague-Dawley rat cerebral cortical membranes, using the voltage-dependent calcium channel blocker [^{3}H]-nitrendipine as radioligand. The rank order of potency obtained for half-maximal displacement of nitrendipine was (IC_{50} values [95% confidence limits] in parentheses): nifedipine (2.8 ± nM [1.2-4.4]) >> TRO (13 μM [4.7-21.3]) > SB-243914 (51 μM [3.9-98.1]) > SB-280789 (110 μM [41.2-174.8]) > RSG (270 μM [137.6-392.4]) > SB-275286 (290 μM [38.0-534.0]) > SB-244675 (560 μM [270.0-906.0]) > SB-237216 (650 μM [113.2-1176.8]). SB-271258 was ineffective (no displacement up to concentrations of 100 μM). None of the metabolites achieved more than 60 % displacement of [^{3}H]-nitrendipine.

In conclusion, we have shown that TRO is a more potent vasorelaxant than RSG, and that RSG is more potent than any of its unconjugated metabolites. In addition, there was a close correlation between vasorelaxant potencies and displacement of nitrendipine binding to calcium channels, suggesting that the vasorelaxant properties of these compounds may be mediated via calcium channel blocking activity. This rank order of potency for vasorelaxation is in sharp contrast to the therapeutic potency of these drugs, with RSG being 100-fold more potent than TRO (Cantello *et al* 1994). This difference in potencies supports the suggestion that RSG is likely to have only limited vascular effects *in vivo*, which may in part account for the low incidence of oedema observed with RSG.

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215P EFFECTS OF CISAPRIDE ON THE CARDIAC ACTION POTENTIAL RECORDED IN SHEEP ISOLATED PURKINJE FIBRES

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Cisapride is a widely used prokinetic agent whose adverse effects include prolongation of the QT interval of the ECG in man (Ahmad & Wolfe, 1995; Bran *et al.*, 1995). In the present experiments we have studied the effects of cisapride in sheep Purkinje fibres.

Methods. Intracellular recordings (at ~36°C) were made from electrically paced (1 Hz) left ventricular Purkinje fibres isolated from hearts of adult Suffolk sheep of either sex. The effects of cisapride (0.01, 0.1, 1 and 10 μM; 30 min at each concentration) were assessed on the following parameters: action potential duration at 90% repolarization (APD_{90}), maximum rate of depolarization (MRD), upstroke amplitude (UA) and diastolic membrane potential (E_m). Cisapride was formulated as a 10 mM stock solution in DMSO, serially diluted to 1, 0.1 and 0.01 mM in DMSO. These stock solutions were diluted directly in the perfusant. Appropriate amounts of DMSO were added to the perfusant of vehicle-treated fibres. Data are presented as means ± s.e. mean.

Baseline values. In the vehicle group (n = 6) the pre-treatment values for APD_{90}, MRD, UA and E_m were 284 ± 17 ms, 428 ± 55 V/s, 115 ± 2 mV and -94 ± 3 mV, respectively. The respective pre-treatment values in the cisapride group (n = 6) were: 278 ± 10 ms, 540 ± 26 V/s, 117 ± 2 mV and -89 ± 2 mV. The baseline values in the 2 groups were not significantly different ($P \geq 0.05$ for each comparison; unpaired 2-tailed Student's t-test).

Effect of cisapride. The table shows the mean differences (± s.e.m.) in these parameters between cisapride- and vehicle-treated fibres:

	Cisapride/vehicle concentration			
	0.01 μM/0.1%	**0.1 μM/0.1%**	**1 μM/0.1%**	**10 μM/0.1%**
APD_{90} (%)	+4.9 ± 2.7	+11.7 ± 3.5*	+13.5 ± 5.3*	-13.5 ± 8.2
MRD (%)	-0.3 ± 6.2	-5.1 ± 7.0	-9.2 ± 9.4	-14.2 ± 10.6
UA (mV)	0.2 ± 1.0	1.0 ± 1.4	-0.7 ± 1.6	-6.5 ± 2.7
E_m (mV)	-0.7 ± 2.2	-1.5 ± 1.5	-0.3 ± 2.8	+2.0 ± 3.4

*$P < 0.02$; (cisapride *vs.* vehicle; unpaired 2-tailed t-test; n = 6 per group)

These experiments indicate that cisapride, at lower concentrations, prolongs action potential duration in sheep Purkinje fibres. This effect is consistent with data obtained in rabbit Purkinje fibres (Puiseux *et al.*, 1996) and is observed over a concentration range which is relevant to the human plasma concentration of cisapride which is associated with QT prolongation (0.1 – 1 µM ;Wiseman & Faulds, 1994). At 10 µM cisapride decreased APD_{90}, an effect associated with a decrease in MRD. Although the present study was not designed to address the molecular basis of the cisapride-induced action potential prolongation, cisapride has been reported to display high affinity blockade of the human cardiac potassium channel, HERG (Mohammad *et al.*, 1997; Rampe *et al.*, 1997). It is therefore probable that an inhibitory action on the rapid component of the delayed rectifier K^+-channel underlies the effect of cisapride on action potential duration observed in this study.

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216P *IN VITRO* DESENSITIZATION OF ATRIAL β_1-ADRENOCEPTOR FUNCTION: EFFECTS OF PDE INHIBITION

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β_1-adrenoceptor-mediated positive inotropic responses of guinea-pig atria undergo desensitization when incubated with isoprenaline (Herepath & Broadley, 1990). One mechanism for β-adrenoceptor desensitization has been suggested to involve an upregulation of phosphodiesterase (PDE) during chronic exposure, which would explain the reduced sensitivity to β-agonists (Giembycz, 1996). Indeed, the slow fade of the positive inotropic response of guinea-pig left atria to isoprenaline has been shown to be reduced by a PDE inhibitor (Argent *et al.*, 1996). In this study we examine the effects of PDE inhibitors on the desensitization of the inotropic responses to isoprenaline induced by prolonged incubation with isoprenaline.

Guinea-pig isolated left atria were set up in Krebs-bicarbonate solution at 37°C gassed with 5% CO_2 in oxygen and electrically paced (2Hz, 5ms, threshold voltage+50%). Isometric tension was recorded and after equilibration, a cumulative concentration-response curve (CRC) to (-)-isoprenaline was constructed to a maximum concentration of 1µM. The tissues were washed and to induce desensitization, isoprenaline (1µM) was returned to the bath and left in contact with the tissue for 4 hours. The tissues were then washed over 1 h and a second isoprenaline CRC obtained. In control experiments, the 1 h washout followed the first CRC and the tissues were sham incubated for 4 h. PDE inhibitors were either (1) added at the first curve maximum and were present with isoprenaline until the 1 h washout, or (2) were present throughout. Responses were measured as the increase in atrial tension, pre-incubation responses being corrected from the controls. Responses were plotted as a percentage of the pre-incubation maximum increase in tension (corrected). Geometric mean EC50 values (with 95% confidence limits) were compared by Student's paired t-test.

Incubation with isoprenaline (1 µM) for 4 h caused desensitization, seen as a rightwards shift of the CRC, the EC50 for isoprenaline being significantly (P<0.05) increased from 0.053(0.046-0.06) to 0.11(0.08-0.16)µM (n=8). When the non-selective PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX, 1µM) was co-incubated with the isoprenaline, there was no shift of the CRC, the EC50s not differing (0.04(0.03-0.05) and 0.04(0.02-0.08)µM, n=5). Similarly, when the selective PDE4 inhibitor, rolipram (0.1µM), was co-incubated with isoprenaline, there was no shift of the curve and the EC50 values were not significantly (P>0.05) different (0.026(0.021-0.032) and 0.034(0.020-0.056)µM, n=8). This suggests that PDE inhibition prevents the desensitization. However, incubation with IBMX or rolipram alone for 4 h followed by washout, caused potentiation of the responses. Thus, the 1 h washout was not effective in reversing the effect of the PDE inhibitor and potentiation of the responses by persistent PDE inhibition could have masked the desensitization.

When the PDE inhibitors were present throughout, desensitization still occurred, seen as a shift of the isoprenaline CRCs to the right by isoprenaline incubation. The EC50 values in the presence of IBMX (n=4) and rolipram (n=6), respectively, were significantly increased (P<0.05) from 0.011(0.005-0.025) and 0.010(0.007-0.015)µM to 0.032(0.015-0.069) and 0.023(0.012-0.046)µM. Thus, an increase in PDE activity during the incubation cannot explain the reduced sensitivity to isoprenaline.

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217P MODIFICATION OF 5-HT-INDUCED VASOCONSTRICTION OF PORCINE ISOLATED CORONARY ARTERIES IN THE PRESENCE OF AN OXYGEN SCAVENGER

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5-Hydroxytryptamine (5-HT) causes constriction of porcine coronary arteries, which is thought to be mediated by $5\text{-HT}_{1B/1D}$ and 5-HT_{2A} receptors (Angus & Cox, 1996). $5\text{-HT}_{1B/1D}$ receptor responses require 'priming' with a subthreashold concentration of agonist (Angus & Cox, 1996). In large coronary arteries, RT-PCR studies have demonstrated the presence of mRNA for all of the 5-HT_1 receptors, and for 5-HT_{2A} and 5-HT_{2C} receptors (Ullmer *et al.*, 1995). In the present study, we have investigated the profile of 5-HT receptors in coronary resistance arteries using RT-PCR. In addition, we have examined the functional mechanisms mediating contraction to 5-HT in these vessels in the absence and presence of an oxygen scavenger, sodium dithionite.

Pig hearts were collected from the local abattoir and transported to the laboratory in ice-cold physiological saline. The left coronary artery and its branches were dissected. Endothelium denuded ring segments (<300 μM) were mounted in a myograph for measurement of changes in isometric tension. Cummulative concentration-effect curves to 5-HT and sumatriptan ($5\text{-HT}_{1B/1D}$ receptor agonist) were constructed in the absence and presence of the selective 5-HT_2 receptor antagonist ketanserin (1μM) and/or the $5\text{-HT}_{1B/1D}$ receptor antagonist GR127935 (100nM) (Skingle *et al.*, 1993). Responses are expressed as a percentage of the maximum response to 80 mM K^+. Hypoxic conditions were simulated by rapidly switching to ungassed Krebs containing an oxygen scavenger sodium dithionite (1 mM, $Na_2S_2O_4$) (Post *et al.*, 1995). For RT-PCR, the oligonucleotide primers for 5-HT_1 and 5-HT_2 receptors were based on Ullmer *et al.* (1995). RNA was extracted from vascular smooth muscle cells and following reverse transcription at 50°C for 30 min and 35 cycles of PCR (94°C for 30 sec, 45°C for 30 sec and 68°C for 45 sec) the PCR products were separated on a 2% agarose gel.

In oxygenated/control Krebs solution, 5-HT stimulated concentration-dependent contractions (10^{-7} to 10^{-5}, Max. 53.4±1.2%, n=5), which were abolished in the presence of ketanserin (1μM). Concentration–dependent contractions to 5-HT and sumatriptan could be obtained in the presence of ketanserin (1μM) if the segments were prestimulated with 20mM K^+ (10^{-6} to $3x10^{-4}$, Max. 81.3±9.5%, n=6; 10^{-6} to $3x10^{-4}$, Max. 54.3±8.5%, n=6, respectively). These responses were attenuated by inclusion of GR127935 (100nM). In the presence of $Na_2S_2O_4$ (1mM) and ketanserin (1μM), but without 20 mM K^+, 5-HT and sumatriptan each stimulated concentration-dependent contraction ($3x10^{-5}$ to $3x10^{-4}$; Max. 53.0±10.1%, n=6; $3x10^{-5}$ to $3x10^{-4}$; Max. 36.6±6.4%, n=6, respectively). The contraction in the absence of any prestimulation was attenuated by GR127935 (100nM) (10^{-4} to $3x10^{-4}$, Max. 12.2±6.5%, n=6; 10^{-4} to $3x10^{-4}$, Max. 10.7±4.6%, n=6, respectively). RT-PCR products corresponding to the expression of receptor subtype mRNAs were detected for $5\text{-HT}_{1A, 1B, 1D, 1E, 1F, 2A}$ and $_{2C}$ receptors.

In conclusion, our data indicate that in porcine small coronary resistance arteries, 5-HT mediated vasoconstriction occurs via 5-HT_2 and $5\text{-HT}_{1B/1D}$ receptors. In oxygenated/control Krebs' vasoconstriction via the $5\text{-HT}_{1B/1D}$ receptor occurs *only* if the tissue is 'primed'. However, in the presence of an oxygen scavenger, and under conditions of presumed hypoxia, a requirement for prestimulation to elicit a $5\text{-HT}_{1B/1D}$ receptor response is removed. This 'uncovering' of the $5\text{-HT}_{1B/1D}$ receptor mediated response may represent an adaptive mechanism in vascular smooth muscle, which may play an important role in adjustment of vascular tone and blood flow during an hypoxic episode.

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218P POSSIBLE ROLE OF HYPOTHYROIDISM IN THE REDUCTION IN ISCHAEMIA-REPERFUSION ARTRHYTHMIAS IN HEARTS FROM STREPTOZOTOCIN-DIABETIC RATS

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Whether or not ischaemic preconditioning (PC) can be demonstrated in the diabetic heart is unclear (Liu et al., 1993; Tosaki et al., 1996). The aims of this work were to assess the susceptibility of the *ex vivo* diabetic heart to regional ischaemia-perfusion arrhythmias and to determine if ischaemic preconditioning could protect the diabetic heart from these arrythmias. Male S.D. rats (~200g n=9) received streptozotocin (STZ -60 mg kg^{-1} i.p.). Age-matched controls (n = 9) were used. 8 weeks later hearts were isolated under pentobarbitone (60 mg kg^{-1} i.p) anaesthesia, perfused (Krebs, 37°C, 7 ml min^{-1}; Langendorff) and subjected to 30 min occlusion (main left coronary artery) and 30 min reperfusion. Some hearts were subjected to 2 cycles of 3 min occlusion and 3 min reperfusion (PC) immediately before the prolonged ischaemia-reperfusion. In control hearts (serum glucose 7.1±0.3mM) PC reduced the incidence of ventricular tachycardia (VT) (Fisher's exact test control 100%; PC 22 % P<0.01) and abolished ventricular fibrillation (VF) (P<0.001) following subsequent occlusion. STZ-diabetes (serum glucose 35.8±1.8mM) mimicked PC in that during occlusion diabetic hearts showed a significantly reduced incidence of VT (control 100%; diabetic 0%, P<0.001), sustained VF and VF (control 89%; diabetic 0%, P<0.001). *In vivo* studies showed hearts of experimental hypothyroid rats to be resistant to ischaemia-reperfusion arrhythmias (Chess-Williams et al., 1989). As STZ-diabetic rats may be hypothyroid we measured serum free thyroxine (FT_4) concentrations in the STZ rats and compared the effects of STZ with those of 4 week-treatment with the antithyroid drug methimazole (MMI, 0.05% in drinking water). FT_4 values (ng ml^{-1}) were 30.8±1.1, 12.3±1.0 (Student's t-test P<0.001) and 2.4±0.3 (P<0.001) in control, STZ-diabetic and MMI-treated rats, respectively. Hearts from MMI-treated rats showed markedly reduced incidence of VF (control 100%, MMI-treated 0% P<0.001) and total number of arrhythmias (control 2711±956, MMI-treated 472±181, P<0.05). Other similarities between STZ-diabetic and MMI-treated rats were reduced heart rate (control 272±6, STZ-diabetic 232±8, MMI-treated 225±11bpm, P<0.001 each) and reduced body temperature (control 37.0±0.1, diabetic 36.2±0.1, MMI-treated 36.5±0.1°C, P<0.001 each). We hypothesis that the marked reduction in the incidence of VT and VF seen in the streptozotocin-diabetic rat may be due to the secondary hypothyroidism seen in these animals.

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219P COLLAR-INDUCED INTIMAL THICKENING IN THE RABBIT CAROTID ARTERY AFTER LOCAL ADMINISTRATION OF ALBUMIN AND GLYCATED ALBUMIN

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Long-term exposure of proteins to glucose leads to the formation of advanced glycation end products (AGEs). Accumulation of these AGEs in the vessel wall has been implicated in atherogenesis. The aim of this study was to examine the effects of local application of glycated bovine serum albumin (AGE-BSA) on collar-induced intimal hyperplasia, a prerequisite for atherosclerosis in humans.

AGEs were prepared by incubating BSA (50mg/ml) with 1M glucose for 8 weeks in the presence of antibiotics and protease-inhibitors. Glycation was monitored by fluorescence-measurements. Control BSA was incubated in the absence of glucose. After extensive dialysis against phosphate-buffered saline (PBS), samples were stored at -80°C. Concentrations were determined using the Bicinchoninic acid method for protein measurements. A non-occlusive silicone collar was placed around the carotid artery of anaesthetised (sodium pentobarbital, 30 mg/kg, iv.) male New Zealand White rabbits (2.5-3 kg) to induce intimal thickening (De Meyer *et al.*, 1997). Via a catheter attached to an osmotic minipump, BSA (15µg/h) or AGE-BSA (15µg/h) were administered locally to the vessel wall. Vessels with a collar unattached to a minipump, and vessels receiving PBS (5µL/h) were used as controls. After 14 days carotid arteries were removed, divided in segments and either formol-fixed or snap-frozen in liquid nitrogen for histological evaluation.

PBS infusion did not influence intimal hyperplasia (Table 1). Local administration of proteins (BSA or AGE-BSA) caused an increase in intimal thickness as compared to control (collar alone or PBS) vessels (Mann-Whitney U, $p<0.05$). Within the protein-treated vessels AGE-BSA was significantly different from BSA (Table 1). Immunohistochemical evaluation showed that the intima of vessels not treated with protein consisted mainly of smooth muscle cells with few leukocytes, whereas the intima of protein-treated vessels contained high numbers of inflammatory cells. The results were semi-quantitatively evaluated by a scoring system for the number of leukocytes (score 0: none, 1: < 10 cells, 2: 11-30 cells, 3: > 30 cells). There was a strong correlation (Spearman test, $p<0.01$) between intimal thickness and immunoreactivity for CD14 (mainly neutrophils, $r_s=0.58$), CD43 (lymphocytes, $r_s=0.65$) and RAM11 (macrophages, $r_s=0.64$). Chi-square tests ($p<0.05$) showed significant differences between the leukocyte scores of protein-treated vessels and vessels treated with PBS or a collar only, but not between those of BSA- and AGE-BSA-treated vessels. A Sirius Red staining for collagen deposition also showed the presence of more extracellular matrix, especially of the fibrillar type in the protein-treated collars.

Table 1. Collar-induced intimal thickening

	collar	PBS	BSA	AGE-BSA
intima (µm)	35 ± 11	54 ± 19	76 ± 26	151 ± 28*

Results expressed as mean ± s.e.mean; n = 5 for all groups.
* Significantly different from BSA (Mann-Whitney U, $p<0.05$)

It is concluded that administration of native BSA evoked an inflammatory response which promoted intimal thickening. This confirms the growing evidence that inflammation is a major contributor to the development of atherosclerotic lesions. Furthermore, the significant enhancement of intimal hyperplasia with AGE-BSA suggests that glycated proteins provide an additional stimulus for intimal hyperplasia. This model therefore provides a possibility to study the contributing components to this process of vessel injury.

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220P Na^+-H^+ EXCHANGE IN HUMAN PLATELETS EXPOSED TO PROPIONATE

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The rate of change in cell size in response to weak organic acid (using the sodium salt of propionic acid, pH 6.7) provides a measure of Na^+-H^+ exchange activity (Grinstein, Goetz, *et al.*, 1984). This method is relatively simple and has been widely used. It is, however, an indirect measure of Na^+-H^+ exchange activity and could be altered by factors which can influence platelet distensibility independent of Na^+-H^+ exchange activity.

In an attempt to investigate the validity of this method we have measured the rate constant of cell swelling (k_s), intracellular sodium ion concentration (k_{Na}) and cell membrane anisotropy (using the probe, trimethylammonium diphenylhexatriene, TMA-DPH) at 24 °C and 37 °C in platelets taken from healthy normotensive subjects. The rate constant of Na^+ flux ($k_{\phi Na}$) was also calculated using k_{Na} and k_s together with estimated values of the ratio of mean platelet volume at the start (V_o) to that at the finish (V_∞) of the swelling reaction.

Platelet rich plasma was prepared from citrated venous blood and gel-filtered in balanced salt solution. For measurement of membrane fluidity, gel filtered platelets were prepared and adjusted to a protein concentration of 40 µg/ml. Platelets were incubated with 5×10^{-7} mol/l of TMA-DPH for ten minutes. Fluorescence polarisation was measured at 24 °C and 37 °C using a Perkin-Elmer LS-50 spectrofluorimeter. Membrane anisotropy was 0.289 ± 0.002 at 24 °C and 0.262 ± 0.002 at 37 °C, mean ± SEM, $P<0.0001$ by Student's paired t-test. Gel filtered platelets were prepared from nine healthy human subjects. Platelet sodium concentration ($[Na^+]_i$) and platelet swelling were measured using the change in optical density to detect swelling and sodium-binding benzofuran isophthalate (SBFI, a Na^+-sensitive fluorophore) to measure $[Na^+]_i$. Platelet suspension was added to balanced salt solution containing propionic acid (140 mM, pH 6.7) in the presence or absence of Na^+ (140 mM) in the temperature controlled chamber of a dual wavelength Deltascan fluorimeter and optical density and cytoplasmic Na^+ concentration measured for 250 sec in paired samples.

At both temperatures the signals changed exponentially, were absolutely dependent on extracellular Na^+ and were abolished by 5-(N,N-hexamethylene)-amiloride (10 µM). k_s at 24 °C was $7.4\pm0.3 \times 10^{-3}$ sec^{-1} and $9.8\pm0.3 \times 10^{-3}$ sec^{-1} at 37 °C ($P<0.01$). k_{Na} at 24 °C was $14.5\pm0.9 \times 10^{-3}$ sec^{-1} and $13.8\pm0.7 \times 10^{-3}$ sec^{-1} at 37 °C ($P=0.923$). k_{Na} was greater than k_s ($P<0.0001$ at 24 °C, $P<0.01$ at 37 °C). $k_{\phi Na}$ calculated using estimates of V_∞/V_o of 1.5-3.0, consistent with published values was similar to k_s.

The difference in membrane anisotropy confirmed that platelet membranes are more fluid at 37 °C than at 24 °C. The finding that $k_{Na}>k_s$ suggests the possibility that k_s may underestimate the rate of Na^+-H^+ exchange; however correction for volume change gives values of $k_{\phi Na}$ that are comparable with k_s. We conclude that the apparent underestimation of Na^+-H^+ exchange by k_s (by comparison with k_{Na} uncorrected for volume change) is in part accountable by the increase in platelet volume following exposure to propionate.

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221P COMPARISON OF EFFECTS OF GTN ON THE DIGITAL VOLUME AND RADIAL PRESSURE PULSE WAVEFORMS

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The radial pressure (RP) pulse waveform may be measured non-invasively by radial tonometry and the digital volume (DV) pulse by photoplethysmography. Vasodilator drugs such as glyceryl trinitrate (GTN) produce profound changes in both RP (O'Rourke & Gallagher, 1996) and DV waveforms (Morikawa, 1967) at doses which produce only minimal changes in heart rate and blood pressure. Both waveforms have been used to assess haemodynamic effects of GTN but the relationship between them is unknown. Effects of GTN on the RP waveform have been attributed to decreased wave reflection; those on the DV waveform are less well understood. We characterised the relationship between RP and DV waveforms by deriving transfer functions (TFs) which allowed RP to be predicted from DV. 20 healthy normotensive men (aged 24 to 46 yrs) were studied at baseline and after administration of 500 μg GTN s/l. RP and DV were recorded by tonometry (Millar tonometer, Millar, USA) and photoplethysmography (Micro Medical, UK) respectively. Signals from at least 7 waveforms were digitised with a sampling interval of 10ms. Both waveforms were calibrated to brachial artery pulse pressure (oscillometric method). Individual TFs were calculated for each subject at baseline and after GTN using fast Fourier transforms. Generalised TFs for baseline conditions and after GTN were calculated by averaging individualised TFs for all subjects at baseline and 3 min after GTN when its effects were maximal. A generalised TF (GTF) for all conditions was obtained by averaging all individual TFs. The generalised TF obtained from baseline data did not differ significantly from that obtained after GTN (figure 1). The GTF allowed prediction of the RP waveform from the DV waveform with a root mean square error, for all digitised time points, for all subjects at baseline and after GTN of 4±2mmHg (mean±SD). GTN produced a fall in height of the inflection point in the DV waveform of 21.9±1.2% (of waveform amplitude). The fall in the corresponding point of the RP waveform was 12.3±1.3% and was predicted by the fall (13.76±0.82%) derived from the RP waveform predicted from the DV waveform (using the GTF). In healthy men the DV waveform contains similar information to the RP waveform and effects of GTN on both waveforms are similar.

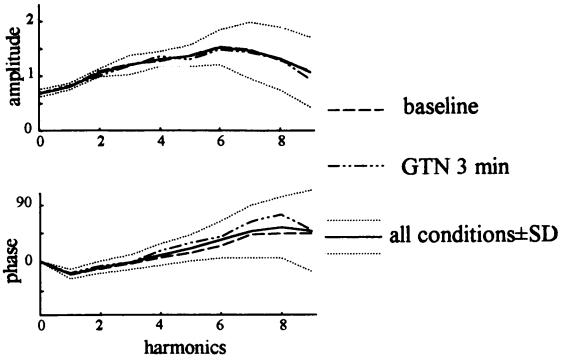

Figure 1 Amplitude and phase of transfer functions obtained at baseline, after GTN and under all conditions.

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222P ROLE OF TYROSINE KINASE IN MEDIATING NORADRENALINE-INDUCED CONTRACTIONS IN HUMAN PROSTATE

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Alpha$_1$-adrenoceptors have been characterised into 3 subtypes (α1A, α1B & α1D). All appear to be G-protein linked to calcium mobilization via the activation of phospholipase C. It has recently become apparent that many G-protein linked receptors can also activate the tyrosine kinase/MAP kinase signalling pathway (Wan et al., 1996). The present study was performed to determine if activation of the tyrosine kinase/MAP kinase pathway plays a role in the α-adrenoceptor induced contraction of the human prostate.

Samples of human prostate were obtained from patients (aged 60-75yr) undergoing transurethral resection of the prostate for benign prostatic hyperplasia. Muscle strips were suspended under 1g tension in Krebs-bicarbonate solution at 37°C and gassed with 5%CO2 in oxygen. Two cumulative concentration-response curves to noradrenaline were obtained on each tissue in the presence of corticosterone (10μM), cocaine (10μM) and propranolol (1μM). Following the initial curve, tissues were washed for 1hr and a second noradrenaline curve constructed in the presence of the test drug. In some tissues responses to KCl (100mM) were also obtained.

Noradrenaline produced a slowly developing tonic contraction, with no phasic component. Nifedipine (1μM) reduced significantly (Students t-test, $P<0.05$, n=14) the maximal response to noradrenaline to 57.4±7.4% of the initial control maximum. The tyrosine kinase inhibitor, genistein (30μM) also significantly ($P<0.05$) reduced the maximal response to noradrenaline to 56.2±3.5% (n=12). Nifedipine and genistein together caused a reduction in the maximal response to noradrenaline to 34.7±7.4% (n=7), which was a significantly ($P<0.05$) greater reduction than either agent alone. The MAP kinase inhibitor PD98059 (50μM) did not reduce the maximal response to noradrenaline (130±19.6% of the initial maximum), but maximal responses to noradrenaline were reduced ($P<0.05$) to 49.0±8.0% by ryanodine (10μM).

The tyrosine kinase activation would appear to be involved in the α-adrenoceptor mediated contraction of human prostate as the response to noradrenaline is partialy inhibited by the tyrosine kinase inhibitor genistein. There does not appear to be activation of the MAP kinase signalling pathway as the MAP kinase inhibitor PD98059 did not inhibit responses.

The maximal response to noradrenaline in the human prostate was only 48.3±2.4% of that to KCl (n=19), suggesting that extracellular calcium influx is not fully activated following stimulation by noradrenaline. Smooth muscle contraction is dependent on calcium mobilization. In the human prostate the inhibition by both nifedipine and ryanodine indicates that noradrenaline mediated contractions utilize both internal and external calcium stores. The additive effects of genistein with nifedipine indicates tyrosine kinase activation mobilizes internal stores. However in some tissues α-adrenoceptor activation of tyrosine kinase has been shown to induce contraction by an action on a capacitance calcium influx which is insensitive to nifedipine (Burt et al., 1995). It is unclear what role, if any, these currents play in contraction of human prostate. The present data suggest that additional sources of calcium may be involved following α-adrenoceptor activation with noradrenaline, since nifedipine and genistein together failed to completely abolish responses to noradrenaline.

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223P NITRIC OXIDE MEDIATES RELAXATION OF RABBIT CORPUS CAVERNOSUM THROUGH ACTIVATION OF cGMP-DEPENDENT PROTEIN KINASES

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Cyclic GMP-dependant kinase (PKG) is postulated to be a key mediator of nitric oxide (NO) induced relaxation of smooth muscle (Sekhar *et al,* 1992). There are three known isozymes of PKG - Iα, Iβ and II (Francis and Corbin, 1994). In this study, we have investigated the effects of selected cyclic nucleotide analogues (CNA) on rabbit corpus cavernosal smooth muscle (CC) and isolated bovine PKG Iα enzyme. The effects of the PKG Iα/Iβ inhibitor, 8-bromo-β-phenyl-1,N^2-ethenoguanosine-3,5-cyclicmonophosphorothioate ((Rp)-8-Br-PET-cGMPS) on the CNA, PET-cGMP- and NO-mediated relaxation (induced by the NO donor sodium nitroprusside (SNP) or electrical field stimulation (EFS)) were also investigated.

Male New Zealand White rabbits (2.5-3.0kg) were killed by cervical dislocation and the CC removed by dissection. Tissues were mounted in organ baths perfused with physiological Krebs buffer (containing 5μM guanethidine and 1μM atropine for EFS studies) at 37°C and gassed with 95%O_2/5%CO_2. For CNA studies, tissues were pre-contracted with phenylephrine (10μM) and cumulative concentration response curves constructed with the CNA. For PKG inhibitor studies, tissues were pre-incubated with (Rp)-8-PET-cGMPS prior to contraction with phenylephrine (10μM). The effects of PET-cGMP, SNP or EFS (45-50 volts, 0.2msec pulse width, 10sec train duration at stimulation frequencies of 1, 2, 4, 8, and 16Hz) on tissue tone were subsequently determined. Activation of PKG Iα by CNA was determined by radiometric measurement of increasing phosphorylation of peptide substrate (RKISASEF) in the presence of 33P-γ-ATP. Reactions were initiated by addition of PKG Iα. Statistical analysis was performed using Students t-tests.

CNA's tested induced complete, concentration dependant relaxation of pre-contracted CC and activation of isolated PKG Iα (Table 1).

Table 1	Relaxation of Rabbit CC		Activation of PKG 1α	
CNA	IC_{50} (μM)	SEM	EC_{50} (nM)	SEM
PET-cGMP	12.0	1.2	17	4.0
8-Br-cGMP	89.7	25.3	21	2.0
8p-CPT-cGMP	50.9	15.7	68	5.0
8-Br-cAMP	731.0*	63.0	6μM*	0.4

(n=3 for all data points; * P<0.01 versus all other compounds)

(Rp)-8-Br-PET-cGMPS (300μM) significantly inhibited (P<0.05) relaxation responses induced by PET-cGMP (EC_{50} increased from 8.1μM ±0.4 to 181.6μM ±31.0), SNP (4.3μM ±0.4 to 43.8μM ±12.0) and EFS (relaxation response observed at 8 Hz reduced from 57% ± 6 to 30% ± 4).

Our results show that cGMP-CNA induce relaxation of pre-contracted rabbit CC and activation of isolated PKG Iα, whilst the activity of cAMP-CNA is significantly lower* (P<0.01). These data indicate that activation of PKG I in rabbit CC induces smooth muscle relaxation. However, the CNA's tested do not show sufficient selectivity for individual PKG I isozymes (Sekhar *et al,* 1992) to enable their relative importance to be determined. We have also shown that an inhibitor of PKG Iα and Iβ attenuates NO-mediated relaxation in rabbit CC. This confirms the role of PKG I activation in NO-mediated relaxation of CC smooth muscle.

In conclusion, the data are consistent with the proposal that PKG is a key mediator of NO/cGMP dependant relaxation of CC and penile erection.

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224P EFFECTS OF SUPEROXIDE GENERATION ON NITRIC OXIDE-MEDIATED RELAXATION RESPONSES IN RABBIT CORPUS CAVERNOSUM

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The key physiological mediator of corpus cavernosal smooth muscle relaxation is nitric oxide (NO; Burnett *et al.*, 1992). Superoxide anions are thought to be involved in the inactivation of NO (Gryglewski *et al.*, 1986). The aim of these studies were to determine if superoxide, generated by the metabolism of xanthine (X), catalysed by xanthine oxidase (XO), inhibits NO-dependent relaxation of corpus cavernosum induced by acetylcholine (ACh) and electrical field stimulation (EFS).

New Zealand white rabbits were killed by cervical dislocation. Strips of corpus cavernosum were mounted in organ baths and perfused with Krebs buffer containing atropine (1μM) and guanethidine (5μM) for the EFS studies and indomethacin (5μM) for the ACh studies. The tissues were pre-contracted with phenylephrine (10μM) and the relaxation responses to ACh (3nM-3μM) and to EFS (1-16Hz, 45-50mV; 0.2 msec pulse width delivered as 10 sec train) determined in the presence of X (100μM) and XO (100mU/ml), either alone, or in combination with diethyldithiocarbamate (DETCA 3mM; an inhibitor of the Zn/Cu superoxide dismutase (SOD) enzyme).

ACh induced transient, concentration-dependent, relaxation of phenylephrine contracted tissue. These relaxation responses were unaffected by X and XO or DETCA alone (data not shown), but in combination they inhibited the relaxation (Table 1). The inhibitory effects of DETCA, X and XO on ACh-induced relaxation were reversed by the addition of SOD (250U/ml) and the XO inhibitor 2-(3-cyano-4-isobutoxyphenyl) -4-methyl-5-thiazole carboxylic acid (TEI-6720; 100nM; Table 1). EFS induced transient, frequency dependent, relaxation of phenylephrine contracted tissues. In the presence of DETCA, X and XO the relaxation responses at 4, 8 & 16Hz were inhibited by 37, 33 and 26% compared to DETCA alone.

