37P THE STABLE NITROXIDE RADICAL TEMPOL REDUCES INFARCT SIZE CAUSED BY REGIONAL ISCHAEMIA AND REPERFUSION IN THE ISOLATED, PERFUSED HEART OF THE RAT

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Several studies correlate reperfusion injury with the generation of reactive oxygen species (ROS), including superoxide anions (O_2^-) and hydroxyl radicals (OH^-) (Bolli *et al.*, 1988). Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), is a stable piperidine nitroxide radical, which reacts with several ROS including O_2^- (Laight *et al.*, 1997). Here we investigate the effect of Tempol on (i) the infarct size caused by regional ischaemia and reperfusion in the isolated, buffer-perfused heart of the rat, and (ii) hydrogen peroxide (H_2O_2)-mediated injury of rat ventricular myoblasts (H9c2 cells).

Male Wistar rats (250-350 g) were anaesthetised with thiopentone sodium (120 $mg \cdot kg^{-1}$, i.p.) and heparinised (1400 $U \cdot kg^{-1}$, i.p.). The heart was rapidly excised and perfused with modified Krebs' solution in Langendorff mode (37°C, 80 mm Hg) and gassed with 95%O_2/5% CO_2. A suture was positioned around the left anterior descending coronary artery (LAD). After 15 min equilibration, the LAD was occluded for 35 min, followed by 120 min reperfusion. Vehicle (Krebs' solution, n=7) or Tempol (3 mM bolus followed by 1 mM infusion, n=6) was administered throughout reperfusion. At the end of the experiment, area at risk (AAR) and infarct size were determined using Evans Blue dye (0.5% w/v) and nitro-blue tetrazolium (0.5$mg \cdot ml^{-1}$, 37°C for 20 min), respectively.
H9c2 cells were cultured in 96-well plates containing DMEM (200 µl) supplemented with L-glutamine (3.5 mM) and 10% foetal calf serum (FCS) until they reached confluence. Cells were preincubated (10 min) in media (1% FCS) alone or media containing Tempol (0.01-30 mM). Cells were then exposed to 1 mM H_2O_2 for 4 h and cell injury was assessed by measuring the reduction of MTT (3-(4,5-dimethyliazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan. Data are expressed as mean ± s.e.mean (*p< 0.05 compared to control, ANOVA followed by Dunnett's test).

AAR was not significantly different between the groups studied (control; 51±2%, Tempol; 57±3%). Reperfusion of hearts with buffer containing Tempol reduced infarct size from 54±4% (n=7) to 33±2%* (n=6) (see Figure 1). H_2O_2 caused an impairment in mitochondrial respiration which was attenuated by Tempol in a concentration-dependent manner (e.g. control; 100%, 1 mM H_2O_2; 10±6%, 1mM Tempol; 72±11%*).

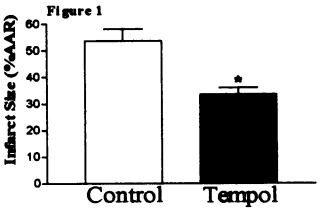

Figure 1 Effect of Tempol on infarct size caused by regional ischaemia (35 min) and reperfusion (2h) in the isolated perfused heart of the rat. Control (vehicle, open column, n=7) Tempol (3 mM bolus + 1 mM infusion, solid column, n=6) *p<0.05

Thus, Tempol, when administered during reperfusion, reduces infarct size caused by regional ischaemia and reperfusion of the isolated buffer-perfused heart of the rat. Tempol also attenuates the reduction in mitochondrial respiration caused by H_2O_2.

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38P AUTORADIOGRAPHIC DISTRIBUTION OF THE α_{1D} ADRENOCEPTOR IN THE RAT CNS

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Although the α_{1D} adrenoceptor (α_{1D}-AR) has been cloned and its mRNA localisation described (Day *et al.*, 1997), the receptor protein itself has not yet been identified in the CNS (Michel *et al.*, 1994).

This study describes the use of a novel α_{1D}-selective piperazine antagonist, SNAP 8493 (8-{2-[4-(2,4,5-trifluorophenyl) piperazin-1-yl]ethyl}-8-azaspiro[4.5]decane-7,9-dione)(Konkel *et al.*, 1998), in the *in vitro* autoradiographic localisation of α_{1D}-AR binding sites in the rat CNS. Brains and spinal cords were removed from 3 adult male Sprague-Dawley rats (250-300g), frozen on dry ice and sectioned coronally at 20 µm. Total α1-AR binding was determined in the presence of 0.3nM ^{3}[H]prazosin in 50 mM TRIS/5 mM EDTA/150 mM NaCl buffer (pH7.4) according to the method of Walden *et al.*, (1997). NS binding was determined in the presence of 10 µM phentolamine and the α_{1D}-AR binding image was determined by subtracting the image obtained in the presence of SNAP 8493 (20nM; K_D at α_{1D}-AR= 1.26 nM) from the total binding. Slide mounted sections were then apposed to a tritium sensitive film for 8 weeks and images analysed by computer assisted densitometry. For each rat five measurements were taken/region and levels of optical density were converted into fmol/g of α_{1D}-AR binding using Amersham tritiated microscales (Table 1).
The highest levels of α_{1D}-AR binding sites were found in the dorsal raphe, lateral amygdala, olfactory bulb, in several thalamic regions, the centromedial and lateral dorsal nuclei as well as in the lateral geniculate nucleus and also in layers IV-V of the somatosensory cortex. Regions with an intermediate number of binding sites include the inferior olives, locus coeruleus, hippocampus and remaining thalamic nuclei. Low α_{1D}-AR binding was found in the nucleus accumbens, caudate putamen and at all levels in the spinal cord, no binding was detected in the corpus callosum.

This is the first report of α_{1D}-AR localisation in the CNS and the distribution pattern strongly indicates a role for this receptor in the modulation and integration of somatosensory information.

Table 1:Regional distribution of $\alpha_{1D\text{-}AR}$ binding sites in rat brain (Mean Bmax, fmol/mg protein ± S.E.M. N=3)

dorsal raphe	112 ± 11.6
lateral amydgala	111 ± 12.2
olfactory bulb (EPL)	90.3 ± 8.8
centromedial thalamus	71.5 ± 13.4
locus ceruleus	51.7 ± 17.5
paravent. thalamic nuc.	43.4 ± 12.4
inferior olives	27.7 ± 6.9
caudate putamen	9.1 ± 2.5
nucleus accumbens	9.2 ± 3.2

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39P THE SODIUM CHANNEL MODULATORS LAMOTRIGINE AND 4030W92 BLOCK ECTOPIC DISCHARGE ORIGINATING IN RAT SCIATIC NEUROMAS

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Sodium channel modulators which have been used in the treatment of neuropathic pain, block the ectopic discharge originating in neuromas induced by ligation and section of rat sciatic nerve (Devor *et al.*, 1992; Yaari & Devor, 1985). The present study extends these observations to the newer sodium channel blocking agents lamotrigine and 4030W92 (Trezise *et al.*, 1998), and examines their effects on different patterns of ectopic nerve activity.

Under halothane/nitrous oxide anaesthesia, the left sciatic nerve of male Sabra rats (390-527g) was ligated and sectioned at mid-thigh level with excision of approximately 5mm distally, followed by wound closure. After 7 to 10 days, the rats were re-anaesthetised with pentobarbitone sodium (50mgkg^{-1} i.p. and 18mgkg^{-1}h^{-1} i.v.). The left carotid artery was catheterised for measurement of blood pressure and withdrawal of blood samples, and the left jugular vein for infusion of drugs. Electrophysiological recordings of nerve activity were made from teased microfilaments containing 2 or 3 spontaneously active fibres originating in the neuroma, selected to represent each of the 2 predominant patterns of firing, viz. rhythmic (interspike interval variation ≤5ms) tonic, and rhythmic 'on-off' bursting. Lamotrigine 0.3mgkg^{-1}min^{-1}, 4030W92 0.1mgkg^{-1}min^{-1} or vehicle 0.3mlkg^{-1}min^{-1} (1.5% or 2% v/v in 0.9% saline of a formulation of 50% tetraglycol, 40% cremophor EL, 10% ethanol v/v) were infused until block of all spontaneous nerve activity (defined as cessation of discharge for 2 min), or up to a maximum of 40 min. Free plasma concentrations of drugs were estimated from arterial blood samples (0.5ml) withdrawn at the time of block, or after 40 min infusion. Results are expressed as mean±s.e.mean for n separate experiments.

None of the spontaneous activity of bursting or tonic fibres was blocked during 40 min of vehicle infusion in 10 rats. Lamotrigine blocked all burst pattern spontaneous firing in 7 rats after 18.9±4.0 min (plasma concentration 13.7±1.7μM, n=5) whereas tonic firing was blocked in only 2 of the 7 rats at 34 min and 38 min (plasma concentrations, 19μM and 21μM, respectively). The plasma concentration of lamotrigine measured in 4 of the remaining rats at 40 min was 18.5±1.1μM. 4030W92 blocked all burst pattern spontaneous firing in 5 rats after 15.3±2.9 min (plasma concentration 1.1±0.2μM, n=5), whereas tonic firing was blocked in only 2 of the 5 rats after 31 min and 36 min (plasma concentrations, 1.2μM and 2μM, respectively). In the remaining rats, the plasma concentration of 4030W92 at 40 min was 1.8±0.2μM (n=3). The compounds resulted in little or no effect on mean arterial pressure at the time of block of burst pattern firing (lamotrigine 0.0±2.7mmHg n=7, 4030W92 +3.8±1.2mmHg n=5, *cf.* pre-dose levels).

The data presented in this study demonstrate block of ectopic discharge in peripheral nerve by lamotrigine and 4030W92. The effective plasma concentration of lamotrigine is within the range resulting in symptomatic relief of neuropathic pain in man (Lunardi *et al.*, 1997). Temporal analysis indicates that spontaneously active fibres firing in bursts are more sensitive to compound induced blockade than fibres firing tonically, possibly because the former are straddling their threshold for rhythmic firing (Matzner & Devor, 1994) making them easier to block. However, involvement of different sodium channel types cannot be ruled out.

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40P DIFFERENTIAL EFFECT OF THAPSIGARGIN ON BICUCULLINE- AND GABAZINE-INDUCED EPILEPTIFORM EXCITABILITY IN DISSOCIATED RAT HIPPOCAMPAL NEURONES

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Both bicuculline (BIC) and gabazine (GBZ; SR-95531), $GABA_A$ receptor antagonists, have been shown to increase neuronal excitability in rat hippocampus both *in vivo* (Margineanu & Wülfert, 1997) and in brain slices (Wülfert & Margineanu, 1998). A 64-channel multiple microelectrode array (MMEA; Mason *et al.*, 1998) was used to investigate the effects of depleting intracellular Ca^{2+} stores with thapsigargin (THG) on BIC- and GBZ-induced epileptiform excitability within hippocampal neuronal networks.

Primary dissociated Wistar rat hippocampal neurones from E18 fetuses were cultured directly onto MMEAs for 21 days. Single-unit extracellular discharge activity was captured using a multi-channel neuronal acquisition processor (Plexon Inc, Texas, USA). Data was analysed off-line using *SpikeWorks* (Plexon Inc), then further processed using spike train analysis software (*Neuronal Explorer*, Nex Technologies, USA). Data are expressed as mean±s.e.mean. Statistical differences between groups were measured using a Student's paired t-test (*: P<0.001).

All cells recorded exhibited spontaneous extracellular discharge activity at 37°C. GBZ (10μM) significantly increased firing rate (7.4±0.5 to 11.8±0.7 Hz; *) and burst rate (32.1±2.3 to 71.3±5.4 bursts min^{-1}; *) in all cells recorded (n=50 cells/3 MMEAs); a burst was defined as a minimum of three spikes with interspike intervals <0.01s. BIC alone (10μM) significantly increased firing rate (10.3±0.7 to 24.7±2.3Hz; *) and burst rate (27.0±3.6 to 112.8±8.8 bursts min^{-1}; *) in only 39/72 cells (4 MMEAs). Firing was decreased in 24/72 cells and remained unchanged in 9/72 cells. THG alone (2μM; n=32 cells/2 MMEA) significantly increased firing rate (11.8±1.1 to 13.8±1.3 Hz; *) and burst rate (58.7±7.5 to 70.6±6.7 bursts min^{-1}; *). Pre-incubation (20min) with THG (2μM; n=50 cells/3 MMEAs) significantly increased the GBZ-induced elevation of firing rate (11.8±0.7 to 13.6±0.8Hz; *) and burst rate (71.3±5.4 to 85.4±5.8 bursts min^{-1}; *). In contrast, (analysing only those cells exhibiting increased firing with BIC), pre-incubation with THG (2μM) significantly decreased BIC-induced increases in firing rate (24.7±2.3 to 15.5±1.7Hz; *) and burst rate (112.8±8.8 to 82.6±10.4 bursts min^{-1}; *).

The ability of THG to increase neuronal excitability in dissociated hippocampal neurones may explain its potentiation of GBZ-induced increases in firing rate and burst rate. THG (2μM) has been reported to deplete intracellular Ca^{2+} stores by more than 90% within 15 minutes (Mathes & Thompson, 1995). Our results therefore suggest that BIC-induced neuronal excitability requires release of Ca^{2+} from intracellular stores, a finding supported by van der Linden *et al.*, (1993) who demonstrated that BIC increased intracellular Ca^{2+} in hippocampal neurones. The results also support the view that the mode of action of the two $GABA_A$ receptor antagonists, GBZ and BIC, differs (Margineanu & Wülfert, 1997; Uchida *et al.*, 1996).

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41P BLOCK OF NEURONAL K_V POTASSIUM CURRENTS BY FLUOXETINE AND IPRINDOLE

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A number of antidepressant drugs have previously been shown to block K_v potassium channels in mammalian neurones (see Mathie *et al.* 1998). In this study, we have investigated the effects of a further two clinically used antidepressant drugs, fluoxetine and iprindole on K_v currents in cerebellar granule neurones and NG108-15 cells.

Cerebellar granule neurones (CGNs) were isolated and cultured from 6-9 day old rats as previously described (Watkins & Mathie, 1996). Recordings were made using whole cell voltage-clamp techniques and amphotericin B as the cell permeant. The bathing solution contained (mM): 120 NaCl, 2.5 KCl, 2 $MgCl_2$, 0.5 $CaCl_2$, 5 glucose, 10 HEPES, pH 7.4, and the pipette solution (mM): 125 KCl, 5 $MgCl_2$, 5 HEPES, 0.1 BAPTA, pH 7.4. K_V currents were usually activated by depolarising the cells to a test potential of +10 mV from a prepulse potential of -50 mV.

Fluoxetine at concentrations of 3, 10, 30 and 100 μM inhibited K_V in CGNs by 24 ± 3% (mean ± s.e.mean; n=6), 45 ± 3% (n=6), 60 ± 5% (n=7) and 78 ± 2% (n=5) respectively, with the IC_{50} calculated as 15 μM. The effects of fluoxetine were less pronounced in NG108-15 cells, as 10 μM and 100 μM caused 20 ± 8% (n=7, $p<0.05$; two-tailed unpaired Student's t-test), and 73 ± 6% (n=8) inhibition respectively.

Iprindole (30 μM) was also effective at inhibiting the K_V current in CGNs (40 ± 2%, n=7), but was not as potent as fluoxetine ($p<0.05$). Inhibition was not significantly greater in NG108-15 cells (45 ± 4%, n=3,) compared to CGNs.

Inhibition by fluoxetine (10 μM) showed little voltage dependence in the range -10 mV to +40 mV. For example, at a test potential of 0 mV, current was inhibited by 37 ± 6%, while at +40 mV, inhibition was 42 ± 4% (n=12). Moreover, inhibition was not use-dependent. Between stopping and resuming depolarising pulses to +10 mV during drug application, K_V was still inhibited by 41 ± 5% (n=6).