Superoxide, generated by the metabolism of X by XO, inhibits both endothelial and neuronal NO-mediated relaxation responses in rabbit corpus cavernosum only when an inhibitor of SOD is present. We therefore postulate that an elevation in XO activity, in conjunction with a reduction of SOD activity, may lead to an increase in superoxide levels in the corpus cavernosum. This may in turn result in impairment of NO-mediated smooth muscle cell relaxation and erectile function.

Table 1. Impairment of relaxation induced by ACh (100nM) in rabbit corpus cavernosum by DETCA, X and XO and its reversal by TEI6720 and SOD. *Results are % relaxation expressed as a geometric mean with 95% confidence intervals (CI; n=3-18; *P<0.01 versus Control [Student's T-test])*

	Control	DETCA	TEI6720	SOD, X & XO
	(DETCA)	X & XO	DETCA, X & XO	DETCA
relaxation	68	34*	62	72
95% CI	59-77	26-46	35 -107	50 - 103

Burnett,A.L. *et al.*, (1992) Science, **257**, 401-403
Gryglewski,R.J. *et al.*, (1986) Nature, **320**, 454-456.

225P INVESTIGATIONS OF THE BLADDER VERSUS CARDIOVASCULAR SELECTIVITY OF TIROPRAMIDE *IN VITRO* AND *IN VIVO*

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Tiropramide (TP) is a broad spectrum antispasmodic, used in the treatment of irritable bowel syndrome (Merck Index, 1996), which has been reported to have therapeutic potential in urinary incontinence (Morita *et al.*, 1992). A series of *in vitro* and *in vivo* pharmacological investigations were undertaken to examine further the possibility reported in the literature (Setnikar *et al.*, 1989) that TP may show selectivity for bladder over vascular smooth muscle.

Tissues for *in vitro* studies were removed from rats (Male, SD, Charles River, 250-350g). Bladders were halved vertically. Aortas were cut into 1-2mm rings. Portal veins were used whole. Each tissue was set up in Krebs-Hensleit solution (37°C, gassed with 95% O_2/5%CO_2) and under 1g tension. The antispasmodic actions of TP (1-100mM) were investigated by cumulative addition to bladder and aorta contracted with 60mM KCl. The effects of TP were similarly determined against spontaneous contractions of rat portal vein (RPV).

For *in vivo* studies rats (Female, SD, Charles River, 200-300g) were anaesthetised (0.5ml.100g^{-1} *ip*, 25% w/v urethane). The carotid artery and the external jugular vein were cannulated with polyethylene cannulae for arterial blood pressure (BP) monitoring and drug administration. The ureters were cut and tied and the bladder was catheterised via the urethra. A bipolar electrode (Harvard design) was applied to the pelvic nerve (PN) fibres and connected to a stimulator. The bladder was filled with saline (0.9% w/v; 21°C) until there was a constant internal basal pressure of 10 mmHg. Body temperature was maintained at 37°C. The PN was stimulated (25V, 20Hz, 0.5 ms for 10 sec) every 15min. Increasing doses of TP or cromakalim were administered *iv* at 15 minute intervals.

TP inhibited KCl induced contractions of the isolated rat bladder and aorta (IC_{50}s=28±1 and 58±7μM) and spontaneously contracting RPV (IC_{50}=23±6mM). All tissues n=4. TP (0.3-10mg.kg-1 iv, n=6-7) had no effect on BP, or on spontaneous or PN stimulated bladder contractions *in vivo*. However, TP was associated with a dose related decrease in heart rate (maximum 19%) with a recovery to baseline rate 15 minutes post dose, (cromakalim inhibited blood pressure and spontaneous and neurostimulated bladder contractions in the same model with ED_{50}s of 29.17±5, 84.77±14.4 and 192.13±10 μg.kg^{-1} *iv*).

TP relaxes rat bladder smooth muscle *in vitro* with a small degree of selectivity over rat aorta but not RPV. No evidence of bladder relaxant or blood pressure activity was detected *in vivo*. The present results do not support the claims of bladder activity or selectivity of TP and question the utility of TP for treating urinary incontinence.

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226P EXPRESSION OF NEUROMEDIN U RECEPTORS IN THE RAT UTERUS AFTER CHRONIC TREATMENT WITH GONADAL STEROID HORMONES

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Neuromedin U (NmU) is a 23 amino acid regulatory peptide characterised by its potent contractile effect on the uterus (Minamino et al., 1985). We have previously demonstrated and characterised specific binding sites for NmU on rat uterine membranes (Nandha et al. 1993). The IC_{50} for binding in uterine membranes (0.35 nM, Nandha et al., 1993) is similar to the EC_{50} for contraction in the isolated rat uterus (0.2 nM, Domin et al., 1989). We propose a role for NmU in uterine contraction/parturition and have examined the effect of 14 day steroid treatment on NmU receptor expression in the rat as a model of the effects of the 2-3 weeks of gonadal steroid action in pregnancy.

Female Wistar rats (groups of 20 intact or ovariectomised, OVX) weighing 200-250g were treated with daily subcutaneous injection of oestradiol (E_2, 2.5 μg) progesterone (P, 2.5 mg), mifepristone (RU486, 2 mg) or combinations for 14 days. Membranes were prepared by differential centrifugation and ^{125}I-NmU binding assays performed as previously described (Nandha et al., 1993). Saturation or competition curves generated were analysed by non-linear regression (Receptor-Fit, Lundon Software, Cleveland, Ohio, USA) to calculate receptor density (Bmax, pmol/mg protein n=4 where n is a membrane preparation from five uteri). Statistical significance was analysed using one-way ANOVA and post hoc Tukey's test.

Study 1: Intact rats. Neither P nor E_2 treatment alone changed NmU receptor density (Control, 0.54 ± 0.08 vs P, 0.64 ± 0.05 or E_2, 0.71 ± 0.11). Mifepristone caused a significant increase in receptor density (Mifepristone, 1.17 ± 0.18, P=0.04 vs control). Mifepristone in combination with P or E_2 also increased uterine NmU receptor density (mifepristone + P, 1.36 ± 0.19, mifepristone + E_2, 1.50 ± 0.15, P<0.005 vs control for both).
Study 2: OVX rats. OVX caused a large decrease in NmU receptor density (Sham operated control, SHC, 0.87 ± 0.10 vs OVX, 0.29 ± 0.04, P<0.003). OVX + E_2 caused a reversal of this effect with increased receptor density (OVX + E_2, 1.26 ± 0.13, P = 0.03 vs SHC). However, neither P nor mifepristone showed this effect (OVX + P, 0.30 ± 0.02, OVX + mifepristone, 0.22 ± 0.01, both P<0.003 vs SHC).

Chronic treatment with the antiprogestin mifepristone caused a 2-fold increase in NmU receptor expression whereas OVX caused a 60% decrease in receptor density which could be reversed by E_2 treatment. Thus NmU receptor expression in this tissue appears to be oestrogen dependent but may also be modulated by progesterone or a progestin-induced factor. Further work is required to determine a direct role of NmU in parturition.

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Minamino N. *et al.* (1985) *Biochem. Biophys. Res. Commun.* 130, 1078-1085.
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227P AN INVESTIGATION INTO THE BLADDER RESPONSES INDUCED VIA PELVIC NERVE STIMULATION IN THE ANAESTHETISED RAT

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Identification of a robust model to investigate the effects of drugs on bladder function *in vivo* has proved very difficult. One of the best methods for mimicking a normal physiological response is to stimulate the pelvic nerve (PN) and record bladder pressure (BLP) changes (Diederichs *et al.*,1992). This study investigates the pharmacology of the neurally-stimulated rat bladder response.

Experiments were performed on urethane anaesthetised (0.5ml.100g^{-1} *ip*, 25% w/v) female rats, (CD, Charles River, 200-300g), which were artificially ventilated. The animals were instrumented for BLP recording as previously described (Diederichs et al 1992). Body temperature was maintained at 37°C. The PN was stimulated (25 V, 20 Hz, 0.5 ms for 10 s) every 15 min and the area under the curve (AUC) for the contractile response measured. For frequency response experiments a 10 min interval with a minimum exposure time of 10 min was used for atropine blockade. Four stimulation cycles were performed prior to compound administration (via external jugular vein) with the fourth taken as the control reading. Drug-evoked responses were expressed as percentage changes from control reading. All values are means ± s.e.mean. At the end of each experiment, xylocaine was applied to the nerve bundle and a further stimulation was administered to verify direct smooth muscle stimulation had not occurred.

The BLP changes following PN stimulation were reproducible throughout the duration of the control experiments (4h). The BLP profile following PN stimulation was biphasic consisting of a phasic and a tonic component. In the presence of atropine (0.3-1mg kg^{-1} *iv*) a dose related decrease in AUC was observed (33 ± 2.86%@1mg kg^{-1}, n=4). Muscarinic blockade by atropine inhibited the tonic but not the phasic component. The phasic response was abolished by administration of alpha,beta-methylene ATP (α-β-mATP). Thus combined pre-treatment with atropine (1mg kg^{-1} *iv*) and α-β-mATP (0.3mg kg^{-1} *iv*) gave 93.4 ± 4.1% (n=4) block of PN stimulation. Frequency/response data show a frequency dependent increase in AUC. The relative contribution of the cholinergic component to the AUC increased with frequency (data expressed as a % maximum frequency response at 30 Hz). At 20 Hz control AUC was 93.3 ± 1.8%, after atropine treatment (1mg kg^{-1} *iv*) the response was down to 26.9 ± 3.1% (n=4), whereas at 3 Hz control response was 25.8 ± 4.2% and after atropine (1mg kg^{-1} *iv*) was 20.4 ± 3.1% (n=4)

In conclusion these results show that the contractile response of rat bladder to PN stimulation *in vivo* consists of a phasic purinergic component predominating at lower frequencies followed by a tonic, cholinergic component predominating at higher frequencies. The model described can be used to investigate the actions of drugs which modulate efferent neuronal drive to the bladder.

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228P EFFECTS OF DIFFERENT STRESS STIMULI ON PARAVENTRICULAR THALAMIC NEURONE DISCHARGE RATES

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The endocrine responses to chronic stress habituate with repeated exposure to the same stimuli (homotypic stressor), but are normal or exaggerated in response to a novel (heterotypic) stressor (Akana *et al.*, 1996). The neuroanatomical mechanisms underlying facilitation of stress responding remains elusive; however, recent data show that heterotypic, but not homotypic, stress exposure selectively increases *cfos* expression in the parvocellular paraventricular thalamus (PvThal) (Bhatnagar and Dallman, in press). As yet, we do not know if stress activates or inhibits PvThal cell activity. In the present study we have examined basal single cell activity from PvThal neurons, and measured their responses to different stressful stimuli. Adult male, Lister hooded rats (350-550g) served as subjects. Under isoflurane anesthesia, rats were implanted with jugular cannulae for delivery of i.v. urethane (0.8g/ml) anesthesia. A hippocampal recording electrode was positioned in the stratum moleculare (A-P -3.3; M-L +2.5; D-V 2.5mm) to record theta activity, and PvThal cells (A-P -4.0; M-L + 0.3; D-V 4-5.5mm) were recorded with glass micropipettes (1-3 MΩ) backfilled with 2M sodium acetate/direct blue dye. Baseline recordings of spontaneous cell firing were obtained over 5 sec, followed by an assessment of cell reactivity to tail pinch or laparotomy. Tail pinch (TP) stress consisted of a 5 sec stimulus applied with a haemostat, sufficient to activate hippocampal theta, a measure of arousal (Smythe, 1992). Laparotomy (LAP) stress consisted of a small incision through the skin into the underlying muscle (Bonaz *et al*, 1997). Independent t-tests were used tocompare basal discharge rates with those in response to TP and LAP applying a randomization process (Edgington, 1987).

A total of 12 PvThal cells were recorded in 11 rats. Table I shows the response of the neurons catagorized on the basis of sensitivity to TP, and Table II shows the response to LAP.

Table I. Cell discharge rates (spikes/sec) of PvThal neurons in response to tail pinch. Means ±sem are shown.

		TAIL PINCH	
NUMBER	**Basal**	**Sensitive**	**Insensitive**
8	7.3±1.7	15.4±2.7*	----------
4	3.5±1.9	----------	2.3±1.7

*significantly different from basal condition (P<.05).

Table II. Cell discharge rates (spikes/sec) of PvThal neurons in response to tail pinch and laparotomy. Means ±sem are shown.

CONDITION	**RATE**	**N**
Basal	8.4±3.1	4
Tail Pinch	16.7±5.0*	
Laparotomy	2.5±1§	

*significantly different from basal condition (P<.05).
§significantly different from basal and tail pinch (P<.05).

These data show that PvThal cell activity responds differentially to various stressors. How these responses relate to stress facilitation or habituation remains to be elucidated.

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Smythe, J.W.(1992) *Neurosci.BioBehav. Rev.*,16: 289-308.

229P AGED, COGNITIVELY-IMPAIRED RATS HAVE REDUCED PLASMA TOTAL CHOLESTEROL LEVELS

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Ageing in humans is often associated with mental decline, consisting of poor short-term memory, cognitive deficits, and confusion (Fibiger, 1991). These are hallmarks of Alzheimer's disease (AD), which has many neuropathological correlates including impaired lipid transportation and uptake (Hartmann *et al.*, 1994). Evidence shows that altered lipid transport influences neuronal membrane content of cholesterol (Mason *et al.*, 1992). Metabolic disturbances of cholesterol synthesis are related to cognitive decline (Benton, 1995), and we decided to investigate whether or not aged, cognitively-impaired rats might express differences in cholesterol levels compared to normal animals.

Aged (18-22 mo), Lister hooded rats (350-650 g; n=19; mixed sex) were tested in a water maze designed to assess cognitive abilities. Rats were given 4 trials of training to locate a submerged platform, with each trial limited to a 60 sec maximum. Four days later, blood samples were obtained from a small incision made in the distal region of the tail, and plasma glucose levels assessed with a glucometer, while cholesterol levels were measured using a test kit (Sigma). Data (latencies to locate the platform, swim speeds, plasma cholesterol and glucose, and body weights) were assessed by ANOVA and Bonferroni t-tests. Animals were designated as impaired (n=13) if they failed on 3 or more trials to locate the platform. All other rats were designated as unimpaired (n=6). Initial screening of cognitive performance revealed two separate groups. Performance of each group is depicted in Figure 1A. Age-impaired rats showed little ability to locate the platform, while age-unimpaired rats exhibited relatively low latencies with $F(1, 17)=30.7$, $P<.001$. However, there were no differences in either swim speeds or body weights that might have confounded performance in these groupings. Total plasma cholesterol levels were significantly reduced in the age-impaired rats compared to the age-unimpaired rats ($P<.05$), although blood levels of glucose were similar. These data are illustrated in Figure 1B and 1C.

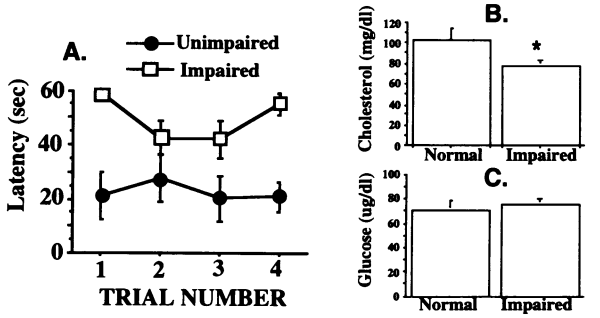

Figure 1. A. Aged rats screened in a water maze separate into impaired and unimpaired groups. B. and C. Plasma cholesterol and glucose levels in aged rats in relation to cognitive performance. Means ±SEM are shown.
*significantly different from normal, aged rats ($P<.05$).

Aged, cognitively impaired rats have lower plasma cholesterol levels than do normal cohorts. Assuming this is functionally related to impaired cognition, augmenting cholesterol may be a relevant therapeutic target for age-related mental decline.

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230P EFFECTS OF THYROXINE ON COGNITIVE FUNCTION AND BRAIN CHOLINESTERASE ACTIVITY

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Recent data show that neonatal thyroid hormone treatment increases hippocampal cholinergic innervation (Schwegler, 1995). In previous reports, we showed that thyroid hormones, administered acutely, enhanced cognitive performance in a water maze task (Shirley *et al.*, 1998), and reduced deficits induced by scopolamine (Smith *et al.*, 1998). In the present study we have examined the effects of chronic thyroxine (T4; tetra-iodothyronine) on cognitive impairments induced by scopolamine, and on brain cholinesterase levels, a measure of cholinergic activity.

Adult male, Lister hooded rats (350-500 g) were injected IP with vehicle (VEH; physiological saline+5% ethanol), 5 or 10.0 mg/kg T4, twice weekly for 4 weeks. On the 5th week rats were given VEH or 1 mg/kg scopolamine, 40 min prior to testing in a water maze (n=7-9/group). Rats were given 4 trials of training to locate a hidden platform, with each trial limited to 60 seconds. Following testing, rats were killed, and frontal cortex and hippocampal areas were dissected out for cholinesterase assay (Sigma Kits). Values were compared with a group of rats acutely injected with T4, at the doses above, and killed one week later. All data (average latencies to locate the platform, and cholinesterase activity) were assessed by ANOVA while *post hoc* testing was performed using Bonferroni corrected t-tests.

ANOVA revealed main effects of T4 and scopolamine on spatial ability with $F(2,42)=4.2$, $P<.05$ and $F(1, 42)=13.5$, $P<.05$, respectively. As shown in Fig. 1A, T4 improved performance an effect most pronounced in the scopolamine-treated rats. Fig. 1B shows the results of the cholinesterase assay. ANOVA revealed significant effects of acute and chronic T4 treatment in the hippocampus with $F(2,54)=3.4$, $P<.05$ and acute effects $F(2,54)=3.5$, $P<.05$ for the frontal cortex.

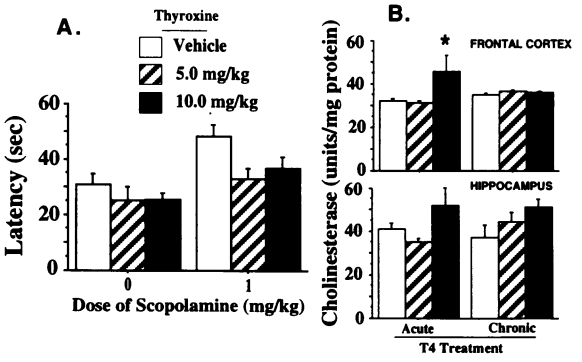

Figure 1. A. Effects of T4 on latencies to locate a hidden platform in a water maze following VEH or scopolamine. B. The effects of T4 on cholinesterase activity in two brain regions. Means ± sem are shown.
*significantly different from VEH group ($P<.05$).

In conclusion, thyroid hormone administration in adulthood improves cognitive performance in rats and this is coincident with increased cholinesterase activity, a marker of cholinergic function. These data are in agreement with those showing that neonatal administration of T4 increases hippocampal cholinergic innervation (Schwegler, 1995).

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231P THE EFFECT OF QUINPIROLE IN A MURINE PLACE CONDITIONING PARADIGM

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In a study investigating the role of dopamine (D_2) receptors in the reinforcing effect of cocaine (Kivastik *et al.*, 1996) it was concluded that a low dose of quinpirole failed to attenuate the conditioned place preference (CPP) response to cocaine in the rat in the absence of an effect *per se*. In an earlier CPP study in the rat (White *et al.*, 1991), the effects of quinpirole were weak and inconclusive over a narrow range of doses. Preliminary findings in our laboratory suggested that quinpirole may affect place conditioning in mice therefore in the present study a wide range of doses was examined in a murine CPP paradigm.

Male adult BKW mice (30-47g) were used. CPP was assessed in a three-chambered apparatus using a method described in detail elsewhere (Ali & Kelly, 1997). Briefly, baseline preferences were calculated by allowing each subject access to the entire apparatus on three separate occasions. The time spent in each outer chamber was recorded over 15min and the mean of the three sessions was taken as the pre-conditioning time (mean±sem; s). For the conditioning phase of the experiment mice (n=10-27) received saline or quinpirole (0.003, 0.01, 0.03, 0.3 and 3.0mg/kg, s.c.) and were placed individually in one of the two outer chambers for 30min. On alternate days they received the other treatment and were confined to the opposing chamber such that each mouse received 4 quinpirole and 4 saline pairings, counterbalanced to the preferred and non-preferred chamber. 24h after the final conditioning trial, mice were tested for 15min as in the pre-conditioning phase in a drug-free state. Differences in the time spent in the drug-paired chamber pre- and post-conditioning were analysed by 2-way ANOVA followed by post-hoc t-test analysis.

Saline/saline conditioning failed to affect the preference response (283±10 s to 258±25 s pre- v post-conditioning). Similarly, the lowest dose of quinpirole (0.003mg/kg s.c.) failed to affect preference (273±13 s compared to 259±33 s). However quinpirole (0.01, 0.03, 0.3 and 3mg/kg, s.c.) significantly reduced the time spent in the drug-paired chamber (311±15 s reduced to 199±27 s $p<0.01$; 288±7 s reduced to 204±18 s $p<0.01$; 287±14 s reduced to 210±18 s $p<0.05$; 278±10 s reduced to 210±20 s $p<0.05$ respectively).

The behavioural changes described here may reflect the hypolocomotion phase of the biphasic profile of effect reported following the acute administration of quinpirole (Eilam & Szechtman, 1989) whilst the behavioural sensitisation which occurs following repeated treatment with quinpirole may have been avoided by the design of the conditioning experiments (Szechtman *et al.*, 1994). Thus in the present study quinpirole induced a place aversion over a range of doses which may be expected to reflect both a pre- and post-synaptic action of the drug (Cory-Slechta *et al.*, 1996) and this warrants further investigation.

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232P REDUCTION OF L-DOPA-INDUCED DYSKINESIAS FOLLOWING INTRAVENTRICULAR ADMINISTRATION OF GDNF IN COMMON MARMOSETS

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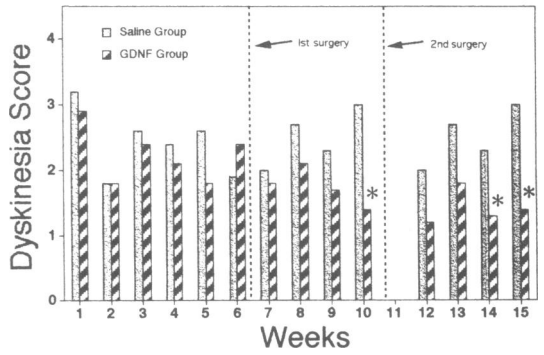
Long-term treatment of Parkinson's disease with L-DOPA is associated with the development dyskinesias. Similarly, chronic L-DOPA treatment of MPTP treated primates produce dyskinesia. The pathophysiology of dyskinesia is not understood, but it is thought to involve an imbalance between striatal output pathways. Glial cell line derived neurotrophic factor (GDNF) promotes the survival and regrowth of dopamine neurons in vitro and in vivo (see Lapchak et al., 1996 for review) and may alter the activity of the striatal output pathways (Iravani et al. *this meeting*). The aim of this investigation was to study the effects of intracerebroventricular (i.c.v.) adminstration of GDNF on L-DOPA induced dyskinesia in common marmosets.

Adult common marmosets of either sex (*Callithrix jacchus*, n=8, 280-380 g) were MPTP-treated once daily (2mg.kg^{-1}, s.c.) for 5 days, resulting in stable parkinsonian state. After 8 weeks of recovery, animals were primed with a combination of L-DOPA / carbidopa (12.5 mg.kg^{-1}, p.o.) for 28 consecutive days, until the animals readily exhibited dyskinesia upon subsequent L-DOPA challanges. The degree of motor dysfunction was assessed on a visible disability score (0-16) and a dyskinesia score (0-4) (Pearce et al., 1995). Animals were divided into two groups, each receiving two 5 μl doses of either saline (n=4) or GDNF (100 μg; n=4; Amgen) i.c.v. 4 weeks apart, under Safan anaesthesia. Disability and dyskinesias were scored after L-DOPA adminstration, weekly, for 4 weeks before, after the 1st and after the 2nd i.c.v. Injections. The data were analysed using Wilcoxon's signed-rank test.

Following daily L-DOPA adminstration, both animal groups developed marked degrees of dyskinesia (Figure 1). Up to 3 weeks after the first injection of GDNF, L-DOPA induced dyskinesia was not significantly reduced, whereas there was a significant reduction in the disability scores compared to saline treated group by the second week. Four weeks after GDNF, there was a significant reduction of dyskinesia score, compared to saline group (Figure 1). The second adminstration of GDNF,

Figure 1. The effectt of GDNF on dyskinesia score in MPTP treted marmosets chronically-treated with L-DOPA. Each bar represents mean dyskinesia score over 240 min. of L-DOPA treatment. * P<0.05, Wilcoxon's signed rank test, n=4.



did not result in further reduction in disability, but significantly reduced the L-DOPA induced dyskinesia score after 2 weeks.

The results of this study indicate that i.c.v. injection of GDNF may be beneficial in ameliorating the side effects of long-term L-DOPA therapy. The physiological basis of this may be the restoration of nigral dopamine neurons or striatal output pathways.

Lapchak, P.A, Miller, P.J., Shoushu, J. et al. (1996) *Neurodegeneration* **5**,197-205.
Pearce, R.K.B., Jackson, M. Smith, L. et al. (1995) *Mov. Disorders* **6**, 731-740.

233P GDNF-INDUCED PROLIFERATION OF NIGRAL DOPAMINE NEURONS AND REDUCTION OF STRIATAL EXPRESSION OF PREPROENKEPHALIN mRNA IN MPTP-TREATED, L-DOPA-PRIMED COMMON MARMOSETS

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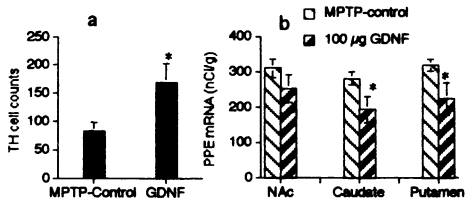
Destruction of the nigrostriatal pathway in MPTP treated primates lead to an imbalance between the direct and indirect output pathways . Thus preproenkephalin (PPE) mRNA is increased (indirect pathway) and preprotachykinin (PPT) mRNA is decreased (direct pathway; Jolkonnen et al., 1995). Chronic L-DOPA treatment normalises the decrease in PPT mRNA but does not affect the PPE mRNA. Glial cell line-derived neurotrophic factor (GDNF) rescues nigral dopamine neurons and also ameliorates motor deficits in MPTP-treated primates. We now report that GDNF influences the expression of PPE mRNA as well as reversing the loss of tyrosine hydroxylase positive cells produced by MPTP treatment of common marmosets displaying dyskinesia following chronic exposure to L-DOPA.

Adult common marmosets of either sex (*Callithrix jacchus*, n=8, 280-380 g) were MPTP-treated once daily (2mg.kg^{-1}, s.c.) for 5 days, resulting in stable motor deficits. After 8 weeks period of recovery, animals were primed with a combination of L-DOPA (12.5 mg.kg^{-1}) / carbidopa (12.5 mg.kg^{-1}) for 28 consecutive days, until the animals displayed L-DOPA-induced dyskinesia. Animals were divided into two groups, receiving two doses of either 5 μl saline or GDNF (100 μg; Amgen) intraventricularly 28 days apart under Safan anaesthesia. Twenty eight days following the last injection, animals were killed by pentobarbitone overdose. Brains were removed and divided into fore- and midbrain. The forebrains containing the striatum (caudate, putamen and nucleus accumbens) were flash frozen, 15 μm coronal sections were incubated with ^{35}S-labelled oligonucleotide probe for human PPE-A or PPT (Jolkkonen et al.,1995). Nonspecific hybridisation was carried out with excess unlabelled oligonucleotide. 30 μm midbrain coronal slices containing the SN were processed for TH-immunocytochemistry. Quantitative evaluation of autoradiograms or TH+ve cells were performed by computerised image analysis (MCID, Imaging Research Inc.).

Following GDNF treatment, there was a significant increase in the number of TH+ve cells in the nigra (figure 1a). GDNF treatment reduced the striatal PPE m RNA expression in both the

Figure 1



* p< 0.05, One-way ANOVA, n=4

caudate and the putamen, (figure 1b). The expression of the PPT mRNA was also decreased in both the caudate and the putamen by 39.5 ± 16% and 33 ± 20 % respectively following GDNF treatment.

Reduction of PPE mRNA, suggests GDNF may be partly acting on indirect striatal output pathway. Whether this effect is direct or mediated through the restoration of nigral dopamine cells remains to be further investigated.

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234P EFFECT OF THE PUTATIVE DOPAMINE D_3 RECEPTOR ANTAGONIST U99194 ON LOCOMOTOR BEHAVIOUR IN THE COMMON MARMOSET

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The putative dopamine D_3 receptor antagonist U99194 has been shown to increase locomotor activity in the rat (Waters et al.,1993). The studies presented here used an ethologically based approach to investigate the effects of U99194 on locomotor behaviour in the marmoset.

Subjects were four adult (290-380g) female common marmosets (*Callithrix jacchus*). Treatments were administered once weekly according to an operator blind randomised schedule. Animals received U99194 (1-32 mg/kg) or vehicle (0.9 % saline) by subcutaneous (s.c.) injection (1 ml/kg) and were placed immediately into individual observation cages (dimensions 75 x 50 x 60 cm fitted with two 50 cm perches). 20 min after drug administration, locomotor behaviour (LA) was analysed by assessment of the number of jumps, rears and perch crossings made over a 15 min period. Data are shown as mean ±s.e.m total locomotor behaviours/15 min (LA/15 min), and mean ±s.e.m. total grooming duration (s/15 min). Emesis was recorded as number of retches+vomits observed over a 30 min period immediately following drug treatment, shown as mean±s.e.m E/30 min. Data were analysed by one-way ANOVA with Dunnett's t-test.

U99194 1-16 mg/kg produced a significant effect on LA ($F(5,23)=4.7$, $p<0.01$). At low doses of 1&2 mg/kg U99194 had no significant effect on LA (50±10&29 ±8 LA/15; compared with vehicle 50±8 LA/15 min) whilst 4 mg/kg of U99194 significantly decreased LA (19±6 LA/15 min; $p<0.05$ compared to vehicle). A small but not significant increase in LA was detected following the highest dose of U99194 (16 mg/kg; 62±8 LA/15 min). U99194 1-16 mg/kg had a significant dose-dependent effect on grooming ($F(5,23)=4.3$ $p<0.01$. At 4-16 mg/kg U99194 significantly decreased grooming (24±8-19±14 s/15 min; $p<0.001$ compared to vehicle 131±45 s/15 min). At 4-16 mg/kg U99194 induced retching/vomiting (4±0.5-14±0.8 E/30 min respectively) of 20-25 min duration with 5-10 min latency. Oral administration of domperidone 2 mg/kg 60 min prior to U99194 8mg/kg significantly ($p<0.001$) reduced the emetic response (2±0.3 E/30 min compared to 8±1.2 E/30 min). At 32 mg/kg U99194 induced 5-12 seizures over a 25 min period with latency of 5-8 mins; thus LA was not assessed.

In these studies U99194 induced a dose-dependent reduction in grooming in the marmoset as previously reported in the rat (Clifford & Waddington, 1998). The reduction in LA following U99194 contrasts with the stimulant effect on locomotion reported in the rat (Waters et al., 1993), although high doses (10-20 mg/kg) inhibit locomotor activity in mice (Gendreau et al., 1997). However, the induction of emesis may have interfered with LA assessment (Löschmann et al., 1991). The emetic effect of U99194 was abolished by domperidone, indicating a possible dopaminergic mechanism. Extension of the dose range of U99194 tested to enable comparison with rodent studies was precluded by the incidence of seizures which may indicate excessive stimulation of central dopaminergic stimulation. Since U99194 has been shown in *in vitro* studies to have selective action at dopamine D_3 receptors, further behavioural studies in the marmoset are necessary to confirm the antagonist properties of U99194 at dopamine D_3 receptors *in vivo*.

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235P EFFECT OF THE PUTATIVE DOPAMINE D_3 RECEPTOR ANTAGONIST U99194 ON PROGRESSIVE RATIO RESPONDING IN THE COMMON MARMOSET

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The dopamine D_3 receptor agonist 7-OH-DPAT has been shown to inhibit cognitive function (Smith et al., 1996a) and locomotor behaviour (Smith et al., 1996b) in the marmoset. In order to examine the role of the dopamine D_3 receptor in reinforced behaviour in the marmoset we investigated the effect of 7-OH-DPAT and the putative dopamine D_3 receptor antagonist U99194 (Waters et al., 1993) on food-reinforced operant responding in a progressive ratio (PR) paradigm.

Subjects were five (3 male, 2 female) adult (290-380g) common marmosets (*Callithrix jacchus*) trained to lever- press for a liquid reward (0.5 ml milk) in daily 30 min sessions. Animals were trained on a PR schedule with response requirements increasing according to the exponential progression 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118 derived from the equation: ratio=[5xe $^{0.2x\ \text{no. reinforcers}}$] (Roberts & Richardson, 1992). The break point was defined as the ratio value corresponding to the final reinforcer delivery. 7-OH-DPAT (1.25-25 µg/kg) or U99194 (1&2 mg/kg) or vehicle (0.9% saline) were given by subcutaneous (s.c.) injection (1 ml/kg) 20 min prior to testing. For antagonist studies, U99194 was injected 30 min prior to 7-OH-DPAT/vehicle. Data are shown as mean ±s.e.m. break point (BPt.) over a 30 min test session. Data were analysed by one-way ANOVA with Dunnett's t-test.

7-OH-DPAT (1.25-25 µg/kg) significantly reduced PR responding in the marmoset [$F(5,29)=11.9$, $p<0.001$]. PR responding was significantly attenuated at doses of 2.5-25 µg/kg 7-OH-DPAT (BPt. 21±4.0 - 5±1.7; $p<0.05$-0.001 compared to vehicle BPt 36±7.3). U99194 (1&2 mg/kg) also had a significant effect on PR responding [$F(2,14)=10.7$, $p<0.001$]. At 2mg/kg U99194 significantly increased lever pressing (BPt 64±11.8; $p<0.001$ compared to vehicle BPt 27±4.3). The significant reduction in lever pressing induced by 7-OH-DPAT 5 µg/kg (BPt 15±2.5; $p<0.001$ compared to vehicle 43±8.2) was significantly ($p<0.01$) antagonised by pretreatment with U99194 2mg/kg (BPt 37±8.3). Doses of U99194 and 7-OH-DPAT tested did not induce nausea or emesis.

These studies in the marmoset show that the dopamine D_3 receptor agonist 7-OH-DPAT induced a dose-dependent reduction in performance on a PR schedule of reinforcement as reported in the rat (Depoortere et al., 1996). Conversely, the putative D_3 receptor antagonist U99194 enhanced PR performance, in agreement with the behavioural activiation induced by U99194 in the rat (Waters et al., 1993). Since U99194 has been shown in *in vitro* studies to have selective action at dopamine D_3 receptors, the ability of U99194 to reverse the inhibitory action of 7-OH-DPAT on PR responding suggests that stimulation of D_3 receptors may result in an inhibitory effect on reinforcement.

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Waters, N., Svensson, K., Haadsma, S.R., et al. (1993) *J. Neural Transm.* 94: 11-19.

236P INTERACTION BETWEEN PREVIOUS SOCIAL HISTORY AND THE EFFECTS OF CHRONIC FLUOXETINE IN MALE GERBILS

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Disruption of established male/female pairs of Mongolian gerbils (*Meriones unguicalatus*) has been shown to have reliable effects on subsequent spontaneous social behaviour in males (Hendrie & Starkey, 1998). The present study was conducted to determine whether these effects could be attenuated by fluoxetine, a clinically active antidepressant.

Seventy vasectomised male gerbils were housed in male/female pairs for 5 weeks. Half these males were then individually housed for a further week (pairs disrupted, PD) whilst the rest remained in their pairs (pairs maintained, PM). Animals were then treated intraperitoneally with 0, 5 or 10mg/kg fluoxetine for the next 14 days (n's = 10-15 per group) whilst kept in these same housing conditions. 1 h after final injection, the spontaneous social responses of each animal were assessed in the presence of an untreated (stimulus) male using detailed behavioural analysis described in Hendrie & Starkey, 1998. Data are presented as means ± s.e. mean and were analysed by Analysis of Variance and orthogonal contrasts as follow-up. * = $p<0.05$ from vehicle control.

In agreement with previous findings (Hendrie & Starkey, 1998), vehicle PD males showed a clear decrease in social interest demonstrated by an increase in their termination of social encounters [PM = 28.8 ± 1.6; PD = 36.7 ± 2.9, $p<0.05$].

Effects of fluoxetine were (i) non-specific, seen in both housing conditions and (ii) specific, seen in one housing condition only.

Non-specific effects were increased locomotion in pair maintained [vehicle =194.4±10.8; 5mg/kg =272.3±16.6*; 10mg/kg =249.9±22.0*] and pair disrupted males [vehicle =213.9±12.0, 5mg/kg =247.2±17.8; 10mg/kg =285.6 ± 15.4*].

Specific effects were decreased measures of social investigation [vehicle =50.3±3.8; 5mg/kg =31.8±5.1*; 10mg/kg = 32.5±5.4*] and offence [vehicle =27.3±12.9, 5mg/kg =0.0± 0.0*, 10mg/kg =0.0±0.0*] seen in PM males only. No specific effects of either 5 or 10mg/kg fluoxetine were seen in PD males.

Together these data show that both 5 and 10mg/kg fluoxetine produce a profile of increased locomotor behaviour and decreased social behaviour in PM males. These effects are probably not independent. By contrast, there were no effects of 5 mg/kg fluoxetine in PD males and the highest dose produced only an increase in locomotor behaviour. The lack of effect on social behaviour in this group cannot be attributed to a floor effect. Therefore, it is concluded that PM and PD males are differentially sensitive to the effects of fluoxetine and hence that there is an interaction between the effects of this known antidepressant and previous social history.

Hendrie CA, Starkey NJ. (1998) Physiol Behav 63: 895-901

237P THE SELECTIVE 5-$HT_{2C/2B}$ RECEPTOR ANTAGONIST SB221284 ENHANCES THE BEHAVIOURAL AND BIOCHEMICAL EFFECTS OF MORPHINE IN MICE

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In vivo electrophysiological studies have shown that 5-HT_2 receptor agonists and antagonists decrease or increase dopamine cell firing in rodents (Lejeune et al., 1997). Furthermore, in vivo dialysis studies have shown that the 5-HT_{2C} receptor antagonist SB206553, increases dopamine release in the nucleus accumbens (Di Matteo et al., 1998) suggesting that 5-HT_{2C} receptors may tonically inhibit mesolimbic dopamine pathways. Given that morphine is known to activate mesolimbic dopamine neurones, the present studies have determined whether the increases in locomotor activity and accumbens dopamine metabolism induced by morphine are enhanced by the 5-$HT_{2C/2B}$ receptor antagonist SB221284.

Male BKTO mice (23-31g, Bantin & Kingman, Hull, U.K.) were habituated to individual activity cages equipped with 2 infra-red beams positioned one at each end of the base of the cage to record cage crossings ie. consecutive beam breaks. Mice (n = 9-10/group) received saline (10 ml/kg, s.c.) or morphine (2.5-20 mg/kg, s.c.) or, in a separate experiment, were pretreated with vehicle (8% cyclodextrin, 25 mM citric acid; 10 ml/kg, i.p.) or SB221284 (3 mg/kg, i.p.) 20 min prior to injection of saline (10 ml/kg, s.c.) or morphine (10 mg/kg, s.c.). Photocell beam breaks were then monitored for 120 min. To assess dopamine metabolism, mice (25-30g; n = 5-8/group) were pretreated with vehicle (10 ml/kg, i.p.) or SB221284 (3 mg/kg, i.p.) 20 min prior to administration of saline (10 ml/kg, s.c.) or morphine (10 mg/kg, s.c.) and sacrificed 60 min later. Brains were removed, the nucleus accumbens dissected and analysed for dopamine and its metabolites, DOPAC and HVA by HPLC with electrochemical detection (Hutson et al., 1991) The ratio [DOPAC] + [HVA]/[DA] provided an index of dopamine metabolism. All data were analysed by analysis of variance followed by Tukey's test (*P<0.05 compared to veh (dose response curve) or veh/sal; † P<0.05 compared to veh/morphine.

Subcutaneous administration of morphine induced a dose dependent increase in locomotor activity (mean ± s.e. mean total cage crosses: sal = 41 ± 21; 2.5 mg/kg = 29 ± 11; 5 mg/kg = 82 ± 21; 10 mg/kg = 228 ± 108; 20 mg/kg = 916 ± 231*) and dopamine metabolism in nucleus accumbens (mean ± s.e. mean: sal = 0.31 ± 0.01; 5 mg/kg = 0.34 ± 0.01 mg/kg; 10 mg/kg = 0.39 ± 0.01*; 20 mg/kg = 0.43 ± 0.02*). Pretreatment with SB221284 (3 mg/kg) significantly increased the hyperlocomotion induced by a submaximal dose of morphine (10 mg/kg) (mean ± s.e. mean total cage crosses: veh/sal = 3.4 ± 0.9†; veh/morphine = 206 ± 55*; SB221284/sal = 5.8 ± 1.3†; SB221284/morphine = 425 ± 85*†) and also significantly enhanced morphine-induced dopamine metabolism in the nucleus accumbens (mean ± s.e. mean: veh/sal = 0.28 ± 0.01; veh/morphine = 0.34 ± 0.01; SB221284/sal = 0.27 ± 0.02; SB221284/morphine = 0.45 ± 0.03*†).

The present results demonstrate that the 5-$HT_{2C/2B}$ receptor antagonist SB221284 enhanced both the hyperactivity and increase in accumbens dopamine metabolism induced by morphine in the mouse and are consistent with the hypothesis that under these conditions 5-$HT_{2C/2B}$ receptors exert an inhibitory tone on limbic dopaminergic neurones.

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238P THE EFFECTS OF THE $GABA_B$ AGONIST BACLOFEN ON VOLAEMIC DRINKING IN RATS

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We have previously reported that the $GABA_B$ agonist baclofen inhibits drinking in response to water deprivation (Ebenezer et al., 1992). Water deprivation leads to a reduction in intracellular and extracellular fluid volumes, both of which can result in thirst (Oatley, 1964). Previous experiments carried out in this laboratory have suggested that the $GABA_B$ agonist inhibits water intake induced by reducing intracellular fluid volume, i.e. cellular drinking (Ebenezer et al., 1992). The present study was undertaken to investigate whether baclofen also inhibits hypovolemic thirst caused by a reduction in the extracellular fluid volume. In this study rats were injected sc with a 30% $^{w}/_{v}$ solution of polyethylene glycol (PEG) which induces hypovolemic thirst by drawing out extracellular fluid (Fitzsimons, 1961).

Three groups of male Wistar rats (b.wt. 340 - 380g; n=6 in each group) were given 3 acclimatization trials in experimental cages where they had free access to water. On the experimental day, the rats received the following treatments: Group 1 - physiological saline followed by saline; Group 2 - PEG followed by saline; Group 3 - PEG followed by baclofen (2 mg kg^{-1}). The first series of injections were given sc (total volume 5 ml, injected at multiple sites) and the second injection was given ip (1 ml). Three hours separated the two injections. Fifteen min after the second injection, the rats were placed in the experimental cages and water intake measured over 240 min. The dose of baclofen used was based on results obtained previously (Ebenezer et al., 1992). The data were analysed by ANOVA.

The rats treated with PEG + saline (Group 2) began drinking almost immediately after they were placed in the experimental cages. The animals consumed most of their water during the first 30 min. PEG treatment significantly increased water intake compared with saline treated control rats (Group 1). Thus, for example, the cumulative water intake (mean ± s.e.mean) at 240 min for rats in Group 1 was 1.6 ± 0.4 ml, while that for rats in Group 2 was 9.2 ± 0.8 ml (P<0.01). Baclofen significantly inhibited PEG-induced drinking during the first 60 min from 7.5 ± 0.3 ml to 2.4 ± 0.8 ml (P<0.01). Thereafter, the inhibitory effects of baclofen on volemic drinking diminished and the rats displayed rebound drinking. At 240 min there was no significant difference in the cumulative amount of water consumed by the rats in Groups 2 and 3.

The results of this study extend previous results (Ebenezer et al., 1992) and show for the first time that, in addition to inhibiting cellular drinking, baclofen also inhibits volemic drinking. The mechanisms by which baclofen inhibits cellular and volemic drinking has yet to be elucidated. However, recent studies have suggested that the drug inhibits water intake by a central mode of action (Houston et al., 1995).

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239P EFFECTS IN THE RAT OF BTS 71 091 ON FOOD INTAKE AND THE BEHAVIOURAL SATIETY SEQUENCE

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BTS 71091 (5-(4-chlorophenyl)-1-azaspiro[5,5]undec-4-ene-hydrochloride) is a potent monoamine reuptake inhibitor which decreases food intake in a dose dependent manner (Jackson *et al.*, 1998). As feeding can be reduced by non specific interference of behavioural or physiological processes a sequence of behaviour termed the 'satiety sequence' is used to identify suppression by natural post-ingestive processes (Halford *et al.*, 1998).

This study was conducted using male Lister hooded rats (275±25g:n=12). BTS 71091, at its ED_{50} to reduce food intake (15.0 mg/kg po), was compared with an equipotent dose of sibutramine (3.0 mg/kg po), and an equivalent prefeeding condition (10 min). Following drug dosing behaviour was continuously classified into 8 categories (eating, drinking, rearing, sniffing, locomotion, grooming, resting and other). The occurrence and duration of each event was recorded on a data collection program.

Table 1 shows the effects of vehicle, BTS 71 091, and prefeeding on food intake and parameters of eating behaviour. Vehicle administration produced a classical satiety profile. BTS 71091 accelerated the decline in feeding by reducing the duration ($P<0.005$) and frequency ($P<0.01$) of eating episodes, and advance the onset and increase the duration of resting ($P<0.05$). Similar changes were brought about by both sibutramine and prefeeding. The effect of the monoamine reuptake inhibitor, BTS 71091, on food intake is consistent with enhancement of the natural development of satiety.

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Jackson H.C., Cheetham, S.C., Harris P.J., et al (1998) Int. J Obesity 22: p45

Table 1 - Effect on food intake (FI), local eating rare (LER), mean eating bout intake (MBI) and mean eating bout length (MBL).

Treatment Group	FI	LER	MBI	MBL
Saline control	12.0g (1.5)	1.12g/min (0.08)	0.87g (0.20)	45.2s (9.39)
BTS 71091 15.0 mg/kg po	4.3g*** (1.1)	0.79g/min** (0.08)	0.44g* (0.09)	36.6s***(7.13)
Sibutramine 3.0 mg/kg po	4.2g*** (0.9)	0.94g/min (0.16)	0.39g (0.09)	30.0s (6.92)
Prefeeding 10 min	4.1g*** (0.7)	1.14g/min (0.16)	0.79g (0.26)	43.3s (12.3)

Derived changes reflect changes in frequency and duration *P<0.05, **P<0.01, ***P<0.005 verses saline (2X8 ANOVA) ( )=s.e.means

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240P THE HYPOPHAGIC EFFECT OF LITHIUM CHLORIDE IN RATS IS ATTENUATED BY PRETREATMENT WITH THE CYCLOOXYGENASE INHIBITOR IBUPROFEN

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Exposure of rats to physical or psychological stressors cause the *de novo* synthesis and release of prostaglandins into the circulation (see Morimoto et al., 1991). It has also been shown that both systemic and central administration of prostaglandins suppress feeding in rats (Morley, 1987). It was therefore of interest to determine whether the hypophagic effects of lithium chloride (LiCl) a pharmacological agents with known aversive actions (see Ebenezer et al., 1992) may be produced by a prostaglandin-mediated mechanism. In the present study we investigated the effect of pretreating rats with a cyclooxygenase (COX) inhibitor (which prevents the *de novo* synthesis of prostaglandins) on the hypophagic response induced by systemic injection of LiCl.

Male Wistar rats (n=16; b. wt. 320 - 400g) were used. Experiment 1. Rats (n=8) were fasted for 22h and injected s.c. with saline (sal) or LiCl (1, 10, 25 or 50 mg kg^{-1}) and placed individually in experimental cages where they had free access to food and water. The amount of food consumed was measured 15, 30 and 60 min after presentation. Experiment 2. Male Wistar rats (n=8) were deprived of food for 22h and injected i.p. with either sal followed by sal, LiCl (50 mg kg^{-1}) followed by sal, sal followed by ibuprofen (Ibu) (100 mg kg^{-1}) or LiCl (50 mg kg^{-1}) followed by ibu (100 mg kg^{-1}). The rats were presented with food and water immediately after the 2nd injection and food intake were measured 15 min later. In both experiments a repeated measures design was used with each animal receiving all treatments. The data were analysed by ANOVA.

LiCl (1 - 50 mg kg^{-1}) produced a dose related decrease in feeding with significant decreases apparent only with the 25 and 50 mg kg^{-1} doses. The greatest inhibitory effects were apparent in the first 15 min after administration. Thus, for example, the 50 mg kg^{-1} dose decreased feeding at 15 min from a mean ± s.e. mean value of 4.5±0.4 g to 0.9±0.2g ($P<0.01$). The results for Experiment 2 are shown in Figure 1. Ibu (100 mg kg^{-1}) had no effects on food intake on its own, but significantly ($P<0.05$) attenuated the hypophagic effect of LiCl (50 mg kg^{-1}) at 15 min. These results indicate that the early inhibition of feeding produced by LiCl may be due to release of prostaglandin as part of a generalized stress response, and suggest that drugs with aversive properties may elicit their hyperphagic actions, at least in part, through a prostaglandin-mediated mechanism.

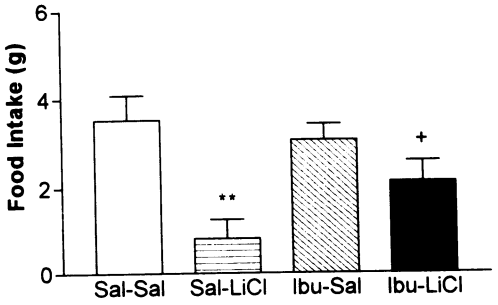

Figure 1. The effect of ibuprofen pretreatment on the hypophagic effect of LiCl. **P<0.01 vs Sal-Sal; +P<0.05 Sal-LiCl vs Ibu-LiCl.

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Morimoto, A. et al. (1991) J. Physiol. (Lond.), 443, 421 - 429.
Morley, J.E. (1986) Endoc. Rev., 8, 256 - 287.

241P EFFECT OF WITHDRAWAL FROM CHRONIC CHLORDIAZEPOXIDE ADMINISTRATION IN THE RAT ELEVATED ZERO MAZE TEST OF ANXIETY

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In humans, physiological dependence on benzodiazepines is accompanied by a withdrawal syndrome which includes increased anxiety (Petursson, 1994). The anxiety component of benzodiazepine withdrawal has been assessed in a variety of animal models including the rat elevated plus maze (Baldwin and File, 1988) and the rat social interaction tests (Baldwin and File, 1989). We have investigated whether the rat zero-maze test of anxiety (Shepherd et al., 1994) is sensitive to anxiogenesis following withdrawal from chronic chlordiazepoxide (CDP) administration.

Male Sprague Dawley rats (Charles River; approx. 300 g when tested) were dosed p.o., b.i.d. for 14 days with CDP (5, 10 or 20 mg/kg) or vehicle (45 % cyclodextrin soln.). 12 or 24 h after their last dose the rats (10/group) were placed onto a closed quadrant of the zero-maze. A 5 min test session was then recorded on video-tape for subsequent analysis. The following behavioural parameters were scored: (a) percentage of test time spent in the open quadrants (%TO); (b) frequency of head dips over the edge of the platform (HDIP); c) frequency of (stretched attend postures (SAP) exhibited when rats, in the closed quadrants, investigated an open area with at least their snouts crossing the closed-open divide; (d) frequency of line crossings (LX).

The results are presented in the table below. 12 and 24 h withdrawal from chronic CDP at 20 mg/kg resulted in a significant decrease in %TO and HDIP, and a significant increase in SAP, indicating anxiogenesis (Shepherd et al, 1994). 24 hr withdrawal from chronic CDP at doses of 5 and 10 mg/kg resulted in a significant reduction in HDIP only, an effect seen also at the 12 hr time point following the lower dose, consistent with anxiogenesis. A reduction in LX was observed 12 h after the last CDP dose at 20 mg/kg only.

These results indicate that the rat elevated zero maze is sensitive to anxiogenic-like effects of withdrawal from chronic chlordiazepoxide administration.

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Petursson, H. (1994) *Addiction* 89, 1455-1459.
Shepherd, J.K., Grewal, S.S., Fletcher, A. et al. (1994) *Psychopharmacol.* 116, 56-64.

CDP dose	12 hr withdrawal				24 h withdrawal			
(mg/kg)	%TO	HDIP	SAP	LX	%TO	HDIP	SAP	LX
0	32.5 ± 4.5	6.5 ± 1.4	2.3 ± 0.7	37.9 ± 3.7	38.7 ± 4.6	11.5 ± 1.9	2.5 ± 1.0	44.6 ± 5.0
5	26.2 ± 6.2	2.4 ± 0.9*	3.3 ± 0.9	30.6 ± 4.8	24.9 ± 4.3	6.2 ± 1.1*	2.7 ± 0.8	32.7 ± 4.1
10	24.6 ± 5.0	4.8 ± 1.1	4.6 ± 1.0	34.9 ± 4.7	27.1 ± 3.5	5.1 ± 1.2*	4.7 ± 0.7	32.1 ± 3.6
20	10.2 ± 3.9*	1.3 ± 0.4*	5.9 ± 1.0*	18.7 ± 3.9*	19.8 ± 5.8*	3.4 ± 0.6*	6.2 ± 1.3*	31.2 ± 5.2

Data presented as mean ± s.e.m.. * $p<0.05$ vs. vehicle group (one-way ANOVA/Dunnett's test). SAP data log transformed prior to analysis.

242P Sch 23390 PREVENTS THE COCAINE ANTI-SENSITISATION EFFECTS OF DIZOCILPINE

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Stimulation of locomotion produced by the systemic administration of dizocilpine is mediated by endogenous DA through the activation of dopaminergic receptors in the nucleus accumbens (Willins *et al.*, 1993). On the other hand, dizocilpine is know to prevent cocaine sensitisation (Wolf & Jeziorski, 1993), but it is not known if the anti-sensitisation effects of dizocilpine are related to the events set into motion by the NMDA antagonist at the level of dopaminergic transmission. The present work examined the effects of pre-treatment with Sch 23390, a selective D_1 receptor antagonist, on the cocaine anti-sensitisation properties of dizocilpine in cocaine-experienced rats. Cocaine-experienced rats were given cocaine (15 mg kg^{-1} day^{-1}, i.p.) from day 1 to day 5; cocaine-naïve rats were given the vehicle. From day 6 to day 15, animals remained drug free in their home cages, and on day 16 rats received a challenge injection of cocaine (15 mg kg^{-1}) and cocaine-experienced animals were tested for sensitisation. On the test day, 2 h before the experiment began, animals were transferred to a dimly illuminated and sound attenuating room separate from the animal colony room, where the test cages were kept; temperature and humidity were the same as in the colony room. Spontaneous locomotor activity was measured using a San Diego Instruments rodent activity monitor (model Flex Field, San Diego Instruments, San Diego, CA) with 48 infrared motion sensors. Ten-minute activity recording begun immediately after placing the test subject at the center of the chamber. Activity was measured automatically with a personal computer using Flex Field software (San Diego Instruments) which provides user-defined intervals of total interruptions. Three parameters of normal spontaneous locomotion were recorded: horizontal activity, vertical activity and center time. In cocaine-naïve rats the acute effect of cocaine was a 25% increase in horizontal activity (from 2038±99 to 2516±107 counts 10 min^{-1}). The locomotor stimulating effect of cocaine in cocaine-experienced rats was twice as great as that observed in cocaine-naïve rats (from 2136±121 to 2999±109 counts 10 min^{-1}). In cocaine-naïve rats, pre-treatment with dizocilpine (50 and 100 μg kg^{-1}) from day 1 to day 5 changed neither spontaneous locomotor activity or cocaine stimulant activity. By contrast, in cocaine-experienced animals that had been given 50 μg kg^{-1} dizocilpine from day 1 to day 5 failed to show the increase in locomotor activity when challenged with cocaine on day 16 (from 2294±137 to 2217±255 counts 10 min^{-1}). This effect of dizocilpine was even more marked at 100 μg kg^{-1} (from 2375±152 to 1813±116 counts 10 min^{-1}). Pre-treatment with Sch 23390 (100 μg $kg^{-1}day^{-1}$, i.p.) from day 1 to day 5 was found to attenuate the cocaine anti-sensitisation properties of 50 μg kg^{-1} dizocilpine (from 2072±124 to 2335±137 counts 10 min^{-1}). Pre-treatment with the D_1 receptor antagonist from day 1 to day 5 failed to prevent cocaine sensitisation in cocaine-experienced rats. It is concluded that prevention of cocaine sensitisation by dizocilpine may be related to the events set into motion by the NMDA antagonist at the level of dopaminergic transmission involving D_1 receptors.

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243P EFFECT OF *d*-AMPHETAMINE ON OPERANT TIMING BEHAVIOUR IN RATS

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d-Amphetamine (*APH*) disrupts timing behaviour in rats; it has been proposed that this effect reflects a reduction of the period of a dopaminergically-regulated endogenous 'pacemaker' that is purported to underlie time perception (see Meck, 1996). We have re-examined the effects of *APH* on rats' timing performance using the interval bisection task (Church & Deluty, 1977). In addition to the standard version of the task, we also used a modified version which enables the effects of treatments on temporal discrimination to be distinguished from effects on the temporal control of movement within the operant chamber (see Ho *et al.*, 1995).

Twenty-four female Wistar rats (250-300 g) received daily 50-min sessions in operant conditioning chambers, in which they pressed levers for a sucrose reinforcer (50 µl, 0.6 M). Sessions consisted of 120 trials; in 100 trials a response on lever A was reinforced after a 2-s presentation, and a response on lever B after an 8-s presentation of a light stimulus; stimuli of intermediate durations were presented in 20 'probe' trials without reinforcement (2.5, 3.2, 4.0, 5.0, 6.4 s; 4 trials each). For 12 rats, a 'nose-poke' response was required between stimulus offset and insertion of the levers into the chamber (modified version); for the remaining 12 rats there was no such requirement (standard version) (Ho *et al.*, 1995). When steady-state performance had been attained, *APH* SO_4 (0.2, 0.4, 0.8 mg kg^{-1}) or 0.9% NaCl vehicle (V) was given i.p. 10 min before the sessions. For each treatment condition, percent choice of lever B (%B) was determined for the range of stimulus durations (*d*); logistic functions ($\%B=100/[1+\{d/T_{50}\}^{\varepsilon}]$) were fitted to the data, and the bisection point, T_{50}, and the slope parameter, ε, were estimated (Ho *et al.*, 1995).

The values of T_{50} and ε are shown in Table 1. In both versions of the interval bisection task, *APH* dose-dependently reduced the slope of the logistic timing function (i.e. ε became less strongly negative: ANOVA, effect of treatment, $F_{3,63}=9.5$, $p<0.01$) without significantly altering the bisection point ($F_{3,63}=1.0$, $p>0.2$). The effect of *APH* did not differ significantly between the two versions of the task (*slope*, ε: effect of task version, $F_{1,21}=1.7$, $p>0.2$; interaction, $F_{3,63}=1.1$, $p>0.2$; T_{50}: effect of task version, $F_{1,21}=0.7$, $p>0.2$; interaction, $F_{3,63}=0.9$, $p>0.2$).

Table 1: Effects of *APH* SO_4 (dose in mg kg^{-1}) on parameters of logistic timing functions (mean ± s.e.mean)

	Slope (ε)				Bisection point (T_{50}: s)			
Timing task	V	0.2	0.4	0.8	V	0.2	0.4	0.8
Interval bisection task								
i. nose poke required	-5.3 ±0.3	-4.1 ±0.9	-3.2 ±0.2	-2.5 ±0.3	4.4 ±0.1	4.6 ±0.2	4.3 ±0.2	4.5 ±0.3
ii. nose poke not required	-5.1 ±0.3	-5.9 ±0.9	-4.0 ±0.7	-3.4 ±0.5	4.2 ±0.1	4.3 ±0.1	4.8 ±0.4	4.9 ±0.7

The dose-dependent effect of *APH* on ε confirms the disruptive effects of this drug on timing behaviour (see Meck, 1996). However the failure of *APH* to reduce the value of T_{50} in either version of the interval bisection task suggests that the drug did not alter the period of the hypothetical 'pacemaker', which is purported to determine the locus of the bisection point in this task (see Meck, 1996). The finding that *APH* had similar effects on timing in the two versions of the interval bisection task suggests that the effect of *APH* on the logistic timing function reflects a disruption of time discrimination, and is not mediated by facilitation of locomotion between the two levers (see Ho. *et al.*, 1995).

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244P EFFECTS OF ZOTEPINE AND COMPARATOR ANTIPSYCHOTICS ON EXTRACELLULAR DOPAMINE IN THE FRONTAL CORTEX OF FREELY-MOVING RATS

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Zotepine is an antipsychotic drug with an atypical profile (Needham *et al.*, 1996). Its clinical profile indicates efficacy against positive and negative symptoms of schizophrenia and a low propensity to induce extrapyramidal side effects. In addition, zotepine has affinity for the noradrenaline (NA) transporter site and we have previously demonstrated that zotepine elevates rat cortical NA levels *in vivo* (Rowley *et al.*, 1998). In the present study, we have investigated the effects of zotepine and the comparator antipsychotics, clozapine, olanzapine and haloperidol on extracellular dopamine (DA) in the frontal cortex of freely-moving rats using *in vivo* microdialysis.

Male, Sprague Dawley-derived CD rats (250-350 g; Charles River) were anaesthetised with isoflurane (5% to induce, 2% to maintain) in an O_2/N_2O mixture and a concentric microdialysis probe (2 mm tip, Hospal AN 69 membrane) was stereotaxically implanted into the frontal cortex (co-ordinates: A +3.2 mm, L 2.5 mm relative to bregma; V -4.0 mm from the dural surface; Paxinos and Watson, 1986). Following surgery, rats were returned to a home cage and allowed to recover for at least 20 h with food and water available *ad libitum*. Probes were continuously perfused with an artificial cerebrospinal fluid at a rate of 1.0 µl/min and samples collected every 20 min into Eppendorf vials containing 5.0 µl 0.1 M perchloric acid. Three 'basal' samples were taken prior to a pharmacological challenge of either drug or vehicle (20 µl glacial acetic acid/ml saline). Functionally equivalent drug doses were based on ED_{50}s to inhibit amphetamine-induced hyperlocomotion (Rowley *et al.*, 1998). Dialysate DA was determined by reverse-phase HPLC with electrochemical detection. Values are mean ± s.e.mean and statistical comparison was made between treatment groups and controls by ANOVA with *post hoc* Dunnett's test.

Basal levels of cortical DA were 8.5 ± 0.3 fmol/20 µl. Treatment with zotepine (1.0 mg/kg, i.p.) resulted in a significant ($p<0.001$), prolonged elevation of DA levels for up to 240 min post-injection ($n = 6$). A maximal rise of 333 ± 26% was observed at 120 min post-dosing, compared to vehicle-treated controls. Clozapine (10.0 mg/kg, i.p.) also evoked a significant ($p<0.001$), sustained increase in DA levels of 240 min duration but the maximal rise was observed at 100 min post-drug (+223 ± 10%; $n = 6$). Furthermore, treatment with olanzapine (1.0 mg/kg, i.p.) significantly ($p<0.01$) increased DA levels above basal values for up to 100 min post-treatment ($n = 6$). A maximal increase was observed 40 min post-treatment (+280 ± 48%), compared to vehicle-treated controls. Haloperidol (0.1 mg/kg, i.p.) had no effect on DA levels compared to vehicle-treated controls.

These data demonstrate that zotepine, clozapine and olanzapine each elevate DA levels in the rat frontal cortex. This action may reflect their atypical properties since the typical antipsychotic, haloperidol, had no effect on cortical DA levels at a functionally equivalent dose.

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245P TOLERANCE DOES NOT DEVELOP TO THE HYPOPHAGIC EFFECTS OF THE $5HT_{1A}$ AGONIST BUSPIRONE IN FOOD-DEPRIVED RATS AFTER CHRONIC ADMINISTRATION

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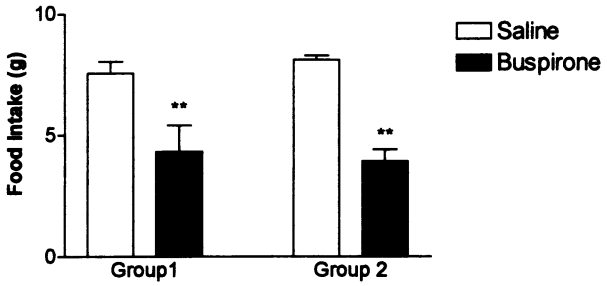
It has been previously shown that while $5HT_{1A}$ agonists increase food intake in non-deprived rats by an action at $5HT_{1A}$ autoreceptors (Dourish, et al., 1986), they decrease feeding in food-deprived animals (Ebenezer, 1992; Ebenezer, 1996). The mechanism responsible for the hypophagic effect of these agents is not known. It has previously been shown that chronic administration of $5HT_{1A}$ agonists lead to a rapid desensitization of $5HT_{1A}$ autoreceptors (Kennett et al., 1987). Thus, if $5HT_{1A}$ autoreceptors are involved in mediating the hypophagic effect of these agents, then rapid tolerance should occur to the hypophagic effect of a $5HT_{1A}$ agonist in food-deprived rats after chronic administration. The present study was undertaken to investigate this possibility.

Male Wistar rats (n=16, b. wt. 350 - 400g) were assigned to 2 equal groups. The rats in Group 1 were fasted for 22h and injected s.c. with saline or buspirone (2 mg kg^{-1}) and placed separately in experimental cages where they had free access to food and water. The amount of food consumed was measured 30 min after presentation. A cross-over design was used with each animal receiving both treatments. The rats in Group 2 were given a single injection of buspirone (2 mg kg^{-1}) each day for 14 days. Following the chronic dosing regimen, the rats were fasted for 22h and food intake measured after s.c. administration of saline or buspirone (2 mg kg^{-1}), as described above. The dose of buspirone used was based on results obtained previously (Ebenezer et al., 1996). The data were analysed by two-way ANOVA.

The results are illustrated in Figure 1. Statistical analysis of the results indicate that (i) buspirone (2 mg kg^{-1}) significantly decreased feeding in both groups of rats ($P<0.01$, in each case), and (ii) tolerance does not develop to the hypophagic effect of buspirone in rats that were chronically treated with the drug. These results extend previous observations with gepirone (Smith and Ebenezer, 1998) and suggest that $5HT_{1A}$ autoreceptors may not play an important role in mediating the inhibitory effect of $5HT_{1A}$ agonists on food intake in food deprived rats.

Figure 1. Effects of buspirone on food intake in (a) rats with no previous experience of buspirone (Group 1) and (b) rats chronically treated with buspirone for 14 days (Group 2). Paired t-test: saline vs buspirone: **P<0.01.



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246P REGIONAL ALTERATIONS IN [^{3}H]AMPA BINDING IN RAT BRAIN AFTER CHRONIC DIAZEPAM TREATMENT

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The neuronal mechanisms underlying dependence on benzodiazepines remain unclear. Changes at the level of the $GABA_A$ receptor are inadequate as an explanation for the changes in neural activity in circuits recruited during chronic benzodiazepine treatment (Pratt et al. 1998). The present study focuses on the hypothesis that the chronic enhancement of GABAergic inhibition by diazepam causes compensatory increases in excitatory glutamatergic mechanisms. The aim of these experiments was to determine if chronic diazepam treatment leads to regionally specific changes in AMPA receptor binding.

Experiments were carried out on male Hooded Long Evans rats (200-300g; n = 9-10 per group). Rats were randomly assigned to 1 of 3 treatment groups, either daily injection of vehicle for 28 days, daily injection of vehicle for 14 days followed by daily injection of diazepam (5mg kg^{-1} i.p.) for 14 days or daily injection of diazepam (5mg kg^{-1} i.p.) for 28 days. Twenty four hours following the last injection, rats were killed and the brains removed and frozen in isopentane before being stored at -70°C. 20µm brain sections were cut at 8-9 selected levels and prepared for [^{3}H]AMPA receptor autoradiography following a protocol modified from Dev & Morris (1994). Sections were incubated with 10nM [^{3}H]AMPA (specific activity = 40.6 Ci/mmol), using 10µM quisqualate to define nonspecific binding. Resultant autoradiograms were analysed using computer based densitometry (MCID). Differences in the levels of specific [^{3}H]AMPA binding in each brain area were analysed by one-way ANOVA, followed by the Student Newman-Keuls multiple range test if appropriate.

Following 28 days treatment with diazepam (5mg kg^{-1} i.p.) there was an increase in specific [^{3}H]AMPA binding in the frontal cortex and in the CA2 field of the hippocampus relative to control. 14 days treatment with diazepam (5mg kg^{-1} i.p.) also produced an increase in specific [^{3}H]AMPA binding in the CA2 region of the hippocampus relative to control (Table 1).

These data provide preliminary evidence which supports the hypothesis of enhanced glutamatergic mechanisms following chronic benzodiazepine treatment.