Thus fluoxetine and iprindole inhibit K_V in CGNs and NG108-15 cells, and block by fluoxetine was neither voltage nor use-dependent. Tytgat *et al.* (1997) have shown that fluoxetine inhibits $K_v1.1$ channels expressed in *Xenopus* oocytes. However fluoxetine was much less potent, in these experiments with an IC_{50} of between 600 and 700 μM at 0 mV. In addition, block of $K_v1.1$ in oocytes was both voltage and use-dependent. It is of interest to determine whether these functional differences reflect structural variations between native and recombinant K_v channels.

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42P MODULATION OF $GABA_A$ RECEPTORS BY MEFENAMIC ACID: INVOLVEMENT OF ASPARAGINE 290 IN THE SECOND TRANSMEMBRANE DOMAIN OF THE β_2 SUBUNIT

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The non-steroidal anti-inflammatory mefenamic acid (MFA) directly activates and modulates neuronal and recombinant $GABA_A$ receptors. These effects are dependent on the type of β subunit within the receptor with β2/3 conferring excitatory, and β1 either zero or an inhibitory sensitivity (Halliwell *et al.*, 1998). This study addresses the link between the molecular action of MFA on $GABA_A$ receptors and its central nervous system side effects. HEK cells were transfected with combinations of cDNAs encoding for α1, β1, β2, β3 and γ2S human $GABA_A$ receptor subunits and whole-cell currents recorded at -40 mV holding potential using the patch clamp technique.

MFA directly activated $\alpha1\beta3\gamma2S$ (EC_{50} 23.5 ± 2.7μM, mean ± s.e.mean; n=13) but did not affect $\alpha1\beta1\gamma2S$ constructs. This differentiation was reminiscent of the action of loreclezole on recombinant $GABA_A$ receptors (Wafford *et al.*, 1994). Application of submaximal concentrations of MFA (10μM) and loreclezole (10μM) yielded an additive activation of $\alpha1\beta3\gamma2S$ receptors; however co-applying MFA (10μM) with saturating levels of loreclezole (300μM), produced no further effect suggesting similar binding sites. To locate the MFA binding site, asparagine (N) 290 in the second transmembrane domain of the β2 subunit, which is pivotal to the action of loreclezole, was mutated to serine (S). For the mutant receptor, $\alpha1\beta2(N290S)\gamma2S$, the maximum MFA (50μM) potentiation of 5μM GABA responses was reduced to 43 ± 15 % (EC_{50} 242 ± 42μM), whereas for the converse mutant, $\alpha1\beta1(S290N)\gamma2S$, MFA (100μM) now potentiated the GABA (1μM)-evoked response by 300 ± 25% (EC_{50} 7.8 ± 1.7μM). Neither β subunit mutation affected receptor sensitivity to GABA (EC_{50}s for $\alpha1\beta2(N290S)\gamma2S$, 7.7 ± 0.5μM and $\alpha1\beta2(S290N)\gamma2S$, 4.3 ± 0.3μM, n=4-6 for all experiments). The bell-shaped concentration response curves for the direct and GABA potentiating effects of MFA on the $\alpha1\beta2/3\gamma2S$ receptors indicated the presence of a potential inhibitory site for MFA. Although the $\alpha1\beta1\gamma2S$ $GABA_A$ receptor was MFA-insensitive, expression of $\alpha1\beta1$ constructs revealed marked inhibition of GABA-activated responses (Halliwell *et al.*, 1998). The MFA inhibition at $\alpha1\beta2$ receptors was assessed by first abolishing the MFA potentiating effect with the mutation, $\alpha1\beta2(N290S)$. MFA (30 - 300 μM) had no direct action but, comparable to $\alpha1\beta1$ receptors, inhibited in a concentration dependent manner (1-1000 μM) the 10 μM GABA response (IC_{50} 67.6 ± 10.3μM, n=3). The modulatory effects of MFA were also assessed in homomeric β1 and β3 subunits. Both subunits formed spontaneously-gated channels with β3 directly activated by MFA (EC_{50} 17.7 ± 0.9μM, n=4) whereas for β1, MFA (5 – 2000μM) caused a dose-dependent block of the spontaneous Cl^- current (IC_{50} 189 ± 12μM, n=3).

Given the similar pharmacological profiles exhibited by MFA, loreclezole and also the general anaestheic etomidate (Belelli *et al.*, 1997), we compared their chemical structures to examine the pharmacophore for this clinically important site. Comparisons were made between the two lowest energy (R) and (S) forms of MFA and the equivalent low energy conformations of loreclezole and etomidate. Superposition of equivalent atoms within each structure revealed two common features: a right-angled conformation with the planes of the two hydrophobic rings positioned approximately orthogonal and the designated reactive electronegative centres forming around the elbow.

These results provide a potential molecular basis for the clinical effects associated with MFA, namely coma and convulsions in overdose. These effects may be attributed to the potentiation and inhibition of the $GABA_A$ receptor channel mediated by MFA, whereby the potentiating effect requires N290 in β2/3 subunits.

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43P ALTERATIONS IN GABA$_A$ α_1, β_2 AND γ_2 RECEPTOR SUBUNIT GENE EXPRESSION IN THE GLOBUS PALLIDUS IN THE 6-HYDROXYDOPAMINE (6-OHDA) LESIONED RAT MODEL OF PARKINSON'S DISEASE

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In Parkinson's disease (PD), striatal dopamine depletion produces downstream changes in GABAergic activity in the basal ganglia. Ligand binding studies have indicated that plasticity of GABA$_A$ receptors accompanies these changes in some regions (e.g. globus pallidus (GP) and substantia nigra (SN)) (Pan *et al.*, 1985). The aim of this study was to determine whether changes in the most often combined GABA$_A$ receptor subunits (α_1, β_2 and γ_2) could underlie this plasticity.

Male Sprague Dawley rats (250-270g) were injected with either 6-OHDA (12.5µg in 2.5 µl) or vehicle into the right median forebrain bundle. Three weeks later rats were sacrificed, the brains frozen (-45°C) and coronal cryosections (15µm) obtained through regions of interest. Sections were stored at -70°C until processing. On the day of hybridisation, sections were fixed, pretreated to reduce non-specific binding, dehydrated and defatted. ^{35}S-labelled oligonucleotide probes complementary to mRNA encoding the GABA$_A$ α_1, β_2 and γ_2 subunits (Wisden et al, 1992) or the 'housekeeping gene' G3PDH (Fort et al., 1985) were diluted in hybridisation buffer to a specific activity of $3x10^6$ cpm ml^{-1}. Sections were hybridised overnight at 37°C. and 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 (Kodak) for periods of 10 to 35 days. Levels of GABA$_A$ subunit expression relative to G3PDH expression were semi-quantified by image analysis (MCID; Imaging Research Inc).

In the 6-OHDA-lesioned rats, changes in expression were restricted to the GP. In this region, a significant reduction in both α_1 (~20%) and β_2 (~17%) gene expression was found (see Table 1). No changes in expression were observed in any region in the sham-lesioned rats (data not shown).

Table 1: GABA$_A$ subunit mRNA level in 6-OHDA-lesioned rats

subunit /G3PDH mRNA levels (OD Units)						
	α1		β2		γ2	
Region	Lesioned	Intact	Lesioned	Intact	Lesioned	Intact
GP	0.40±0.02*	0.51±0.02	0.11±0.00*	0.13±0.01	0.15±0.01	0.16±0.01
STN	0.63±0.12	0.58±0.11	0.13±0.02	0.13±0.02	0.19±0.04	0.17±0.04
SNr	0.86±0.05	0.89±0.08	0.19±0.01	0.18±0.02	0.16±0.01	0.17±0.01

Values are means ± SEM (n=6). * p<0.01; lesioned vs. intact; paired t-test. GP = globus pallidus, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata. OD = optical density.

The decrease in α_1 and β_2 gene expression in the lesioned GP is consistent with earlier flunitrazepam binding studies and may reflect a compensatory response to the marked overactivity of striatopallidal projections in PD. The regional selectivity of these changes indicates that GABA$_A$ receptor composition is altered in a topographical manner in PD. The relevance of these findings for therapeutic manipulation in PD remain to be seen.

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44P DOPAMINE CELL LOSS AND EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE IMMUNOREACTIVITY FOLLOWING INTRANIGRAL INJECTION OF LIPOPOLYSACCHARIDE

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Parkinson's disease (PD) is characterised by loss of dopamine cells in the substantia nigra. There are reports that link inflammatory processes (characterised by proliferation of microglia and reactive macrophages) to PD (McGeer et al., 1988).

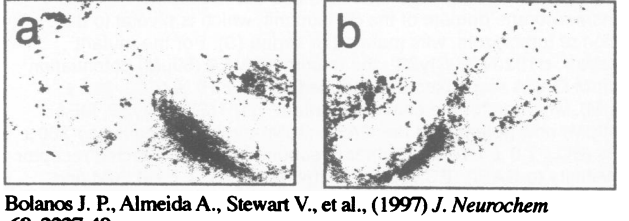
The bacterial endotoxin, lipopolysaccharide (LPS), an inflammatory mediator, is also a potent inducer of the nitric oxide synthase (iNOS). Post mortem analysis of PD patients has shown that iNOS is expressed in cells displaying the morphological characteristics of activated macrophages, and that these cells also exhibit iNOS-like immunoreactivity (Hunot et al., 1996). Although the release of nitric oxide by iNOS has been implicated in cell death (Bolanos et al., 1997), it is not yet clear whether the NO mediated cell death is a consequence of an inflammatory process. In this study we have looked at the effects of intranigral injection of LPS on the expression of iNOS-immunoreactivity (-ir) and further studied its effects on the population of tyrosine hydroxylase (TH)-positive neurons in the ventral mesencephalon.

Male Wistar rats (180-250 g) were anaesthetised with sagatal (60mg.kg^{-1}, i.p.). LPS (serotype 026:B6, *E.coli;* Sigma) was dissolved (1 mg.ml^{-1}) in phosphate buffered saline (PBS) and 2 µl of either LPS or vehicle (PBS) was injected into the left substantia nigra (A, 5.3 mm; L, 2.2 mm; V, 8.3 mm) at rate of 1 µl.min^{-1} using standard stereotaxic techniques. After 16 hours, animals were anaesthetised, perfused with 4 % paraformaldehyde and contiguous 30 µm sections were cut through the midbrain using a cryostat. Free-floating sections were processed for NADPH-diaphorase histochemistry or iNOS-, GFAP- and TH-ir. The population of dopamine neurons (characterised by TH-ir) in the substantia nigra pars compacta (SNc) or the ventral tegmental area (VTA) were counted at the level of the 3rd nerve, using quantitative image analysis.

Following intranigral injection of vehicle, no NADPH-positive or iNOS-ir cells could be seen in either the SN or the VTA and the number of TH positive cells in either the SNc or the VTA were not significantly different from the contralateral side. Occasionally there were some GFAP-ir seen along the injection tracts. Injection of LPS resulted in an intense expression of NADPH-diaphorase positive and iNOS-ir in non-neuronal, macrophage-like cells, accompanied with a significant reduction in the number of TH-ir neurons in the SNc (see figure 1; PBS control: 174 ± 20; LPS: 76 ± 16; $P < 0.006$, n=5, unpaired t-test) but not in the VTA (PBS control: 273 ± 38; LPS: 282 ± 72; n=5, NS). There was also a very intense expression of GFAP-ir in the SN but the pattern of distribution of GFAP-ir did not correspond to the distribution of either the NADPH-positive or iNOS-ir cells, suggesting that astrocytes were not the source of iNOS.

This study suggests that the reduction of TH-ir neurons following LPS injection may be NO-mediated but other actions of LPS (independent of iNOS-induction) on these cells cannot be ruled out and this requires further experiments.

Figure 1. TH-ir in the rat ventral mesencephalon, after LPS (**a**) or vehicle (**b**) injection.



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45P GLUTAMATE AUTORECEPTORS OF THE $mGlu_5$ SUB-TYPE ENHANCE NEURONAL GLUTAMATE RELEASE IN THE RAT FOREBRAIN *IN VITRO*

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The metabotropic glutamate (mGlu) receptors are divided into three groups based on their sequence homology, agonist pharmacology and signal transduction mechanisms. Group I mGlu receptors ($mGlu_1$ & $mGlu_5$) are coupled to phospholipase C and are selectively activated by (S)-3,5-dihydroxyphenylglycine ((S)-3,5-DHPG). Group II ($mGlu_{2\&3}$) and group III ($mGlu_{4,6,7\&8}$) mGlu receptors are both negatively coupled to adenylate cyclase. There is much evidence to support a role for group II and group III mGlu receptors in decreasing excitatory synaptic transmission via presynaptic mechanisms, whilst it has been suggested that group I mGlu receptors mediate *enhancement* of neuronal glutamate release (e.g. Croucher et al., 1997). We now report that electrically stimulated release of [^{3}H]D-aspartate ([^{3}H]D-asp) (a non-metabolized marker for glutamate) from rat forebrain slices is enhanced by activation of group 1 mGlu receptors which have the pharmacological profile of the $mGlu_5$ sub-type.

Serial forebrain slices were cut and incubated as previously described (Patel & Croucher, 1997). After loading with [^{3}H]D-asp (40nM final concentration) slices were superfused with oxygenated Krebs buffer and the influence of drugs on basal and electrically stimulated release of label was examined using a standard protocol (Patel & Croucher, 1997). Release in this preparation is Ca^{++}-dependent and tetrodotoxin-insensitive, consistent with its origin from presynaptic nerve terminals. Results are means of at least 5 independent observations. (S)-3,5-DHPG, 0.1-3μM, dose-dependently enhanced the stimulated efflux of [^{3}H]D-asp (maximum release 337%; graphically estimated ED_{50} 0.9μM) whilst having no effect on basal release. Application of the agonist at 10μM resulted in desensitization of the receptor which was lifted upon co-administration of cyclothiazide (CYZ), 10μM, a desensitization inhibitor which has been reported to selectively potentiate $mGlu_5$-mediated responses (Sharp et al., 1994). The non-selective mGlu receptor antagonist (S)-α-methyl-4-carboxyphenylglycine ((S)-MCPG), 0.01-100μM, inhibited the responses to (S)-3,5-DHPG, 1μM ($P<0.05$), with an IC_{50} 0.05μM. However, the $mGlu_1$-selective antagonists (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA), 1-500μM, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester (CPCCOEt), 0.1-100μM and (+)-LY367385, 0.1-10μM, all failed to attenuate the (S)-3,5-DHPG, 1μM, mediated enhancement of evoked release. None of the antagonists used in this study influenced basal or stimulated efflux of [^{3}H]D-asp when given alone. The novel $mGlu_5$-selective agonist (RS)-2-chloro-5-hydroxyphenylglycine ((RS)-CHPG), 10-300μM, also dose-dependently increased electrically stimulated [^{3}H]D-asp release in the rat forebrain (maximum release 326%; graphically estimated ED_{50} 50μM). Receptor desensitization at high agonist concentrations (1-3mM) was again apparent with this ligand, as was inhibition of the agonist-induced response by (S)-MCPG, 10μM ($P<0.05$).

These results are consistent with the presence of $mGlu_5$ glutamate autoreceptors on presynaptic nerve terminals in the rat forebrain acting to enhance the release of neuronal glutamate.

We thank the Wellcome Trust (Project Grant No. 040256/Z/93/Z) and the Trustees Research Committee for generous financial support and Mr. John Harris for the kind gift of (+)-LY367385.

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46P THE GROUP II METABOTROPIC GLUTAMATE RECEPTOR AGONIST DCG-IV INCREASES GENERALIZED SEIZURE THRESHOLDS IN THE AMYGDALA KINDLING MODEL OF EPILEPSY

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Presynaptic mechanisms of seizure generation and spread have recently become a focus of epilepsy research. Of particular interest in this respect is the role of presynaptic metabotropic glutamate (mGlu) receptors. Activation of these receptors has been reported to attenuate epileptiform seizure activity (Attwell *et al.*, 1995; Abdul-Ghani *et al.*, 1997). One of the most potent agonists at mGlu receptors *in vitro* is DCG-IV, a ligand which shows clear selectivity for group II mGlu receptors. Here we have examined the actions of this drug in the amygdala kindling model of epilepsy. The influence of DCG-IV on depolarization-induced glutamate release from isolated synaptosomes was also studied using the glutamate tracer [^{3}H]D-aspartate.