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Brain area	Control (Vehicle treated)	14 days 5mg Kg^{-1} Diazepam	28 days 5mg Kg^{-1} Diazepam
Frontal Cortex	1883 ± 313.9	2396 ± 258.5	3024 ± 223.0*
Cingulate Cortex	1628 ± 296.0	1716 ± 299.5	1571 ± 341.4
CA1 Field Hippocampus	3159 ± 255.4	3588 ± 527.2	3311 ± 490.2
CA2 Field Hippocampus	1071 ± 169.9	2864 ± 573.7*	2608 ± 444.3*

Table 1. [^{3}H]AMPA specific binding (nCi/g) in rat brain. Data expressed as mean ± s.e. mean (n = 4-9 per group). * $p<0.05$ vs vehicle treated animals.

247P IMMUNOHISTOCHEMICAL LOCALISATION OF THE NMDA RECEPTOR NR1 AND NR2C/D SUBUNITS IN THE MAMMALIAN HIPPOCAMPUS AND CEREBELLUM

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The N-methyl-D-aspartate (NMDA) subtype of excitatory glutamate receptors is a family of hetero-oligomeric, ligand-gated ion channels. NMDA receptor subunits are classified according to sequence homology into two classes, NR1 and NR2. The NR2 subclass comprises four closely related genes encoding subunits, NR2A-NR2D. Recombinant studies have shown that the type of NR2 subunit co-expressed with NR1, dictates the functional and pharmacological properties of cloned NMDA receptors. Autoradiographical information is available for the distribution of NR1/NR2A and NR1/NR2B subtypes in the brain, but there is a paucity of pharmacological ligands which selectively distinguish NR1/NR2C and NR1/NR2D receptors. To localise these, we have adopted an immunohistochemical approach using anti-NR1 and anti-NR2C/D subunit-specific antibodies. Peptide-directed anti-NMDA receptor subunit-specific antibodies were generated, affinity-purified and characterised (Chazot and Stephenson, 1997). Anti-NR1 (17-35) antibodies recognise all splice forms of the NR1 subunit in immunoblots. Anti-NR2D (1307-1323) antibodies recognise both NR2C and NR2D, but not NR2A or NR2B subunits expressed singly in human embryonic kidney 293 cells. Moreover, anti-NR2D (1307-1323) antibodies recognise a single protein, M_r 152,000, in both murine forebrain and spinal cord membranes, and two proteins, M_r 145,000 and M_r 152,000, in murine cerebellar membranes consistent with the known distribution of the NR2C and NR2D subunits in the murine central nervous system.

Mouse brains were perfusion-fixed with 4% paraformaldehyde: 0.05% glutaraldehyde in phosphate buffer, pH 7.4. Free-floating, 20 μm thick, horizontal and saggital sections were developed for immunoreactivity by standard methods using the VECTASTAIN Elite ABC kit with diaminobenzidine as horseradish peroxidase substrate. Affinity-purified anti-NR1 (17-35) and anti-NR2D (1307-1323) antibodies were used at 0.25-1 μg protein/ml. Anti-NR1 immunoreactivity in the hippocampus was localised to the pyramidal cell bodies with CA3 labelling > CA1, to granule cells of the dentate gyrus and to cell bodies in the polymorphic layer of dentate gyrus. Apical dendrites of the CA subfields were also labelled. Anti-NR1 immunoreactivity in the cerebellum was strongest in the cell bodies and dendrites of Purkinje neurons with less intense, punctate, staining in the molecular and granule cell layers. Golgi, basket and stellate interneurons, and cell bodies in the cerebellar nuclei were also immunopositive. Anti-NR2C/D immunoreactivity in the hippocampus was diffuse and restricted to the oriens layer of CA1 and CA2, the stratum radiatum of CA3, the molecular layer of the dentate gyrus and the inner third of the lacunosum moleculare. Pyramidal and granule cell bodies were not immunoreactive. In the cerebellum, anti-NR2C/D immunoreactivity was strongest in granule cells but intense immunostaining was also found in cerebellar nuclei. Strong but diffuse staining was observed in the molecular layer. Immunoreactivity was not detected in Purkinje neurons.

In conclusion, we have localised in hippocampus and cerebellum, two regions important in excitatory neurotransmission, NMDA receptor NR1, NR2C and/or NR2D subunits.

Chazot, P.L. and Stephenson, F.A. (1997) *J.Neurochem.* **69** 2138-2144.

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248P CHARACTERIZATION OF SIB-1757 AND SIB-1893: HIGHLY SELECTIVE ANTAGONISTS AT METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5

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Based on amino acid sequence identity, the eight identified metabotropic glutamate receptors (mGluRs) can be divided into three groups (I, II and III). Group I mGluRs includes both mGluR1 and mGluR5, and activation of these G-protein-coupled receptors stimulates phospholipase C. Understanding the role of group I mGluRs in normal physiology and pathophysiology has been hampered by the lack of potent and selective ligands for these receptor subtypes. Here we report the identification of structurally novel, highly selective mGluR5 antagonists.

We have previously reported the establishment of stable cells lines expressing recombinant human mGluR1b (hmGluR1b/L13-23-7 cells) and mGluR5a (hmGluR5a/L38-20 cells) (Daggett *et al.*, 1995; Lin *et al.*, 1997). These cell lines give robust increases in inositol phosphates (IP) and intracellular Ca^{2+} when activated by group I mGluR agonists such as dihydroxyphenylglycine (DHPG). The activity of compounds obtained from a random library of small molecules was evaluated on both cell lines using an automated high throughput screening system that detects changes in Ca^{2+} (Veliçelebi *et al.*, 1998). One compound, SIB-1757 (6-methyl-2-(phenylazo)-pyridin-3-ol), was identified as an antagonist at hmGluR5 with an IC_{50} of 0.4 (0.2, 0.7) μM (geometric mean, (lower, upper SD), N=5), and an IC_{50} >30 μM at hmGluR1b (N=5).

Testing of analogues of SIB-1757 led to the identification of an equi-potent compound, SIB-1893 ((E)-6-methyl-2-styryl-pyridine). SIB-1893 selectively inhibited glutamate-stimulated Ca^{2+} signals at hmGluR5 with an IC_{50} of 0.3 (0.1, 0.6) μM (N=5), compared to an IC_{50} of >30 μM at hmGluR1b. The activities of SIB-1757 and SIB-1893 were evaluated at additional glutamate receptor subtypes. Using cAMP measurements, the agonist and antagonist potencies of SIB-1757 and SIB-1893 at group II and III mGluRs were >30 μM at recombinant hmGluR2, hmGluR4, hmGluR6, hmGluR7 and hmGluR8 (N=4-6).

Ca^{2+} measurements were used to determine the agonist and antagonist activities of SIB-1757 and SIB-1893 at recombinant AMPA receptors ($hGluR1_i$, $hGluR2(Q)_i$, $hGluR3_i$, $hGluR4_i$), kainate receptors (hGluR5 and hGluR6) and NMDA receptors (hNR1/2A and hNR1/2B). The agonist and antagonist potencies of SIB-1757 and SIB-1893 were >30 μM at these ionotropic glutamate receptors (N=3).

The potency of these compounds was examined in rat neonatal (8-12d) brain regions. In striatal tissue slices, the group I selective agonist DHPG (10 μM) evoked an increase in IP accumulation. SIB-1757 inhibited 68 ± 9 % of the DHPG-induced IP accumulation with an IC_{50} of 3.3 (1.5, 7.3) μM (N=3). In contrast, in the cerebellum, a brain region that has a low expression of mGluR5 and a higher expression of mGluR1 (Testa *et al.*, 1994), 100 μM SIB-1757 inhibited a maximum of 4 ± 10 % of DHPG-induced IP accumulation.

In conclusion, this is the first report of potent, subtype-selective antagonists at mGluR5 that can markedly discriminate between mGluR5 and mGluR1. SIB-1757 and SIB-1893, and further analogues (see Gaspirini *et al.*, this meeting) are valuable tools for investigating the role of mGluR5 in models of pain (see Bowes *et al.*, this meeting) and CNS disorders.

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Gaspirini, F., Lingenhoehl, K., Flor, P., *et al.*, *This meeting.*

Bowes, M., Panesar, M., Gentry, C., *et al.*, *This meeting.*

249P 2-METHYL-6-(PHENYLETHYNYL)-PYRIDINE (MPEP): A NOVEL POTENT, SUBTYPE-SELECTIVE AND SYSTEMICALLY ACTIVE ANTAGONIST AT METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5

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Metabotropic glutamate receptors (mGluR) are a heterogeneous family of G-protein-coupled receptors linked to multiple second messengers and modulation of ion channel functions in the nervous system. However, mGluR pharmacology is hampered by the lack of subtype-specific compounds. Here, we report on the selectivity of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a novel antagonist for the mGluR subtype 5 (mGlu5) identified from chemical derivatisation around SIB-1893 (2-methyl-6-styryl-pyridine, Varney *et al.*, this meeting).

The inhibitory effect of MPEP on quisqualate-stimulated phosphoinositide (PI) hydrolysis was studied in cells expressing human mGlu5a and hmGlu1b receptors, respectively (methods described by Lin *et al.*, 1997). MPEP inhibited quisqualate-stimulated PI hydrolysis in hmGluR5a expressing cells with an IC_{50} of 36 nM (pIC_{50} = 7.44 ± 0.09, n=3), but not in cells expressing the human mGluR1b (EC_{50} and IC_{50} values were > 100 µM, n=3). Schild analysis indicated that MPEP (10 to 300 nM) acted in a non-competitive manner at the hmGluR5a by dose-dependently decreasing the efficacy of glutamate-induced PI hydrolysis (e.g., 60 ± 7 % of maximal response at 30 nM MPEP, n=3) without significantly affecting the EC_{50} value or Hill coefficient of glutamate. MPEP (up to 100 µM) did not affect cAMP levels in human mGluR2, -4a, -7b and -8a expressing cell lines (methods described by Flor *et al.*, 1995a, b; 1997). Electrophysiological recordings in *Xenopus laevis* oocytes demonstrated that MPEP (up to 10 µM) did not affect the agonist-induced currents mediated by recombinant human $NMDA_{1A/2A}$, $NMDA_{1A/2B}$, rat AMPA (GluR3) or human kainate (GluR6) receptors (0.2 ± 1.1, -2.8 ± 4.8, 0.6 ± 1.3 and -6.5 ± 1.8% change, respectively; n=3). MPEP inhibited (1S,3R)-ACPD-induced PI hydrolysis with an IC_{50} of 160 nM (pIC_{50} = 6.80 ± 0.08, n=4) and a maximal inhibition of 62 ± 13% in hippocampal slices from neonatal rats (Sprague-Dawley, postnatal days 8 to 10). Extracellular recordings from hippocampal CA1 neurons of adult anaesthesized rats (male Sprague-Dawley, 170 – 300 g, 1.9% isoflurane in 70:30 O_2/N_2O) demonstrated that MPEP (6 mg/kg, i.v.) inhibited microiontophoretically applied DHPG-induced excitation (40 ± 8, % inhibition, n=6), but not the excitation induced by AMPA (n=6). Measurements of intracellular [Ca^{2+}] in HEK293 cells transiently transfected with hmGluR1b, hmGluR5a or chimeric hmGluR1/5a and hmGluR5/1b receptors revealed that the inhibitory activity of MPEP is mediated by the C-terminal part of the mGluR5 receptor containing the transmembrane segments II to VII and the cytoplasmic tail.

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250P ANTI-HYPERALGESIC EFFECTS OF THE NOVEL METABOTROPIC GLUTAMATE RECEPTOR 5 ANTAGONIST, 2-METHYL-6-(PHENYLETHYNYL)-PYRIDINE, IN RAT MODELS OF INFLAMMATORY PAIN

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Excitatory amino acids, including glutamate, are released at the site of inflammation and glutamate is hyperalgesic when injected into the rat skin (Lawand *et al.* 1997). Group I metabotropic glutamate receptors (mGluR) are expressed in the rat dorsal root ganglion and spinal dorsal horn (Valerio *et al.* 1997). Gasparini *et al.* (this meeting) and Varney *et al.* (this meeting) have demonstrated that 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and SIB-1893 are selective antagonists of the human and rat mGluR5. We now show that MPEP inhibits the development of mGluR group I agonist-induced mechanical hyperalgesia. Furthermore, we have examined the effects of mGluR5 blockade with MPEP in rat models of inflammatory hyperalgesia.

Rat hind paw withdrawal thresholds (PWT) to a mechanical stimulus were measured in Sprague-Dawley rats (160-200 g n=6/group) using a Ugo Basile Analgesymeter with a blunt, wedge-shaped probe (cut-off threshold of 180 g). The effects of glutamate receptor agonists and antagonists were examined 5 to 60 min after intraplantar (i.pl.) injection into the hind paw of naïve rats. The effects of MPEP were also tested against the mechanical hyperalgesia produced either 24 hours after a 25 µl i.pl. injection of Freund's complete adjuvant (FCA), or 1 and 3 hours after a 50 µl i.pl. injection of turpentine/paraffin oil (1:1) into a rat hind paw. The anti-hyperalgesic effects of MPEP were compared following oral (p.o.), i.pl. or intrathecal (i.t.) routes of administration. Compounds were dissolved in phosphate buffered saline, MPEP was suspended in 10% DMSO in a 1% solution of tragacanth for p.o. administration and 10% ethanol, 10% Tween 80 in saline for i.pl. or i.t. injections (injection volume = 10 µl). Data were analysed by ANOVA followed by Tukey HSD test ($p<0.05$).

Glutamate, NMDA, AMPA and the group I mGluR agonists DHPG and CHPG produced a dose-dependent decrease in PWT. Maximal decreases in PWT were produced by glutamate (1-100 nmoles, i.pl.) 30-60 min following injection (51 ± 2 % decrease in PWT, 100 nmoles i.pl., n=6). The rank order of potency for the mechanical hyperalgesic effects of glutamate receptor agonists was: glutamate > CHPG = DHPG > NMDA = AMPA > LY314582 (group II mGluR agonist) > L-AP4 (group III mGluR agonist). The mechanical hyperalgesia induced by the group I mGluR agonists DHPG and CHPG was dose-dependently inhibited by i.pl. co-administration of MPEP (10-300 nmoles), but not by the mGluR1 antagonist (S)-4C-PG (1-300 nmoles). Repeated testing from 1-5 h following administration of MPEP (10-100 $mgkg^{-1}$, p.o.) showed at least 63 ± 6 % reversal of mechanical hyperalgesia in the FCA-inflamed hind paw. MPEP reversed FCA-induced mechanical hyperalgesia when administered i.pl. but not when it was administered i.t. (dose range: 10-300 nmoles). Pre-treatment of the rat hind paw with MPEP (10-300 nmoles, i.pl.) 30 min prior to turpentine treatment inhibited the development of mechanical hyperalgesia.

These data show that blockade of peripheral mGluR5s with the selective antagonist MPEP inhibits mechanical hyperalgesia in the inflamed rat hind paw. Group I mGluR agonists induce the development of mechanical hyperalgesia when injected into the naïve rat hind paw and this hyperalgesia is inhibited by MPEP but not a mGluR1 antagonist.

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251P THE EFFECTS OF METABOTROPIC GLUTAMATE RECEPTOR AGONISTS ON NEURONES IN THE SUPERFICIAL LAMINAE (LI III) OF THE DORSAL HORN OF THE RAT LUMBAR SPINAL CORD *IN VITRO*

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Several studies have shown that metabotropic glutamate receptor (mGluR) activation alters synaptic transmission and neuronal excitability in the spinal cord (e.g. Boxall *et al.*, 1996; King & Liu, 1997). Immunohistochemical studies have revealed $mGlu_{1\alpha}$ receptors in LI-III, $mGlu_2$ and $mGlu_3$ in LII (Yung, 1998) and $mGlu_7$ presynaptically on C- fibre terminals in LII (Li *et al.*, 1997). In the present study the effects of selective mGluR agonists on neuronal excitability and synaptic transmission of neurones in LI-III of the dorsal horn of the rat lumbar spinal cord have been investigated *in vitro*.

Neonatal (12-18 day) rats were anaesthetized with ether and decapitated. A para-sagittal slice was prepared from the lumbar spinal cord with attached dorsal root ganglia and the peripheral nerves. The slice was arranged in a multicompartment tissue bath perfused with gassed ACSF. Intracellular recording was made with sharp microelectrodes. Peripheral nerve stimulation was via two pairs of platinum electrodes and stimulus parameters of > 80 V, 0.5 ms to recruit both A- and C- fibres. The effects of the Group I ($mGlu_1$ and $mGlu_5$) agonists (S)-3,5-Dihydroxyphenylglycine ((S)-3,5-DHPG), Group II ($mGlu_2$ and $mGlu_3$) agonist (2S,3S,4S)-CCG/(2S,1'S,2'S)-2-(Carboxy-cyclopropyl)glycine (L-CCG-I), and Group III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) agonist L(+)-2-Amino-4-phosphonobutyric acid (AP4) were tested. Paired *t*-test was used for statistical analysis.

It was found that all mGluR agonists could alter the neuronal excitability. (S)-3,5-DHPG (25-40 μM) consistently induced depolarisations in 8 out of 11 cells tested (7.6 ± 1.3 mV, mean ± s.e.m, n=8, peak amplitude). L-CCG-I (25 μM) also induced small depolarisations (6 out of 12, 4.8 ± 0.4 mV), however in 3 neurones hyperpolarisations were observed (-7.3 ± 1.9 mV). In contrast, none of the cells (n=13) tested with AP4 (25 μM) showed any depolarisation, although four of them exhibited hyperpolarisations (-7.1 ± 2.1 mV). In addition, (S)-3,5-DHPG (25-40 μM) and L-CCG-I (25 μM) typically increased input resistances (23.4 ± 4.6 %, $P < 0.05$ in 8 out of 11 cells; 27.4 ± 5.2 %, $P < 0.05$ in 6 out of 9 cells, respectively), whilst in contrast, AP4 (25 μM) predominately decreased the input resistances (21.0 ± 2.9 %, $P < 0.01$, 7 out of 14). All the mGluR agonists attenuated the evoked-EPSPs even when the membrane potential was returned to its pre-drug level by current injection. (S)-3,5-DHPG (25-40 μM), L-CCG-1 (25 μM) and AP4 (25 μM) produced reductions of 27.5 ± 5.2 % ($P < 0.05$, n=8), 24.1 ± 2.1 % ($P < 0.01$, n=7) and 31.6 ± 4.4 % ($P < 0.01$, n=8) in mean amplitude respectively.

In conclusion, activation of mGluRs can modulate synaptic transmission and neuronal excitability. More detailed analysis is in progress to assess whether this could be exploited to influence nociception.

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252P DOCOSAHEXAENOIC ACID AFFECTS GLUTAMATE-INDUCED CELL DEATH IN PRIMARY CORTICAL CULTURES

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Glutamate is an excitatory neurotransmitter released during cerebral ischaemia. Glutamate acts via NMDA receptors to increase calcium influx, which activates calcium dependent phospholipases A_2 (PLA_2). Arachidonic acid (AA), released by PLA_2, is metabolised to inflammatory mediators to initiate necrotic and apoptotic mechanisms. AA also potentiates the effects of glutamate by inhibiting its reuptake and enhancing its release. In contrast, 21 day pretreatment i.p. with docosahexaenoic acid (DHA) has been shown to be neuroprotective (Okada *et al.*, 1996), and is therefore prophylactic. However, it is not known if administration of DHA post-ischaemia reduces glutamate induced neurotoxicity. To determine if DHA would be useful as a treatment for stroke in the clinic we investigated its effect when given before or after glutamate shock on neuronal cell death in rat primary cortical cultures.

Cultures were prepared from 18d Sprague Dawley rat foetuses, essentially as described by Halliwell *et al.*, (1989). Cells were grown in 24 well Primaria treated culture plates in media at 37°C in a 5% CO_2/95% air atmosphere. Glutamate was prepared in cortical cell buffer. Cells were treated with 10μM to 1mM glutamate for 15min at room temperature then incubated for 24h in serum free media. For pretreatments, 0.5μl of 0.01mM to 10mM DHA in absolute ethanol was added to 500μl serum free media in each well to give final concentrations of 0.01μM to 10μM DHA. After 24h cells were treated with 200μM glutamate. For post-treatments, 3h after glutamate shock, DHA was added and the cells incubated for 21h. Cell death was measured 24h after glutamate shock by lactate dehydrogenase (LDH) release, using the Sigma LDH kit by the method of Cabaud & Wroblewski (1958).

Glutamate increased LDH release dose dependently with an ED_{50} of 127μM (95% C.I. 103μM to 158μM). Therefore, 200μM glutamate was used in all subsequent experiments. This gave LDH values of 687.1±111.4 B-B units/ml (n=7) compared to buffer control, which gave LDH values of 76.1±28.8 B-B units/ml (n=7). DHA pretreatment for 24h increased cell death, whereas 0.01μM DHA, 3h post glutamate, was neuroprotective (Table 1). AA (0.1μM to 100μM) tested in parallel was neurotoxic given either pre or post-treatment.

Table 1 LDH release with DHA given before or after 200μM glutamate

DHA (μM)	0.01	0.1	1.0	10.0
Pretreatment	174±16.2**	155±6.3	158±8.3	187±14.5**
Post-treatment	77.4±4.4**†	114±3.6	117.5±5.8	99.4±7.0

Values are expressed as percent of LDH release induced by 200μM glutamate. Data were analysed by one way analysis of variance with treatment as factor; n=4, except †, where n=20; significance from control, **p<0.01.

In our study we have shown that 3h post-treatment with DHA had a direct neuroprotective effect. This is probably due to DHA interfering with AA metabolism, as delayed ischaemic cell death is, in part, due to sustained AA release and consequent metabolism by cyclooxygenases and lipoxygenases to diffusible inflammatory mediators (Weber, 1988). In contrast, we found that 24h pretreatment with DHA increased glutamate induced cell death, suggesting that longer term pretreatment is necessary to confer neuroprotection. Thus, in conclusion, DHA may be useful in stroke therapy not only as a dietary prophylactic but also after the event to limit deficits.

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253P EXTRACELLULAR GLUTAMATE LEVELS FOLLOWING INTRAHIPPOCAMPAL INFUSION OF α-DENDROTOXIN AND DENDROTOXIN-K IN THE RAT

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Dendrotoxins are a family of 7kDa polypeptides, isolated from mamba snake venoms, which can block specifically a variety of voltage-gated K^+ channels. Microinfusion of two of these compounds, α-dendrotoxin (α-DTx) and dendrotoxin K (DTx-K), into the CA1 region of the rat hippocampus has been shown to produce motor seizures and selective neuronal loss, but apparently by different mechanisms. Seizure induction and CA1 pyramidal cell loss resulting from α-DTx infusion are not prevented by prior treatment with NMDA and non-NMDA antagonists (Bagetta et al., 1994). In contrast, these antagonists do protect against seizures and CA1 hippocampal damage induced by DTx-K (Bagetta et al., 1996). This suggests that DTx-K neurotoxicity may result from pre-synaptic blockade of K^+ channels leading to glutamate-mediated excitotoxicity whereas α-DTx acts at the postsynaptic level. In this study we have used microdialysis to measure glutamate release following infusion of α-DTx or DTx-K into the CA1 region of the rat hippocampus.

Male Wistar rats (255 - 315 g) were anaesthetised with chloral hydrate (400 mg/kg i.p.) and a microdialysis probe (concentric design, 2mm regenerated cellulose membrane) and guide cannula were implanted in the CA1 region of the hippocampus (4.0P, 2.0L, 2.4V, mm, relative to bregma). After surgery, anaesthesia was maintained with halothane (1.5%) in O_2. The microdialysis probe was perfused with artificial CSF (mM: NaCl, 125; KCl, 2.5; $MgCl_2$, 1.18; $CaCl_2$, 1.26; NaH_2PO_4, 0.2; pH adjusted to 7.0) at 2 µl/min. An internal cannula was placed into the guide cannula leaving the tip within 0.5 mm of the dialysis membrane surface. The animal was allowed to stabilise for 2h after which 6x5 min dialysis samples were collected to establish basal levels. α-DTx or DTx-K (35 pmol), or vehicle (0.05% bovine serum albumin) was then infused through the cannula (volume, 1µl; rate, 1µl/min) and a further 6x5 min dialysis samples collected. Glutamate levels were determined by HPLC with fluorimetric detection.

Infusion of vehicle did not significantly increase dialysate glutamate levels above basal values (n=5, $p>0.05$, repeated measures ANOVA). Infusion of 35 pmol α-DTx produced a significant increase in glutamate in the sample collected within 5 min of infusion (956±535% of basal, n=3, $p=0.03$, repeated measures ANOVA with post-hoc Dunnett's test), which then returned to basal level. In contrast, infusion of 35 pmol DTX-K produced increases in glutamate level in the first and second samples collected after infusion (728±132% and 631±116% of basal respectively, n=3, $p<0.0001$, repeated measures ANOVA with post-hoc Dunnett's test) before returning to basal level.

These findings confirm that glutamate release is enhanced by both α-DTx and DTx-K. The apparent difference in the time course of release patterns may be a consequence of differing pre- or post-synaptic mechanisms.

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254P A COMPARISON BETWEEN THE BINDING AFFINITIES OF TWO NOVEL GLYCINE SITE ANTAGONISTS TO CLONED NMDA RECEPTOR SUBTYPES

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The identification of the co-agonist glycine binding site on the N-methyl-D-aspartate (NMDA) receptor as a potential therapeutic target has led to the development of novel glycine site antagonists. These include MDL105,519, ((Z)-2-(phenyl)-3-(4,6dichloroindol-3-yl-2-carboxylic acid) propenoic acid), GV150,526A (3-[2-(phenylaminocarbonyl)ethenyl]-4,6-dicloroindole-2-carboxylic acid sodium salt) and GV196,771A (E-4,6-dichloro-3-(2-oxo-1-phenyl-pyrrolidin-3-ylidenemethyl)-1H-indole-2-carboxylic acid sodium salt) (Gaviraghi *et al.*,1998). We have previously reported that the glycine site antagonists, MDL105,519 and GV150,526A bind with high affinity to the NR1 NMDA receptor subunit and that they both display no subtype selectivity between NR1a/NR2A or NR1a/NR2B receptors (Chopra *et al.*, 1997). Here, we investigate further the subtype selectivity of GV150,526A and GV196,771A to cloned NMDA receptors expressed transiently in human embryonic kidney (HEK) 293 cells by radioligand binding. HEK 293 cells were transfected with NMDA receptor clones by the calcium phosphate method. Cells were collected 24 h post-transfection and well-washed cell homogenates prepared. Non-specific binding was defined using 1 mM glycine. The Inhibitory Constants (K_I) were determined by the displacement of [^{3}H] MDL105,519 radioligand binding by the filtration method with an incubation time of 90 min at 4°C (Chazot et al., 1998). Competition curves were analysed by non-linear least squares regression analysis using GraphPAD Prism. Table 1 summarises the results where the K_I values were obtained from a sigmoidal fit. Both GV150,526A and GV196,771A bound to the NR1 subunit expressed alone, with no significant difference in affinity between the NR1-1a and NR1-4b splice forms. No specific binding was detected to NR2 subunits expressed alone. The binding of both GV150,526A and GV196,771A to heteromeric receptors was best fit by a two-site binding model. GV196,771A showed an apparent four-fold lower affinity for NR1a/NR2A. Detailed analyses revealed that this was attributable to the low affinity binding component ($p<0.05$). These results substantiate that the major determinants for the binding of glycine site antagonists resides on the NR1 subunit. As for GV150,526A, the N1 exon of the NR1 subunit has no effect on the affinity for GV196,771A. Furthermore, GV150,526A has a higher affinity for the NMDA receptors investigated compared to GV196,771A.

Table 1 Comparison of the inhibitory constants

Receptor Subtype	GV150,526A K_I (nM)	GV196,771A K_I (nM)
NR1-1a	3.4 ± 1.5	30 ± 5
NR1-4b	5.0 ± 3.0	39 ± 7
NR1-1a/NR2A	5.9 ± 2.2	157 ± 40
NR1-1a/NR2B	6.8 ± 2.3	35 ± 15
NR1-1a/NR2D	2.4 ± 1.3	38 ± 18

Results are mean ± SD for 4 separate determinations.

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255P 8-OH-DPAT LABELS 5-HT_{1A} RECEPTORS AND 5-HT UPTAKE SITES AS WELL AS OTHER SEROTONERGIC BINDING SITES IN THE RAT BRAIN RAPHE AREA

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The 5-HT_{1A} agonist 8-OH-DPAT has been widely used to label 5-HT_{1A} receptors. This compound, however, has additional 5-HT uptake inhibiting properties (Assié & Koek, 1996a). The present work was undertaken to examine further the binding of [^{3}H]8-OH-DPAT in the raphe area of the rat brain, a region rich in 5-HT_{1A} receptors and 5-HT uptake sites.

Binding assays were carried out in membrane homogenates from rat brain raphe area (Sprague Dawley 180-200 g) prepared in Tris (50 mM, HCl pH 7.4 at 25°C) as described previously (Assié & Koek, 1996b). [^{3}H]8-OH-DPAT (0.25 nM) alone or in the presence of the 5-HT_{1A} antagonist WAY100635 (1 µM), and test compounds were incubated with membranes (5 mg/ml Tris buffer) for 30 min. The 5-HT uptake inhibitor [^{3}H]citalopram (0.5 nM) and test compounds were incubated with membranes (0.5 mg/ml Tris containing NaCl 120 mM, KCl 5 mM) for 60 min. For both assays, the incubation, carried out at room temperature, was stopped by filtration (GF/B filters).

5-HT inhibited [^{3}H]8-OH-DPAT binding in a biphasic manner (pKi1: 8.82 ± 0.01, pKi2: 6.07 ± 0.05, n = 4) with the low affinity site representing 36 ± 4 % of the total population. In contrast, the inhibition curve of the 5-HT_{1A} antagonist, WAY100635 was monophasic (pKi: 8.5, n = 2). In the presence of 1 µM WAY100635, 5-HT inhibited [^{3}H]8-OH-DPAT binding in a monophasic manner (pKi: 6.04 ± 0.07, n = 3). Affinity of various compounds for sites labelled by [^{3}H]8-OH-DPAT in the presence of 1µM WAY100635 and for sites labelled by [^{3}H]citalopram are reported in Table 1. For the compounds examined to date, there was a significant correlation between their pKi values at 5-HT uptake sites and at non-5-HT_{1A} sites labelled by [^{3}H]8-OH-DPAT ($r = 0.87$, $P<0.01$, $n = 9$), suggesting these latter sites to be 5-HT uptake sites. The ratio of Ki values at both sites, however, varied markedly among the compounds tested. The non-5-HT_{1A} binding sites labelled by [^{3}H]8-OH-DPAT are unlikely to be 5-HT_7 receptors, because 5-HT has much higher affinity for 5-HT_7 receptors than for the non-5-HT_{1A} sites. Thus, in addition to 5-HT_{1A} receptors and 5-HT uptake sites, 8-OH-DPAT might recognise other serotonergic binding sites as yet not characterised.

Table 1: Affinity of various compounds for the sites labelled by [^{3}H]8-OH-DPAT in the presence of 1µM WAY100635, and by [^{3}H]citalopram, expressed as pKi ± sem of 3 determinations.

Compound	[^{3}H]8-OH-DPAT	[^{3}H]citalopram	ratio
8-OH-DPAT	7.32 ± 0.15	6.52 ± 0.10	5.85
ritanserin	6.60 ± 0.13	6.30 ± 0.04	1.82
risperidone	6.21 ± 0.12	6.01 ± 0.03	1.50
TFMPP	6.36 ± 0.08	6.31 ± 0.08	1.13
5-HT	6.04 ± 0.07	6.45 ± 0.01	0.378
RU 24969	6.45 ± 0.13	7.17 ± 0.02	0.174
fluoxetine	7.19 ± 0.01	8.43 ± 0.09	0.060
paroxetine	8.38 ± 0.10	10.21 ± 0.05	0.014
indatraline	7.57 ± 0.13	9.58 ± 0.05	0.009

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256P CELLULAR LOCALISATION OF THE 5-HT_{2C} RECEPTOR IN THE RAT BRAIN

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The neurotransmitter 5-HT (5-hydroxytryptamine; serotonin) interacts with at least 14 different mammalian receptor subtypes (for review see Hoyer et al., 1994). The structure of the 5-HT_{2C} receptor indicates that it is a member of the 7 transmembrane domain superfamily, and is G-protein coupled. Consistent with this classification activation of the 5-HT_{2C} receptor increases inositol phosphate turnover (Hoyer et al., 1989). The 5-HT_{2C} receptor has been implicated in a number of physiological/pathological processes including hypolocomotion, hypophagia, anxiety, depression and temperature regulation (Koek et al., 1992). These responses mediated presumably via the CNS.
Whilst previous studies have demonstrated the regional distribution of the 5-HT_{2C} receptor in the rat brain, little information is available concerning the cellular distribution of the 5-HT_{2C} receptor. The present study utilises a previously described polyclonal antibody (serum 522; Abramowski et al., 1995), that recognises selectively the 5-HT_{2C} receptor protein, to study the cellular distribution of the 5-HT_{2C} receptor in the rat forebrain.

Rats (male Wistar, 150-200 g) were subject to an in *vivo* tissue fixation process. Briefly, rats were anaethetised terminally with barbituate before trans-cardiac perfusion (10 ml/min) with 100 ml saline-heparin followed by 4% paraformaldehyde + 0.1% glutaraldehyde (400 ml). The whole brain was subsequently removed and stored at 4°C in 4% paraformaldehyde for at least 20 hrs. Coronal brain sections (70 µm) were cut using a vibratome and sections stored in PBS (phosphate buffered saline) + 1% paraformaldehyde at 4°C.

Free floating tissue sections were processed for immunohistochemistry using an affinity purified antibody directed against the C-terminus of the 5-HT_{2C} receptor. The sections were incubated with the primary antibody (diluted 1:500 in PBS + 1% normal goat serum + 0.1% Triton X-100) over night at 4°C and then a biotinylated anti-rabbit secondary antibody (Vector) the next day (for 2 hrs) before being processed with a TSA (tryamide signal amplification) kit (NEN Life sciences). Sections were then developed with 3,3`-diaminobenzidine (0.025% DAB, 0.35% nickel ammonium sulphate, 0.0006% H_2O_2) mounted onto slides, counterstained with cresyl violet, dehydrated and coverslipped with DPX before viewing under a light microscope.

5-HT_{2C} receptor-like immunoreactivity was associated with cells in numerous brain regions (e.g. choroid plexus, striatum, septum, cerebral cortex). Apart from the immunoreactivity in the choroid plexus, the immunoreactive cells displayed a neuronal morphology with the majority of the positive cells 10-15 µm in size (cell body diameter). The immunoreactivity was associated with both the cell body and associated proximal processes.