Male Sprague-Dawley rats (290-330g) were anaesthetised with a halothane-N_2O mixture and implanted with guide cannula/bipolar electrode units in the vicinity of the right basolateral amygdala. At least one week after surgery, afterdischarge thresholds (ADTs) were determined and animals were kindled by daily electrical stimulation with 125% of their threshold current. The development of seizure activity was rated on a 5 point scale, based on that of Racine (1972) and continued until 3 consecutive stage 5 seizures were obtained, at which time the generalised seizure thresholds (GSTs) were determined. The effect of DCG-IV on fully kindled seizure activity was studied by injecting the drug (in 0.5μl buffer) close to the kindling site 20min prior to redetermining the GST. Synaptosomes were prepared from rat cerebral cortex and the effect of drug treatment on veratridine (50μM)-evoked [^{3}H]D-aspartate release was studied using a standard superfusion procedure, as previously described (Hughes *et al.*, 1993).

DCG-IV dose-dependently increased the GST in fully kindled animals ($P<0.001$; ANOVA), this effect reaching significance compared to control at 0.1nmol (63.5 ± 21.6% increase; $P<0.05$; Tukey's test). The estimated GST_{100} (dose required to increase GST by 100%) for DCG-IV was 0.22nmol. The agonist did not influence seizure score, afterdischarge duration, motor seizure duration or latency to onset of generalised seizure when evoked at the higher threshold. Co-administration of the selective group II mGlu receptor antagonist MCCG (40nmol) significantly inhibited the anticonvulsant effect of DCG-IV ($P<0.05$; Tukey's test), whilst showing no effect on kindled seizure parameters when administered alone. Superfusion of synaptosomes with Krebs-buffer containing DCG-IV fully inhibited veratridine-evoked release of [^{3}H]D-aspartate with an IC_{50} value of 0.39 ± 0.08μM (mean ± 95% confidence intervals; n=3 in duplicate). The IC_{50} value for the clinically effective anticonvulsant drug lamotrigine (Lamictal), tested in the same system, was approximately 70-fold greater (27.7 ± 2.07μM) than that for DCG-IV.

These findings demonstrate the very high potency of DCG-IV as an anticonvulsant agent and support the concept of the involvement of group II mGlu receptors in the control of seizure activity via their modulatory action on neuronal glutamate release.

We thank the Wellcome Trust for their generous financial support.

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47P APCD DEPOLARISES RAT CHOLINERGIC INTERNEURONES THROUGH ACTIVATION OF GROUP I METABOTROPIC GLUTAMATE RECEPTORS

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It has been previously reported that group I metabotropic glutamate receptors are expressed in medium spiny projection neurones of the rat neostriatum (Testa *et al.* 1994). Recent work suggests that they are also expressed in striatal interneurones but their presence and role in these cells remains controversial (Kerner *et al.* 1997). In the present study we have tested for the presence of metabotropic glutamate receptors in identified striatal cholinergic neurones and examined their functional role.

300μm coronal brain slices containing the striatum were prepared from 13-21 day-old Wistar rats in physiological saline. Cholinergic interneurones were visually identified using infra-red video microscopy and were preferentially targeted for whole-cell patch-clamp recordings. Retrospective immunocytochemical processing in a number of cells confirmed their cholinergic nature. The physiological saline contained (mM) 125.0 NaCl, 25.0 $NaHCO_3$, 10.0 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgCl_2$ and was bubbled with a 95%, 5% O_2/CO_2 gas mixture. Physiological saline was also used as the extracellular solution whilst the intracellular (pipette) solution comprised (mM) 120.0 Kgluconate, 10.0 NaCl, 2.0 $MgCl_2$, 0.5 K_2EGTA, 10.0 HEPES, 4.0 Na_2ATP, 0.3 Na_2GTP, cascade blue 1mg/ml, pH 7.2

In whole-cell current clamp recordings, bath application of 50μM (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) depolarised all neurones tested by 12.3 ± 0.2mV (n=9; mean ± SEM) in a tetrodotoxin (TTX)-insensitive manner (1μM TTX; 11.2 ± 1.2mV depolarisation (n=5)). The effects of ACPD were mimicked by the group I metabotropic agonist 3,5-dihydroxyphenylglycine (50μM DHPG; 11.5mV ± 3.3mV depolarisation (n=5)). In whole-cell voltage clamp recordings, 50μM DHPG induced an inward current (101.1 ± 15.5pA at -60mV (n=4)) which was unaffected by the removal of extracellular Ca^{2+} with the addition of 10mM $MgCl_2$, 5μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F)quinoxalone (NBQX) and 50μM D-2-amino-5-phosphovalerate (D-AP5, 99.3 ± 18.4pA (n=3)) indicating that these effects are mediated post-synaptically.

The magnitude of the current was significantly inhibited by pre-incubation with the selective group I metabotropic antagonist (RS)-1-aminoindan-1,5-dicarboxylic acid (400μM caused a 60.3 ± 7.2% reduction in control current (n=3, $P<0.05$ using Student's t-test)). Replacement of the external NaCl with Tris-HCl also caused a significant reduction in the magnitude of the current (by 80.2 ± 6.3% at -60mV(n=4, $P<0.05$)).

Finally, to confirm the identity of the metabotropic glutamate receptor responsible for these effects, the cytosolic contents were aspirated from three cells and subjected to RT-PCR using primers specific for choline acetyl transferase (ChAT) and the mGluR subunits. In each cell, ChAT was detected together with mGluR1 and mGluR5 metabotropic glutamate receptors.

In conclusion, we have demonstrated that metabotropic glutamate agonists depolarise cholinergic interneurones in the rat striatum via a group I receptor mediated inward current which is mediated at least in part by influx of extracellular Na^+ ions.

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48P NICOTINIC ACETYLCHOLINE RECEPTORS ON GLUTAMATERGIC NERVE TERMINALS CAN ENHANCE DOPAMINE RELEASE IN RAT STRIATAL SLICES

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Presynaptic nicotinic acetylcholine receptors (AChR - Kaiser *et al.*, 1998) and ionotropic glutamate receptors (iGluR - Chéramy *et al.*, 1996) modulate the release of dopamine (DA) from rat striatal synaptosomes. Evidence from *in vivo* and slice experiments support an indirect nicotinic stimulation of DA release via an increase in glutamate efflux. Here, by using the superfusion technique, we studied the indirect nicotinic enhancement of striatal DA release.

Slices (0.25 mm) were prepared from the striata of male Sprague-Dawley rats (250-280 g), loaded with [^{3}H]-DA and perfused as previously described (Marshall *et al.*, 1996). After 20 min washing with Krebs bicarbonate buffer, and a further 10 min with normal buffer or buffer containing antagonist (kynurenic acid (KYNA), 500 μM; 6,7-dinitroquinoxaline-2,3-dione (DNQX), 100 μM; mecamylamine, 10 μM; or α-conotoxin-ImI (α-CTx-ImI), 1 μM), the potent nicotinic agonist (±)-anatoxin-a ((±)AnTx-a, 0.1-25.0 μM – Figure 1 inset) was applied for 40 s. Two min fractions were collected and counted for radioactivity. Data were analysed using SigmaPlot™ for Windows, and responses were compared using the one way ANOVA test. The results are expressed as percentage of mecamylamine-sensitive responses (mean ± SEM).

In striatal slices, the dose response curve for (±)AnTx-a-evoked [^{3}H]-DA release was biphasic with EC_{50} values of 180.4 ± 0.1 nM and 4.5 ± 0.5 μM (Figure 1). KYNA (500 μM), a broad spectrum iGluR antagonist, inhibited responses to 1, 10 and 25 μM (±)AnTx-a by 23.0±2.4% (n=3), 53.0±1.8% (n=3) and 45.9±5.1% (n=4) respectively (Figure 1). DNQX (100μM), a non-NMDA receptor antagonist, inhibited 25 μM (±)AnTx-a-evoked DA release by 42.6±8.0% (n=5). KYNA (500 μM) and DNQX (100 μM), when applied together, decreased 25 μM (±)AnTx-a-evoked response by 42.5±10.0 % (n=3).

These results suggest that the second phase of the dose-response curve is, at least partially, a consequence of (±)AnTx-a-evoked glutamate release that in turn enhances DA release.

α-CTx-ImI (1μM), an antagonist selective for the α7 subtype of nicotinic AChR, decreased the response to 25 μM (±)AnTx-a by 35.2±9.5% (n=4). In striatal synaptosomes (±)AnTx-a-evoked DA release is insensitive to α-CTx-ImI (Kulak *et al.*, 1997) consistent with the absence of α7-type nicotinic AChR on dopaminergic terminals. The α-CTx-ImI sensitivity in slices supports the involvement of α7-type nAChR on non-DA terminals.

These data suggest that striatal dopamine release is directly and indirectly modulated by nicotinic AChR, directly via presynaptic nicotinic AChR on dopaminergic terminals and indirectly by evoking glutamate release. We also propose that the nicotinic AChR on the glutamatergic nerve terminal contains the α7 nicotinic AChR subunit.

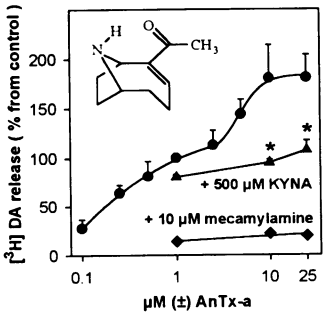

Figure 1. Effect of KYNA on (±) AnTx-a-evoked [^{3}H]-DA release from striatal slices. CONTROL: 1 μM (±) AnTx-a (* $P<0.05$)

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49P L-NAME REDUCES FOS EXPRESSION IN TRIGEMINO-CERVICAL COMPLEX AFTER SUPERIOR SAGITTAL SINUS STIMULATION IN THE ANAESTHETISED CAT

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Previously, we have examined the transmission of nociceptive inputs of the trigeminal nerve within lamina I and II, of the C1 and C2 cervical spinal cord and the outer lamina of the trigeminal nucleus caudalis, by stimulating the superior sagittal sinus (SSS) and using Fos as a marker of neuronal activation (Kaube *et al.*, 1993). Furthermore we have examined the effect of various anti-migraine drugs on Fos expression following SSS stimulation and found Fos levels to be reduced (Hoskin & Goadsby, 1998). It is possible that inhibiting noxious transmission between primary trigeminal afferent fibres and 2nd order neurones in the spinal cord and trigeminal nucleus caudalis contributes to the therapeutic properties of these drugs. Given the positive clinical result in a recent study, treating migraine patients with a nitric oxide synthase (NOS) inhibitor L-N^{G}methylarginine hydrochloride (Lassen et al 1998), we set out to examine the role of nitric oxide in central activation following SSS stimulation using the NOS inhibitor L-NAME.

Cats (2.1-3.5kg) were anaesthetised with α-chloralose (60mg/kg i.p.), 1-2% halothane and i.v. doses of 20mg/kg of α-chloralose in dextrin. A circular midline craniotomy was performed to expose the SSS, which was then isolated by lateral and ventral incisions in the dura matter surrounding it. Cats were maintained for 23.5 hrs under anaesthesia, following which L-NAME was infused 100mg/kg i.v. over a 30min period. The SSS was then stimulated (150V, 250μs duration, 0.3 Hz) for 2 hrs and the cat maintained for a further 1hr before being perfused-fixed with 4% paraformaldehyde. The brain was removed and stored in 30% sucrose. 40μm sections were cut including the trigeminal nucleus caudalis and C1 and C2 spinal cord, and the sections processed for Fos immunoreactivity, which was visualised by diaminobenzidine (DAB) reactivity.

Fos immunoreactivity was observed to be significantly reduced in lamina I, II of C1 and C2 spinal cord and outer lamina of the trigeminal nucleus caudalis following L-NAME pre-treatment compared to values following stimulation of the SSS alone (see table 1). Pretreatment with L-NAME increased blood pressure from 85±3mmHg, to 131±17mmHg (n=3, mean ± SD).

Table 1

	TNC median25%75%	C1 median25%75%	C2 median25%75%
Stimulated n=7	65 51 76	59 4 83	39 29 80
L-NAME n=3	10* 4 17	15* 9 20	14* 5 23

Numbers of FOS positive cells (median and interquartile range) counted on the right hand side of 10 sections, values for lamina I/II of C1 and C2, outer lamina in TNC. * denotes significance $P<0.05$ using Mann-Whitney U-test.

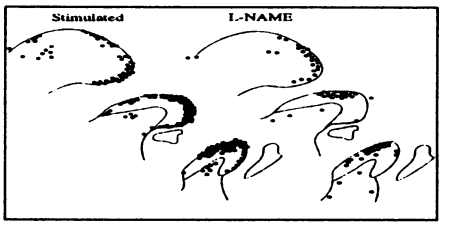

Figure 1: Fos positive cells (•) in the superficial laminae of the TNC (top), C1 (middle) and C2 (bottom) spinal cord of two representative cats following stimulation of the SSS (stimulated) and sinus stimulation preceded by *i.v.* administration of 100mg/kg L-NAME (L-NAME).

Results demonstrate L-NAME reduces FOS labelling following SSS stimulation in pain conducting outer laminae of the spinal cord and TNC, and hence nitric oxide appears to play a role in the central transmission of nociceptive trigeminal inputs. This may form part of the basis for the therapeutic actions of NOS inhibitors in migraine.

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50P THE SPINAL EFFECTS OF INTERLEUKIN-1β AND AN INTERLEUKIN-1 CONVERTING ENZYME INHIBITOR, SDZ-224-015, ON THE RESPONSES OF DORSAL HORN NEURONES IN THE ANAESTHETIZED RAT

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The aim of this study was to observe the contribution of interleukin-1β on the processing of somatosensory inputs at the spinal cord level. Rat recombinant interleukin-1β (rrIL-1β) and an interleukin-1 converting enzyme (ICE) inhibitor, SDZ 224-015 (Elford et al. 1995) were evaluated on the electrically evoked responses of dorsal horn wide-dynamic range (WDR) neurones in the anaesthetized rat. Male Sprague Dawley rats were anaesthetized with enflurane in a mixture of O_2/N_2O (33/66% respectively) and held in a stereotaxic frame (see Dickenson & Sullivan, 1987). A laminectomy was performed over the lumbar segments (L_1 - L_3) to expose the spinal cord. The cord was clamped rostral and caudal to the laminectomy for stability. Transcutaneous electrical stimulation, into the peripheral receptive field, was employed to evoke Aβ-, Aδ-, C-fibre, post-discharge and wind-up responses of the WDR-neurones. rrIL-1β (0.5-500pg/50μl) and SDZ-224-015 (0.05-10μg/50μl) were applied intrathecally onto the spinal cord, in a cumulative manner, in separate experiments. Each dose was followed for one hour. rrIL-1β (n=8) was made up in phosphate buffered saline (PBS) with 0.1% bovine serum albumin. Vehicle controls were done for each experiment. SDZ-224-015 was made up in saline (n=7). Results are expressed as percentage of control ± standard error of the mean. Statistical analysis was carried out using a Student's t-test, paired, 2 tailed, and significance set at $P=0.05$.

SDZ-224-015 had no effect on the Aβ-fibre evoked responses compared with controls. In contrast the Aδ- and C-fibre evoked responses were inhibited significantly by SDZ-224-015 (10μg) to 66±8 and 70±6% of control ± standard error of the mean, respectively ($P=0.05$). The enhanced neuronal responses of post-discharge and wind-up were reduced to 61±8 and 45±14% of control respectively ($P=0.05$) with the same dose of SDZ-224-015.

rrIL-1β application produced a dose related enhancement of Aδ-fibre evoked responses, post-discharge, wind-up and input responses. Thus the Aδ-fibre evoked responses were facilitated to 131±8 and 143±10 % of control with 50 and 500pg IL-1β respectively ($P=0.05$). Marked increases in post-discharge to 188±31 and 207±54% of control were observed following 50 and 500pg IL-1β respectively ($P=0.05$). Similarly, wind-up showed a facilitation to 137±17 and 176±17 % of control ($P=0.05$), and input responses were increased to 160±18 and 190±32% of control following 50 and 500pg IL-1β respectively ($P=0.05$ for 50μg only). In contrast, Aβ- and C-fibre evoked responses showed no significant change from controls.

This study demonstrates an involvement of interleukin-1 in the processing of noxious inputs in the dorsal horn of the spinal cord.

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51P MODULATION OF SYNAPTIC TRANSMISSION IN THE RAT SPINAL CORD *IN VITRO* BY 4030W92, A NOVEL ANTIHYPERALGESIC AGENT

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4030W92 (2,4-diamino-5 (2,3-dichlorophenyl) 6-fluoromethyl pyrimidine) is a new antihyperalgesic agent that is effective in animal models of neuropathic and chronic inflammatory pain (Clayton *et al.*, 1998; Collins *et al.*, 1998). We have recently demonstrated potent voltage- and use-dependent inhibition of Na^+ channels in nociceptive sensory neurones by 4030W92 and have surmised that such an action may underlie the *in vivo* properties of this agent (Trezise *et al.*, 1998). In order to further understand possible sites and mechanisms of action of 4030W92, we have studied in detail the effects of this compound on synaptic transmission in the rat spinal cord *in vitro*.

Electrophysiological techniques were used to record dorsal root-evoked ventral root potentials (DR-VRPs) from hemisected preparations of rat spinal cord. Preparations isolated from 7-12 day old rats (CD, either sex), were placed in a recording chamber and perfused with a modified Krebs solution (3-4ml min^{-1}, 20-25°C), gassed with 95% O_2/ 5% CO_2. Glass suction electrodes were used to record d.c. potentials from a lumbar ventral root (L4-L6) and to stimulate afferent input from the ipsilateral dorsal root of the same segment. Supramaximal intensity (500μs, 500μA) single shock stimulation every 60s elicited stable DR-VRPs with clear monosynaptic-, polysynaptic (A-fibre) and long lasting high threshold C-fibre components. The mean latencies and peak amplitudes were 8.8±0.7ms and 1.23±0.4mV for the monosynaptic peak and 23.6±2.2ms and 0.43±0.09mV for the polysynaptic peak, respectively (n=6). The C-fibre component, measured as the area of the long lasting depolarisation (up to 20s) was 911±345mV s (n=6).

4030W92 (0.3-100μM) produced concentration-dependent inhibition of the C-fibre and monosynaptic components of the DR-VRP, with geometric mean IC_{50} values [and 95% confidence limits] of 32μM [12-89μM] and 45μM [30-68μM], respectively. Depression of the polysynaptic component was only observed at high concentrations of 4030W92 (mean IC_{50} value 171μM [58-495μM], $P<0.05$ *vs.* C-fibre). Repetitive dorsal root stimulation (1Hz for 10s or 10Hz for 10s) produced summation of the long lasting VRP which can be considered an index of action potential 'wind up'. The control rates of rise (slope value) and areas of the cumulative VRP were 0.007±0.002mV s^{-1} and 2114±595mV s for 1Hz and 0.010±0.001mV s^{-1} and 2718±302mV s for 10Hz (n=4). 'Wind-up' was very sensitive to inhibition by 4030W92; at 30μM, 4030W92 reduced the rate of rise (1Hz) by 79±2% and the cumulative VRP area by 72±13% (n=4). This was significantly greater than the inhibition by 30μM 4030W92 of both the single shock C-fibre and monosynaptic components (43±15% and 34±8% inhibition, respectively, $P<0.05$). Interestingly, the inhibitory effects of 4030W92 (30μM) were even greater at the 10Hz stimulation frequency where 'wind up' was abolished (112±6%; n=4; $P<0.05$ *vs.* 1Hz). Inhibition of the cumulative VRP (10Hz) by 4030W92 was also observed in the presence of either strychnine (100μM) or bicuculline (20μM).

In summary, 4030W92 attenuates nociceptive neurotransmission and 'wind up' in the rat spinal cord, via a mechanism that is independent of glycine or $GABA_A$ receptor activation. The frequency-dependent inhibition of long lasting spinal nociceptive reflexes by 4030W92 is consistent with the voltage and use-dependent Na^+ channel blocking properties of this agent in sensory neurones. Further studies are in progress to determine the site of action of 4030W92 in the spinal cord and the relevance of these findings to antihyperalgesia.

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52P INFLUENCE OF GABAPENTIN ON C-*FOS* EXPRESSION IN RAT SPINAL CORD FOLLOWING INTRAPLANTAR FORMALIN INJECTION

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Gabapentin (Neurontin®), a novel anticonvulsant which binds to the α2δ subunit of voltage dependent calcium channels (Gee *et al.*, 1996), has recently been shown to be an effective agent in a number of animal pain models including the formalin test (Field *et al.*, 1997). The mechanism of action of gabapentin is unknown but is likely to be centrally mediated, possibly at the level of the spinal cord. Formalin injection into the hind paw results in the induction of the immediate early gene c-*fos* in the dorsal horn of the spinal cord (Abbadie *et al.*, 1997). We have examined whether the behavioral effects of gabapentin in the formalin test are reflected in changes in the level of fos expression in the spinal cord following formalin injection.

Male Sprague-Dawley rats (200-300 g) received a single injection of formalin (0.1mls, 5%, s.c.) into the plantar surface of the right hind paw. The behavioural response was scored as the number of paw licks over a 45 min period post-formalin. 75 mins later rats were deeply anaesthetised with sodium pentobarbitone (120 mg/kg i.p.) and perfused with 4% paraformaldehyde. Spinal cord (L3-5) was removed and frozen sections (14μm) processed for immunocytochemistry using an antibody to fos protein (Genosys). The number of cells expressing immunoreactivity (FLIR) was established by averaging the count on 4 sections every 300μm, 9 counts were added together to give a total number over 2.7 mm of cord. A minimum of 6 animals was used per dose group.

Injection of formalin into the paw produced the well described bi-phasic licking and biting response. Gabapentin (30-300 mg/kg s.c. 60 min pretreatment) had no significant effect on the early phase nociceptive response but dose dependently inhibited the late phase with a minimum effective dose of 100 mg/kg (64 % inhibition) (Table 1). In contrast morphine (10 mg/kg s.c. 20 min pretreatment) abolished both early and late phase responses. In saline treated animals intraplantar formalin induced FLIR in neurones in the dorsal horn of the ipsilateral spinal cord (Table 1). Gabapentin reduced the number of cells expressing FLIR in a dose dependent manner in superficial laminae but did not reach significance (37 % inhibition at 300 mg/kg). Gabapentin did however significantly reduce FLIR in deeper laminae with a minimum effective dose of 300 mg/kg (50 % inhibition). Morphine significantly decreased the FLIR in both superficial and deeper laminae (74 and 87 % respectively).

Table 1. Comparison between the behavioural response to formalin injection and the number of cells expressing FLIR in the spinal cord. *P<0.05, **P<0.01

	Duration of licks/ bites		FLIR cells in d. horn	
Dose (mg/kg)	Early phase	Late phase	Lm. I/II	Lm. III/IV/V
Saline	60 ± 13	305 ± 35	189 ± 25	153 ± 22
Gabapentin 30	73 ± 13	229 ± 32	172 ± 36	114 ± 23
Gabapentin 100	86 ± 19	110 ± 19**	139 ± 22	123 ± 21
Gabapentin 300	58 ± 5	35 ± 11**	121 ± 24	77 ± 15*
Morphine 10	2 ± 1**	0 ± 0**	49 ± 8**	20 ± 8**

The observation that gabapentin selectively blocks the second phase formalin response is consistent with previous studies (Field *et al.*, 1997). Gabapentin pretreatment resulted in a moderate decrease in the number of dorsal horn neurones expressing FLIR. It is interesting to note that this only reached significance in the deeper laminae. It is known that both the early and the late phases of the formalin response contribute to increased fos expression in dorsal horn neurones (Abbadie *et al.*, 1997) so that this apparently weak effect of gabapentin on fos expression may be obscured by the fos response to the uninhibited first phase.

In conclusion the selective inhibitory effect of gabapentin on the second phase of the formalin response in rats is associated with a moderate but significant decrease in the number of cells expressing fos in the spinal dorsal horn. The significance of this observation to the mechanism of action of gabapentin remains to be established.

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53P GABAPENTIN BINDING IN THE RAT SPINAL CORD: EFFECT OF RHIZOTOMY AND CAPSAICIN

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Gabapentin, a novel anticonvulsant, has recently been shown to be an effective antihyperalgesic agent. Its mechanism of action is unknown but it has been shown to bind to $\alpha_2\delta$ subunit of voltage dependent calcium channels (VDCCs) (Gee *et al.*, 1996). In the rat, low dose intrathecal gabapentin has been shown to reverse the thermal hyperalgesia induced by intraplantar carrageenan whereas the same dose administered intraplantar is ineffective, indicating a central site of action possibly at the level of the spinal cord (Field *et al.*, 1997). Such an effect could be mediated via a presynaptic action on primary sensory neurones or on (postsynaptic) sensory processing in the dorsal horn. Autoradiography studies have revealed a wide distribution of gabapentin binding sites in the central nervous system (Hill *et al.*, 1993) but the binding profile in the spinal cord has not been systematically studied. Here we describe the distribution of gabapentin binding in the spinal cord of normal rats, rats treated neonatally with the neurotoxin capsaicin, and rats subjected to unilateral dorsal rhizotomy.

Two day old rat pups (Sprague Dawley) were treated with capsaicin (50 mg/kg in 10% ethanol, 10% Tween 80 and 80% saline, subcutaneous) or vehicle. After 2 months animals were killed and the spinal cord removed. Frozen tissue was 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) and fixed tissue (4% paraformaldehyde (PFA)) from the same animal was processed for immunocytochemistry using antibodies raised to substance-P or CGRP (both were raised in rabbits and used at a dilution of 1:1000).

Unilateral dorsal rhizotomy, was performed on male Sprague Dawley rats anaesthetised with sodium pentobarbitone (40 mg/kg). A laminectomy was performed at lumbar 2 and 3. Two to three adjacent dorsal roots were sectioned close to the point of entry to the spinal cord and a short (4-5mm) length removed. 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 region of cord displaying evidence of rhizotomy was removed and frozen at -30°C. Frozen sections were processed for gabapentin autoradiography and post-fixed (4% PFA) for immunocytochemistry using antibodies to CGRP as previously described.

Gabapentin binding was present in the normal spinal cord with an uneven distribution. The most dense binding was seen in laminae I and II of the dorsal horn, extending medial and ventral into lamina X.

In rats treated neonatally with capsaicin, there was a significant decrease (25%, Mann Whitney $p<0.05$) in the intensity of the immunolocalisation of substance-P and CGRP consistent with the reduced number of small diameter primary afferent terminals in these animals. However, the distribution of gabapentin binding in capsaicin treated animals (nCi/mg:- 22.9 ± 2.9, n=8) was indistinguishable to that seen in vehicle controls (26.3 ± 3.2, n=8).

Spinal cords taken from rats following unilateral dorsal rhizotomy displayed the expected absence of cgrp-immunoreactivity in the dorsal horn. However, adjacent sections revealed a similar distribution of [^{3}H]-gabapentin binding in superficial lamina of both ipsi- (nCi/mg:- 27.0 ± 2.4, n=8) and contralateral (30.5 ± 3.0, n=8) dorsal horns.

The failure of either (neonatal) capsaicin or dorsal rhizotomy to significantly reduce gabapentin binding in the dorsal horn of the spinal cord suggests that very few of the binding sites are present presynaptically on sensory terminals. If the binding sites visualised in these studies correspond to the $\alpha_2\delta$ subunit, which is thought to be common to all VDCCs, these data seem somewhat surprising. However, it may be that gabapentin binds to $\alpha_2\delta$ subunits associated with a specific subtype of channel which is predominantly present on cell bodies and dendrites.

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54P DIFFERING EFFECTS OF PAROXETINE AND FLUOXETINE ON 5-HT RELEASE AND REUPTAKE IN THE RAT DORSAL RAPHE NUCLEUS

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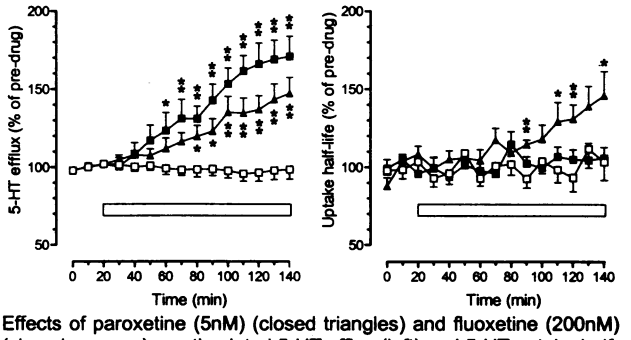
It is widely held that the antidepressant actions of selective serotonin reuptake inhibitors (SSRIs) such as paroxetine and fluoxetine are mediated by blockade of the 5-HT transporter (Feighner & Boyer, 1991). In this study we have used fast cyclic voltammetry (FCV) to compare the extent to which pharmacologically comparable concentrations of paroxetine and fluoxetine affect 5-HT efflux and reuptake in the dorsal raphé nucleus (DRN), a key midbrain 5-HT cell body region.

DRN slices (350μm thick) obtained from male Wistar rats (150-200g) were superfused with artificial cerebrospinal fluid at 32°C for 1h prior to stimulation and throughout the experiment. Slices were electrically stimulated (20 pulses, 0.1ms, 10mA, 100Hz) every 10min and real-time 5-HT efflux and reuptake were measured at carbon fibre microelectrodes using FCV (Stamford, 1990). Following 3 stable release events, paroxetine (5nM) or fluoxetine (200nM) were administered via the superfusate for a period of 2h. Each drug was used at a concentration 100 times greater than its K_i value for rat cortex 5-HT transporter, but which was still selective for 5-HT uptake (Owens *et al.*, 1997).

Paroxetine increased stimulated 5-HT efflux to 147 ± 10% of pre-drug values at maximum (mean ± s.e.mean, n=5) and significantly lengthened the half-life of reuptake to 146 ± 16% (Figure 1). In contrast, fluoxetine increased 5-HT efflux to 171 ± 13% (n=5) but had no effect on uptake.

Figure 1.



Effects of paroxetine (5nM) (closed triangles) and fluoxetine (200nM) (closed squares) on stimulated 5-HT efflux (left) and 5-HT uptake half-life (right) in the DRN. Drugs were applied during the period indicated by the horizontal bar. *$P<0.05$, **$P<0.01$ (one-way ANOVA with Dunnett's post hoc test) vs vehicle control (open squares). Values are means ± s.e.mean (n=4-7).

In conclusion, paroxetine was able to increase both 5-HT efflux and uptake half-life, whilst fluoxetine seemingly elevated 5-HT efflux without affecting reuptake. These data suggest that, whilst the effect of paroxetine on 5-HT efflux is wholly explicable by uptake blockade, fluoxetine increases extracellular levels of 5-HT in the DRN via mechanism(s) which are independent of its ability to block the 5-HT transporter.