In summary, the present study has demonstrated the cellular distribution of 5-HT_{2C} receptor-like immunoreactivity in a number of brain regions. Subsequent studies are attempting to phenotype the 5-HT_{2C} receptor expressing cells in the rat brain.

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257P CO-ADMINISTRATION OF PHENTERMINE WITH *d*-FENFLURAMINE CAUSES ADDITIVE EFFECTS ON RAT BRAIN 5-HT RELEASE *IN VIVO* AND *IN VITRO*

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Administration of *d*-fenfluramine (Fen) either alone or in combination with phentermine (Phen) is associated with the production of valvular heart disease in man, but the mechanism of action is uncertain (Connolly *et al.*, 1997). The present study examined the effect of the antiobesity agents, Fen and Phen, alone and in combination on neuronal 5HT function *in vivo* and *in vitro*.

5HT efflux from rat anterior hypothalamus was measured using *in vivo* microdialysis while *in vitro* release of [^{3}H]5HT was measured from superfused slices of rat frontal cortex (see Heal *et al.*, 1998) because of the small size of the hypothalamus. In dialysis studies, Fen and Phen were administered i.p. at doses approx. 3 x oral ED_{50} values for the inhibition of food intake obtained 1 h after drug (Data on File, Knoll Pharmaceuticals).

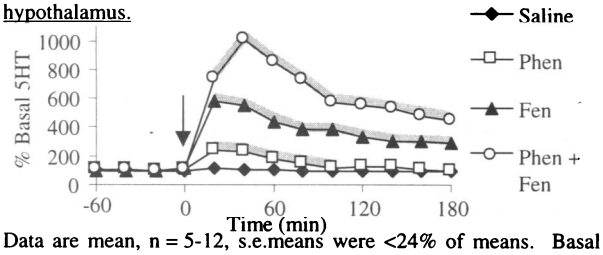
In vitro, Fen caused a concentration-related release of preloaded [^{3}H]5HT from frontal cortex slices (Table 1). Phen alone was without effect and did not interact with the response to Fen when the two drugs were combined (Table 1). *In vivo*, both Fen and Phen alone caused significant efflux of endogenous 5HT from rat hypothalamus (Fig. 1). In combination, there was no statistical evidence for an interaction between Fen and Phen that was anything other than additive (Fig. 1).

Table 1 Effect of Fen, Phen or the Fen/Phen combination on [^{3}H]5HT release from CD rat frontal cortex slices.

KCl (50 mM)	Drug Conc	Fen	Phen	Fen/Phen[a]
296 ± 37***	1 μM	70 ± 8***	10 ± 9	78 ± 9***
240 ± 45***	10 μM	135 ± 19***	-7 ± 22	120 ± 17***

[a]Each drug applied at the concentration stated. Data are mean ± s.e.mean % change from control fractional release values, n=6. Significant change from control *** p<0.001 Dunnett's test.

Figure 1 Effect of saline (2 ml kg^{-1}), Phen (5.7 mg kg^{-1}), Fen (3.0 mg kg^{-1}) or Phen + Fen (5.7 + 3.0 mg kg^{-1}, respectively) on extracellular 5HT in the freely moving Wistar rat anterior hypothalamus.



Data are mean, n = 5-12, s.e.means were <24% of means. Basal pre-drug 5HT levels were 9.0 ± 1.1 fmol/20 μl (n = 34). Drug was injected at the arrow and shading shows significant changes from saline (p<0.05 ANCOVA followed by Dunnett's test).

The lack of 5HT-releasing action of Phen *in vitro* may result from the absence of long loop neuronal feedback systems in the deafferented slices. The *in vivo* data accord with previous microdialysis findings from rat nucleus accumbens indicating that these antiobesity agents act additively on monoaminergic systems (Shoaib *et al.*, 1997).

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258P PRETREATMENT WITH 8-OH-DPAT OR GEPIRONE DOES NOT ATTENUATE THE DEPRESSANT EFFECT OF CCK ON OPERANT FOOD INTAKE IN PIGS

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Poeschla et al. (1992) have previously reported that pretreatment with the $5HT_{1A}$ agonist 8-hydroxy-2 (di-n-propylamino) tetralin (8-OH-DPAT) attenuates the hypophagic action of CCK in rats. As $5HT_{1A}$ agonists decrease 5HT function in the CNS, these workers have argued that the inhibitory effect of peripherally acting CCK is dependent on intact central 5HT systems. However, this interpretation is controversial, as in a more recent study, Ebenezer and Brooman (1994) failed to replicate their results. The present experiments were undertaken to investigate whether peripheral exogenous CCK recruits central 5-HT processes to elicit its suppressant effect on food intake in a species other than the rat, namely, the pig.

Prepubertal Large White boars (b.wt. 30 - 40 kg) were chronically prepared under halothane anaesthesia with jugular vein cathethers and housed in metabolism cages where they were trained to perform operant responses to obtain food and water. The pigs were maintained on the following feeding schedule: at 9.30h the animals were given 400g of their pelleted food to eat and at 15.00h a buzzer signalled that the feeders in their cages were activated for 60 min and that they could make operant responses for food. One hour prior to the start of the afternoon feeding session (i.e. 14.00h), the pigs (n=7) were injected iv with either saline or 8-OH-DPAT (25 or 50 μg kg^{-1}). Five minutes after the start of the feeding session the animals received a second iv injection of either saline or CCK (1 μg kg^{-1}).The number of food reinforcements obtained by each pig was monitored on a data logger. The results were analysed by ANOVA for repeated measures. A second experiment was conducted using a similar experimental protocol, except that the pigs (n=7) were pretreated with the $5HT_{1A}$ agonist gepirone (0.5 mg kg^{-1}; iv).

CCK (1 μg kg^{-1}) produced a significant reduction in food intake (P<0.01) during the first 15 min after injection. Pretreatment with 8-OH-DPAT (25 and 50 μg kg^{-1}; iv) did not attenuate the depressant effect of CCK on food intake and neither of these doses produced significant alteration in feeding compared with vehicle. Thus, the number of food reinforcements (mean ± s.e. mean) obtained by the pigs in the first 15 min after the various treatments were as follows: Saline-Saline, 32.4±4.7; Saline-CCK, 10.9±4.1; 8-OH-DPAT (25 μg kg^{-1})-Saline, 27.0±4.0; 8-OH-DPAT (25 μg kg^{-1})-CCK, 12.9±4.9; 8-OH-DPAT (50 μg kg^{-1})-Saline, 25.4±2.2; 8-OH-DPAT (50 μg kg^{-1})-CCK, 7.3±2.8. Similarly, pretreatment with gepirone (0.5 mg kg^{-1}) did not attenuate the depressant effect of CCK (1 μg kg^{-1}) on feeding. It is noteworthy that both 8-OH-DPAT and gepirone induced a number of behavioural changes in the animals, which included snout-rubbing and head-nodding. However, these abnormal behaviours were only apparent during the first 10 - 15 min after drug administration.

The results of this study show that pretreatment of pigs with the $5HT_{1A}$ agonists 8-OH-DPAT or gepirone does not attenuate the depressant effect of iv CCK on operant food intake.These results confirm and extend previous observations in the rat (Ebenezer and Brooman, 1994) and suggest that peripheral CCK in the pig does not recruit 5-HT systems in the central nervous system to elicit its hypophagic effect.

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259P IMMUNOCHEMICAL INVESTIGATION OF A NOVEL PEPTIDE, OREXIN-A: ANATOMICAL DISTRIBUTION IN RAT BRAIN AND SPINAL CORD AND ITS RELATIONSHIP TO OTHER HYPOTHALAMIC NEUROTRANSMITTER SYSTEMS

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Energy homeostasis is co-ordinated in the lateral (LH), ventromedial, arcuate (ARC) and paraventricular (PVN) hypothalamic nuclei by a host of neuromodulators, including neuropeptide Y (NPY), leptin, melanin concentrating hormone, cholecystokinin and glucagon-like peptide 1 (Wilding *et al.*, 1997). Orexin-A and orexin-B are novel peptides discovered in the LH which are up-regulated with fasting and stimulate feeding when injected i.c.v. (Sakurai *et al.*, 1998). In this study, we employed immunochemistry to determine the distribution of orexin-A throughout the rat CNS, and its possible co-localization with other hypothalamic peptides or signalling systems.

Adult male Wistar rats were anaesthetized (Sagatal; 150 $mg.kg^{-1}$, i.p.), perfusion fixed and coronal sections (80 µm thick) of brain and spinal cord were taken. Free-floating sections were sequentially incubated with: 2% donkey serum, rabbit anti-orexin-A antiserum (1:2000; raised against synthetic orexin-A_{15-33} fragment) and anti-rabbit FITC (1:100; Jackson, USA). Double-labelling was performed by co-incubation of antisera to orexin-A and one of (goat- or sheep-raised) NPY (1:500), leptin (Ob) receptor (1:200; Santa Cruz, USA), neuronal nitric oxide synthase (nNOS; 1:1000) or neurophysin (1:200), followed by anti-rabbit FITC, anti-goat/sheep biotin (1:500; Jackson) and strepavidin-Cy3 (1:100; Jackson). Sections were washed (3-5x, 15 min each) with 0.1 M phosphate buffered saline prior to each step; all washes and incubations (60-90 min duration) were at room temperature, except for the primary antisera (20-48 h, 4°C).

Orexin-A-immunoreactive cells were found only in the hypothalamus, concentrated in the LH/perifornical nuclei and more sparsely distributed in the dorsal aspect of the anterior and dorsomedial hypothalamic areas. Immunoreactive fibres were localized throughout the brain and spinal cord, with prominent projections to sites in the hypothalamus (e.g. LH, perifornical, dorsomedial, PVN and ARC nuclei), thalamus (e.g. paraventricular, paracentral and central medial nuclei) and elsewhere (e.g. locus coeruleus, central grey, septal and raphe nuclei); the presence of apparent terminal fibres near the ventricular ependymal cell layers and pia suggests that orexin-A may be released into the cerebrospinal fluid. The extensive fibre projections into nuclei known to contain additional feeding regulators, e.g. NPY (PVN, ARC) or the Ob receptor (ARC), and other factors, e.g. neurophysin (PVN) or nNOS (LH), prompted investigation into the possibility of co-expression with orexin-A. Orexin-A neurones or fibres did not appear to be co-localized with cells or processes which were immunoreactive for NPY, Ob receptor, neurophysin or nNOS.

The wide-spread distribution of orexin-A fibres and potential release into the cerebrospinal fluid indicates that orexin-A may have, in addition to increasing food intake, more global behavioural implications. Furthermore, the lack of co-localization of orexin-A with either NPY or the Ob receptor in the hypothalamus suggests that orexin-A is disparate from the NPY-leptin system which modulates energy balance.

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260P AFFINITY OF β-ADRENOCEPTOR LIGANDS FOR PRE- AND POST-SYNAPTIC 5-HT_{1A} RECEPTORS IN RAT BRAIN

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There is considerable interest in the use of drugs which block presynaptic 5-HT_{1A} receptors in the adjunctive treatment of major depressive illness. A number of 5-HT_{1A}/β-adrenoceptor ligands including, pindolol, tertatolol and penbutolol, are available for use in man, and antidepressant properties of pindolol have been detected in some clinical trials (Zanardi et al., 1998). Although these drugs have high affinity for 5-HT_{1A} binding sites (Langlois et al., 1993), little is known of their affinity for presynaptic 5-HT_{1A} receptors compared to 5-HT_{1A} receptors located postsynaptically in regions such as hippocampus. In functional studies, some authors claim that pindolol acts selectively at presynaptic 5-HT_{1A} receptors in the midbrain raphe nuclei (Romero et al. 1996) but this was recently disputed (Corradetti et al., 1998). Here we report receptor autoradiographic studies using the novel 5-HT_{1A} receptor antagonist radioligand [^{3}H]WAY 100635 (Khawaja et al., 1995) to determine the affinity of pindolol, tertatolol and penbutolol for raphe versus hippocampal 5-HT_{1A} binding sites in the rat.

Cryostat-cut brain sections (14 µm) obtained from adult Sprague Dawley rats (250-270 g) and stored at -20 °C before use. Sections were preincubated in 50 mM Tris-HCl buffer (pH 7.5) for 30 min at room temperature. For competition studies, sections were incubated with 3 nM [^{3}H]WAY 100635 in the presence of 10 µM pargyline for 2 h at room temperature, with or without displacement agents. For saturation studies, the sections were incubated with increasing concentrations of [^{3}H]WAY 100635 (0.25 - 12 nM). Non-specific binding was defined by 10 µM unlabelled 5-HT. Following incubation, sections were dipped and rinsed twice for 2 min in cold buffer, briefly dipped in cold water, and then dried in a cold air stream. Sections were exposed to photographic films for about 6 weeks before quantification by image analysis.

In saturation studies, the binding of [^{3}H]WAY-100635 was specific, saturable and showed nM affinity in the dorsal raphe nucleus and all regions of hippocampus (table 1).

Table 1. Parameters of specific [^{3}H]WAY 100635 binding

Region	K_d (nM)	B_{max} (fmol/mg tissue)
CA1	1.65 ± 0.03	218.1 ± 12.0
CA3	1.71 ± 0.21	209.1 ± 23.5
DG	1.69 ± 0.18	283.2 ± 21.9
DRN	1.50 ± 0.15	220.9 ± 20.4

Data are mean ± s.e.mean. values for 4 determinations. DRN- dorsal raphe nucleus, DG - dentate gyrus.

In competition studies, the specific binding of [^{3}H]WAY 100635 was approximately 90 % of the total binding. All compounds exhibited nM affinity (table 2) and produced monophasic displacement of the binding in all regions with Hill coefficients close to unity.

Table 2. K_i values (nM) of β-adrenoceptor ligands for 5-HT_{1A} sites

Compound	CA1	CA3	DG	DRN
(±) Pindolol	8.5 ± 1.1	8.7 ± 1.8	7.9 ± 1.0	6.5 ± 1.2
(-) Penbutolol	11.6 ± 1.7	11.9 ± 2.0	14.3 ± 1.1	8.1 ± 2.1
(-) Tertatolol	24.2 ± 2.8	25.4 ± 2.1	23.1 ± 1.4	28.4 ± 2.4

Data are mean ± s.e.mean. values for 4 determinations. Affinity values were not statistically significant between regions (1 way ANOVA).

In summary, our data show that in rats, pindolol, penbutolol and tertatolol exhibit high affinity for 5-HT_{1A} binding sites and do not discriminate between sites in the DRN and hippocampus. Any regional differences in the effects of pindolol observed in some functional studies cannot be ascribed to a greater affinity for presynaptic (somatodendritic) versus postsynaptic 5-HT_{1A} sites.

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261P EFFECT OF SELECTIVE MONOAMINE UPTAKE INHIBITORS ON EXTRACELLULAR 5-HT AND NORADRENALINE IN THE HIPPOCAMPUS OF THE AWAKE RAT

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When administered repeatedly, monoamine reuptake inhibitors are effective in the treatment of depression whether they are selective for 5-HT or noradrenaline (NA). However, there is much evidence that the 5-HT and NA systems in the brain interact at the functional level. For example, in microdialysis studies we have found evidence for a facilitatory effect of 5-HT on extracellular NA mediated via 5-HT_{1A} receptors (Done and Sharp, 1994; Hajós-Korcsok and Sharp, 1996). In similar studies, NA has been found to exert an inhibitory effect on extracellular 5-HT mediated via α2-adrenoceptors (Tao and Hjorth, 1992). The aim of the present study was to investigate the effect of paroxetine and desipramine (selective inhibitors of 5-HT and NA uptake, respectively) on both extracellular 5-HT and NA levels. Drugs were administered repeatedly to mimic their therapeutic application.

Groups of male Sprague-Dawley rats (260-280 g) were administered one of the following treatments for 14 days: paroxetine (5 mg/kg, s.c. once or twice daily) desipramine (10 mg/kg s.c. once daily) or 5 % glucose vehicle (once daily). On day 14 microdialysis probes were implanted in the ventral hippocampus under halothane anaesthesia. On day 15 microdialysis probes were perfused with artificial CSF (2 µl/min). Samples were collected every 30 min and analysed for both 5-HT and NA by HPLC-ECD. After establishing stable basal levels, animals were challenged with a further dose of paroxetine, desipramine or vehicle, as appropriate. Mean basal levels of NA and 5-HT were calculated as the average of the last four samples collected before drug/vehicle challenge.

The data are summarised in Table 1. Rats injected twice daily with paroxetine showed a 2-fold increase in basal dialysate 5-HT levels. In the same animals basal dialysate NA levels also increased about 2-fold. Neither 5-HT nor NA increased after a further paroxetine challenge. In comparison, basal 5-HT and NA were not significantly altered by once daily paroxetine.

Repeated administration of desipramine caused a 4-fold increase in basal dialysate NA levels but did not change 5-HT. A subsequent challenge with desipramine had no further effect on NA; 5-HT increased but the effect was small (+22.5 ± 8.9 %, t=30 min, n=7).

Table 1. Basal levels of 5-HT and NA in rat hippocampal dialysates following repeated administration of paroxetine or desipramine.

Treatment	5-HT fmol/sample	NA fmol/sample
Treatment naive	15.5 ± 3.72	19.6 ± 4.21
Repeated vehicle	15.5 ± 2.15	17.0 ± 2.48
Repeated paroxetine (5 mg/kg, once daily)	21.7 ± 3.32	14.9 ± 1.72
Repeated paroxetine (5 mg/kg, twice daily)	30.4 ± 4.31*	30.1 ± 2.98*
Repeated desipramine (10 mg/kg, once daily)	13.9± 2.28	75.7± 10.41*

Values are mean ± s.e.mean of n=6-9. * P<0.05 compared to repeated vehicle group (1-way ANOVA, post-hoc Dunnett's test).

In summary, we report that when administered repeatedly, the selective 5-HT uptake inhibitor, paroxetine, enhanced not only extracellular 5-HT but also NA in rat hippocampus. In contrast, the selective NA reuptake inhibitor, desipramine, markedly increased NA but not 5-HT. The paroxetine-induced increase of extracellular NA may indicate the facilitatory effect of 5-HT on NA detected in our recent studies (Hajós-Korcsok and Sharp, 1996). An indirect action on NA might be involved in the therapeutic effect of paroxetine and possibly other selective 5-HT reuptake inhibitors.

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262P RS-127445, A NOVEL, SELECTIVE 5-HT RECEPTOR ANTAGONIST, INHIBITS NEUROGENIC INFLAMMATORY RESPONSES IN RAT DURA MATER

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5-HT_{2B} receptors are proposed to play a role in the initiation of migraine. Activation of cerebral vascular endothelial 5-HT_{2B} receptors may result in the production of nitric oxide, sensitization of perivascular sensory fibers and extravasation of plasma proteins thus producing inflammation in the cerebral meninges and consequently migraine (Fozard, 1995; Schmuck *et al.*, 1996)

To examine the role of 5-HT_{2B} receptors in these neuro-vascular events, we developed a selective 5-HT_{2B} receptor antagonist suitable for *in vivo* studies. This antagonist was then used to test whether 5-HT_{2B} receptors are involved in experimentally-evoked inflammatory reactions in the rat cerebral vasculature.

Radioligand binding studies, performed as previously described (Bonhaus *et al.*, 1995), demonstrated that RS-127445 (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropyl pyrimidine) has high affinity for the human recombinant 5-HT_{2B} receptor (pKi = 9.5 ± 0.1, N = 9) and 1,000-fold selectivity for this receptor as compared to numerous other binding sites including 5-HT_{2A} and 5-HT_{2C} receptors (pKi < 6.0, N=9). In HEK-293 cells expressing human 5-HT_{2B} receptors, RS-127445 (10 µM, N = 3) had no intrinsic activity but blocked the 5-HT-evoked formation of inositol phosphates (as measured by the conversion of [^{3}H]myoinositol to [^{3}H]inositol phosphates) (pK_B = 9.5 ± 0.1) and the 5-HT-mediated increases in intracellular calcium (as quantified by the increase in fluorescence in cells preloaded with Fura-2) (pIC_{50} = 10.4 ± 0.1, N = 11). RS-127445 was also a potent antagonist at 5-HT_{2B} receptors in the rat stomach fundus (pA_2 = 9.5 ± 1.1) and at 5-HT_{2B}-like receptors mediating endothelium-dependent relaxation in rat jugular vein (pA_2 = 9.9 ± 0.3). RS-127445 (5 mg kg^{-1}, N ≥ 8 animals per group) was absorbed via the oral (F = 14%) or intraperitoneal (F = 60%) routes The terminal elimination plasma half-life of RS-127445 (as determined by measuring plasma levels of RS-127445 in eight animals at 7-9 different times after dosing) was approximately 1.6 hours

RS-127445 (1.0 or 2.5 mg kg^{-1} IP, N = 5 animals per dose) abolished plasma protein extravasation in dura mater of anesthetized rats evoked by the 5-$HT_{2B/2C}$ receptor agonist meta-chlorophenylpiperizine (1.0 mg kg^{-1} IV). RS-127445 (2.0 or 5.0 mg kg^{-1} IV, N ≥ 6 animals per dose) also attenuated (30 and 41% respectively) capsaicin-evoked (10 nmol, IC) expression of c-fos protein in the cervical trigeminal nucleus caudalis of anesthetized rats (P < 0.05, ANOVA). These finding are consistent with 5-HT_{2B} receptor involvement in neurogenic inflammatory responses in the rat cerebral vasculature, regardless of whether the receptors are stimulated directly or indirectly following activation of capsaicin-sensitive sensory fibers.

These findings demonstrate that RS-127445 is a selective, orally bioavailable, high affinity 5-HT_{2B} receptor antagonist, devoid of intrinsic activity at both human and rat 5-HT_{2B} receptors. They also provide the first direct evidence of a role for 5-HT_{2B} receptors in neuro-vascular processes postulated to underlie the initiation of migraine.

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263P AGONIST-STIMULATED GTPγ[^{35}S] BINDING TO 5-HT1$_A$ RECEPTORS IN HUMAN POST-MORTEM HIPPOCAMPUS

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Stimulation of GTPγ[^{35}S] binding to neuronal membrane preparations has proved useful as a measure of agonist activity at receptors. In particular, 5-HT1$_A$ receptors have been studied in this way in both cloned human cell lines and rat hippocampus (Newman-Tancredi *et al.*, 1998; Alper & Nelson, 1998). As part of a study to determine the action of several atypical antipsychotic drugs at the 5-HT1$_A$ site, we have applied this technique to the naturally-occurring receptor in human brain tissue taken post-mortem. We have also studied the receptor by conventional radioligand binding techniques employing the selective antagonist [^{3}H]MPPF.

Briefly, for [^{3}H]MPPF ligand binding assays, frozen hippocampal tissue from human post-mortem samples was prepared in Tris-HCl buffer as described by Kung *et al.* 1996. Experiments were conducted at 37°C for 20 mins in a volume of 0.5 ml and a tissue concentration of 1.8 mg/ml. The incubation was terminated by rapid filtration and washing with ice-cold buffer. GTPγ[^{35}S] (0.1 nM) binding assays were conducted in a similar manner, in the presence of 30 μM GDP unless otherwise stated, but at the lower tissue concentration of 1 mg/ml.

[^{3}H]MPPF saturation binding yielded a B_{max} of 16.9 ± 3.4 fmol/mg tissue and a K_D of 0.63 ± 0.28 nM (n = 4). The affinity of the agonists 5-HT and 8-OH DPAT, the selective antagonist MPPI and the atypical antipsychotic clozapine, were determined and gave K_i values of 3.5 ± 1.1, 0.40 ± 0.05, 1.75 ± 0.09 and 158 ± 44 nM respectively (n = 3 - 4). Other antipsychotics that were tested, i.e. chlorpromazine, fluphenazine, olanzapine, haloperidol and loxapine were unable to displace more than 50 % of [^{3}H]MPPF (0.49 nM) at a concentration of 1 μM.

5-HT and 8-OH DPAT stimulated GTPγ[^{35}S] binding to an equal extent with EC_{50} values of 323 ± 25 and 65 ± 10 nM, respectively. These effects were confirmed to be 5-HT1$_A$ mediated as MPPI (1 μM) completely inhibited stimulated binding, which was typically 100 - 250 %. As described previously, decreasing the concentra-tion of GDP in the GTPγ[^{35}S] assay buffer is found to increase the amount of basal binding, but decreases the percentage of agonist-stimulated binding (Alper & Nelson, 1998). In this system, lowering the concentration of GDP to 3 μM also significantly decreased the EC_{50} of 5-HT to 116 ± 24 nM ($p < 0.05$).

Thus this method allows us to identify agonist effects at the natural human 5-HT1$_A$ receptor. We are also able to determine functional antagonism by inhibition of 5-HT stimulated GTPγ[^{35}S] binding. Clozapine shows both partial agonist effects as well as substantial antagonism of 5-HT's action.

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264P SELECTIVE NEUROANATOMICAL CHANGES IN 5-HT$_{1A}$ AND 5-HT$_{2A}$ RECEPTORS AND REUPTAKE SITES IN DIETARY-OBESE RATS

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5-Hydroxytrytamine (5-HT; serotonin) agonists produce satiety when injected into rodent brains (Kennett *et al.*, 1987) and drugs which increase 5-HT availability, such as sibutramine, inhibit food intake in humans (Finer, 1998). However, it is not presently known why the endogenous 5-HT system fails to prevent the development of obesity in animal models or humans. It is possible that sustained 5-HT synaptic activity in obese subjects results in the down-regulation of 5-HT receptors, thereby attenuating the satiety effects. Studies were performed to examine for neuroanatomical changes in 5-HT receptor densities in the dietary-obese rat, a model which closely resembles human obesity. Male Wistar rats (200g) were housed in groups of three and fed a highly palatable diet for 7 weeks during which they became obese, as compared to controls which were fed a standard pellet laboratory diet (Widdowson *et al.*, 1997). Obese and control rats were killed by CO_2 inhalation, the brains removed and frozen in cold isopentane (-35°C). Quantitiative receptor autoradiography was performed on cryostat cut sections (20μm) using 2 nM [^{3}H]8-OH-DPAT (non-spercific binding defined with 1 μM 5-HT) for 5-HT$_{1A}$ receptors, 2nM [^{3}H]ketanserin (non-specific binding defined with 1 μM cinanserin) for 5-HT$_{2A}$ receptors and 0.5nM [^{3}H]paroxetine (non-specific binding defined with 10 μM fluoxetine) for 5-HT transporters (Radja *et al.*, 1991). Obese rats exhibited marked hperinsulinaemia and hyperleptinaemia, a moderate hyperglycaemia and increased epidiymal and perirenal fat pad masses. Western blotting for tryptophan hydroxylase in 1 mm^3 blocks containing the dorsal (DR) and median raphe (MR) did not reveal a significant change between dietary-obese rats and controls (DR, optical density in arbitary units; controls = 49.3±5.5, obese = 53.7±5.4; mean ± S.E.M., n = 7). Specific [^{3}H]8-OH-DPAT binding revealed a significant increase in 5-HT$_{1A}$ receptor density in both DR (controls = 16.1 ±2.1 fmol/mg tissue; obese= 30.8±3.6 fmol/mg tissue; $P<0.01$, Student's t-test) and MR, but a significant reduction in the enterorhinal cortex (controls = 40.5±1.7 versus obese = 34.7±0.9 fmol/mg tissue; $P<0.05$). In contrast, [^{3}H]ketanserin binding to 5-HT$_{2A}$ receptors in the hypothalamic arcuate nuclei (controls = 21.5±2.4 versus obese = 30.9±2.0 fmol/mg tissue, $P<0.05$) and lateral hypothalamic area (LH) were significantly incrased in obese rats. Specific [^{3}H]paroxetine binding was significantly increased in DR, MR, enterorhinal cortex, hypothalamic anterior, paraventricular and ventromedial (obese = 70.1±9.8 versus 43.6±6.6 fmol/mg tissue; $P<0.05$) hypothalamic nuclei and lateral hypothalamic area. In conclusion, we have demonstrated marked regional changes in 5-HT autoreceptors, reuptake sites and postsynaptic receptors in dietary-obese rats that is consistent with increased seotonergic activity. At present, we do not know why increased 5-HT activity in obese rats fails to curb the hyperphagia associated with feeding the highly palatable diet, which leads to obesity.

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265P CONTINUOUS SUBCUTANEOUS DELIVERY OF CLOZAPINE DECREASES 5-HT$_{2A}$ RECEPTOR BINDING IN RAT BRAIN

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Many studies have been performed to investigate the effect of chronic clozapine treatment on 5-HT$_{2A}$ receptors (eg. O'Dell et al., 1990; Wilmot and Szczepanik., 1989). However, these studies have used repeated daily dosing, which would lead to intermittent receptor occupancy, as clozapine has a half life of 1.5 hrs in rat. To determine whether similar changes in 5-HT$_{2A}$ receptors and mRNA occur, the present study used osmotic minipumps to deliver chronic clozapine or haloperidol to mimic as closely as possible the clinical dosing regime and achieve constant receptor occupancy.

Osmotic minipumps were implanted (s.c.) at day 1 in male Long Evans hooded rats (186-248g) under halothane anaesthesia. Acute and vehicle treated animals were implanted with their respective vehicle pump (for clozapine 0.9%saline/3% acetic acid/water and haloperidol 3% acetic acid/water). For chronic treatment, pumps were filled with either clozapine or haloperidol to give daily doses of 20 mgKg^{-1} or 1 mgKg^{-1} respectively. At day 22 all vehicle and chronic treated animals were injected with vehicle (1mlKg^{-1}; i.p.). Acute treated animals were injected with either clozapine (20mgKg^{-1}) or haloperidol (1mgKg^{-1}) in volumes of 1mlKg^{-1}. All animals were killed 45 min later. The brains were removed, rapidly frozen and stored at -70°C. All brains were sectioned (20 µm) at -20°C, for both mRNA and receptor determination.

Sections for receptor studies were stored at -70°C and sections for in-situ hybridisation (ISH) were prepared as previously described (Wisden and Morris, 1994). For receptor studies, sections were pre-incubated at room temperature for 2x10 min in 50mM Tris pH 7.4 buffer, then incubated for one hour in [^{3}H]ketanserin (0.84nM) Tris buffer which included tetrabenazine (1µM) and prazozin (1µM). Specific binding was defined by 50nM spiperone. The sections were washed 2x10 min in ice-cold Tris buffer then rapidly dried. They were exposed to Amersham Hyperfilm for 1 month then analysed using an MCID imaging system. For ISH a 5-HT$_{2A}$ oligo (45mer) was used complementary to bases 1480-1424 as previously (Wisden and Morris, 1994). Data were analysed using one-way ANOVA followed by Student Newman-Keuls multiple range test where appropriate.

Acute and chronic clozapine treatment decreased 5-HT$_{2A}$ receptor binding in all areas by 30-50% compared to vehicle (Table 1). With the exception of the prefrontal cortex, there were no changes in haloperidol treated animals. There were no significant changes in 5-HT$_{2A}$ mRNA levels in prefrontal cortex for either clozapine (vehicle 0.0994±0.0078, chronic clozapine 0.1099±0.0081) or haloperidol (vehicle 0.1187±0.0078, chronic haloperidol 0.1191±0.0151 (all data represents relative optical density; n = 5; p>0.05)) treated animals.

In conclusion, continuous administration of clozapine produces similar decreases in 5-HT$_{2A}$ receptors to those observed previously with daily injections.

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	Clozapine			Haloperidol		
Brain Region	Vehicle	Acute	Chronic	Vehicle	Acute	Chronic
prefrontal cortex	0.317±0.016	0.103±0.038*	0.062±0.006*	0.297±0.017	0.218±0.023*	0.305±0.026
cingulate cortex	0.220±0.011	0.083±0.023*	0.053±0.005*	0.204±0.014	0.171±0.021	0.199±0.016
nucleus accumbens shell	0.088±0.011	0.042±0.013*	0.031±0.003*	0.093±0.009	0.071±0.007	0.078±0.011
nucleus accumbens core	0.115±0.017	0.055±0.017*	0.040±0.005*	0.125±0.007	0.100±0.012	0.118±0.009

Table 1[^{3}H]Ketanserin specific binding (pmol/mg) in the rat. All data are expressed as mean ± sem (n=5-6 per group). * p<0.05 Vs vehicle treated animals.

266P INHIBITORY EFFECT OF NOCICEPTIN ON [^{3}H]-5HT RELEASE FROM THE RAT CEREBRAL CORTEX

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The wide distribution and localization of the opioid-like receptor (ORL$_1$) suggests that its endogenous ligand, nociceptin (NC), may modulate a variety of central processes (Meunier, 1997). In particular, NC has been implicated in pain perception and anxiety, conditions in which the importance of serotonin (5-HT) has long been known. Since high levels of ORL$_1$ have been demonstrated in the rat cerebral cortex, the effect of NC on 5-HT release in this brain area has been examined.

Fronto-parietal cortices from male Sprague-Dawley rats (200-300 g) were isolated and slices (Beani et al., 1984) or synaptosomes (Morari et al., 1998) prepared. Tissues were incubated, at 37°C for 30 min, in Krebs' solution containing ascorbic acid 50 µM, EDTA 30 µM, and [^{3}H]-5-HT50 nM, bubbled with 95% O_2 5% CO_2. and then superfused at 0.25 ml/min. Samples were collected every 5 min from the 30th to the 95th min. Slices were electrically stimulated (3 Hz, 100 mA/cm^2, 2 msec, for 2 min) at the 45th (St_1) and the 75th (St_2) min of superfusion; synaptosomes were stimulated by a 1-min pulse of 10 mM K^+ at the 45th min. Drugs were added 5 min before St_2 or K^+ stimulation. The radioactivity was determined by liquid scintillation spectroscopy. Net tritium overflow evoked by stimulation and, for slice experiments, the St_2/St_1 ratios, were calculated. Data are expressed as mean ± s.e. mean of n experiments and have been analyzed statistically using the Mann-Whitney U test.

In control slices electrical field stimulation (EFS) evoked a net [^{3}H]-5-HT overflow equal to 2.83 ± 0.17% of the tritium content (n=20). NC (0.1-3 µM) inhibited in a concentration dependent manner EFS induced [^{3}H]-5-HT release. Under the same experimental conditions, morphine was ineffective. Naloxone neither modified *per se* [^{3}H]-5-HT release nor affected the inhibitory action of NC (Table 1). In control synaptosomes, 10 mM K^+ evoked a net fractional overflow of 2.01 ± 0.04% (n=10). NC (1µM) reduced K^+-evoked net fractional overflow to 1.01± 0.07% (n=10, P<0.05). NC effect was not significantly modified by 1µM naloxone (1.06±0.09%, n=6).