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55P CO-LOCALISATION OF 5-HT$_7$ RECEPTOR mRNA WITH GAD$_{67}$-LIKE IMMUNOREACTIVITY IN RAT BRAIN NEURONES

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The 5-HT$_7$ receptor has been implicated in the control of circadian rhythms since 5-HT$_7$-like ligands cause a phase shift in the spontaneous neuronal activity of the rat suprachiasmatic nucleus (SCN) within the hypothalamus (Lovenberg et al., 1993) possibly via GABA neurones (Kawahara et al., 1994). *In situ* hybridisation and receptor autoradiographic studies have demonstrated that the 5-HT$_7$ receptor is expressed differentially in rat brain (Gustafson et al., 1996; Ruat et al., 1993). However, neither the 5-HT$_7$ receptor nor mRNA have been localised to the SCN (Lovenberg et al., 1993; Gustafson et al., 1996).This study performed *in situ* hybridisation in rat brain to detect 5-HT$_7$ receptor mRNA with cellular resolution and combined this with immunohistochemistry to phenotype the neurones expressing 5-HT$_7$ mRNAwith particular focus on the SCN.

Rat whole brains from male Wistar rats (250-300g) were frozen in isopentane at -40°C for 5 mins and stored at -80°C until sectioning. Cryostat sections (12 µm) were cut at -15 to -20°C and mounted onto microscope slides. Sections were fixed with 4% paraformaldehyde in ice-cold phosphate buffered saline (PBS) pH 7.2 for 5 min, washed in fresh PBS, dehydrated through a series of alcohols and stored in 95% ethanol at 4°C until hybridisation. Sections were removed from alcohol, allowed to dry and hybridised with an [^{35}S]-labelled antisense 5-HT$_7$ receptor oligonucleotide probe (45-mer; Lovenberg et al., 1993) overnight at 42°C. Sections were then washed, dehydrated and exposed to photographic emulsion. Following *in situ* hybridisation, sections were processed for immunohistochemistry using an antiserum to glutamate decarboxylase 67 (GAD$_{67}$; Kaufman et al., 1986). After incubating (20 hours at 4°C) with the primary antibody, sections were processed with an ABC kit (Vector laboratories). Sections were subsequently counterstained with haematoxylin, dehydrated, cleared and mounted.

5-HT$_7$ mRNA was discretely localised in rat brain (n=6). Analysis of emulsion dipped slides revealed silver grains over cells which displayed a neuronal morphology in many brain regions including the cingulate cortex, retrosplenic granular cortex, piriform cortex, CA3 region of the hippocampus, septum, red nucleus, thalamus, hypothalamus (including SCN) and the amygdala particularly cortical, medial, and central nuclei. Areas with weak hybridisation signals included layers 1-3 of the cortex, CA2 of the hippocampus, striatum and cerebellum. The non-specific hybridisation signal, assessed with radiolabelled sense probe or radiolabelled antisense probe in the presence of 100-fold excess of unlabelled probe was uniformly distributed, just above background.

Double labelling studies demonstrated the co-localisation of 5-HT$_7$ receptor mRNA with GAD$_{67}$-like immunoreactivity in individual neurones throughout many regions of the rat brain including cortex, hippocampus, thalamus, hypothalamus, septum and amygdala (n=6). In addition, 5-HT$_7$ receptor mRNA and GAD$_{67}$-like immunoreactivity were also co-localised in approximately 80% of the 5-HT$_7$ mRNA expressing neurones of the SCN.

These findings suggest that 5-HT$_7$ receptor expression is evident in some GABA neurones in rat brain. Furthermore, the demonstration of 5-HT$_7$ mRNA localisation within the SCN and co-localisation with GAD$_{67}$-like immunoreactivity concurs with electrophysiological studies (Lovenberg et al., 1993; Kawahara et al., 1994).

R.L.Stowe is recipient of a Wellcome Trust Prize Studentship.

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56P 5-HT$_{1A}$ RECEPTOR BINDING *IN VIVO* USING [^{3}H]-MPPF, A SELECTIVE 5-HT$_{1A}$ RECEPTOR LIGAND

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4-(2'-methoxy-phenyl)-1-[2'-(N-2"-pyridyl)-*p*-fluorobenzamido]-ethyl-piperazine) (*p*-MPPF) has recently been described as a selective, high affinity 5-HT$_{1A}$ receptor antagonist (Kung *et al.*, 1996) which, as a [^{11}C] radiolabel may be of value in visualising central 5-HT$_{1A}$ receptors in man using positron emission tomography (Plenevaux *et al.*, 1997). The aim of the present study was to determine if [^{3}H]-MPPF could be used to estimate 5-HT$_{1A}$ receptor binding in the mouse brain *in vivo*.

Male Swiss Webster mice (28-30g) were pre-treated with either vehicle (10 ml/kg, s.c.) or test compound: 8-OH-DPAT (0.05-5 mg/kg, s.c.), NAN 190 (1-(2-methoxyphenyl)-4-[4-(2-[Phthalimido) butyl]piperazine) (0.03-3 mg/kg, i.p.), (-)pindolol (0.1-10 mg/kg, s.c.), WAY 100635 (4-(2'-[N-22-pyridinyl)cyclohexanecarbox amido[ethyl] piperazine)) (0.003-1 mg/kg, s.c.), fluoxetine (10 mg/kg, i.p.), paroxetine (3 mg/kg, i.p.), idazoxan (1-20 mg/kg, i.p.), SCH 23390 (R (+) - 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro 1H-3-benzazepine hydrochloride) (0.3 mg/kg, s.c.), haloperidol (0.3 mg/kg, s.c.), or prazosin (3 mg/kg, s.c.) 30 min before intravenously administering [^{3}H]-MPPF (Amersham, UK 79.9 Ci/mmol), (4 µCi in 100 µl/mouse). Ten min later mice were humanely killed, the cortex removed and homogenised in 40 vol of 50mM Tris/HCl (pH 7.4 @ 4°C). Aliquots (500 µl) of homogenates were counted or filtered through Whatman GF/B filters to determine the total and bound fractions respectively. Non-specific binding was determined using WAY 100635 (1 mg/kg, s.c.). Specific binding represented approximately 80% of total binding.

In vivo, 8-OH-DPAT, WAY 100635, (-)pindolol, NAN 190 and idazoxan dose-dependently inhibited [^{3}H]-MPPF binding in mouse cortex (Table 1). No effect on binding was observed with mice pre-treated with fluoxetine, paroxetine, SCH 23390, haloperidol or prazosin.

Table 1. *In vivo* binding of [^{3}H]-MPPF in mouse cortex

Compound (route)	ED $_{50}$ (mg/kg)
WAY100635 (s.c.)	0.02 (0.02 - 0.03)
8-OH-DPAT (s.c.)	0.55 (0.51 - 0.61)
(-)pindolol (s.c.)	0.88 (0.79 - 0.97)
NAN 190 (i.p.)	0.15 (0.11 - 0.18)
Idazoxan (i.p.)	11.75 (11.5 - 12. 0)

ED$_{50}$ = dose (mg/kg) giving 50% of the maximal inhibition of [^{3}H]-MPPF binding and are geometric means for 3 separate experiments with 3 mice per dose. Values in parenthesis are high and low errors of the mean.

Results indicate that [^{3}H]-MPPF can be used to determine 5-HT$_{1A}$ receptor occupancy *in vivo*. Interestingly, the α_2-adrenoceptor antagonist, idazoxan weakly displaced [^{3}H]-MPPF from mouse cortex which is consistent with its binding affinity at the 5-HT$_{1A}$ receptor, Ki = 63 nM (Kawai *et al.*, 1994)

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57P 5-HT_{1A} RECEPTOR ANTAGONISM OR UPTAKE INHIBITION ENHANCE THE EFFECTS OF 5-$HT_{1B/1D}$ AUTORECEPTOR BLOCKADE ON EXTRACELLULAR 5-HT IN GUINEA-PIG FOREBRAIN

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There is evidence to suggest that both 5-HT_{1B} and 5-HT_{1D} receptors are terminal autoreceptors (Hoyer and Middlemiss, 1988; Davidson and Stamford, 1996), while somatodendritic autoreceptors encompass 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor subtypes (Starkey and Skingle, 1994; Davidson and Stamford, 1995). Potentially all 3 autoreceptor subtypes are important in modulation of 5-HT extracellular levels but literature reports infer that blockade of only one autoreceptor does not result in a maximum increase in 5-HT. Therefore, the aim of this study was to investigate the effect of combinations of selective 5-HT_{1B} and mixed 5-$HT_{1B/1D}$ receptor antagonism with 5-HT_{1A} receptor antagonism and 5-HT uptake site blockade, in both the frontal cortex and dentate gyrus. This was investigated using the technique of *in vivo* microdialysis in the freely-moving guinea-pig.

Male guinea-pigs (300-400g) were anaesthetised with isoflurane and dialysis probes implanted in the frontal cortex (AP+4.5mm; ML±2mm, V-3mm) or dentate gyrus (AP-4.1mm, ML±2.1mm, V-4.5mm from Bregma). Animals were allowed to recover overnight before probes were perfused with a.CSF at 2µl/min. After 2 hours perfusion, samples were collected every 20 min into 10µl of a.CSF. 5-HT was separated by HPLC and detected by ECD (electrode potential of 0.65V).

Basal levels of extracellular 5-HT were 16±3 (n=16) and 9±2 (n=11) fmol/sample in the frontal cortex and dentate gyrus respectively. In the frontal cortex a 5-HT_{1A} (WAY 100635, 1mg/kg i.p.), 5-HT_{1B} (SB-224289, 4mg/kg i.p.) and a mixed 5-$HT_{1B/1D}$ (GR 127935, 0.3mg/kg i.p.) receptor antagonist were unable to increase extracellular levels of 5-HT. However, paroxetine (10µM) significantly increased ($p<0.05$) extracellular 5-HT to 353±73% of basal (n=6) when perfused down the probe. In contrast, in the dentate gyrus while 5-HT_{1A} receptor antagonism had no effect, both 5-HT_{1B} and mixed 5-$HT_{1B/1D}$ receptor blockade significantly increased 5-HT levels ($p<0.05$) reaching 151±19% (n=4) and 146±11% (n=5) of basal respectively.

In the frontal cortex, co-administration of WAY 100635 and SB-224289 had no effect on extracellular 5-HT. In contrast, co-administration of WAY 100635 and GR 127935 or paroxetine and SB-224289 significantly increased ($p<0.05$) extracellular 5-HT to 285±103% (n=7) and 629±151% (n=5) of basal respectively, potentiating the effect of compounds alone. In the dentate gyrus, co-administration of WAY 100635 with both SB-224289 and GR 127935 potentiated the effect of the compounds alone, significantly increasing extracellular 5-HT levels to 151±19 (n=4) and 255±61 (n=5) of basal respectively.

These data demonstrate that to increase extracellular 5-HT in the frontal cortex, concurrent 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor blockade is required; this effect is enhanced with paroxetine. In the dentate gyrus, 5-HT_{1B} receptor blockade alone increases extracellular 5-HT, although this effect was enhanced with 5-HT_{1A} receptor blockade.

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58P 5-HT RECEPTOR AGONIST-ANTAGONIST BINDING AFFINITY RATIOS AS A MEASURE OF INTRINSIC ACTIVITY, USING [^{3}H]8-OH-DPAT AND [^{3}H]MPPF AS RADIOLIGANDS

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G-protein coupled receptors can exist in alternative affinity states. These states have high and low affinity for agonists but both states can display similar affinity for antagonists. The difference in affinity exhibited by a compound between these states can give an indication of the compounds intrinsic activity (Freedman et al. 1988). [^{3}H]8-OH-DPAT(DPAT) has been used extensively as an agonist radioligand for 5-HT_{1A} receptors (Sijbesma et al (1991)). Recently [^{3}H]MPPF has been used as an antagonist radioligand for these receptors (Kung et al. (1996)). We have compared compound inhibition of these two ligands with compound efficacy.

Human 5-HT_{1A} receptors were stably expressed in HEK293 cells. Cells were homogenised and the membranes washed twice in Tris buffer (50mM Tris, 10mM $MgCl_2$, pH 7.4). Rat cerebral cortex was excised, homogenised in Tris buffer and membranes incubated at 37°C for 15 mins, prior to washing twice. Membranes (from $5x10^5$ cells or 10mgs wet weight tissue/incubation well) were incubated in Tris buffer containing [^{3}H]DPAT (2nM) or [^{3}H]MPPF (0.3nM), with/without compounds, at 37°C for 45 min. Incubations were terminated by filtration over GF/B filters. Non-specific binding was defined by buspirone (10µM). [^{35}S]GTPγS binding was performed as described previously (Watson et al 1996). Intrinsic activity was calculated as % stimulation of basal [^{35}S]GTPγS binding and expressed as a fraction of the maximal 5-HT response.

In saturation binding studies in the cell line, [^{3}H]MPPF labelled over 3 times as many sites as [^{3}H]DPAT (Bmax = 8.67 and 2.34pmoles /mg protein, respectively) but a similar number of sites in rat cortex (0.36 and 0.34 pmoles/mg protein, respectively).

In the recombinant system, agonists displayed greater potency in inhibiting [^{3}H]DPAT than [^{3}H]MPPF and in the latter, inhibition curves were biphasic (see Table 1). Antagonists displayed monophasic inhibition curves with either radioligand. In rat cortex, both agonists and antagonists gave monophasic inhibition curves with either radioligand.

The ratio of compound affinity (pKi) at the agonist high affinity state ([^{3}H]DPAT) with the agonist low affinity state ([^{3}H]MPPF) paralleled compound intrinsic activity measured by [^{35}S]GTPγS binding.

Table 1. Compound inhibition of 5-HT_{1A} receptor binding.

	[^{3}H]MPPF			[^{3}H]DPAT		HEK293	
	HEK 293		Rat Cx	HEK 293	Rat Cx	Ag:Ant Ratio	IA
5-HT	8.12	6.52#	8.31	8.17*	8.30	44.7	1.0
DPAT	8.43	7.11#	8.44	8.51*	8.53	25.1	1.0
Pindolol	7.08#		7.46	7.43*	7.34	2.2	0.4
WAY100635	9.07#		9.29	8.87*	8.91	0.6	0

Data = mean pKi, n = 3; IA = intrinsic activity in [^{35}S]GTPγS
Ag:Ant = pKi ([^{3}H]DPAT)*:pKi([^{3}H]MPPF)#

In conclusion, [^{3}H]MPPF labels both high and low agonist affinity states of the 5-HT_{1A} receptor and where both states exist, agonist:antagonist ratios are a useful measure of intrinsic activity. In rat cortex only the agonist high affinity state of the 5-HT_{1A} receptor was observed. This may be accounted for by differences in the level of receptor expression.

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59P IS KOJIC AMINE A $GABA_B$ RECEPTOR AGONIST IN THE RAT ISOLATED ANOCOCCYGEUS?

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Based on structure and activity both *in vivo* (e.g. Martin & Bendesky, 1981) and *in vitro* (e.g.Yarbrough et al., 1979) Kojic amine has been proposed to be a GABA mimetic. Only a few studies, however, have studied the effects of GABA receptor antagonists on the response to kojic amine. The rat isolated anococcygeus possesses only $GABA_B$ receptors, which are located on sympathetic nerve terminals (Muhyaddin et al., 1983). In this study we have compared the agonist activity of Kojic amine with the known $GABA_B$ agonists - baclofen, SKF97541 (see Bowery, 1993) and CGP47656 (see Knight and Bowery, 1996) on electrically-stimulated contractions of the rat anococcygeus and have also studied the effects of the $GABA_B$ receptor antagonists 2-OH saclofen and CGP55845 (see Bowery 1997) on these responses.

Rats (male CD, 250-300g) were killed by cervical dislocation, the anococcygeus dissected and mounted under 1g tension in tissue baths containing Krebs' buffer (mM: NaCl 118.0; KCl 1.4; $MgSO_4$ 1.2; KH_2PO_4 1.17; $NaHCO_3$ 25.0; glucose 11.0; $CaCl_2$ 2.0) aerated with 95%O_2/5%CO_2 and maintained at 37°C. Twitches were evoked by 1 second trains of pulses (10 Hz, 0.5 msec pulse width) every 10 sec at supramaximal voltage and were recorded isometrically. A partial serial concentration-effect curve to baclofen (1-10 μM) was constructed on all tissues. Following washout, tissues were incubated for 15 min with either antagonist or vehicle and responses to ligands studied. Agonist responses were expressed as a percentage of the 10 μM baclofen response and E_{max} and pEC_{50} values determined. Antagonist affinity (pK_B±SEM) was determined from the Gaddum equation (pK_B=log(CR-1)-log[B]).