Table 1. Inhibitory effect of nociceptin on EFS-evoked ^{3}H-5-HT efflux from rat fronto-parietal cortex slices (*P<0.05 vs control)

Treatment (µM)	*St_2/St_1*	*% inhibition*	*n*
Control	1.03 ± 0.05	-	20
NC 0.1	0.90 ± 0.13*	13	7
NC 0.3	0.73 ± 0.08*	32	6
NC 1	0.52 ± 0.07*	54	6
NC 3	0.62 ± 0.09*	35	4
Morphine 30	1.18 ± 0.15	-	5
Naloxone 1	1.10 ± 0.10	-	4
NC 1 + naloxone 1	0.54 ± 0.16*	51	5

The present data clearly indicate the presence, in the rat cerebral cortex, of NC functional sites inhibiting 5-HT release. This inhibitory action was neither shared by morphine nor affected by naloxone, ruling out the involvement of classical opioid receptors. Moreover, NC inhibitory action appears to be due to the activation of NC receptors located on serotoninergic terminals. Since the brain 5-HT system has been implicated in anxiety states (Hamon, 1994) and NC was reported to act as an anxiolytic agent (Jenck et al., 1997), the inhibitory effect of NC on cortical 5-HT release could be a component of the anxiolytic action of this peptide.

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267P CHANGES IN NOCICEPTIN RECEPTOR DENSITY IN DIETARY-OBESE RATS

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The endogenous 17-amino acid ligand at the opioid-like receptor, nociceptin (orphanin FQ), increases food intake when injected into the hypothalamus or nucleus accumbens, (Pomonis *et al.*, 1996; Stratford *et al.*, 1997) suggesting a role of this neurotransmitter in the regulation of energy balance. We have examined regional changes in nociceptin receptors in rat brains from two models of altered energy balance that may occur in response to sustained changes in endogenous nociceptin synaptic activity, leading to either up- or down-regulation. Eighteen male Wistar rats (150g) were fed a highly palatable diet, rich in fat, over an 8 week period, during which they were hyperphagic and became obese, as compared to 8 controls rats fed standard laboratory pellet diet (control body weight = 379± 16g; obese = 465 ± 15g; P<0.01, Student's t-test) (Widdowson *et al.*, 1997). In addition, 8 rats were dietary-restricted to 60% of their normal daily requirements of pellet diet for 10 days, whilst controls were allowed free access to the pellet diet *ad libitum*. Rats were killed by CO_2 inhalation, their brains removed and frozen in cold isopentane (-40°C). Quantitative receptor autoradiography was performed on 20μm cryostat cut sections through the nucleus accumbens and hypothalamus using 70 pM [^{125}I]nociceptin and 1 μM nociceptin to define the non-specific binding. Autoradiograms were analysed by densitometry. Specific [^{125}I]nociceptin binding to the hypothalamic arcuate (ARC; controls = 0.21 ±0.03 fmol/mg tissue; obese = 0.44 ±0.06 fmol/mg tissue; P<0.01, Student's t-test; mean ± S.E.M.), and ventromedial nucleus (VMH; controls = 0.64 ±0.04 fmol/mg tissue; obese = 1.08 ±0.13 fmol/mg tissue; P<0.01), was significantly higher in dietary-obese rats, versus controls. In contrast, specific [^{125}I]nociceptin binding to the nucleus accumbens (controls = 0.64 ±0.04 fmol/mg tissue; obese = 0.41 ±0.4 fmol/mg tissue; P<0.01), lateral septum (controls = 1.48 ±0.14 fmol/mg tissue, obese = 0.93 ±0.08 fmol/mg tissue; P<0.01) and motor cortex (controls = 1.36 ±0.08 fmol/mg tissue, obese = 0.86 ±0.07 fmol/mg tissue; P<0.01) of obese rats were significantly lower than in control brains. The up-regulation of nociceptin receptors in the VMH was confirmed by increased nociceptin-stimulated (1μM) [^{35}S]GTPγS binding in adjacent sections through the hypothalamus. (controls =28.5 ± 4.0 fmol/mg tissue, obese = 49.9 ± 3.9 fmol/mg tissue; P<0.01). The density of [^{125}I]nociceptin binding in the hypothalamus and forebrain of dietary-restricted rats was not significantly different from controls, showing that endogenous nociceptin activity is not altered during negative energy balance. In conclusion, we have demonstrated a marked reduction in nociceptin receptor density in forebrains of dietary-obese rats, whereas receptors in the hypothalamus display a modest up-regulation. The marked down-regulation of nociceptin receptors in forebrain regions may be related to a nociceptin-mediated reinforcement produced by highly palatable food, which is overconsumed, leading to the development of obesity.

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268P NOCICEPTIN AND THE PUTATIVE ORL1 ANTAGONIST [Phe^{1}Ψ(CH_2-NH)Gly^2]NOCICEPTIN(1-13)NH_2 ACT AS ANTI-OPIOID PEPTIDES IN FREUND'S ADJUVANT-INDUCED ARTHRITIS, A CHRONIC MODEL OF PAIN IN THE RAT

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Nociceptin has been shown to modify analgesic responses when administered intracerebroventricularly (i.c.v.). Also, an anti-opioid role has been proposed for nociceptin, based on its ability to reverse stress and opioid-induced antinociception (for reviews see Darland et al., 1998; Henderson & McKnight, 1997; Meunier, 1997). Thus far all studies have focused on acute models of pain, whereas the role of nociceptin in chronic inflammation remains to be clarified.

The present study was undertaken to evaluate the effects of nociceptin itself and in conjunction with morphine. The Freund's adjuvant-induced arthritis model in the Lewis rat (180-200 g) was used and thermal hyperalgesia was assessed using the plantar test. Furthermore, we have studied the effects of the first proposed ORL1 antagonist, [Phe^{1}ψ (CH_2-NH)Gly^2]-nociceptin-(1-13)-NH_2 (Guerrini et al., 1998).

Nociceptin at the doses of 1, 3, 10 and 30 nmol/icv, was unable to induce either analgesia or hyperalgesia, while the nociceptin antagonist, [Phe^{1}ψ(CH_2-NH)Gly^2]-nociceptin-(1-13)-NH_2, at each concentration studied (1, 3 and 10 nmol/i.c.v.) induced hyperalgesia in the arthritic paw (50% reduction vs vehicle; p<0.01; MANOVA analysis). The effects of this peptide were relatively long-lasting, from 15 to 60 min after i.c.v. injection.

Nociceptin, given at 1-30 nmol/i.c.v., 30 min after morphine injection (3 mg/kg s.c.), i.e., at the peak effect of morphine, was able to induce an immediate and short-acting reversal of the drug (30% reduction vs morphine; p<0.01; MANOVA analysis), demonstrating an anti-opioid activity. Likewise, 1 nmol/i.c.v. [Phe^{1}ψ(CH_2-NH)Gly^2]-nociceptin-(1-13)-NH_2, reversed the analgesic effects of morphine (40% reduction vs morphine; p<0.01; MANOVA analysis). However, the effects of this pseudo-peptide were higher in comparison with nociceptin effects.

The present results show that nociceptin acts as an anti-opioid peptide in Freund's adjuvant-induced arthritis in the rat, a chronic model of inflammation. Furthermore, [Phe^{1}ψ(CH_2-NH)Gly^2]-nociceptin-(1-13)-NH_2, which is known to be an antagonist in the periphery and an agonist in the central nervous system (Calò et al.,1998; Hao et al., 1998), like nociceptin, induces hyperalgesia and inhibits the morphine evoked analgesia. These data will aid the further elucidation of the functions of nociceptin and related peptides on mechanisms of pain.

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269P ENKEPHALINERGIC MODULATION OF STRIATAL GLUTAMATE AND GABA RELEASE *IN VIVO* FOLLOWING A NIGROSTRIATAL LESION IN THE RAT

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There are reciprocal interactions between several neurotransmitter systems within the striatum. A unilateral lesion of the nigrostriatal pathway with 6-hydroxydopamine (6-OHDA) increased the content and release of glutamate (Glu), GABA and met-enkephalin (met-enk) in the striatum (Thal et al., 1983;Segovia et al., 1986;Lindefors et al., 1990). So far the possible modulation of amino acidergic systems by met-enk is unknown. We have studied *in vivo* striatal neurotransmitter release in rats following a 6-OHDA lesion and the modulation of Glu and GABA release induced by met-enk.

Male Wistar rats (250-280 g) were lesioned with 6-OHDA injected into the substantia nigra (12 µg/4 µl ascorbic ac.); controls (sham-operated) received vehicle. Success of the lesion was assessed by circling behaviour (apomorphine: 0.5 mg/kg, sc and amphetamine 3 mg/kg ip). Four months postlesion microdialysis probes were implanted in the striatum of anaesthesized rats (Equitesin) and perfused with cerebrospinal fluid (2 µl/min) and 15 min fractions were collected after 1h stabilisation. Basal and K^+-induced (100 mM K^+,10 min) release was measured in the absence or presence of met-enk (100 nM). Amines and amino acids were measured by standard reverse phase HPLC methods.

Lesioned rats had significantly decreased striatal levels of DA (95%), and its metabolites HVA (95%) and DOPAC (84%) ipsilateral to the lesion. Extracellular levels were also markedly decreased in lesioned rats (DA:undetected; HVA:87%; DOPAC: 92%; Fig.1 A). Striatal Glu content was significantly reduced (73.7%), while its metabolite, glutamine (Gln) and GABA were unaltered ipsilateral to the lesion. In contrast, extracellular Glu and GABA increased around a 70% (Fig.1 B). Met-enk reduced the release of both amino acids (around a 50%) in sham-operated rats. Interestingly, met-enk reduced Glu release to the same extent in lesioned animals, while its effect was lower on GABA release (29 %).

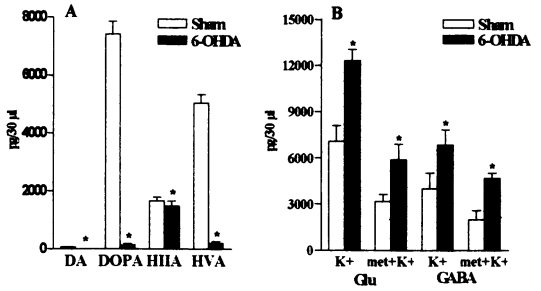

Fig.1. A:Extracellular amines; B:extracellular amino acids.*:P<0.05 (Student's t test) 6-OHDA (n=6) vs.sham (n=6).

In summary, met-enk is able to modulate Glu and GABA release in the striatum. In rats with a severe 6-OHDA lesion met-enk modulation of GABA release is reduced, while that of Glu release is maintained. Subsensitivity of opiod receptors due to increased met-enk release may be responsible for that effect.

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270P EFFECTS OF [Phe$^1\Psi$(CH$_2$-NH)GLY^2]NOCICEPTIN(1-13)NH$_2$ AND Ac-RYYRWK-NH$_2$ ON cAMP FORMATION IN CHINESE HAMSTER OVARY CELLS EXPRESSING THE RAT NOCICEPTIN RECEPTOR

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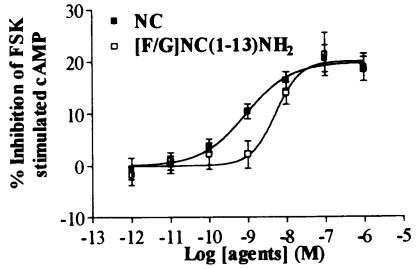
Nociceptin (NC) is the endogenous ligand of the opioid receptor like-1 receptor (NC-receptor) and a hexapeptide Ac-RYYRWK-NH$_2$ (CAM) is a potent agonist for this receptor [Meunier, 1997 for review]. [Phe$^1\psi$(CH$_2$-NH)Gly^2]Nociceptin(1-13)NH$_2$ ([F/G]NC(1-13)NH$_2$) is reported as both an antagonist and an agonist depending on the preparations examined and the levels of expression of this receptor [Guerrini *et al.*, 1998, Butour *et al.*, 1998, Toll *et al.*, 1998]. In this study, to examine the binding and functional properties of these agents in tissues with low levels of NC-receptor, we have performed binding and cAMP assays in CHO cells expressing low levels of rat CNS NC-receptors ($CHO_{LC\text{-}132}$).

The binding of [^{125}I]-Tyr^{14}-NC (≈1pM) was measured in cell membrane suspensions in Tris (50mM) with $MgSO_4$ (5mM) and BSA (0.5%), pH7.4 at room temperature. Following 30 mins of equilibration, bound and free radioactivities were separated by vacuum filtration [Okawa *et al.*, 1998]. cAMP was measured in whole cell suspensions in Krebs/HEPES buffer with BSA (0.5%), IBMX (1mM) and forskolin (FSK: 1µM), pH7.4. After 15 min of incubation at 37°C, cAMP was measured in the supernatant using a radio-receptor assay [Okawa *et al.*, 1998]. In both assays, peptidase inhibitors (30µM of captopril, amastatin, bestatin and phosphoramidon) were included. Data are mean±s.e.mean (n≥5).

The binding of NC assessed using ≈1pM of [^{125}I]-Tyr^{14}-NC and increasing concentration of unlabelled NC (pseudo-isotope dilution) was concentration-dependent and saturable, with B_{max} and pK_d values of 10.50±2.60 fmol/mg protein and 10.24±0.10 (0.06nM) respectively. The binding of [^{125}I]-Tyr^{14}-NC was displaced by NC, [F/G]NC(1-13)NH$_2$ and CAM with pK_i (nM) values of 9.67±0.11 (0.21), 9.47±0.09 (0.34) and 10.20±0.08 (0.06) respectively. NC and [F/G]NC(1-13)NH$_2$ inhibited FSK stimulated cAMP formation, with pIC_{50} and I_{max} (%) values of 9.06±0.24 and 19.8±1.5, 8.29±0.21 and 20.0±2.0, respectively (Fig.1). CAM inhibited cAMP formation <10% up to 1µM.

Fig.1 Effects of NC and [F/G]NC(1-13)NH$_2$ on cAMP formation



In rat vas deferens (rVD) [F/G]NC(1-13)NH$_2$ is an antagonist whereas centrally an agonist profile has been suggested. This could result from differences in levels of NCR expression as suggested by Toll et al (1998). In this study using $CHO_{LC\text{-}132}$ cells with low levels of expression a full agonist profile for [F/G]NC(1-13)NH$_2$ was described. It is interesting to note that B_{max} in $CHO_{LC\text{-}132}$ cells was similar to that of cerebellar membranes [Okawa *et al.*, 1998]. To date no binding data in rVD are available. As CAM is reported to be a partial agonist [Dooley *et al.*, 1997] the lack of effect on cAMP formation at low levels of expression is not surprising.

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271P INTRACELLULAR Ca^{2+} IN SINGLE ADHERENT CHINESE HAMSTER OVARY CELLS IS INCREASED THROUGH ACTIVATION OF RECOMBINANT μ, δ AND κ OPIOID RECEPTORS

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We have previously shown that in fura-2 loaded whole cell suspensions, activation of recombinant μ, δ, and κ opioid receptors expressed in Chinese hamster ovary (CHO) cells increases intracellular calcium (for review see Harrison *et al.*, 1998). To further characterize these stimulatory responses, the aim of this study was to examine the effect of opioid receptor activation in single fura-2 loaded adherent cells.

CHO cells grown on glass coverslips in supplemented Hams F-12 media were incubated with 2μM fura-2 AM for 2hours at room temperature. Cells were positioned onto the stage of an inverted Nikon microscope with a x40 objective and perfused with Krebs-HEPES buffer to remove any free fura-2 AM and to allow hydrolysis of intracellular fura-2 AM. Cells were subjected to excitation at 340 and 380nm using a spectral Wizard monochromater with a 700ms exposure time, with emission measured at 510nm. Cells were perfused at room temperature with 1μM of the appropriate agonist (CHOμ, fentanyl; CHOδ, [D-Pen^{2,5}]Enkephalin (DPDPE); CHOκ, spiradoline) for 1 minute and imaged using a cooled charge-coupled device camera and Merlin software (Life Science Resources). Follwing subtraction of basal, 340/480 ratios are expressed as mean±s.e.mean.

Activation of μ, δ, and κ opioid receptors by appropriate agonist produced an increase in $[Ca^{2+}]_i$ in a proportion of cells, (Table 1). As a positive control, cells were challenged with 3μM UTP, to which all produced an increase in $[Ca^{2+}]_i$.

Table 1. Percentage of cells responding to opioid agonist, with mean Δ340/380 ratio in responders.

Cell Line (agonist)	No. of cells tested	% responding	Δ340/380
CHOμ (fentanyl)	129	12.4	0.45±0.07
CHOδ (DPDPE)	904	9.6	0.37±0.04
CHOκ (spiradoline)	523	7.6	1.05±0.07

These data show that single adherent CHOμ, δ and κ cells appear to have a low response rate to opioid agonist. This may be the underlying reason why, when $[Ca^{2+}]_i$ is measured in the same cell lines in whole cell suspensions, the increase in $[Ca^{2+}]_i$ is relatively small (Harrison *et al.*, 1997; Smart *et al.*, 1997). A low response rate to opioid in adherent cells has also been demonstrated in another study using cells expressing endogenous opioid receptors (Sarne & Gafni, 1996), however synchronization was found to increase the probability of detecting a response. Therefore further studies investigating the experimental conditions that affect the coupling of recombinant opioid receptors expressed in CHO cells to Ca^{2+}, are clearly warranted.

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272P EFFECT OF 6-HYDROXYDOPAMINE-LESIONING ON THE FULL COMPLEMENT OF $GABA_A$ RECEPTOR SUBUNIT GENE EXPRESSION IN THE RODENT BASAL GANGLIA AND THALAMUS

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In the 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease (PD), loss of striatal dopamine innervation produces downstream changes in GABAergic activity in motor regions of the basal ganglia and thalamus. We have previously shown that α_1 and β_2, but not γ_2, subunit gene expression is decreased in the lesioned globus pallidus (Chadha & Duty, 1998). The aim of this study was to determine the full complement of changes in $GABA_A$ receptor subunit genes in the parkinsonian basal ganglia and thalamus. Such a full analysis will enable the functional significance of these changes to be established.

Male Sprague Dawley rats (250-270g) were injected with either 6-OHDA (12.5μg in 2.5μl) or vehicle into the right median forebrain bundle. Three weeks later rats were sacrificed, the brains frozen (-45°C) and cryosections (15μm) obtained through regions of interest. Sections were fixed, dehydrated and defatted. ^{35}S-labelled oligonucleotide probes complementary to mRNA encoding the $GABA_A$ α_1-α_4, β_1-β_3, γ_1-γ_3 and δ subunits (Wisden et al., 1992) or the housekeeping gene G3PDH (Clontech) were diluted in hybridisation buffer to a specific activity of $3x10^6$ cpm ml^{-1}. Sections were hybridised overnight at 37°C then washed in a series of standard saline citrate solutions (SSC) to a maximum stringency of 60°C and 0.1x SSC. Sections, together with ^{14}C standards, were exposed to Biomax MR film for periods of 10-42 days. Levels of $GABA_A$ receptor subunit expression were semi-quantified by image analysis (MCID; Imaging Research Inc). Statistical significance between lesioned and intact sides was demonstrated by the Student's t-test with a significance level of $p<0.05$.

Loss of mazindol binding confirmed a full lesion was produced in all 6-OHDA-lesioned rats used in this study (data not shown). In 6-OHDA-lesioned rats, changes in $GABA_A$ receptor subunit gene expression were found only in the globus pallidus (GP), substantia nigra pars reticulata (SNr), parafasicular nucleus (PFN) and striatum of the lesioned side. In GP, SNr and PFN only α_1 and β_2 subunit genes, out of all eleven studied, were changed (Table 1).

Region	GP	SNr	PFN
α_1	18.1 ± 2.9 % ↓	10.7 ± 3.8 % ↑	18.7 ± 2.5 % ↓
β_2	13.6 ± 1.9 % ↓	20.1 ± 1.7 % ↑	13.2 ± 3.6 % ↓

Table 1. Percentage changes (mean ± sem; n= 6) in α_1 and β_2 subunit gene expression in lesioned versus intact sides of 6-OHDA-lesioned rat globus pallidus (GP), substantia nigra pars reticulata (SNr) and parafasicular nucleus (PFN). ↓ = decrease; ↑ = increase.

In the striatum, only small but significant increases (<10%) in α_4 and γ_2 gene expression were found. No changes in G3PDH expression occurred in any of these regions, confirming specificity of the subunit changes. In addition, no changes in the expression of any subunits were found in sham-lesioned rats.

The present data indicate that only four out of the eleven $GABA_A$ receptor subunits studied display plasticity in the parkinsonian basal ganglia. The nature of these changes in GP, SNr and PFN suggests that they occur in response to the known alterations in GABAergic transmission in these regions (Pan et al., 1985). Moreover, since these changes are specific for the α_1 and β_2 subunits that contribute to benzodiazepine type I binding, they are consistent with earlier evidence of altered benzodiazepine pharmacology in these areas (Pan et al., 1985). The relevance of the α_4 and γ_2 subunit changes in the striatum remain to be seen.

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273P $GABA_B$ MODULATION OF GABA RELEASE IS APPARENTLY ALTERED IN A RAT MODEL OF ABSENCE EPILEPSY

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Genetic Absence Epilepsy Rats from Strasbourg (GAERS) are a validated animal model of absence epilepsy (Vergnes *et al*, 1982). Absence seizures are generated by the cortex and thalamus, and $GABA_B$ receptors have been implicated in the causation of these seizures (Marescaux *et al*, 1992). As increased levels of extracellular GABA have been reported in the thalamus of GAERS (Richards *et al*, 1995), we have now investigated GABA release *in vitro* from thalamus of GAERS and non-epileptic control (NEC) rats to establish whether release, and modulation by $GABA_B$ receptors are altered in GAERS.

GABA release was measured in vitro, using a crude preparation of thalamic synaptosomes. Synaptosomes were prepared according to Hornsby *et al* (1992) and resuspended in artificial CSF (aCSF) before being aliquoted onto GF/F filters in individual wells of a perfusion chamber (0.5mg protein per chamber. Gassed ($95\%O_2/5\%CO_2$) aCSF was perfused at 0.4ml/min and, after 30min equilibration, perfusate samples were collected every 2 minutes. Depolarisation was induced from 36-37.5min using aCSF containing 30mM KCl. (-)Baclofen, where used, was included throughout perfusion. GABA levels in perfusates were determined using HPLC with fluorescence detection, as described by Richards *et al* (1995). Statistical analysis was by Student's t-test. Values are given as mean±s.e.mean, n=4.

Neither basal (59±7 vs 50±9 pmol/mg protein/min for NEC and GAERS respectively), nor 30mM KCl-stimulated GABA release from thalamic synaptosomes differed between the two strains. (-)Baclofen (10μM) had no effect on basal levels of release in either strain (50±7 and 56±13 pmol/mg protein/min for NEC and GAERS respectively). However, in the presence of (-)baclofen, stimulated release from GAERS was significantly lower than that of NEC (Table 1).

Table 1 Stimulated GABA release from thalamic synaptosomes (pmol/mg protein/min; mean±s.e.mean; n=4)

	NEC	GAERS
Control stim	134±15	114±7
Stim + bac (10μM)	154±20	93±15*

* significantly different from 'NEC + (-)baclofen' ($P<0.05$)

Similar results were obtained using 15mM KCl as the depolarisation stimulus.

The apparent inhibition of GABA release in GAERS thalamic synatosomes, by the $GABA_B$ receptor agonist (-)baclofen, suggests that presynaptic $GABA_B$ receptor function may be increased in the thalamus of these animals. Whether this is a cause or a consequence of absence seizures in these animals remains to be elucidated.

R.S. is an MRC student.

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274P THE EFFECTS OF THE $GABA_B$ AGONIST CGP35024 IN MODELS OF NEUROPATHIC AND INFLAMMATORY PAIN AND IN THE RAT ISOLATED HEMISECTED SPINAL CORD

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The GABAergic system may play an important role in the modulation of nociceptive input in the spinal cord. $GABA_B$ receptor expression in the spinal cord is altered by inflammation and axotomy and the $GABA_B$ agonist baclofen is antinociceptive in models of chronic pain. (Hwang *et al* 1997). Here we have examined the antinociceptive activity of a novel $GABA_B$ agonist, CGP35024 in models of inflammatory and chronic neuropathic pain in the rat, and its inhibitory action on spinal transmission *in vitro*. The effects of CGP35024 were compared with L-baclofen and gabapentin, a novel anticonvulsant reported to possess antinociceptive activity (Hunter *et al* 1997).

Chronic neuropathic hyperalgesia was induced by partial ligation of the left sciatic nerve (Seltzer *et al*, 1990). Inflammatory hyperalgesia was induced by intraplantar injection of 25μl Freund's complete adjuvant (FCA), into the left hind paw. 10-21 days following surgery and 24 hours following FCA mechanical hyperalgesia was assessed by measuring paw withdrawal thresholds using an Ugo Basile Analgesymeter. Mechanical thresholds were determined on both hind paws prior to and then up to 6 h following s.c. and i.t. drug or vehicle administration. Drug effects are expressed as D_{50} values (dose producing a 50% reversal of predose hyperalgesia) and efficacy (maximal percentage reversal of hyperalgesia). Separate experiments examined the effects of drugs on tactile allodynia by measuring paw withdrawal thresholds to von Frey hairs with a cut-off of 20.9g. *In vitro* hemisected spinal cord preparations were set up as previously described (Thompson *et al*,1994) and capsaicin-evoked ventral root potentials examined prior to and following drug perfusion.

CGP35024, L-baclofen and gabapentin produced a dose related inhibition of capsaicin-evoked depolarization in the isolated spinal cord, with IC_{50} values of 0.08, 0.18 and 1.85 μM respectively. CGP35024 and L-baclofen also produced a dose-dependent reversal of neuropathic mechanical hyperalgesia following single administration which was maintained for at least 6 h. Motor effects were not evident at the doses shown in Table 1. In contrast, L-baclofen and CGP35024 did not affect inflammatory mechanical hyperalgesia following s.c. administration. Gabapentin only moderately affected neuropathic hyperalgesia following a single administration by either route (Table 1) and produced a weak (20%) reversal of inflammatory hyperalgesia. However, following repeated dosing (250mg/kg, s.c. once daily for 5 days), gabapentin produced a significant reversal of neuropathic hyperalgesia (40%) although it remained ineffective against inflammatory hyperalgesia. Co-administration of CGP56433A, a selective $GABA_B$ receptor antagonist prevented the anti-hyperalgesic effects of both L-baclofen and CGP35024 but did not affect gabapentin activity following repeat administration. L-baclofen and CGP35024 did not affect tactile allodynia following single s.c. administration, whilst gabapentin produced a significant increase in withdrawal threshold from 2.4g to 12.3g.

In summary, the $GABA_B$ agonist CGP35024 is potent and efficacious in reversing neuropathic mechanical hyperalgesia. It is ineffective in reversing tactile allodynia and inflammatory mechanical hyperalgesia.

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Table 1: Effects of $GABA_B$ agonists and gabapentin on neuropathic mechanical hyperalgesia.

Compound	intrathecal administration			subcutaneous administration		
	dose range(μg)	D_{50} (μg)	efficacy	dose range (mg/kg)	D_{50} (mg/kg)	efficacy
L-baclofen	0.03-1.0	0.17 (1h)	76.5±21.2*	0.1-3.0	2.1 (0.5h)	54.1±4.6*
CGP35024	0.001-0.03	0.005 (3h)	106.1±16.5*	0.003-0.1	0.039 (2h)	58±4.9*
Gabapentin	10-300	>300 (3h)	30.1±6.1	3.0-100	>100 (2h)	9.1±3.4

All D_{50} values taken at time of peak drug effect; in paranthesis. * $p< 0.05$ compared to vehicle group, (ANOVA followed by Tukey's HSD test on raw data).

275P [^{3}H]-GABAPENTIN BINDING IN THE RAT SPINAL CORD FOLLOWING LOCAL IBOTENIC ACID INJECTIONS

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Gabapentin is a novel anticonvulsant with antihyperalgesic properties in animal models of pain and has recently been shown to bind to specific sites in the rat spinal cord. We have recently shown that neither (neonatal) capsaicin or dorsal rhizotomy significantly reduce the level of gabapentin binding in the spinal dorsal horn suggesting that only a small proportion of the sites are located on presynaptic terminals of primary sensory afferents (Blyth *et al.*, 1998). These studies suggested that the dorsal spinal gabapentin binding sites may either be located on the soma/dendrites of local dorsal spinal neurones, or on presynaptic terminals originating in other regions of the CNS. We now report on the effect of local injection of ibotenic acid on gabapentin binding in the dorsal horn of the spinal cord.

Rats were anaesthetised using pentobarbitone (20 mg/kg, i.p.), diazepam (2.5 mg/kg, i.p.) and Hypnorm (0.3 ml/kg, i.m.). A hemilaminectomy was performed from T13 to L1 and five, 0.2 µl injections of either ibotenic acid (75 nmol/µl dissolved in PBS, pH 7.4) or vehicle were administered into the right dorsal horn at 1 mm intervals rostro-caudally. The wound was sutured and treated with topical antibiotics. Animals were allowed to recover and after 14 days were anaesthetised with fluothane and perfused with phosphate buffered saline (PBS). The lumbar cord was removed and frozen at -30°C. Frozen sections were processed for gabapentin autoradiography (25nM [^{3}H]-gabapentin, in the absence and presence of 10 µM (S)-(+)-3-isobutyl GABA, a gabapentin analogue which also binds to $\alpha_2\delta$) (Hill *et al.*, 1993). In order to confirm the extent of the lesion these sections were then stained with cresyl violet and to confirm the sparing of axons, adjacent sections were processed for immunocytochemistry using an antibody raised to calcitonin-gene-related peptide (CGRP).

Following local injections of ibotenic acid into the rat spinal cord, autoradiographic analysis of [^{3}H]-gabapentin binding revealed an 80% loss of binding site density in laminae I and II of the dorsal horn ipsilateral to the injection sites, compared to the contralateral side. The difference in binding between sides in these animals was significant. Mean [^{3}H]-gabapentin binding density (nCi/mg) contralateral to the ibotenate injection 16.75 ± 1.18 (6); ipsilateral density 3.65** ± 0.63 (6) ($p < 0.01$). Animals injected with saline showed no equivalent loss of signal ipsilateral to the injection. Mean [^{3}H]-gabapentin binding density (nCi/mg) contralateral to saline injection 16.08 ± 0.67 (3); ipsilateral density 15.84 ± 0.89 (3). Cresyl violet staining of sections after autoradiographic binding analysis confirmed the tissue integrity but loss of cell bodies in areas showing reduced binding. Adjacent sections to those used for autoradiographic analysis showed clear staining for CGRP-LIR in laminae I and II both ipsilateral and contralateral to the lesion, indicating that afferent fibres entering the dorsal horn had not been destroyed by the lesion.

Intrathecal administration of low concentrations of gabapentin has been shown to be effective in reducing inflammation-induced hyperalgesia (Field *et al.*, 1997) suggesting that at least part of its effects are mediated at the level of the spinal cord. The precise mechanism of action is unknown but it has been shown to bind to the $\alpha_2\delta$ subunit of voltage dependent calcium channels (VDCCs) (Gee *et al.*, 1996).

The present data suggest that the majority of gabapentin binding sites are located on the somata or dendrites of neurones within the dorsal horn. It is possible that gabapentin binds selectively to $\alpha_2\delta$ subunits associated with VDCCs which are preferentially located on postsynaptic, somal or dendritic regions of spinal neurones thereby modulating nociceptive processing at the spinal level.

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276P CONTINUOUS SUBCUTANEOUS DELIVERY OF HALOPERIDOL INCREASES D2 RECEPTOR BINDING IN RAT STRIATUM

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Chronic treatment with typical but not atypical antipsychotics has been shown to upregulate D2-like receptors (Tarazi *et al.* 1997). However, the contribution of D3 receptors to these changes remain unclear. In addition, many studies have employed chronic daily injection regimes which may not be representative of the clinical situation. The aim of the present study was to investigate the effects of continuous subcutaneous release of clozapine and haloperidol upon D2 receptor protein and mRNA levels simultaneously in rat brain using receptor autoradiography and in situ hybridisation respectively.

Groups of male hooded Long Evans rats (175-200g) were treated with either clozapine (20mg kg^{-1} day^{-1}; n=6), haloperidol (1mg kg^{-1} day^{-1}; n=6) or vehicle (3% acetic acid in saline for clozapine (n=12) and 3% acetic acid in water for haloperidol (n=12)) administered subcutaneously via Alzet osmotic minipumps (implanted on day 1 under halothane anaesthesia) for 21 days. On day 22 the chronic drug treated rats and six animals from each vehicle group were given an i.p injection of corresponding vehicle. The remaining rats receiving chronic vehicle were given an i.p injection of clozapine (20mg kg^{-1}) or haloperidol (1mg kg^{-1}). 45 minutes after i.p injections animals were killed and brains prepared for D2 receptor autoradiography based upon a modification of the method of Tarazi *et al.* (1997). Binding was determined by incubating sections in either 3nM [^{3}H] raclopride with 3µM of the D3 selective ligand U91994A (5,6-Dimethoxy-2-(di-n-propylamino)indan maleate), either alone (total) or in the presence of 1µM spiperone (non-specific) at room temperature for 60 minutes. Sections were exposed to film for 5 weeks. D2 receptor mRNA levels were determined as outlined by Wisden & Morris (1994) using an oligo (45mer) complementary to bases 116-160. The resultant autoradiograms were analysed using computer based densitometry. Data were analysed using one-way ANOVA followed by Student Newman-Keuls multiple range test where appropriate.

Chronic haloperidol, but not chronic clozapine treatment, produced a significant elevation of D2 receptor binding within the dorsolateral and ventolateral striatum (Table 1). Acute haloperidol produced a significant decrease in binding within the dorsolateral striatum. The use of U99194A removes any possibility that the changes observed could be due to some previously undetectable effect upon the D3 receptor. Interestingly neither antipsychotic produced a significant effect upon D2 receptor mRNA levels suggesting that the effect of chronic haloperidol treatment is a result of post transcriptional modification. These data suggest that continuous administration of haloperidol produces adaptive processes in D2 receptor binding similar to those observed previously with daily injection procedures.

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	Specific Binding (nCi mg^{-1})					
Brain Region	Cloz Veh	Acute Cloz	Chronic Cloz	Hal Vehicle	Acute Hal	Chronic Hal
DL Striatum	10.05±0.47	9.58±0.51	10.67±0.58	10.39±0.69	8.46±0.31^{+}	12.97±0.61*
VL Striatum	10.22±0.38	9.93±0.50	11.01±0.35	9.86±0.76	9.48±0.86	12.83±0.22*
			ROD			
DL Striatum	0.093±0.017	0.084±0.009	0.086±0.0087	0.088±0.0127	0.071±0.006	0.107±0.0175
VL Striatum	0.088±0.018	0.084±0.007	0.103±0.0108	0.094±0.0163	0.076±0.008	0.106±0.0099

TABLE 1: Specific binding of [^{3}H] raclopride in the presence of U99194A and D2 receptor mRNA levels expressed as relative optical density (ROD). All data are expressed as mean±sem. *$p<0.05$ chronic haloperidol vs haloperidol vehicle. $^{+}p<0.05$ acute haloperidol vs haloperidol vehicle.

277P ADMINISTRATION OF 6-OHDA ABOLISHED GIRK2 EXPRESSION IN SUBSTANTIA NIGRA

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G-protein activated inward rectifier K'-channels (GIRKs) are coupled to a large number of neurotransmitter receptors, including those of dopamine D_2 and $GABA_B$ receptors (Hill, 1994). It has been shown that the GIRK2 subunit is highly expressed in dopamine cell body regions of substantia nigra pars compacta (SNc) (Liao *et al.*, 1996). In contrast, the dopamine terminal regions of striatum are lacking in GIRK2 mRNA but have a moderate level of GIRK2 protein expression (Murer *et al.*, 1997). The aim of the present study was to determine, by unilateral 6-OHDA lesion, whether GIRK2 subunits are expressed by dopamine neurones, and their neuroanatomic localization, i.e., at somato-dendrites or axon-terminals.

Male Sprague-Dawley rats (270 -300g) were used in all experiments. 6-OHDA (8µg dissolved in 3µl of saline containing 2µg ascorbic acid) was unilaterally injected into the left median forebrain bundle (A4.3, L1.5, V8.5) under halothane anaesthesia. Control rats were given an equal volume of vehicle. Rats were allowed to recover from the anaesthetic with access to food and water. All rats were killed 3 weeks after lesion and brains were processed for *in situ* hybridization (ISH) and immunocytochemistry as previously described (Pei *et al.*, 1997). The relative GIRK2 mRNA abundance was determined by densitometric quantification (MCID Image Analysis System). The optical densities were converted to nCi/g tissue and expressed as Mean ± SEM. Statistical significance was tested using ANOVA and Dunnett's *t*-test.

Unilateral administration of 6-OHDA abolished GIRK2 mRNA signals in SNc of injection side (left) while on the unlesioned side (right) it was similar to that in vehicle control rats (see Table 1). No significant differences between lesioned and unlesioned sides were observed in other brain regions such as the parietal cortex, dentate gyrus (DG) and CA1 subfield of the hippocampus (Table 1). In these lesioned rats GIRK2-IR in SN was undetectable, while in striatum no difference in optical density between lesioned and non-lesioned sides were observed.

In summary, we have shown that 6-OHDA lesion abolished both mRNA and protein expression of GIRK2 in the SN, suggesting that the dopamine neurones in these regions express GIRK2 subunits and GIRK2 subunits are localized at soma-dendrite of theses neurones. In contrast 6-OHDA lesion did not affect GIRK2-IR in striatum, suggesting that GIRK2 are not localized at the axon terminals of dopamine neurones.

Table 1. Effect of 6-OHDA lesion on GIRK2 mRNA abundance (expressed as nCi/g tissue) in the rat brain.

	6-OHDA		Vehicle	
	left (lesioned)	right (intact)	left	right
SNc	23± 4.1**	129± 6.6	123 ± 5	119 ± 6
cortex	53 ± 5.2	58 ±7.1	61 ±5.6	65 ± 5
CA1	169 ± 16	157 ±16	159 ± 13	168 ±17
DG	264 ± 23	281 ± 17	249 ± 21	255 ±19

**p<0.001 ANOVA Dunnett's *t*-test, DG dentate gyrus.

Hill B. (1994) *Trends Neurosci.* 16, 3559-3570.
Liao *et al.*, (1996) *J. Neurosci.* 15, 7132-7150.
Murer *et al.*, (1997) *Neurosci.* 80, 345-357.
Pei, Q. *et al.*, (1997) *Neurosci.* 78, 343-350.

278P THE RELEASE OF DOPAMINE AND ITS METABOLITES IN THE STRIATUM OF CONSCIOUS AS/AGU RATS

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The AS/AGU rat is a spontaneously occurring mutation of the parent Albino Swiss strain, which exhibits locomotor abnormalities and reduced tyrosine hydroxylase levels in the substantia nigra, together with lower extracellular levels of dopamine. We have now examined the effects of several agents known to modify amine neurotransmission in order to determine the locus of any transmission defect.

Animals of the mutant strain were compared with controls using microdialysis to measure the extracellular levels of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum. Guide probes were implanted under anaesthesia (ketamine/xylocaine) and dialysis performed at least 48 hours after recovery from anaesthesia. Statistical comparisons were made using a *t* test between different groups of control and mutant animals.

The basal levels of dopamine in AS/AGU rats were around 10% of those in controls, one control group having levels of 18.8 ± 1.45 $pmols.20\mu l^{-1}$ compared with the mutant group of 166.3 ± 21 $pmols.20\mu l^{-1}$ (n = 5). Perfusion of a high potassium medium through the dialysis probe evoked a 4.6-fold rise of dopamine levels in control rats but double this in the mutant strain ($p<0.01$, n = 3). Amphetamine ($5mg.kg^{-1}$) increased extracellular dopamine in control rats by 7.1-fold and in mutants by 14.8-fold ($p<0.01$, n = 5).

The monoamine oxidase-A inhibitor clorgyline ($15mg.kg^{-1}$) produced a modest 1.8-fold increase of dopamine levels in control rats but an 11.7-fold increase in AS/AGU animals ($p<0.001$, n = 5). In contrast, the uptake inhibitor nomifensine increased dopamine levels by 9.1-fold in control AS rats but only 2.7-fold in the mutant strain ($p<0.05$, n = 4). After subchronic treatment of animals for 4 weeks with benserazide 2.5 $mg.kg^{-1}.day^{-1}$ plus L-dopa $25mg.kg^{-1}.day^{-1}$, an acute injection of L-dopa $100mg.kg^{-1}$ during dialysis evoked a release of dopamine which was twice as large in the mutant AS/AGU rats compared with the controls ($p<0.05$, n = 4).

The results confirm earlier data showing a reduced extracellular level of dopamine in AS/AGU rats at a time when the locomotor disorder is apparent but there has been little loss of tyrosine hydroxylase immunoreactivity (Campbell et al., 1998). The response to clorgyline in particular supports this view in that the present results are qualitatively different form those obtained using the 6-hydroxydopamine model of nigrostriatal degeneration. The greater effect of potassium may indicate the presence of a larger pool of newly synthesised amine in the mutants, an idea which could also explain the increased levels of metabolites. Such an increased pool could arise from a failure of release, a situation which could explain the effects of nomifensine. Overall the results are interpreted to suggest the existence of a defect in the systems associated with the release of dopamine from striatal terminals.

Campbell, J.M., Gilmore, D.P., Russell, D. et al. (1998) Neuroscience, 85, 229-237.

279P EFFECT OF ACUTE NICOTINE ON STRIATAL DOPAMINE OUTPUT AND METABOLISM IN RATS KEPT AT DIFFERENT AMBIENT TEMPERATURES

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It is well established that nicotine and related drugs enhance the release and metabolism of dopamine (DA) in the brain. This effect occurs readily in the mesolimbic brain areas, where it has been associated with addictive properties of nicotine. On the other hand, several investigators found that the nigrostriatal dopaminergic system is less sensitive to nicotine (Brazell *et al.*, 1990; Grenhoff & Svensson, 1988). The findings that Parkinson's disease is less prevalent among tobacco smokers than among non-smokers, stress the importance of the nigrostriatal dopaminergic system as a target site for the actions of nicotine. Haikala *et al.* (1986) showed that the nicotine-induced changes in striatal DA metabolism of mice depend on body temperature. In the present experiments *in vivo* microdialysis was used to characterize the effect of elevated ambient temperature on nicotine-induced striatal dopamine release in freely moving rats.

Microdialysis probes were implanted under general halothane anaesthesia in the striatum of male Wistar rats (body weight 250-350 g). The rats were placed at the ambient temperatures of 20-23°C or 30-33°C, and the experiments were performed 2 days later. Nicotine (0.3, 0.5 or 0.8 mg kg^{-1}) or saline were administered s.c. Mecamylamine (5.0 mg kg^{-1}) and dihydro-β-erythroidine (2.8 mg kg^{-1}) were administered i.p. 30 or 10 min before nicotine, respectively. Dialysate samples were collected every 20 min and analysed for DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), using HPLC with electrochemical detection. Brain nicotine concentrations were estimated using gas chromatography-mass spectrometry. Data were analysed with two way ANOVA for repeated measures.

The basal striatal extracellular concentrations of DA or its metabolites were not altered in the rats kept at the elevated ambient temperature. Neither did the elevation of ambient temperature alter the brain concentrations of nicotine at 60 min after administration of 0.5 mg kg^{-1} of nicotine. At the ambient temperature of 20-23°C nicotine at the doses used did not elevate DA concentration significantly, but at the temperature of 30-33°C the doses 0.5 & 0.8 mg kg^{-1} significantly ($P<0.05$) increased the DA concentration. The extracellular concentrations of DOPAC and HVA were elevated at 20-23°C by 0.5 & 0.8 mg kg^{-1} of nicotine. At 30-33°C already the dose of 0.3 mg kg^{-1} elevated extracellular DOPAC and HVA concentrations significantly.

At the ambient temperature of 30-33°C the non-competitive ion channel blocker, mecamylamine, and the competitive nicotinic receptor antagonist, dihydro-β-erythroidine, blocked or reduced the observed increases of the concentrations of extracellular DA and its metabolites suggesting that the observed temperature-dependent enhancement of nicotine's effects on striatal DA release and metabolism is mediated by cholinergic nicotinic receptors.

We conclude that the functional state of nicotinic cholinergic receptors *in vivo* can be modulated by altering the ambient temperature. This modulation may underlie processes such as changes in the conformational state/desensitization properties of these receptors.

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280P REPEATED ADMINISTRATION OF VENLAFAXINE, MAPROTILINE AND MIANSERIN INCREASES DOPAMINE D_2 BUT NOT D_1 RECEPTOR EXPRESSION IN THE NUCLEUS ACCUMBENS OF THE RAT

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Dysfunction of neurotransmission within the mesolimbic system may underlie some of the core symptoms of major depression, and changes in transmitter function in this system may contribute to the therapeutic effect of antidepressant treatment. We have recently reported that repeated administration of certain antidepressant drugs increases the expression of dopamine D_2 receptors in the nucleus accumbens (Ainsworth *et al.*, 1998a,b). Specifically, the 5-HT reuptake inhibitor fluoxetine, and the noradrenaline reuptake inhibitor desipramine, both increased D_2 mRNA and binding site density in the nucleus accumbens (and not striatum) and did not alter D_1 mRNA. Here we extend these observations by studying the antidepressant drugs, venlafaxine (VEN; 5-HT/noradrenaline reuptake inhibitor), maprotiline (MAP; noradrenaline reuptake inhibitor) and mianserin (MIAN; 5-HT_2 receptor/α_2-adrenoceptor antagonist).

Male Sprague-Dawley rats (200-250 g) were injected i.p. twice daily for 14 days with saline vehicle, VEN, MAP or MIAN (all at 10 mg/kg). Brains were dissected out 24 h after the final injection and sectioned The abundance of mRNA encoding D_2 and D_1 receptors was determined by in situ hybridization histochemistry (Ainsworth *et al.*, 1998a,b). D_2-like and D_1-like binding densities were determined by receptor autoradiography using [^{3}H]YM-09151-2 (Yokoyama *et al.*, 1994) and [^{3}H]SCH 23390 (Mansour et al., 1992). Image analysis of the autoradiograms was performed on four regions; the nucleus accumbens shell and core (Shell and Core), and the ventromedial and dorsolateral striatum (VMS and DLS).

The results are summarised in Table 1. Relative to vehicle controls, VEN, MAP and MIAN all enhanced D_2-like binding in the Shell and Core (+22-47%). MAP also enhanced to a lesser degree (+16%) D_2-like binding in the VMS and DLS whereas neither VEN nor MIAN had a significant effect. Generally speaking, increases in D_2-like binding density coincided with increases in D_2 mRNA. None of the treatments had a consistent effect D_1 mRNA/binding density.

Table 1. Effect of antidepressant drugs on D_1/D_2 mRNA (μCi/g tissue) and binding site density (fmol/mg tissue).

	Vehicle	VEN	MAP	MIAN
D1 mRNA				
Shell	75.2±9.0	76.0±9.5	88.2±9.1	88.1±11.1
Core	63.9±4.9	48.5±4.6	66.3±5.3	56.6±4.3
VMS	65.1±4.7	58.5±4.3	82.0±7.7*	65.4±4.6
DLS	76.9±5.1	65.1±4.2	87.4±8.2	94.6±14.3
D2 mRNA				
Shell	62.8±3.2	63.2±10.6	81.9±4.8**	79.9±4.2**
Core	53.7±2.0	65.4±5.7**	78.7±2.8**	80.4±3.5**
VMS	63.3±3.1	65.2±3.1	77.7±3.2**	85.3±2.3**
DLS	85.6±3.1	82.8±4.5	97.6±3.6**	98.9±1.6**
D1 -like binding density				
Shell	1137.8±32.7	1124.8±36.7	1150.8±39.8	1338.8±82.6
Core	1154.0±39.9	1188.7±50.1	1248.1±43.2	1340.4±88.6
VMS	1317.6±19.0	1292.0±45.4	1403.6±50.0	1565.9±127.0
DLS	1314.6±30.9	1234.8±66.9	1397.0±36.2	1579.9±119.8
D2 -like binding density				
Shell	346.9±10.9	449.3±17.4*	511.2±12.1**	488.5±12.5*
Core	377.9±19.3	460.4±46.4**	502.0±20.3**	476.5±13.5**
VMS	505.6±12.0	521.9±21.7	585.6±20.4**	543.2±26.9
DLS	636.0±15.1	662.0±27.6	738.3±21.3**	695.3±34.0

Data expressed as mean ± s.e.mean, N=4-6 per group. * $P<0.05$, ** $P<0.01$ *vs* vehicle control (one-way ANOVA and *post-hoc* Dunnett's test).

In summary, the present data show that, in keeping with our studies of fluoxetine and desipramine, VEN, MAP and MIAN increase D_2 receptor expression in the nucleus accumbens. Since D_2 dopamine receptors in this region are known to facilitate reward and motivated behaviour (Koob,1992), these effects may be relevant to the antidepressant actions of these drugs.

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281P INTRACELLULAR STUDY OF AN ADENOSINE RECEPTOR INTERACTION IN HIPPOCAMPAL PYRAMIDAL CELLS

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We have previously reported the suppression by adenosine A_2 receptors of activity mediated by A_1 receptors using an analysis of excitatory postsynaptic potential/ spike coupling (O'Kane & Stone, 1997). In the present study this phenomenon has been examined in more detail using intracellular techniques.

Male Wistar rats (150-250g) were deeply anaesthetised with urethane and the brain rapidly removed to ice-cold artificial cerebrospinal fluid (aCSF) of composition (mmol.l^{-1}) KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5 $MgSO_4$ 1.2, glucose 10 saturated with 95% O_2 and 5% CO_2. The hippocampi were cut into slices 450μm thick and maintained in an incubation chamber saturated with 95% O_2/5% CO_2 for at least one hour before being transferred to a recording chamber. Drugs were added to the superfusion fluid. Intracellular recordings were made from neurones in the CA1 pyramidal cell layer using sharp microelectrodes filled with 1M KCl or potassium acetate (90-120 M Ω). Potentials were amplified using a Neurolog NL 102 amplifier or Axoclamp-2A operated in bridge balance mode. Neurones were used if they displayed a stable resting potential greater than 60 mV and a spike of at least 70 mV amplitude. Depolarising current pulses were used to determine the threshold for spike initiation.

At concentrations of 50 or 100 nM, N^6-cyclopentyladenosine (CPA) produced very little or no change of resting membrane potential (mean hyperpolarisation of 1.7 ± 0.9 mV at 100 nM, n=12), but raised the threshold for spike initiation in 11 of 12 neurones. The change of threshold was reflected in the blockade of spike initiation and as a reduction in the number of evoked spikes at higher current intensities. 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 100 nM) prevented this effect in 4 cells tested. When 2-p-(2-carboxyethyl) phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS21680) 30 nM was perfused for 15 minutes before and together with CPA, the change of spike threshold was prevented in 9 of the 11 cells. The A_{2A} receptor antagonist 4-(2-[7-amino-2-{2-furyl}{1,2,4,}triazolo{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl)phenol (ZM 241385) at 100 nM blocked the antagonistic effect of CGS21680 and restored the inhibitory effect of CPA.

The present results strongly support our earlier conclusion that postsynaptic A_1 receptors elevate spike threshold. It is unlikely that a change of inhibitory transmitter tone is involved as there was no significant evidence of postsynaptic hyperpolarisation in the intracellular recordings. The A_{2A} receptor agonist CGS 21680 attenuated the effect of adenosine A_1 receptor activation, showing there is cross-talk between the two classes of receptor. It is unlikely that there is any functional antagonism between CPA and CGS21680 since no excitatory response was seen with an A_{2A} receptor-selective concentration of CGS 21680 used alone and no significant change of resting potential was produced.

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282P BARIUM PREVENTS ADENOSINE A_1 RECEPTOR CHANGES OF EPSP-SPIKE COUPLING

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We have previously shown an inhibitory effect of adenosine A_1 receptors on EPSP-spike (E-S) coupling, and the ability of adenosine A_{2A} receptor activation to attenuate this effect (O'Kane & Stone, 1997). The population excitatory postsynaptic potential (popEPSP) gives primarily a measure of presynaptic function while the population spike (PS) reflects the excitability of the postsynaptic neurone. E-S coupling gives an indication of the ability of a given level of synaptic depolarisation to induce the postsynaptic cell to fire an action potential. Here, we examine the role of potassium channels, second messengers and nitric oxide in the A_1 receptor effects on these parameters.

Slices (450μm thick) of rat hippocampus were prepared as described by Higgins & Stone (1996). Test stimulation (0.05Hz) was delivered via a concentric bipolar electrode placed in the stratum radiatum. After 45mins, recordings of orthodromic extracellular popEPSPs and PSs were made from the stratum radiatum and stratum pyramidale respectively. Input/output (I/O) data were obtained by varying the stimulus intensity and measuring the responses via a CED interface. Five stimuli were delivered at each stimulation intensity and the average computed. These I/O data were normalised and E-S relationships computed for each slice. The results are expressed as mean ± s.e. mean.

Blocking K^+ channels with Ba^{2+} (0.5mM, n=5) reduced the inhibitory effects of the specific adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) on PS amplitude but not popEPSP slope, and attenuated the inhibitory effect of adenosine A_1 receptor activation on E-S coupling in a similar manner to adenosine A_{2A} receptor activation (O'Kane & Stone, 1997). CPA 50nM decreased the ratio PS/EPSP from 0.79±0.1 in controls to 0.15±0.05 on addition of drug, a change of 80.1%±6.4. The change in PS/EPSP ratio when Ba^{2+} was present was 51.4%±9.9, significantly different from CPA used alone ($p<0.05$). In the presence of the adenosine A_{2A} receptor agonist 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS21680 30nM, n=5) this value is 44.7%±8.7, significantly different from CPA ($p<0.05$), but not from CPA in the presence of Ba^{2+}. In contrast, manipulation of several second messenger systems had no effect on the actions of CPA. The agents tested included the membrane permeable analogue of cyclic AMP, 8-Bromoadenosine-3',5'-cyclic monophosphate (8BrcAMP; 100μM, n=3), the adenylate cyclase activator forskolin (10μM, n=3) and inhibitor 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ 22536; 10μM, n=3), the specific protein kinase C inhibitor 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl)maleimide (GF109203X; 100nM, n=5) and the protein kinase A inhibitor, N-[2-methylamino)ethyl]-5-isoquinolinesulfonamide hydrochloride (H8; 30μM, n=4). The selective inhibitor of brain nitric oxide synthase 7-nitroindazole (7NI; 40μM, n=5) also had no significant effects on the inhibitory CPA effects on popEPSP slopes, PS amplitudes or E-S coupling.

These results suggest that the decrease in E-S coupling observed upon adenosine A_1 receptor activation is a result of increased postsynaptic K^+ conductance, an effect which could underlie the antagonistic effect of adenosine A_{2A} receptor activation.

O'Kane, E.M. & Stone, T.W. (1997) *Brit. J. Pharmacol.* 122, 287P.
Higgins, M.J. & Stone, T.W. (1996) *Eur. J. Pharmacol.* 317, 215-223.

283P THE ADENOSINE A_{A2} RECEPTOR BLOCKER, SCH 58261, IS EFFECTIVE OVER A 2-WEEK TREATMENT IN HALOPERIDOL-INDUCED CATALEPSY

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Increasing evidence suggests that interactions between the adenosine A_{2A} receptor and the dopamine D_2 receptor in the brain are important in the regulation of motor behavior (Ferrè et al., 1997). Indeed, A_{2A} receptors are highly concentrated in the striatum (Jarvis and Williams, 1989) and, particularly, they are co-localized with D_2 receptors in the GABAergic striato-pallidal neurons (Schiffmann et al., 1991; Fink et al., 1992). With the aim to assess the opposite interaction between A_{2A} and D_2 receptors, we tested the effects of the selective adenosine A_{2A} receptor antagonist, SCH58261 (Ongini, 1997) on haloperidol-induced motor inhibition after both acute or repeated dosing schedule. Catalepsy was induced by subcutaneous administration of haloperidol (1 $mgkg^{-1}$) in male Sprague-Dawley rats (175-200 g). Animals were placed on a vertical grid and the descent latency (cut-off 120 sec) was measured 90 min after haloperidol injection.

Dose-response studies: the acute, intraperitoneal (ip) injection of SCH58261, 30 min before testing, either alone or with a subthreshold dose of L-dopa (25 $mgkg^{-1}$, ip) plus benserazide (12.5 $mgkg^{-1}$, ip), significantly reduced the descent latency on the vertical grid test (Table 1).

Repeated drug treatment: animals were treated with SCH58261 (1 $mgkg^{-1}$, ip) or vehicle twice daily for 14 days. On the 15th day, rats were challenged with haloperidol and SCH58261 (1 $mgkg^{-1}$, ip) alone or in combination with L-dopa plus benserazide. The administration of SCH58261 maintained its anticataleptic effect even after 14-day repeated treatment (Table 2).

Table 1. Dose-response curve of SCH58261 (SCH) in the haloperidol-induced catalepsy model in rats

Treatment ($mgkg^{-1}$, ip)	Descent latency (sec)	
	Without L-dopa	With L-dopa
Vehicle	109±7	119±5
SCH 0.1	112±3	98±7*
SCH 0.3	84±10	73±8**
SCH 1	73±9**	50±8**

**p<0.01, *p<0.05 vs vehicle: Dunnett's *t*-test.

Table 2. The effect of SCH58261 (SCH) on descent latency after 14-day repeated treatment

Acute treatment	Repeated treatment	
	Vehicle	SCH (1 $mgkg^{-1}$, ip)
Vehicle	108±6	111±7
SCH 1	84±10	86±12
L-dopa+Vehicle	88±13	102±9
L-dopa+SCH 1	53±12*	54±12**

Data were analyzed by ANOVA.

These results further support the concept that blockade of A_{2A} receptors by a selective antagonist, such as SCH58261, has a therapeutic potential for the treatment of motor disorders such as Parkinson's disease.

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284P GR205171, A SELECTIVE NK_1 RECEPTOR ANTAGONIST ATTENUATES STRESS-INDUCED INCREASE OF DOPAMINE METABOLISM IN RAT MEDIAL PREFRONTAL CORTEX

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Previous studies have demonstrated that stress induced increase of dopamine metabolism in the medial prefrontal cortex is blocked not only by $GABA_A$ receptor agonists including diazepam and zolipidem but also by the infusion of substance P monoclonal antibody into the ventral tegmental area (Bannon *et al.*, 1983). In the present study we have investigated the effects of (S)-GR205171 (3(S)-(2-methoxy-5-(5-tri fluoromethyltetrazol-1-yl)-phenylmethylamino)-2(S)-phenylpiperidine), a selective, potent antagonist at the rat neurokinin$_1$ (NK_1) receptor (Gardner *et al.*, 1996) and its less active enantiomer ((R)-GR205171) on the increase of prefrontal cortex DA metabolism following acute stress.

Male Sprague Dawley rats (weight range 250-300g, B & K Ltd, U.K.) were pretreated with either vehicle (8% cyclodextrin, 25mM acetic acid, 1 ml/kg, i.p.), (S)-GR205171 (5 or 10 mg/kg, i.p.) or (R)-GR205171 (10 mg/kg i.p.). Thirty min later rats were either left in the home cage or stressed by immobilisation on a wire grid for 20 min following which they were humanely killed. The medial prefrontal cortex was dissected and frozen on solid CO_2, before being stored at -80°C until required for analysis of dopamine and the acidic metabolite dihydroxyphenylacetic acid (DOPAC) by HPLC with electrochemical detection (Barton & Hutson 1997).

Data were subjected to analysis of variance followed, where significant, by Tukey's t-test. A value of $P<0.05$ was considered significant.

Dopamine metabolism, as indicated by the concentration of DOPAC, in medial prefrontal cortex was significantly increased following 20 min immobilisation stress. Pretreatment of rats with the NK_1 receptor antagonist, (S)-GR205171 (10 but not 5mg/kg, s.c.) attenuated the stress induced increase of cortical DOPAC concentration (Table 1). In contrast, this response to stress was not affected by pretreatment with the less active enantiomer (Table 1). The concentration of dopamine in the medial prefrontal cortex was not significantly affected by either stress or drug treatment (data not shown).

Results in the present study demonstrate that activation of mesocortical dopamine neurones by acute stress is attenuated by the selective NK_1 receptor antagonist (S)-GR205171.

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Table 1. Effects of GR205171 on the stress-induced increase of DOPAC concentration in rat medial prefrontal cortex

Treatment	DOPAC (ng/g)	Treatment	DOPAC (ng/g)	Treatment	DOPAC (ng/g)
Veh/NS	18.7 ± 1.8	Veh/NS	12.5 ± 1.6	Veh/NS	50.2 ± 3.5
Veh/S	32.6 ± 3.5*	Veh/S	29.5 ± 3.1*	Veh/S	92.9 ± 3.6*
(S)GR(5)/NS	18.4 ± 1.6	(S)GR(10)/NS	12.7 ± 1.6	(R) GR(10)/NS	45.4 ± 3.3
(S)GR(5)/S	33.2 ± 3.2*	(S)GR(10)/S	18.6 ± 3.0'	(R)GR(10)/S	84.5 ± 12.0*

Veh = Vehicle, GR = GR205171(5 or 10 mg/kg i.p.), S = stress, NS = non-stress. Values are mean ± s.e.mean, n = 6/8. * $P<0.05$ compared with appropriate non-stressed animals, ' $P<0.05$ compared with vehicle treated stressed animals.

285P CHANGES IN SPINAL CORD NEUROPEPTIDES IN THE ADJUVANT MODEL OF CHRONIC INFLAMMATORY HYPERALGESIA IN THE PRESENCE AND ABSENCE OF THE NK1 RECEPTOR ANTAGONIST GR205171

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Afferents present within joint nerves in both animals and man, contain pro-inflammatory peptides including substance P, which can increase during inflammation and arthritis (Scott et al., 1994.). Injection of these inflammatory mediators can cause hypersensitivity, observed as hyperalgesia and/or allodynia. It has also been shown that there are increases in expression of pro-inflammatory peptides in the spinal cord in models of inflammation (Neumann et al., 1996). We have previously shown that the selective NK_1 neurokinin receptor antagonist GR205171 (rat pKi 9.5, human pKi 10.5; Gardner et al 1996) dosed for 17 days (10mg/kg t.i.d s.c.), exhibited anti-hypersensitive and anti-inflammatory activity in model of chronic inflammatory hyperalgesia induced by intraplantar Freunds Complete Adjuvant in the rat. (Clayton et al., 1997). From the same experiment we have now also investigated the effect of GR205171 on neuropeptide changes in the lumbar spinal cord. Freunds complete adjuvant (FCA) was injected intraplantar (100µl) into the left paw of male Random Hooded rats (180-220g) to induce the arthritis. GR205171 10mg/kg s.c. or vehicle (mannitol), were administered 1 hr before adjuvant and then three times a day for 17 days. After 18 days, rats were perfused with 4% buffered formaldhyde and the cords removed and processed for immunohistochemistry. Expression of NPY in laminae 1-2 and 3-4 and CGRP were unchanged by FCA and by FCA +GR205171. There was also no statistically significant change in NK1 expression in FCA alone or FCA+GR205171 groups. Substance P expression in the laminae 1-2 of the dorsal horn was unchanged following FCA treatment; however, substance P expression was significantly increased on the ipsilateral side compared to the contralateral side following FCA+GR205171 treatment (Table 1). These results suggest that NK_1 antagonists may increase substance P release through inhibition of an inhibitory presynatic receptor. This may reduce the analgesic efficacy of an NK_1 receptor antagonist. In conclusion, The results suggest the presence of presynaptic NK_1 receptors similar to that previously described (Hu et al., 1997).

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Table 1. Effect of GR205171 on the percentage peptide staining in the ipsilateral versus the contralateral dorsal horn

peptide	Data presented as percentage in peptide staining in ipsilateral dorsal horn vs the contralateral side. (mean±sem, * $p<0.05$, ANOVA)		
	Control group	FCA group	FCA+GR205171 10mg/kg s.c. t.i.d for 17 days.
NPY 1-2	1.5±4.1(n=3)	2.1±1.8(n=6)	-1.9±1.9(n=7)
NPY 3-4	4.1±3.6(n=3)	1.8±3.2(n=6)	1.9±2.7(n=7)
NK1	0.5±0.5(n=3)	13±3.5(n=5)	10.7±5.9(n=7)
SP	1.1±2.2(n=6)	2.7±5.0(n=6)	28±7.2(n=6)*
CGRP	1.2±1.5(n=5)	3.8±3.1(n=7)	7.0±1.5(n=8)

286P AGONIST-DEPENDENT COUPLING OF THE NK_1-RECEPTOR

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Evidence is emerging for the concept of agonist-dependent trafficking of receptor signals, where different patterns of agonist pharmacology can be obtained depending on the signalling pathway activated. In the present study we have investigated the evidence for agonist-specific alternative coupling of the tachykinin NK_1 receptor after activation by the natural agonist substance P (SP) and its synthetic analogue septide ([pGlu^6, Pro^9]SP(6-11). We made parallel measurements in Chinese hamster ovary cells stably transfected with the human NK_1 receptor (CHO-hNK_1) of three response endpoints, progressively more distal from the recognition site: increases in intracellular levels of inositol 1,4,5-trisphosphate (IP_3), or of Ca^{2+} (Fura-2-loaded cells), or the increased extracellular acidification-rate response, using the technique of microphysiometry (Cytosensor®).

In the Cytosensor SP and septide, and also neurokinin A (NKA) and neurokinin B (NKB), all caused a concentration-dependent increase in the acidification rate of CHO-hNK_1 cells; the order of potency (n=3-6) was septide (pEC_{50} 9.0±0.3) ≥ SP (8.7±0.2) > NKA (7.6±0.3) ≥ NKB (7.1±0.3). Senktide and [β-Ala^8]NKA(4-10) at 100nM had no effect in those cells, and substance P, NKA or NKB produced no response at 1µM in parental CHO-K1 cells. The NK_1 antagonist CP-99994 produced concentration-dependent rightward shifts of the response curve to SP without affecting the maximum response (pA_2 9.7 by Schild regression; slope 0.8), but with septide a non-surmountable block was observed (maximum response 78% and 65% of control in the presence of 10nM and 30nM CP-99994 respectively). The response to a sub-maximal concentration of substance P (3nM) was almost abolished in the presence of thapsigargin (10µM), whereas that with an equieffective concentration of septide (3nM) was only reduced, by about 40%. The concentration-response curve for SP was not affected by verapamil (10µM) (pEC_{50} 9.1±0.2 in control, and 8.9±0.3 after verapamil), whereas with septide a rightward shift and a reduction in the maximum (≈37%) was noted. The effect was the same with nifedipine (10µM).

In cells loaded with Fura-2, SP and septide were equipotent (pEC_{50} values 10.0±0.3 and 10.2±0.4 respectively, n=3-6). The increased [Ca_i] response to a sub-maximal concentration of SP (100pM) was not significantly reduced by the removal of external Ca^{2+} from the medium, while the response to 100pM septide was almost abolished. Again the response to substance P (100pM) was abolished by thapsigargin (10µM), whereas responses to septide (100pM) was only partially reduced (≈50%).

With measurements of the generation of IP_3 in the CHO-hNK_1 cells, SP was almost ten times more potent than septide (pEC_{50} values 7.9±0.2 and 7.0±0.3 respectively, n=3-6). The response to both agonists was significantly reduced in the presence of the phospholipase C inhibitor, U73122 (10µM).

In conclusion, our findings point to a crucial importance of an increase in the level of intracellular Ca^{2+} in the regulation of the integrated cellular response to activation of the NK_1 receptor. The means by which this increase in [$Ca^{2+}i$] is achieved, however, may differ depending on the agonist. With substance P liberation of IP_3-mobilised stores is enough to account for the response, while with septide there is an additional requirement of influx through L-channels. Although responses to both agonists are profoundly blocked by the NK_1 antagonist CP-99994, the nature of the interactions is different. This feature of the pharmacology of the two NK_1 agonists presumably points to differences in the modes of agonist interaction at the receptor recognition site, and may point to some flexibility in the transduction mechanism employed by the NK_1 receptor.

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287P INABILITY OF THE TACHYKININ NK_1 RECEPTOR ANTAGONIST GR 205171 TO MODIFY ENDOTOXIN-INDUCED FEVER AND CORTISOL RELEASE IN PIGS

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Bacterial endotoxin (lipopolysaccharide, LPS) induces prostaglandin-dependent fever and cortisol release in pigs (Parrott et all., 1995). Such effects may, or may not, involve the vagus, depending upon the dose and route of LPS administration (Sehic and Blatteis, 1996; Goldbach et al., 1997; Romanovsky et al., 1997). Circulating prostaglandins might also act at the area postrema, lesions of which reduce LPS fever (Takahashi et al., 1994). Moreover, substance P (SP) neurones in the vagus and/or brainstem may also be involved as capsaicin has been reported to attenuate LPS fever (Szekely et al., 1997). Therefore, since brainstem SP neurones can be inhibited by the potent NK_1 receptor antagonist GR 205171 (Fukuda et al., 1994), the drug may also modify the febrile effects of LPS in pigs.