Kojic amine and the $GABA_B$ receptor agonists evoked monophasic concentration-dependent inhibition of the twitch response with a rank order of potency of SKF97541>baclofen=CGP47656>Kojic amine.

The two antagonists inhibited the activity of the $GABA_B$ agonists but had no effect on the response to Kojic amine. Results are in Table 1.

Table 1 Agonist and antagonist effects in rat isolated anococcygeus (mean ± SEM, n=3-18).

Agonist	pEC_{50}	E_{max}	pK_B CGP55845	pK_B 2-OH saclofen
Baclofen	5.61±0.03	146±5	8.29±0.05	4.78±0.17
SKF97541	6.19±0.07	176±12	8.26±0.07	4.37±0.19
CGP47656	5.56±0.07	76±4	8.09±0.08	4.37±0.10
Kojic amine	4.49±0.10	>161±65	<6.0	<4.0

Results with SKF97541, baclofen and CGP47656 coupled with effects of the antagonists confirm that the biological effects seen are mediated via activation of $GABA_B$ receptors. The resistance of Kojic amine, however, to block by the two antagonists suggests that it may not be acting at $GABA_B$ receptors in this preparation. This suggests that Kojic amine is inhibiting twitch either by a mechanism unrelated to $GABA_B$ receptor activation, or alternatively is acting at a site on the receptor different to that recognised by the agonists and antagonists used in this study.

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60P CHARACTERISATION OF THE 5-HT RECEPTOR MEDIATING THE CONTRACTION RESPONSE TO DOI IN THE PROXIMAL REGION OF THE *SUNCUS MURINUS* INTESTINE

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5-Hydroxytryptamine 5-HT_2 receptors mediate a 5-HT induced contraction response in different regions of the *Suncus murinus* intestine (Javid *et al.*, 1996, 1997). This study investigated the 5-HT receptors mediating the contraction response to the 5-HT2 receptor agonist DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, Hoyer *et al.*, 1994) in the proximal region of the *Suncus murinus* intestine.

Segments (1 cm length) taken from the intestine (1 cm distal to the pyloric sphincter) of adult Japanese House Musk shrew, *Suncus murinus* (30-68 g) of either sex were mounted in 10 ml organ baths containing Krebs' solution (37°C, 95%O2, 5%CO2). The tissues were allowed to equilibrate for 60 min and washed every 20 min. The contractions were recorded isometrically with a resting tension of 0.5 g . Non-cumulative concentration-response curves to DOI (0.003-30μM) were established with a 1 min contact time and 22 min intervals. The procedure was repeated in the presence of SB206553 (SB, 0.01, 0.1, 1μM), methysergide (methy, 1μM), ketanserin (ket, 1μM), a combination of ondansetron (ond, 1μM) plus SB204070 (KB, 1nM) and a combination of methysergide plus SB206553 (1μM). The control responses to DOI alone and in the presence of antagonist were established using a randomised experimental design. Tension changes were expressed as the mean+s.e.mean of absolute values and as a percentage of KCl-induced contraction (0.12mM) of n=6 and analysed using one-way ANOVA followed by Bonferroni-Dunnett's t-test.

DOI (0.003-10μM) produced a bell-shaped concentration-dependent contraction curve. Methysergide (5-HT1/2 receptor antagonist) but not ketanserin (5-HT2A receptor antagonist) or a combination of ondansetron (5-HT3 receptor antagonist) plus SB204070 (5-HT4 receptor antagonist, Wardle *et al.*, 1994) significantly ($p<0.05$) reduced or abolished the responses to DOI. In the presence of SB206553, a selective 5-HT2C receptor antagonist (Forbes *et al.*, 1996), the responses to DOI (1-30μM) were significantly ($p<0.05$) greater than the control responses to DOI; the contraction curve was shifted to the right by a combination of methysergide plus SB206553 (Figure 1).

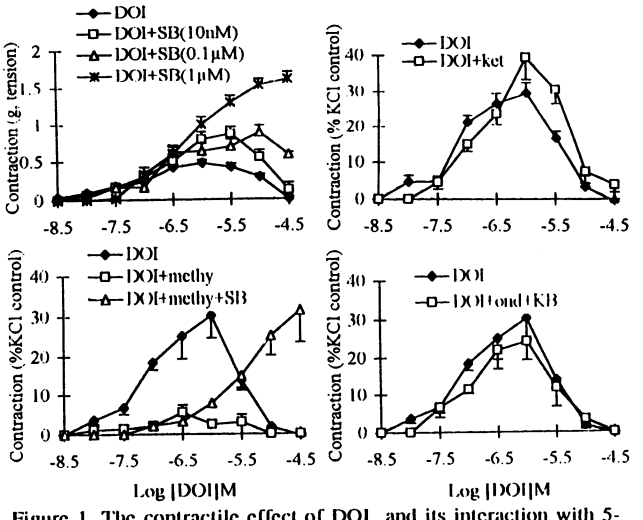

Figure 1. The contractile effect of DOI and its interaction with 5-HT receptor antagonists in the proximal region of *Suncus murinus* intestine.

The data indicate that the contractile effects of DOI are mediated via 5-HT_1 receptors whilst HT_{2C} receptors are involved in mediating the decreasing contractions observed at higher concentrations of DOI. 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptors are not involved.

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61P INHIBITORY EFFECTS OF SUMATRIPTAN AND ZOLMITRIPTAN IN THE RAT ELECTRICALLY STIMULATED VAS DEFERENS

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The presence of inhibitory 5-HT_{1B} receptors in the epididymal portion of the rat isolated vas deferens was reported by Borton & Docherty (1990). We have used the 5-$HT_{1B/D}$ receptor agonist, anti-migraine drugs, sumatriptan and zolmitriptan (311C90; Martin *et al.*, 1997) and the 5-$HT_{1B/D}$ receptor antagonist GR 127935 (GR, Skingle *et al.*, 1993) to further characterise this receptor mechanism.

Male, Wistar rats (200-250g) were killed by stunning and cervical dislocation. The vasa deferentia were removed and divided into two equal portions (prostatic and epididymal). The tissues were mounted between platinum ring electrodes in 10 ml tissue baths in Krebs' solution (2.5 mM Ca^{2+}), gassed with a 95% O_2/5% CO_2 mixture, at 35.5°C and stimulated at 0.05 Hz, 1 msec pulse width at 100 mA. Tissue responses were recorded isometrically. Cumulative concentration-response curves (CRCs) to sumatriptan (0.3-200 μM) and zolmitriptan (30 nM-10 μM) were constructed with 4 min contact time at each concentration. Two CRCs were obtained in each tissue with a 1h recovery period between curves. Those tissues exposed to GR (10 nM) were equilibrated for 40 min with the antagonist. CRCs were analysed using the Allfit programme. Mean values ± s.e. mean are reported.

In preparations of the epididymal portion of the rat vas deferens electrical stimulation evoked an increase in tension of 0.186 ± 0.025 g (n=10). Responses of the epididymal portion of the vas deferens to electrical stimulation were reduced in the presence of sumatriptan and zolmitriptan with pIC_{50} values of 5.4±0.08 (n=10) and 6.4±0.08 (n=6) and maximum inhibition of 87.3±3.6 and 72.9±6.0% respectively. In preparations equilibrated with GR (10 nM) CRCs to sumatriptan were displaced to the right with an apparent pA_2 value for GR of 8.6±0.09 (n=3). The mean maximum inhibition evoked by sumatriptan was 91.3±5.8% before and 69.7±8.3% after equilibration with GR. In control preparations not equilibrated with antagonist the apparent geometric mean concentration-ratio for two repeated sumatriptan CRCs was 1.56 (n=3). The results from antagonist treated tissues were not corrected for this small change. In preparations from the prostatic portion of the vas deferens sumatriptan evoked a concentration-related inhibition of responses to electrical stimulation with a pIC_{50} value of 5.3±0.06 and a maximum inhibition of only 21.0±2.4% (n=4). The CRC for inhibition of the prostatic portion of the vas deferens by zolmitriptan was bell-shaped with a maximum of 11.0±1.8% (n=4) inhibition at 3 μM.

The potencies of sumatriptan and zolmitriptan in inhibiting responses of the rat epididymal vas deferens and the antagonist potency of GR are consistent with an interaction at 5-$HT_{1B/D}$ receptors (Martin *et al.*, 1997; Razzaque *et al.*, 1995). The apparent pA_2 value for GR suggests a 5-$HT_{1D\alpha}$ (5-HT_{1D}) rather than 5-$HT_{1D\beta}$ (5-HT_{1B})-receptor mechanism (Skingle *et al.*, 1993). The inhibitory mechanism was much less evident in the prostatic portion of the rat vas deferens. The bell-shaped nature of the CRC to zolmitriptan in the prostatic region of the vas deferens requires further study.

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62P COMPARISON OF MUSCARINIC RECEPTOR SUBTYPES IN THE SHEEP CILIARY MUSCLE AND IRIS SPHINCTER USING A RADIOLIGAND BINDING APPROACH

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Anti-muscarinic agents are employed in ophthalmic practice to produce mydriasis and cycloplegia, by acting upon the iris sphincter (IS) and ciliary muscle (CM) respectively. These drugs often produce both effects simultaneously (albeit in varying degrees) since both tissues are believed to contain the same muscarinic receptor subtype (M_3: Gabelt & Kaufman, 1994; German *et al*, 1997a). However, in light of possible M_3 receptor heterogeneity (Eglen & Watson, 1996), the current study compared the affinities of some muscarinic antagonists for receptors in the sheep IS and CM under identical conditions using a radioligand binding technique adapted for intra-ocular muscles (German *et al*, 1997b). The binding characteristics of several anti-muscarinic agents used clinically in the eye were also examined.

ISs and CMs were dissected from adult female sheep (≤6 hours post mortem) and soaked in 10mM chloroquine + 10mM EDTA + 10mM benzamidine at 4°C for 16 hours, to reduce non-specific binding (NSB) levels and stabilize receptors. Membrane preparation and binding procedures were carried out as described by German *et al* (1997a), approximately 0.32 mg/ml protein being incubated for 45 mins with ^{3}H-quinuclidinyl benzilate (QNB, 0.5nM, 47Ci/mmol), Hepes-Krebs' buffer and varying concentrations of competing drug (10^{-9}-10^{-4}M). The drugs used are shown in Table 1 (HHSiD: hexahydrosiladifenidol, pfHHSiD: p-fluoro analogue of HHSiD, AFDX-116: 11-({2-[(diethylamino)-methyl]-1-piperidyl}acetyl)-5,11-dihydro-6H-pyridol[2,3-b][1,4]benzodiazepine-6-one, 4-DAMP: 4-diphenylacetoxy-N-methyl-piperidine methiodide, AQ-RA 741: 11-({4-[4-(diethylamino)-butyl]-1-piperidinyl}acetyl)-5,11-dihydro-6H-pyridol[2,3-b][1,4]benzodiazepine-6-one, UH-AH 37: 6-chloro-5,10-dihydro-f{[1-methyl-4-piperidinyl]acetyl)-11H-dibenzo-(b,e)(1,4)diazepine-11-one chloride). Following rapid filtration, the bound radioactivity was counted and competition curves analysed to yield affinity estimates (mean pK_i ± s.e.m., n=4) for all compounds.

Competition between the drugs and ^{3}H-QNB for muscarinic binding sites is given in Table 1. No significant difference in the pK_i value between the IS and CM was found with most compounds, the correlation between the affinities for IS and CM tissue being high ($r=0.90$, $p<0.05$) and the rank order of potencies being similar and generally characteristic of the M_3 receptor. However, a significantly greater affinity for the IS compared with the CM was observed with zamifenacin, UH-AH 37 and tropicamide ($p<0.05$, t-test).

Table 1. Affinity estimates of muscarinic antagonists for receptors in sheep IS and CM tissue (mean ± s.e.m, n=4).

	IS pK_i	CM pK_i	Ki_{IS}/Ki_{CM}
atropine	7.31 ± 0.03	7.35 ± 0.04	1.11 ± 0.14
hyoscine	7.07 ± 0.17	6.97 ± 0.05	1.17 ± 0.61
4-DAMP	6.79 ± 0.02	6.74 ± 0.04	0.90 ± 0.05
zamifenacin	6.52 ± 0.08	5.59 ± 0.17	0.16 ± 0.08
cyclopentolate	6.41 ± 0.20	6.48 ± 0.05	1.38 ± 0.43
telenzepine	6.39 ± 0.04	6.36 ± 0.10	1.02 ± 0.20
AQ-RA 741	6.17 ± 0.10	6.16 ± 0.08	1.18 ± 0.36
UH-AH 37	6.00 ± 0.05	5.22 ± 0.07	0.18 ± 0.04
HHSiD	5.72 ± 0.05	5.76 ± 0.13	1.37 ± 0.54
homatropine	5.69 ± 0.10	5.88 ± 0.03	1.65 ± 0.33
tropicamide	5.51 ± 0.09	4.72 ± 0.11	0.17 ± 0.03
pfHHSiD	5.41 ± 0.12	5.28 ± 0.03	0.83 ± 0.19
pirenzepine	5.11 ± 0.05	5.08 ± 0.11	1.10 ± 0.34
AFDX-116	4.93 ± 0.11	4.70 ± 0.03	0.65 ± 0.14
methoctramine	4.67 ± 0.03	4.57 ± 0.05	0.81 ± 0.06
gallamine	4.37 ± 0.01	4.35 ± 0.02	0.95 ± 0.06

The results indicate that, although the muscarinic receptor subtype in both the IS and CM is likely to be the same (M_3 subtype), the compounds zamifenacin, UH-AH 37 and tropicamide are capable of selectively targeting the IS to some extent. The potential clinical implications are considerable; it should be possible to achieve adequate pupillary dilation with a minimal influence upon accommodation, an outcome currently achievable in a clinical setting using tropicamide alone.

Drugs were donated by Chauvin (tropicamide), Martindale (homatropine), Boehringer Ingelheim (cyclopentolate, UH-AH 37, AQ-RA 741), Pfizer (zamifenacin) and R.B.Barlow (AFDX-116, 4-DAMP).

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63P THE EFFECTS OF CHRONIC ADMINISTRATION OF THE $5HT_{1A}$ AGONIST GEPIRONE ON FOOD INTAKE IN FOOD-DEPRIVED RATS

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$5HT_{1A}$ receptors agonists, such as 8-hydroxy-2-(di-N-propylamino)-tetralin and gepirone, have previously been shown to increase food intake in non-deprived rats by an action at $5HT_{1A}$ autoreceptors (see Ebenezer, 1992a). However, chronic administration of these drugs lead to desensitization of the $5HT_{1A}$ autoreceptors and loss of the hyperphagic response (Kennett et al., 1987). By contrast, it has been demonstrated that acute administration of these agents decrease feeding in food deprived rats (Ebenezer, 1992b; Ebenezer and Tite, 1997). The mechanism for this effect is not known, but we reasoned that if $5HT_{1A}$ autoreceptors were involved, 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=8, b. wt. 350 - 400g) were deprived of food for 22h a day and trained to press a lever in an operant chamber for food pellets on a fixed ratio of 5. The rats were assigned to 2 equal groups and each animal received one s.c. injection of either saline (Group 1) or gepirone (2 mg kg^{-1}; Group 2) each day over a 10 day period. The rats were placed separately in the operant chamber for 60 min immediately after their 1st, 3rd, 8th and 10th injection of saline or gepirone and operant food intake was measured during this period, as described previously (Ebenezer, 1992a). After a 4 week dry-out period, the procedure described above was reversed, with rats in Group 1 receiving gepirone and those in Group 2 receiving saline.