The hypothesis was tested in prepubertal boars (b. wt. 35 kg; n=6) living in metabolism cages and surgically implanted with venous catheters, and blunt ending cannulae abutting the carotid artery for the insertion of thermistor probes. Animals were injected intravenously (iv) with saline (Sal), 5 mg ($\approx$0.14 mg kg^{-1}), or 20 mg ($\approx$0.57 mg kg^{-1}) GR 205171 20 min before a low iv dose (20 µg; $\approx$0.6 mg kg^{-1}) of LPS. Body temperature was measured using a digital thermometer at 10 min intervals throughout the 180 min experimental period. Blood samples were also collected at various intervals before and after LPS administration for determination of plasma cortisol concentrations.

Core temperature (^{0}C, mean change ± s.e. mean) rose rapidly during the first 50 min following LPS injection (Sal, 1.45 ± 0.21, 5 mg, 1.45 ± 0.11; 20 mg 1.34 ± 0.24). During the remaining 100 min period, averaged core temperature increments were virtually identical in each treatment condition (Sal, 1.84 ± 0.21; 5 mg, 1.83 ± 0.10; 20 mg, 1.83 ± 0.24) and greater ($P<0.01$, 80-180 min) than in the period before LPS injection. In like manner, the net increase in plasma cortisol (nmol/l; mean ± s.e. mean) following LPS injection was significant ($P<0.02$, 50-170 min) but similar in each case (Sal, 66.12 ±11.00; 5 mg, 64.09 ± 8.85; 20 mg, 54.12 ±14.15).

These results show that doses of GR 205171 within the range (0.01-1.0 mg kg^{-1} iv) that inhibit emetic responses in pigs (Grelot et al., 1998) fail to modify the febrile and neuroendocrine effects of a low dose of LPS. Thus, although emetic agents may activate central SP neurones, these findings provide no support for a role of SP in mediating the effects of peripherally-administered LPS in this species.

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288P DIFFERENTIAL EXPRESSION OF NITRIC OXIDE SYNTHASE ISOFORMS AND NADPH-DIAPHORASE STAINING IN SPINAL CORD FOLLOWING CARRAGEENAN-INDUCED PERIPHERAL INFLAMMATION

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Nitric oxide (NO) contributes to nociceptive processing in spinal cord following inflammation (Meller *et al.*, 1994). The NO catalysing enzyme NO synthase (NOS) exists in three isoforms, neuronal NOS (nNOS) and endothelial NOS (eNOS) which are constitutively expressed and a cytokine-inducible isoform (iNOS). This study examined the expression of all three NOS isoforms following carrageenan-induced inflammation by immunohistochemistry and NADPH-diaphorase (NDP)-staining.

Carrageenan (0.5mg, 500 µl) was injected intradermally into the left forelimb (interdigital space) of five adult female sheep (60 - 70 kg). Forelimb withdrawal thresholds to noxious mechanical stimulation were measured (in Newtons) as described previously (Nolan *et al.*, 1987) over a 4 hour period. Animals were euthanased after 6 hours and spinal cords removed. Spinal cords were also collected from five untreated control sheep. Spinal segments C6 - T2 were sectioned (30 µ m) and processed for NDP-staining (Dawson *et al.*, 1991), or nNOS (polyclonal, 1:1000), eNOS (monoclonal, 1:500) or iNOS (polyclonal, 1:2000) immunoreactivity (-ir). Optical density (O.D.) of tissue staining was measured using a computerised image analysis system (image, v.5.2 NIH). Values reported are mean ± SEM. Data were analysed using an ANOVA with post-hoc Dunnett's or Tukey's test.

Carrageenan induced a significant reduction in mechanical withdrawal thresholds from pre-injection baseline responses, in both ipsilateral and contralateral legs (decrease 51.8 ± 6% and 43.2 ± 9%, respectively) after 3 hours ($p < 0.05$). The O.D. for nNOS-ir and eNOS-ir in lamina I-II was significantly greater in carrageenan treated animals compared to controls (62.5 ± 8% and 33.1 ± 11% higher, respectively) ($p < 0.01$). No difference was detected in iNOS-ir in these layers. In contrast, NDP-staining was significantly lower in carrageenan treated animals compared to controls (32.0 ± 5% lower) ($p < 0.01$).

Carrageenan induced an increase in sensitivity to noxious mechanical stimuli, which was accompanied by differential changes in NOS expression. Both nNOS and eNOS were rapidly up-regulated in superficial dorsal horn following inflammation. However, the decrease in NDP-staining, which appears to measure dynamically active NOS (Morris *et al.*, 1997) suggests that there is not a direct correlation between NOS protein levels and enzyme activity.

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289P CLOZAPINE AND HALOPERIDOL EVOKE DIFFERENTIAL PATTERNS OF LOCAL CEREBRAL GLUCOSE UTILISATION IN THE RAT

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The atypical antipsychotics such as clozapine represent clear improvements in the treatment of schizophrenia as compared to the typical antipsychotics. However, the neural circuitry important for mediating these improved actions remains elusive. The aim of the present study was to determine the neural systems differentially recruited in response to typical (haloperidol) and atypical (clozapine) antipsychotics. This was achieved using the technique of 2-deoxyglucose autoradiography (2-DG) in freely moving conscious rats (Crane and Porrino, 1989).

Male Long Evans hooded rats (250-350g) were randomly allocated to one of eight treatment groups. Cannulae were implanted into one femoral artery and one femoral vein under halothane anaesthesia and exteriorised at the nape of the neck. The animals were allowed to recover for 2 hours before the administration of drugs or vehicle.

The 2-DG procedure was initiated one hour after i.p. injection of haloperidol (0.1-1 mg kg^{-1}), clozapine (2-20 mg kg^{-1}) or vehicle (0.5% glacial acetic acid in saline) by a bolus i.v. injection of ^{14}C-2-deoxyglucose (125 µCi kg^{-1}). Local Cerebral Glucose Utilisation (LCGU) was determined in over 70 brain structures using a computer based densitometry system (MCID). For each brain region, data was analysed using one-way ANOVA followed by Student Newman-Keuls multiple range test where appropriate.

Clozapine produced markedly different patterns of change in LCGU as compared to haloperidol. In particular, clozapine evoked dose dependent decreases in cortical regions (table 1) whereas haloperidol was without effect. Within the basal ganglia, both haloperidol and clozapine reduced LCGU within the striatum (table 1). Interestingly, LCGU within structures comprising the Papez circuit, which has been implicated in both emotion and cognition, such as the prefrontal and the cingulate cortices, anterior thalamus, mammillary body and hippocampus was reduced by clozapine but not haloperidol (table 1). It is therefore possible that this neural circuit is important in mediating the superior effects of the atypical antipsychotic clozapine.

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Table 1: LCGU (µmol /100g /min) in the rat. All data are expressed as mean ± sem (n=5-6 per group). * $p<0.05$ vs vehicle treated animals.

	haloperidol				clozapine			
Brain Region	vehicle	0.1mg kg^{-1}	0.3mg kg^{-1}	1mg kg^{-1}	vehicle	2mg kg^{-1}	6mg kg^{-1}	20mg kg^{-1}
prefrontal cortex	120±5	114±6	100±8	107±14	117±3	108±6	71±6*	69±4*
cingulate cortex	111±7	105±6	100±9	90±6	108±4	100±7	76±4*	71±5*
anteroventral thalamus	128±3	128±8	129±14	95±9	146±8	119±10*	94±10*	72±10*
mammillary body	125±5	122±6	107±10	99±6	124±3	98±5*	89±4*	73±4*
CA1 molecular layer	88±2	89±3	89±6	86±5	94±2	83±5	78±6	69±4*
dorsolateral striatum	110±3	107±4	92±5*	78±3*	110±3	99±5	79±7*	70±7*

290P ALLOSTERIC MODULATION OF FUNCTIONAL RESPONSES AT THE hM1 AND hM3 RECEPTORS BY BRUCINE AND GALLAMINE

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Radioligand binding experiments indicate that the affinity of muscarinic receptors for their agonists may be enhanced by allosteric modulators such as brucine (Lazareno *et al* 1998). We have investigated if brucine can enhance the effect of carbachol on human muscarinic (hM) hM1 and hM3 receptors expressed in CHO cells in a functional assay using the Cytosensor microphysiometer (Molecular Devices; Baxter *et al*, 1994) and compared its actions with gallamine.

Changes in extracellular acidification were determined using the microphysiometer. Cells, at a density of 300 000 per transwell cup, were perfused at a flow rate of 100 µl/min with a low buffered DMEM medium (bicarbonate-free DMEM, Gibco 52100-021), 2 mM glutamine, 44 mM NaCl, pH7.4, during which time the pH of the microenvironment surrounding the sensor was kept constant. The rate of output of acid from the cells (acidification rate) was periodically halted, allowing a build up of acid metabolites and, therefore, a change in chamber pH. This on-off cycle was repeated throughout the experiment and the effect of compounds determined by adding the compound to the chamber through a valve. Acidification rate measurements and agonist exposure times were optimised as: hM_1 - pump cycle time 1 min 30 sec, pump on 1 min 13 sec with a 32 sec exposure to test compounds, starting 15 sec prior to pump off and data collection for 13 sec starting 2 sec after pump off; and hM_3 - pump cycle time was 1 min 30 sec, pump on 1 min with a 1 min 30 sec exposure to test compound, data collection for 20 sec starting 8 sec after pump off. Data are mean ± s.e.m. from 7 separate experiments.

Typically basal acidification rates were 200 and 150 µvolts/sec at hM_1 and hM_3 receptors respectively and this was increased by a maximal concentration (30 µM and 10 µM) of carbachol to 500 and 230 µvolts/sec. Brucine (100 µM) increased the potency of carbachol at hM1 receptors, from $pEC_{50} = 5.42 \pm 0.08$ (n = 7), to $pEC_{50} = 5.75 \pm 0.04$ (n = 7)* and decreased the intrinsic activity, from 94.4 ±1.8 (n = 7) to 78.4 ± 2.5 (n = 7)*. Brucine at 100 µM had no effect on the response to carbachol at hM3 receptors. Gallamine, 300 µM, decreased the carbachol response in both hM1, from $pEC_{50} = 5.55 \pm 0.05$ (n = 7), to $pEC_{50} = 4.21 \pm 0.03$, (n = 7)* , and in hM3 cells from $pEC_{50} = 7.15 \pm 0.08$ (n = 7), to $pEC_{50} = 6.46 \pm 0.08$ (n = 7)*. (* $P<0.01$ Student's t-test).

These results demonstrate that it is possible to both enhance and diminish the functional effects of muscarinic agonists by allosteric modulators. This may represent an alternative site of action for the modulation of muscarinic neurotransmission.

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291P THE NEUROPROTECTIVE AGENT BW619C89 INHIBITS NEURONAL EXCITABILITY AND SYNAPTIC TRANSMISSION IN RAT CORTICOSTRIATAL SLICES

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In animal models of cerebral ischaemia and traumatic brain injury, the lamotrigine-derivative BW619C89 (4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl)-pyrimidine) affords potent neuroprotection (Meldrum et al., 1994). In electrophysiological studies on isolated cells it inhibits voltage activated Na^+ (Xie and Garthwaite 1996) and Ca^{2+} (Macnaughton et al., 1997; Stefani et al., 1998) channels. Here the actions of BW619C89 in a brain slice preparation were investigated.

Corticostriatal coronal slices were prepared from adult male Wistar rats (150-250 g) and intracellular current clamp recordings made from medium spiny neurones, as described previously (Calabresi et al., 1997). Slices were superfused with Krebs solution bubbled with 95% O_2, 5% CO_2 (35°C) containing known concentrations of BW619C89 and intracellular recording electrodes were filled with 2M KCl (30-60 MΩ). BW619C89 (mesylate salt) was synthesized in the University of Greenwich. Depolarizing current commands (0.7s, typically 0.9 nA) applied to the neurone of study via the recording electrode induced repetitive action potential firing (approx. 30 Hz). Excitatory post-synaptic potentials (EPSPs) were recorded in medium spiny neurones in response to low frequency stimulation of the corticostriatal pathway. Langmuir-Hill curves (IC_{50} values given below) were fitted by least-squares analysis to pooled concentration-inhibition data.

BW619C89 reduced the number of action potentials elicited in response to a depolarizing current command (IC_{50} = 4.6 μM; n=22) with abolition of firing at concentrations above 30 μM. The baseline conductance of the cells was unaffected by the drug. The amplitude of corticostriatal EPSPs was also depressed by BW619C89 (IC_{50} 2.0 μM; n=25). EPSP inhibition was incomplete, the highest concentration used (300 μM) inhibiting to only 49±3.9% amplitude (mean ±SEM, n=4). No effect of BW619C89 (3-30 μM) on the depolarizing response to exogenous application of 0.3-1.0mM L-glutamate (10-20 s), in the presence of 1 μM TTX, was observed (n=6).

These data demonstrate that BW619C89 inhibits neuronal excitability in medium spiny neurones, consistent with previous findings, and is a potent but incomplete inhibitor of excitatory synaptic transmission *in vitro*.

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292P CAFFEINE BLOCKS THE POST-STIMULUS AFTERDEPOLARISING TAIL CURRENT (I_{ADP}) INDUCED BY MUSCARINIC RECEPTOR ACTIVATION IN OLFACTORY CORTICAL NEURONES *IN VITRO*

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Activation of muscarinic receptors on mammalian olfactory cortical neurones results in a sustained increase in neuronal excitability accompanied by a prolonged membrane depolarization and induction of a slow post-stimulus afterdepolarization (sADP); the sADP, and its underlying tail current (I_{ADP}), are strongly dependent on calcium entry, and are believed to involve a novel *decrease* in potassium conductance (Constanti et al., 1993). Caffeine has been shown to *enhance* depolarizing afterpotentials (DAPs), while calcium-induced calcium release (CICR) blocking agents reduce DAPs in supraoptic nucleus neurones (Li & Hatton, 1997). The present study investigates the effects of caffeine on the cortical I_{ADP} using conventional single-electrode voltage clamp recording techniques.

Transverse slices of adult guinea-pig olfactory cortex were prepared and maintained as previously described (Libri et al., 1997). Stable intracellular recordings were made from neurones in the deep pyramidal cell layer (III) using microelectrodes (60-80 MΩ) filled with 4M K acetate or 2M CsCl. Measurements were performed before and during bath-application of drugs so each neurone served as its own control. Data are shown as mean ± S.E.M.

Bath-application of the muscarinic receptor agonist oxotremorine-M (Oxo-M, 10 μM; 10 min; n=35) resulted in a reversible membrane depolarization (8.3 ± 0.4 mV), and induction of a slow post-stimulus afterdepolarization (sADP) (8.7 ± 0.4 mV; 1.6 s, 2 nA stimulus) (all at -70 mV). The mean underlying inward tail current (I_{ADP}) measured under 'hybrid' voltage clamp at -70 mV was 0.35 ± 0.02 nA. The amplitude of I_{ADP} was reversibly reduced by co-application of caffeine (51.5 ± 15.5 % at 1 mM caffeine, n=3; 74.5 ± 9.1 % at 2 mM caffeine, n=3, and 74.2 ± 8.1 % at 3 mM caffeine, n=8), while muscarinic depolarizations were unaffected by 3 mM caffeine (n=3). Application of the L-type calcium channel blocker nifedipine (10 μM) resulted in a reduction of I_{ADP} amplitude (46.6 ± 1.7 %, n=4). The I_{ADP} was also reduced by ryanodine (10 μM), a blocker of CICR (9.9 ± 4.2 %, n=7;), although no inhibition of I_{ADP} was recorded in the presence of the CICR inhibitor dantrolene (10 μM, n=4), or the intracellular calcium store depleting agent thapsigargin (3 μM, n=3). 3-isobutyl-1-methylxanthine (IBMX; 0.1 mM), an inhibitor of phosphodiesterase, also failed to affect I_{ADP} (n=3); moreover, adenosine (100 μM), applied in the presence of Oxo-M, failed to reverse the antagonism of I_{ADP} by 3 mM caffeine (n=3). In neurones recorded with CsCl-filled electrodes, evoked action potentials displayed prolonged calcium plateaus; the spike widths at half amplitude were reduced by 10 μM nifedipine (40.0 ± 15.0 %,) or 3 mM caffeine (45.0 ± 15.0 %) (n=3). All above reductions were significant ($p<0.05$; paired t-test).

We conclude that in these neurones, caffeine interfered with the mechanism(s) underlying I_{ADP} generation. This was unlikely to involve inhibition of phosphodiesterase, or direct antagonism of adenosine receptors by caffeine, or CICR. Caffeine did, however, exert some block of voltage-activated calcium conductances, although this could not account for the full inhibition of I_{ADP}. It is thus possible that caffeine directly blocks the proposed potassium conductance underlying the I_{ADP} in this system.

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293P EFFECTS OF ANXIOGENIC/PANICOGENIC DRUGS ON FOS EXPRESSION IN THE LOCUS COERULEUS, PERIAQUEDUCTAL GRAY AND DORSAL RAPHE NUCLEUS

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Drugs capable of inducing anxiety in animals and humans are pharmacologically diverse and include receptor agonists for 5-HT (mCPP) and CCK (CCK4), receptor antagonists for adenosine (caffeine) and noradrenaline (yohimbine), and benzodiazepine inverse agonists (FG-7142). Whether there are any common neuroanatomical substrates important for the anxiogenic action of such drugs is little investigated. Previous studies have used behavioural models combined with measures of *c-fos* gene expression to map neuronal pathways associated with anxiety (eg. Beck and Fibiger, 1995; Carrive et al., 1998). Here we have used Fos immunocytochemistry to explore neuronal pathways activated following administration of different anxiogenic drugs at doses known to be active in various rodent animal models of anxiety. We have focused on Fos expression in lower brain areas thought to be important in anxiety and fear, specifically the locus coeruleus (LC), the periaqueductal gray (PAG) and the dorsal raphe nucleus (DRN). The drugs selected have also been used in human studies to provoke panic attacks experimentally.

Male Sprague-Dawley rats (250-300g) were habituated to the procedures room and their experimental cages at least 24 h before the experiment. Rats were injected i.p. with one of the following: BOC-CCK4 (0.1 mg/kg), mCPP (5 mg/kg), caffeine (50 mg/kg), yohimbine (5 mg/kg), FG-7142 (7.5 mg/kg) or saline vehicle. Two h after injection the rats were anaesthetized with sodium pentobarbitone and perfused transcardially with 4% paraformaldehyde. Brainstem sections were processed for the localization of Fos-like immunoreactivity (Fos-LI) as reported (Moorman et al., 1995). Fos positive cells were counted in 2 consecutive sections/region in an area of 0.025 mm^2.

Results are summarized in Table 1. In brief, compared to saline controls, all anxiogenic drugs investigated increased the expression of Fos-LI in the LC. This effect was greatest with FG-7142. In addition, all drugs enhanced Fos-LI in the caudal ventrolateral PAG (VL-PAG), but had no effect in the caudal dorsolateral PAG (DL-PAG). FG-7142, yohimbine and caffeine also increased Fos-LI in the caudal DRN while other treatments were without effect.

Table 1. Effect of anxiogenic drugs on Fos-LI.

	LC	VL-PAG	DL-PAG	DRN
SALINE	2.4±0.6	10.1±1.8	14.8±0.9	2.0± 0.6
BOC-CCK4	9.8±1.9*	24.8±3.5*	16.3±1.4	5.8±2.2
mCPP	15.0±4.0*	25.3±2.0*	15.0±2.0	4.7±1.2
CAFFEINE	16.2±2.8**	25.3±.9*	19.0±.0.6	7.3±1.2*
YOHIMBINE	20.2±5.2**	25.3±1.5*	15.7±2.	5.3±.0.9*
FG-7142	30.6±3.2**	31.3±1.9**	15.9±1.0	8.7±0.5**

Values (means ± s.e.m, N=4-6) are counts of cells with Fos-LI/0.025 mm^2 . * $p<0.05$, ** $p<0.01$ vs saline, Kruskal-Wallis one-way ANOVA, Mann-Whitney U Test.

In summary, we report that anxiogenic drugs with different pharmacological properties commonly induce Fos expression in the LC and the PAG. Interestingly, in behavioural models these areas (amongst others) display Fos expression in response to fear (Beck and Fibiger, 1995; Carrive et al. 1997). Therefore, activation of the LC and PAG may be important for the anxiogenic effects of the drugs tested.

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294P CLOMETHIAZOLE ATTENUATES HYPOXIA-INDUCED DAMAGE TO RAT CEREBELLAR GRANULE CELLS *IN VITRO*

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When cultured cerebellar granule cells are exposed to a hypoxic medium intracellular [$^{45}Ca^{2+}$] rises with a peak occurring after 2h. This increase is inhibited by several glutamate antagonists which are known to be neuroprotective against hypoxic-ischaemic damage in experimental models of stroke in rats (Anderson *et al.*, 1996). We have now investigated the effect of clomethiazole (CMZ), another compound which is neuroprotective in a wide range of experimental models of stroke (see Green, 1998), but which is not a glutamate antagonist (Green *et al.*, 1998).

Rat cerebellar granule cells were prepared as described by Schousboe *et al.* (1989) and calcium flux and IC_{50} values measured as described by Anderson *et al.* (1996). In addition, we examined the effect of exposure of the cells to the hypoxic medium on morphological damage (measured by a visual scoring system, 0 - 4) 16 h later and the effect of CMZ on this damage.

Exposure of cerebellar granule cells to the hypoxic medium (O_2<7%; glucose=5.6mM) resulted in an increase in [$^{45}Ca^{2+}$] uptake of 190% compared to cells in the normoxic medium. CMZ (300 μM) did not alter [$^{45}Ca^{2+}$] uptake in cells in the normoxic medium, but inhibited uptake by 65% in cells incubated in the hypoxic medium. This inhibition was concentration dependent with a Log IC_{50} of -3.70 ± 0.37M (mean ±s.e. mean).

CMZ also concentration dependently inhibited the hypoxia-induced damage with an approx. Log IC_{50} of -4.1M (Figure).

These data demonstrate that clomethiazole inhibits hypoxia-induced [$^{45}Ca^{2+}$] uptake in cerebellar granule cells and protects these cells from the damage resulting from exposure to a hypoxic insult *in vitro*. The results are consistent with evidence that CMZ is an effective neuroprotective agent *in vivo* (Green, 1998).

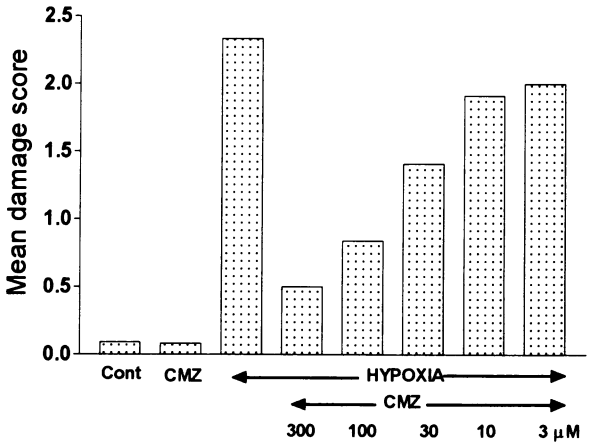

Figure. Mean damage score of cerebellar granule cells (n≥6 observations) after 16 h exposure to a hypoxic medium and effect on damage of clomethiazole (CMZ).

Green, A.R. (1998) *Pharmacol. Ther.* **80**, 123-147.
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295P ADDITIONAL EVIDENCE FOR AN *EEG AROUSAL* EFFECT OF THE MEDIAN RAPHE NUCLEUS IN THE RAT

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The neocortical and hippocampal electroencephalogram (EEG) are controlled to a large extent by nuclei of the brainstem reticular formation. Electrical stimulation of most of these nuclei results in hippocampal theta, indicative of EEG arousal. Conflicting data have been reported regarding the median raphe nucleus (MnR) and the hippocampal EEG, with some groups reporting an increase in hippocampal theta (Peck & Vanderwolf, 1991; Costall *et al.*, 1998) and others a decreased EEG amplitude (e.g. Vertes, 1981). Our objective was to substantiate previous findings (Costall *et al.*, 1998) that the MnR can mediate hippocampal theta in anaesthetised rats, a reliable index of EEG arousal.

Under urethane anaesthesia male Hooded Lister rats (400-500 g) were implanted with a stimulation electrode in the MnR (AP -7.8 mm, L 0.0 mm, DV -8.5 mm from bregma), and a recording electrode in the hippocampus (AP -3.5 mm, L 2.5 mm, DV -3.3 mm from bregma). EEG effects of electrical stimulation were quantified and analysed in both the time and the frequency domain. The total power in the theta band was calculated, and a Student's *t* test was used to test for a difference between the presence and absence of MnR stimulation.

Using standard histological methods electrode placements were verified in 9 rats. In four of these rats electrical stimulation of the MnR produced a significant power increase in the theta band (4-8 Hz) (rat 2: $t(9)=2.318$, $p<0.05$; rat 6: $t(17)=2.409$, $p<0.05$; rat 8: $t(2)=16.925$, $p<0.01$; rat 9 $t(5)=3.401$, $p<0.05$). In 2 rats there was a non-significant increase, and the remaining 3 rats showed a non-significant reduction of theta band power.

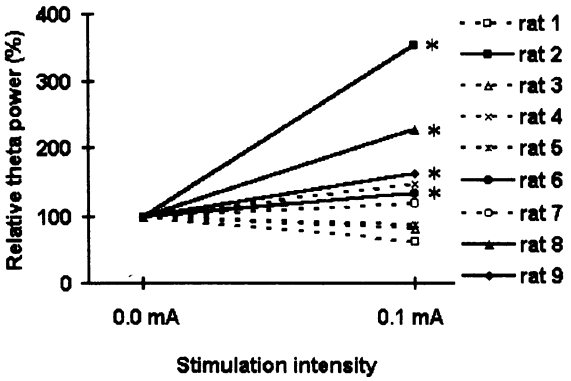

Figure 1: Effects of electrical stimulation of the median raphe nucleus in 9 rats. Relative theta power (*EEG arousal*) is significantly increased in 4 rats marked with *. (SEMs omitted for clarity.)

We thus provide further evidence that the MnR can have a stimulating effect on hippocampal theta, indicative of EEG arousal. However this effect is not observed in all cases, and we propose that slight differences in stimulation electrode placement are responsible for the differences observed. This would imply that the MnR consists of at least two subpopulations of neurones mediating different effects upon the hippocampal EEG, and this could account for the inconsistencies in the literature. The role of serotonergic neurones in the observed effect remains to be determined.

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296P AMINO ACID EFFLUX FROM THE RAT SPINAL CORD *IN VIVO* IN RESPONSE TO THERMAL STIMULATION OF THE HINDPAW

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Neurotransmission mediated by amino acid transmitters is proposed to play an important role in the processing of sensory information at the level of the spinal cord (Dickenson *et al.*, 1997). Using microdialysis sampling *in vivo*, we have studied amino acid efflux in the dorsal horn of the rat lumbar spinal cord. Preliminary characterisation of amino acid efflux is reported together with the effect of thermal stimulation of the hind paw.

Methodology was adapted from Gerin & Privat (1996). Briefly, male Wistar rats (250-350 g; Charles Rivers) were anaesthetised under halothane (4% induction, 1.5% maintenance in O_2) and mounted on a stereotaxic frame. Vertebra T_{13} was exposed and immobilised with the use of a vertebral clamp such that the dorsal surface of the underlying spinal cord lay on the horizontal plane. A burr hole was then created in the dorsal aspect of the vertebra through which the microdialysis probe was introduced at an angle of 16° from horizontal 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 position of the probe was fixed by application of dental cement around the probe and anchorage screws located in the T_{13} and L_1 vertebrae and anaesthesia reduced to 0.75-1% halothane in O_2/N_2O (1:1). The probe was perfused at a rate of 1 $\mu l.min^{-1}$ with an artificial extracellular fluid solution (aECF) for 120 min post implantation before 20 min samples of dialysate were collected. After 60 min to estimate basal efflux, separate groups received either 20 min perfusion with high K^+ (100 mM) aECF or thermal stimulation of the hind paw ipsilateral to the probe whilst perfusing with normal aECF. Heat was applied for 20 s every 2 min for 20 min at 40-42°C between 60-80 min of dialysate sampling and at 44-46°C between 120-140 min. A control group was perfused with normal aECF throughout (0-180 min). Samples were analysed for aspartate (ASP), glutamate (GLU), glycine (GLY) and citrulline (CIT) content using HPLC with fluorescence detection. Data are presented as mean percentage difference from basal efflux (mean concentration in the initial three samples (0-60 min)).

Basal efflux: aspartate 2.21 ± 0.33, glutamate 13.31 ± 1.71, glycine 13.83 ± 1.37 and citrulline 5.28 ± 0.68 $pmol.20\mu l^{-1}$, mean ± s.e.mean, n=13 in each case. Perfusion with high K^+ ECF induced a marked increase in ASP, GLU and GLY efflux ($P<0.01$ ANOVA with repeated measures, n=5 in each case) of 460 ± 95, 560 ± 205 and 249 ± 33% above basal efflux respectively. CIT efflux was not significantly altered by high K^+ perfusion. Thermal stimulation of the hind paw at 40-42°C did not alter amino acid efflux. However, application of heat at 44-46°C evoked a significant 1.5-2 fold increase in spinal cord ASP and GLY efflux ($P<0.05$, two-way ANOVA with post hoc T-test compared to equivalent time point in the control group, n=4 in each case). The efflux of GLU and CIT showed a tendency to increase, although this failed to reach significance ($P=0.24$ and 0.07 respectively, n=4 in each case).

These results indicate that the endogenous release of putative neurotransmitter amino acids can be monitored *in vivo* in the rat spinal cord and that changes in their extracellular level occur in response to thermal stimulation.

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297P MONOCULAR DEPRIVATION ENHANCES CITRULLINE CONTENT IN THE LATERAL GENICULATE NUCLEUS OF NEW-BORN RATS AND THIS IS PREVENTED BY N^{ω}-NITRO-L-ARGININE METHYL ESTER (L-NAME)

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In mammals, monocular deprivation (MD), during early postnatal development, causes cell shrinkage and decreases the number of Y cells in the lateral geniculate nucleus (LGN), leading to loss of visual responses in cortical neurones and reduction in visual acuity (Rauschecker, 1991). We have recently shown that MD for 2, 7 and 14 days produces apoptosis in the LGN of new-born rats and this is prevented by L-NAME, an inhibitor of nitric oxide synthase (NOS) (Nucci *et al.*, 1998). Here we report that, under similar experimental conditions, MD for 24 h and 48 h increases LGN content of citrulline, the co-product of nitric oxide (NO) synthesis, and this is abolished by treating the animals with L-NAME. The right eyelids of Long Evans new-born rats (post-natal day=14 (P14); 20±5 g; n=6 per group) were sutured for 24 h, 48 h and 7 days. Age-matched, non-deprived rats (n=6 per group) were used as control. Test groups received injections of L-NAME (3mg/kg^{-1}, i.p. twice daily during MD), or of D-NAME (same treatment schedule), a less active inhibitor of NOS. The LGN levels of citrulline were determined by high-performance liquid chromatography (see Bagetta *et al.*, 1995) and expressed as nmol of citrulline/g of wet tissue weight. The resulting means±s.e.m. from MD and control rats were evaluated statistically for differences. As shown in table 1, LGN citrulline levels increase in control rats from P15 to P21, in agreement with previous data indicating that, in the LGN, mature NOS expression is achieved at the third postnatal week (Bertini and Bentivoglio, 1995). MD for 24 and 48 h enhances LGN concentrations of citrulline by 47% and 32% respectively ($p<0.05$ vs control), suggesting that MD induces excessive NO accumulation in the LGN of new-born rats, and this may be implicated in the mechanisms of MD-evoked apoptosis. In accordance to this hypothesis, treatment with L-NAME, but not with D-NAME, reduces the increased levels of citrulline (present data) and prevents MD-induced apoptosis (Nucci *et al.*, 1998). After 7 days of MD the citrulline content does not differ from control, suggesting that early NO accumulation induced by 24 and 48 h of MD may trigger biochemical alterations leading to the expression of MD-induced apoptosis in new-born rats.

Table 1: Citrulline content in the LGN of new-born rats

	24 h (P15)	48 h (P16)	7 days (P21)
Control	124±7	138±12	178±11
MD	182±7*	182±10*	177±10
MD+L-NAME	153±8	140±7	
MD+D-NAME	175±5*	169±6*	

*$p<0.05$ vs age-matched controls (Student's "t" test)

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298P A COMPUTER-BASED INTERACTIVE TUTORIAL TO TEACH THE PHYSIOLOGY AND PHARMACOLOGY OF THE NEUROMUSCULAR JUNCTION TO UNDERGRADUATE STUDENTS

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Computer-based learning is now a feature of most undergraduate courses and is used to support or even substitute for traditional lectures and laboratory classes. Here we demonstrate a computer-based interactive tutorial covering the essentials of the neuromuscular junction. Learning by this method is non-intimidating, is independent of time and place, may be self-paced and may take place either individually or in small groups. It is designed for undergraduates from a range of courses e.g. medicine, pharmacology, physiology and the biosciences.

The program is divided into several sections:
Introduction which gives an overview of content and approach of the program;
Neuromuscular Transmission which uses animated stepwise sequences to describe synthesis of acetylcholine, transmitter release mechanisms, action of acetylcholine at receptors and transmitter inactivation;
Acetylcholine Receptors which describes the function of and action of acetylcholine at both pre- and post-synaptic nicotinic receptors;
Pharmacology which gives examples of, and describes the characteristics and mechanism of action of depolarising and non-depolarising neuromuscular blocking agents and anti-cholinesterases;
Clinical Aspects which covers the clinical use of neuromuscular blocking agents and anticholinesterases (particularly for treatment of myaesthenia gravis). This section describes how depth of blockade may be monitored, and the pharmacokinetics, characteristics, side-effects and drug interactions of clinically used drugs.

It was developed using Multimedia Toolbook ® (Asymetrix) to run on IBM PC compatibles, capable of running Windows™ version 3.1 or better (Microsoft), with a 256 colour VGA monitor and a mouse.

The approach is to combine succinct textual/factual descriptions with graphics and to use features such as animation and hotwords where appropriate. Hotwords function either to define terms which may be unfamiliar to the student or to provide additional, sometimes more detailed or advanced, information. Some experimental data which illustrates the different actions of neuromuscular blocking agents in animal models is also used. The program contains numerous self-assessment questions e.g. multiple choice and true/false questions with feedback, drag and drop exercises (to test e.g. knowledge of stepwise sequences), and clinically-related scenarios. These are designed primarily to promote and reinforce learning rather than to test students.

It is estimated that the program would occupy students for perhaps three to four hours of self-directed study. It could be used for revision, primary learning or as a tutorial aid.