The results are shown in Table 1. The main hypophagic effect of gepirone was observed during the 1st 30 min after administration. Analysis of the data at 30 min indicated significant main effects of treatment ($F_{(1,7)}$=32.8465, P<0.01), measurement day ($F_{(3,21)}$=3.5976, P<0.05) and treatment x measurement day interaction ($F_{(3,21)}$ =11.6968, P<0.01). The latter observation suggests the development of tolerance to the depressant effect of gepirone on food intake. However, further statistical analysis indicated that, while there was a significant attenuation of the hypophagic effect of gepirone between the 1st and 3rd injection, there was no significant changes in the hypophagic responses after the 3rd, 8th and 10th injection. This suggests that while there is some attenuation of the inhibitory effect of gepirone on food intake in hungry rats after their 1st experience of the drug, further tolerance does not occur with chronic treatment. These observations tentatively suggest that the hypophagic effect of gepirone on food intake in food deprived rats may not be due solely to stimulation of $5HT_{1A}$ autoreceptors.

Table 1. Effects of chronic administration of gepirone on operant food intake. n = 8 rats Dunnett's t-test, saline vs gepirone: **P<0.01, *P<0.01

	Food Reinforcements ± s.e.m.			
	0-30 min		0-60 min	
Day	Saline	Gepirone	Saline	Gepirone
1	137.4±9.0	13.5±2.9**	192.5±14.7	92.1±8.9**
3	120.4±10.1	59.4±13.9**	168.9±15.6	170.1±17.4
8	138.3±14.7	64.0±11.2**	189.8±16.8	166.9±16.8
10	127.9±15.6	87.4 ±16.6*	166.4±20.0	190.6±23.4

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64P EFFECTS OF THE HISTAMINE-3 (H-3) RECEPTOR AGONIST GT2016 ON FOOD INTAKE AND CORTISOL SECRETION IN PIGS

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It has previously been demonstrated that histamine inhibits food intake in rodents by a central mechanism of action (Ookuma, et al., 1993) Histamine-3 (H-3) receptors are mainly located presynaptically on histamine and non-histamine neurones in the CNS and drugs that block these receptors increase extracellular concentrations of histamine, as well as other neurotransmitters (Schlicker, et al., 1989). The present study was undertaken to investigate the effects of a novel H-3 receptor antagonist, GT2016 (5-Cyclohexyl-1-[4-(1H-imidazol-4-yl]-pentan-1-one), on food intake in pigs.

Male Large White pigs (n = 6; b.wt: 28 - 35 kg) were housed separately in metabolism cages and were trained to press operant switch panels with their snouts to obtain food and water reinforcements. Each pig was surgically prepared, under halothane anaesthesia, with a catheter in the external jugular vein. The animals were maintained on the following feeding schedule: At 10.15h a buzzer signalled that the feeder was activated for 120 min and that the pigs could press the food panel for reinforcement during that period. At 16.45h the buzzer signalled that the feeder was activated for 30 min. Water was available ad libitum throughout the experiment. During experimental sessions, the animals were injected with either vehicle (tartaric acid + proplylene glycol) or GT 2016 (0.25, 0.5, 1 or 5 mg kg^{-1}, iv) 30 min prior to start of the morning feeding session. The number of reinforcements obtained by each pig was monitored on a computer based data logger. In a second experiment, blood samples were taken from pigs (n=8) via indwelling jugular catheters 15 and 0 min prior to and 15, 30, 60 and 90 min after iv administration of vehicle or GT 2016 (1.0 mg/kg) and assayed for cortisol. Data were analysed by ANOVA for repeated measures and *post-hoc* Tukey Test.

GT 2016 (0.25 - 5 mg kg^{-1}) produced a dose-related inhibition of food intake. The lower doses (0.25 and 0.5 mg kg^{-1}) were without significant effect, while the higher doses (1 and 5 mg kg^{-1}) produced significant decreases in feeding. For example, the number of food reinforcements obtained by the pigs at 60 min was reduced from 65.7 ± 6.3 after vehicle to 33.0 ± 8.4 (P<0.01) after GT2016 (1 mg kg^{-1}) and 21.2 ± 9.6 (P<0.01) after GT 2016 (5 mg kg^{-1}). All doses caused behavioural effects upon injection, which included salivation, flushing, increased heart rate, and ataxia. The pigs normally recovered from these effects within 5 to 10 min following injection. GT 2016 (1.0 mg kg^{-1}) significantly increased cortisol release [for example, plasma cortisol concentration at 30 min: Vehicle: 35.8±5.5 nmol L^{-1} ; GT 2016 (1.0 mg kg^{-1}): 71.2 ± 13.1 nmol L^{-1}, P<0.01]

The results of this study show the H-3 receptor antagonist GT 2016 administered iv to hungry pigs produces a dose-dependent reduction in operant food intake. These results are consistent with observations in rodents that H-3 receptor antagonists inhibit food intake by increasing extracellular histamine (Ookuma, et al., 1993). However, the observation that iv administration of the drug increases plasma concentrations of the stress hormone cortisol may also suggest the possible involvement of the pituitary-adrenocortical axis.

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GT 2016 was a gift from Dr. Erik Nielsen at Novo Nordisk, Denmark.

65P 5-HT_{2C} RECEPTOR ANTAGONISTS, BUT NOT A 5-HT_{2A} OR 5-HT_{2B} RECEPTOR ANTAGONIST, ATTENUATE HALOPERIDOL-INDUCED CATALEPSY IN RAT

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Catalepsy induced in the rat is used as a preclinical screen to predict whether neuroleptics will cause extrapyramidal side-effects. "Atypical" neuroleptics such as clozapine cause less side-effects and this trait may be due to their additional ability to block receptors from the $5HT_2$ family. Indeed, it has been suggested that the $5HT_{2A}$ antagonist, MDL-100907 may act as an atypical neuroleptic. To test this hypothesis, we have examined the ability of a series of $5HT_{2A, 2B}$ and $_{2C}$ antagonists to block haloperidol-induced catalepsy in the rat.

Male Sprague Dawley rats (200-250g; Charles River) were housed under a 12hour light/dark cycle. A horizontal bar, 1 cm diameter, was raised 10 cm above bench level. In tests, the rats were positioned so that their hindquarters were placed on the bench with their forelegs resting over the bar. The interval that the rats kept their forelegs over the bar was recorded up to a maximum of 120 sec. This procedure was repeated for each rat at 30, 60 and 90 min after drug administration as catalepsy increases with repeated tests. Rats were judged to be cataleptic if they maintained their position on the bar for 30 sec or more; analysis was performed on the 3rd (90 min) test results using a logistic regression analysis statistical package in SAS-RA©. Haloperidol, was dissolved in water with tartaric acid and injected, 1 ml/kg i.p. The $5HT_{2A}$ antagonist, MDL-100907, $5HT_{2B}$ antagonist SB215505 (6-chloro-5-methyl-1-(5-quinolylcarbamoyl) indoline ($5HT_{2B}$ pKi = 8.3), and the $5HT_{2C}$ antagonists, SB-228357 (1-[5-fluoro-3-(3-pyridyl)phenylcarbamoyl]-5-methoxy-6-trifluoromethylindoline) ($5HT_{2C}$ pK_i = 9.0), and SB-242084 (Kennett et al,1997) were ground with 1 drop of BRIJ-35 and 1% methylcellulose and administered @ 2ml/kg po. Treatment groups were n=6. Haloperidol caused a dose related increase in catalepsy between 0.038 and 3.8 mg/kg. At 1.13 mg/kg, haloperidol produced catalepsy in 5/6 rats ($P<0.01$) compared to 0/6 after vehicle. This dose was used in future studies. In the MDL-100907 study, haloperidol produced catalepsy in 6/6 rats. MDL-100907 (0.003-0.1 mg/kg) did not reverse haloperidol-induced catalepsy ($P>0.05$ at each dose). In the SB-215505 study, haloperidol produced catalepsy in 6/6 rats. SB-215505 (0.1-3.2 mg/kg) did not reverse haloperidol-induced catalepsy ($P>0.05$ at each dose). In the SB-242084 study, haloperidol produced catalepsy in 5/6 rats. SB-242084, although not dose-related, significantly attenuated this effect to 1/6 cataleptic rats at a dose of 3.0 mg/kg; $P<0.05$). In the SB-228357 study, haloperidol produced catalepsy in 6/6 rats. SB-228357 partially reversed this effect at 0.32, 3.2 and 10 mg/kg (catalepsy in 3/6 rats, $P<0.05$ at these doses). Results suggest that blockade of $5\text{-}HT_{2C}$ receptors may be responsible for the anticataleptic effects of $5\text{-}HT_2$ receptor antagonists, and this property may be useful for improving the side-effect profile of neuroleptics.

Kennett, G.A., Wood , M.D., Bright, F. *et al.* (1997) *Neuropharmacol.* 36, 609-620

66P THE ANTIPARKINSONIAN ABILITY OF BUPROPION IN MPTP-TREATED COMMON MARMOSETS

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The inhibitors of the dopamine transporter (DAT), NS 2214 and BTS 74 398, reverse motor disability in MPTP treated primates without evoking established dyskinesias (Pearce *et al.*, 1995; Smith *et al.*, 1998). Another such compound, bupropion, is already available for clinical use and has been tested in Parkinson's disease (PD) with some success (Goetz *et al.*, 1984). We now evaluate the ability of bupropion to reverse motor deficits in MPTP treated common marmosets in comparison to L-DOPA and BTS 74 398.

Adult common marmosets (350-488g, n=12) were treated with MPTP (10 mg/kg over 5 days, s.c.) to induce motor deficits. Animals were treated with L-DOPA (10 mg/kg, after 12.5 mg/kg carbidopa, n=12), bupropion (6-25 mg/kg, n=11), BTS 74 398 (5-20 mg/kg, n=4) or 10% sucrose vehicle (n=12). Dosing was separated by a one week period. Behavioural disability/10 min was scored on a scale 0-18 (normal-markedly impaired) and locomotor activity/10 min was automatically recorded.

Administration of L-DOPA reversed motor deficits and increased locomotor activity (Figure 1). As previously reported, BTS 74 398 also reversed motor impairment and disability (Figure 1). Similarly, administration of bupropion increased locomotor activity and reduced motor disability. The intensity of peak activity of bupropion was not different to that of BTS 74 398. However, bupropion had a shorter duration of action (3hrs) than BTS 74 398 (10 hrs), but was similar to L-DOPA (4hrs). Both reuptake blocking compounds also elevated total locomotor activity (Figure 1, inset). Bupropion and BTS 74 398 improved checking and grooming, and induced climbing and jumping activity. Stereotypy and hyperactivity were absent. Total disability scores/4hrs were significantly ($P<0.05$) reduced for bupropion (152±13, n=11), L-DOPA (109±7, n=12) and BTS 74 398 (93±24, n=4) relative to vehicle (206±9, n=12). Bupropion appeared less effective than BTS 74 398 and L-DOPA.

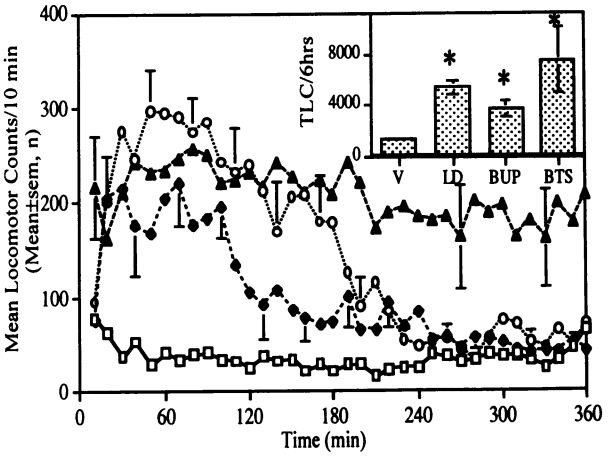

Figure 1 Effect of drug treatment on total (TLC/6hrs, inset) and mean locomotor Counts/10 min. *P<0.05 (Mann Whitney U Test) relative to vehicle. □, vehicle (V, n=12); ○, L-DOPA (LD, n=12); ◆, Bupropion (BUP, n=11) 25 mg/kg; ▲, BTS 74 398 (BTS, n=4) 20 mg/kg. Data is mean±sem, n.

In conclusion, bupropion reversed the motor deficits in MPTP treated primates, although, not to the extent seen with BTS 74 398 and L-DOPA at the doses used. DAT blockade, however, may form a novel means of treating early stage PD.

Goetz, C.G., Tanner, C.M. and Klawans, H.L. (1984) Neurology 34: 1092 -1094.
Pearce, R.K.B., Banerji, T., Scheel-Krüger, J. *et al.*, (1995) Brit. J. Pharmacol. 116 (suppl): 304P
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67P *IN VIVO* EVIDENCE FOR OLEAMIDE AS A POTENTIAL ENDOCANNABINOID LIGAND

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cis-9,10-octadecenoamide (ODA, oleamide) has been identified as a putative sleep-inducing agent in cat cerebro-spinal fluid (CSF) (Cravatt *et al*, 1995) that potently and selectively modulates $5HT_2$ receptor activity *in vitro*. Furthermore, ODA exhibits molecular charateristics that closely resemble those of endogenous cannabinoid ligands as it is a amidrohydrolase-resistant ligand at CB1 (central) receptors (Huidobro-Toro and Harris, 1996; Cadogan *et al*, in press). The apparent potentiation of $5HT_2$-receptor function can be assessed *in vivo* by means of two $5HT_2$-mediated peripheral reflexes commonly known as Wet-dog Shakes (WDS) and Back Muscle Contractions (BMC) (Fone *et al*, 1989). WDS are thought to be mediated by $5HT_2$ (Beddard and Pycock, 1977) receptors and other (non-5HT) receptors whereas BMC have been shown to be mediated exclusively by $5HT_2$ receptors. The present study determined whether ODA's hypolocomotor properties as well as the modulation of $5HT_2$-receptor mediated responses are CB1-mediated.

Male Wistar (250-300 g) rats were given the selective $5HT_2$-receptor agonist DOI (0.5 $mg.kg^{-1}$) alone, or 5 min before administration of either the potent cannabinoid agonist HU210 (20 $\mu g.kg^{-1}$) or saline (all drugs given i.p.). DOI-induced behaviours (WDS and BMC) were counted every 5 min for 50 min post-injection after either saline or HU210 injection by a person "blind" to the treatment protocol. To further determine whether ODA's modulation of the $5HT_2$ behaviour involves cannabinoid receptors, rats were given the cannabinoid antagonist SR141716A (3.0 $mg.kg^{-1}$) or saline 15 min before DOI (1.0 $mg.kg^{-1}$) followed 5 min later by ODA (2.0 $mg.kg^{-1}$). Finally, we wished to determine whether ODA's hypolocomotor properties are cannabinoid-receptor mediated. Rats were given SR141716A (3.0 $mg.kg^{-1}$) followed by ODA at a dose known to decrease locomotor activity (10 $mg.kg^{-1}$) 20 min later.

Table 1. Effects of HU210 on DOI-induced behaviours.

Behaviour	DOI alone	DOI + HU210
BMC	65.8 ± 17.9	91 ± 13.4*
WDS	18.67 ± 5	10.3 ± 4.5*

*$p < 0.01$ Mann-Whitney U Test, n=6

HU210 respectively enhanced and inhibited DOI-induced BMC and WDS (Table 1). Both the ODA-induced potentiation of BMC (but not WDS) and decrease in locomotor activity were significantly inhibited by SR141716A ($p < 0.01$ and $p < 0.05$ respectively) at a dose that effectively blocked HU210 (100 $\mu g.kg^{-1}$)-induced catalepsy.

The similarity between the effects of HU210 and ODA and their sensitivity to SR141716A further support the mediation of the effects of ODA by CB1 receptors possibly interacting with serotonergic systems.

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Huidobro-Toro J.P. and Harris R.A. (1996) *Proc. Natl. Acad. Sci* USA, 93, 8078-8082.

68P USE OF TELEMETRIC RECORDING OF EEG, EMG AND EOG TO ASSESS SLEEP PARAMETERS IN THE COMMON MARMOSET

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Common marmosets *(Callithrix jacchus)* are small diurnal primates which have a similar sleep-wake cycle to that of man. The marmoset may, therefore, provide a useful laboratory species for the study of primate sleep and pharmacological modification of this behaviour (Crofts *et al* 1997). Few reports of sleep behaviour in the marmoset exist in the literature. This may be due to the fact that measurement of electroencephalogram (EEG) by classical methodology presents technical difficulties in this species. We have utilised miniature multichannel telemetric transmitters (Data Sciences Int.) to record remotely EEG, electromyogram (EMG) and electro-oculogram (EOG) in the marmoset. This system allows, for the first time, accurate and unequivocal recording of rapid eye movement (REM) sleep in this species. The aim of the present study was to investigate the effects of hypnotic compounds on sleep architecture in this species.

Adult marmosets (3 male, 2 female) were anaesthesised using Saffan (1.8ml/kg i.m.) and intraperitoneal implantation of the telemetric transmitter was carried out using standard surgical techniques. Recording electrodes were transported subcutaneously and positioned to record cortical EEG, EMG from the neck region and EOG.

Animals were housed in pairs (one implanted, one unoperated companion) in standard cages in a holding room maintained at 25 ±1°C and 60% humidity on a 12 hour light dark cycle (lights off at 1800). Prior to lights-on and lights-off a 30min period of simulated dawn and twilight was applied using fading lights.

Drug studies were carried out according to a randomised, paired crossover design where, in separate experimental sessions, each animal received control and drug treatments. Both animals of each pair received identical treatments immediately prior to lights off. To assess the effects of hypnotic agents, basal sleep of the animals was reduced for the five hour test period using the disturbing influence of "white noise" (70dB, equivalent noise level to normal daytime activity in the colony). This reduced total sleep time by approximately 30%. EEG recordings were transformed into hypnograms using Fast Fourier Transformation (FFT) and four sleep parameters were analysed; awake, stage 1-2 (light) sleep, stage 3-4 (deep) sleep and REM sleep. The effects of triazolam (0.1 and 0.3mg/kg p.o.) and zolpidem (1 and 3mg/kg p.o.) or vehicle (5% methocel). Experiments were separated by a 7 day rest period which allowed drug washout and ensured that the sleep routine of the animals was disturbed only minimally.

Effect of triazolam and zolpidem on sleep stage duration (min±SEM).

Treatment (mg/kg)	Awake	Stage 1-2	Stage 3-4	REM
normal/untreated	45.7±8	88.6±11	146.1±9	19.6±3
noise+VEHICLE	114±22*	76.4±8	105±25*	4.3±2*
noise+TRIAZ 0.3	91±34	55.5±12	151.3±22#	2.15±1
noise+VEHICLE	64.6±12	128.3±25*	100.6±31*	6.5±4*
noise+ZOLP 3.0	45.7±2	87.5±18	162.8±18#	4.0±2

(5h test period, significant differences: *P<0.05 w.r.t. normal/untreated and #P<0.05 w.r.t. noise+VEH, paired t test n=4-5).

The white noise reduced both the quantity and quality of sleep in the animals. This effect was particularly clear for stage 3-4 and REM sleep. The hypnotic effects of triazolam and zolpidem were seen as a significant reversal of the reduction in stage 3-4 sleep. The compounds failed, however, to reverse the REM deficit.

In conclusion, telemetric EEG/EMG/EOG recording combined with the disturbed sleep model may provide an invaluable method to investigate drug effects on sleep parameters in the primate.

Crofts H, Nutt DJ, Pearce PC, Scott EAM and Wilson S. J. Psychopharmacol 11 A53 1997

69P THE EFFECT OF CHOLINERGIC DRUGS ON A RANGE OF PARAMETERS IN THE GUINEA-PIG

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Studies in the rat and mouse have shown that organophosphates modify the cholinergic effects of drugs like oxotremorine and nicotine (Clement, 1993; Gordon, 1994). If carbamates were used as a pretreatment against organophosphate poisoning they may themselves produce such effects. It is now generally accepted, however, that the guinea pig is the best non-primate model for predicting the efficacy of pretreatments against organophosphate poisoning in primates (Inns & Leadbeater, 1983).

The aim of this study was to determine whether oxotremorine or nicotine produced hypothermic and behavioural effects in the guinea pig. This species could then be used to assess how carbamate pretreatments affect cholinergic parameters.

Male Dunkin-Hartley guinea pigs (190-300g) were maintained on a 12:12 h light:dark cycle, with food and water *ad libitum* throughout. All experiments were performed according to the Animals (Scientific Procedures) Act, 1986. Plexx transponders were implanted subcutaneously and temperature recorded twice daily. The animals were weighed daily as an indicator of general health.

Animals were dosed subcutaneously with oxotremorine (0.0125–0.200 mg/kg), nicotine (2.5–7.5 mg/kg) or saline. Dose groups generally consisted of 4–6 animals. Temperatures were recorded every 10 mins for 1 h prior to and 4 h following injection. The animals were closely observed during this period and overt cholinergic and behavioural signs including salivation, chewing, tremors, eating and drinking were noted.

Saline-treated guinea pigs showed no cholinergic signs. Chewing behaviour was only associated with eating and drinking, which occurred throughout the observation period. Temperature remained stable in this control group.

Oxotremorine produced a dose-dependent hypothermia. Compared with mean baseline values, 0.0125 mg/kg oxotremorine reduced temperature by 0.023±0.08 °C at 30 mins; 0.2 mg/kg reduced temperature by 4.73±0.55 °C at 70 mins. A dose of 2.5 mg/kg nicotine reduced temperature by 0.87±0.09 °C at 20 mins, while a dose of 7.5 mg/kg reduced temperature by 1.75±0.06 °C at 30 mins. All temperatures had returned to the normal range by the end of the 4h monitoring period.

Oxotremorine, but not nicotine, produced writhing in all dose groups. This may be explained by activation of gastrointestinal tract muscarinic receptors. The incidence of tremor and salivation were dose dependent. Nicotine recovery was more rapid, in that tremor had ceased in all dose groups 90 mins following administration. Oxotremorine-induced tremor was apparent up to 180 mins in some dose groups. During the first 90 mins, the oxotremorine treated animals showed chewing not associated with eating or drinking. For the animals given nicotine chewing was only observed in the two highest dose groups. In all groups, at later times, chewing was only associated with eating and drinking. These findings are consistent with those reported for the rat and mouse.

Co-workers have demonstrated a significant correlation between core temperature measured by telemetry and subcutaneous temperature recorded by transponders in the rat (Bowditch *et al.*, pers comm). The advantage of transponders is that repeated handling during the observation period is avoided. The rectal probe technique, (Dilsaver *et al.*, 1992), though accurate, is time-consuming, interferes with behaviour and introduces additional handling stress. It would therefore be unsuitable for use in the present study.

We have shown that the guinea pig is an appropriate species in which to study cholinergic hypothermia and behavioural signs. Having developed and validated these techniques, future studies will include investigations into the effects of reversible and irreversible acetylcholinesterase inhibitors.

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70P TIME COURSE OF SPONTANEOUS BEHAVIOURAL EFFECTS OF ANTISENSE OLIGONUCLEOTIDES TARGETING THE α_{2D}-ADRENOCEPTOR IN THE RAT

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At least four subtypes of α_2-adrenoceptor have been identified based on their pharmacology, amino acid sequence and chromosomal location (Mackinnon *et al.*, 1994). As yet there are no highly selective ligands available to determine the *in vivo* function of these subtypes. Previous studies using a phosphorothioate oligonucleotide (S-ODN) have shown that antisense to the α_{2D}-subtype significantly reduced α_2-adrenoceptor expression in specific brain areas, following a three day infusion (Robinson *et al.*, 1997). The aim of the present study was to investigate the behavioural consequence of inhibiting expression of the α_{2D}-adrenoceptor using the same antisense sequence (Nunes, 1995).

Behavioural observations were made daily during a two day baseline period, during the infusion and also for three days after the end of the infusion. Rats were observed every minute for a total of 60min and behaviours scored to give a maximum frequency score for each behaviour of 60. On day 3, male Wistar rats (270-310g) were anaesthetised with isoflurane and stereotaxically implanted with an i.c.v. cannulae to the left lateral ventricle (0.92mm caudal to bregma, 1.4mm lateral and 3.5mm below the dura). S-ODN antisense or mismatch (toxicity control) or vehicle were administered using an osmotic mini-pump combined with a brain infusion kit (8μg μl^{-1} hr^{-1}, i.c.v.). The treatments remained coded until the end of the experiment. Behavioural observations and body weight data were analysed using the Cochran-Mantel-Haenszel test and two-way ANOVA followed by Dunnett's test *post hoc* respectively.

A significant increase in behavioural activity (locomotion, grooming, wall climbing, rearing and eating) was seen in the antisense-treated rats on day 4, 5 and 6 of the time course study. In particular, the increase in activity was due to an increase in both locomotion and grooming. In both the mismatch and vehicle infused controls, behavioural scores were not significantly different from the naive group. The behavioural activation in the antisense-treated group returned to baseline values 2 days after the end of the infusion (Fig 1). Total body weight gain was also significantly reduced in the antisense-treated group compared to the controls at the end of the study (vehicle 51.4 ± 4.4g, antisense 33.9 ± 6.2g*†, mismatch 48.5 ± 3.1g and naive 47.2 ± 6.9g, mean ± s.e.mean, *p< 0.05 compared to vehicle, † p<0.05 compared to mismatch, n=8 animals per group). There was no effect of treatment on the daily or total food and water intake, therefore, the reduction in body weight gain may reflect the behavioural activation induced by the antisense infusion. These behavioural effects appear to be a consequence of the antisense-mediated knockdown of α_2-adrenoceptors in the rat CNS.

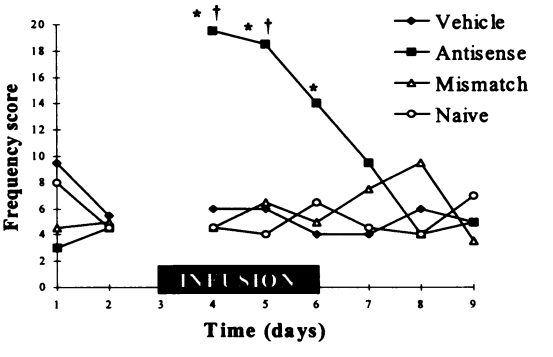

Fig 1: Median frequency score for total activity (range 60-141% of median). *p<0.05 antisense vs vehicle, †p<0.05 antisense vs mismatch (n=8 rats per group).

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Nunes, (1995) *Eur. J. Pharmacol.*, 278, 183-185
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71P THE USE OF THE LEECH (*HIRUDO MEDICINALIS*) RETZIUS CELL AS A MODEL FOR STUDYING THE ACTION OF CONVULSANTS AND ANTICONVULSANTS

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The aim was to examine the possibility that identified Retzius (R) cells of the leech *Hirudo medicinalis* could be used as a model for studying epileptiform activity. Paired giant R cells are serotonergic neurones which modulate leech feeding behaviour (Leake, 1986). They normally fire regularly at 0.1-3Hz but may be induced to undergo epileptiform activity, defined as paroxysmal depolarising shifts (PDSs) of membrane potential accompanied by bursts of action potentials (Dean and Leake, 1988).

Intracellular recordings were made from R cells in isolated leech ganglia bathed in normal or high (20mM) magnesium leech Ringer. All drugs were applied to the bath dissolved in Ringer, except for carbamazepine which was dissolved in ethanol (2-22 mM).

Bemegride (2-10mM) and cobalt chloride (0.01-1mM) induced severe PDS activity in R cells, time to onset being dose-dependent. Bemegride action was abolished using high magnesium Ringer and was inhibited by anticonvulsants sodium valproate (10^{-5} -$^{-3}$M) and carbamazepine (CBZ: 10^{-6} -$^{-4}$ M). Cobalt chloride action was not blocked by high magnesium Ringer and was inhibited by CBZ (10^{-5} -$^{-3}$M) but not by valproate (10^{-7} -$^{-3}$M). 5-Hydroxytryptamine (5-HT) hyperpolarised the R cells and reduced the frequency of PDSs induced by both convulsants in a dose-dependent manner (10^{-6}-$^{-3}$ M). Table 1 gives a summary of the results (means sampled for 3 mins at 10mins after drug application / at 3 mins for 5-HT) at one concentration of each of the drugs.

R cells could also be induced to fire PDSs by application of the nitric oxide (NO) donor, S-nitroso-N-acetyl penicyllamine (SNAP) and the NO synthase substrate, arginine, both at 10^{-7}-$^{-3}$ M. Time to onset of PDSs was dependent on dose (n=4). Both effects were blocked by CBZ (10^{-5} -$^{-3}$M) but not by sodium valproate (10^{-5} -$^{-3}$M).

Thus, R cells can discriminate between two effective convulsants which operate through different modes of action (i.e. cobalt chloride,which has a direct effect on the neuronal membrane, and bemegride whose effect is mediated via synaptic activity); thus making the R cell a useful model for studying epileptiform activity and for testing convulsant and anticonvulsant drugs. Epileptiform activity induced by both mechanisms is modulated by 5-HT. Further, nitric oxide may be implicated in epileptiform behaviour of neurones as arginine and SNAP both induce PDSs which can be blocked by carbamazepine.

Dean J.A. and Leake L.D. (1988) Comp. Biochem. Physiol., 89C, 31-38. Pharmacological control of the pattern of activity of leech Retzius cells.
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M.K. acknowledges support by a Wellcome Toxicology Studentship.

Table 1. Effects of anticonvulsants and 5-HT on amplitude, duration and frequency of PDSs induced by two convulsants (mean ± S.E., n=15, Ps<0.01)

Convulsant (M)	Anticonvulsant (M)	PDS					
		Amplitude (mV)	S.E.	Duration (s)	S.E.	Frequency (Hz)	S.E.
Bemegride ($5x10^{-3}$)		12.0	0.31	5.6	0.27	0.16	0.007
	Na^+ Valproate (10^{-4})	0.8	0.31	0.4	0.15	0.6	0.008
	Carbamazepine (10^{-4})	0.0	0.0	0.0	0.0	0.0	0.0
	5-HT (10^{-4})	16.5	0.33	5.9	0.28	0.08	0.006
$CoCl_2$ (10^{-5})		11.9	0.33	2.2	0.08	0.13	0.007
	Na^+ Valproate (10^{-3})	11.0	0.41	2.0	0.16	0.14	0.006
	Carbamazepine (10^{-4})	0.0	0.0	0.0	0.0	0.0	0.0
	5-HT (10^{-4})	0.5	0.30	0.2	0.10	0.04	0.002

72P THE SELECTIVITY OF FUROSEMIDE FOR α6-CONTAINING $GABA_A$ RECEPTORS IS DETERMINED IN PART BY Ile228

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Furosemide has previously been shown to be a subtype-selective $GABA_A$ receptor non-competitive antagonist eliciting approximately 100-fold greater sensitivity for α6β2/3γ2s receptors than for α1β2/3γ2s receptors (Korpi *et al.*, 1995). The aim of this study was to identify the amino acids within the α6 subunit that are responsible for this difference using α1/α6 chimeric receptors and α1 point mutations.

Recombinant human α1, α6 or chimeric cDNA were coexpressed with β3γ2s in *Xenopus* oocytes and the IC_{50} for furosemide determined against a GABA EC_{50} concentration using the two-electrode voltage-clamp method. Results are given as mean (95% CL) n=4 or more.

Three α1/α6 chimeras (C1, C2 and C3) were initially constructed (numbering according to mature polypeptide): C1 was α6 from the N-terminal until amino acid 118, C2 was α6 from 119 until 279 and C3 was α6 between 92 and 131 with the remainder being α1. The furosemide IC_{50} for C1 and C3 were similar to α1 (1.38 (1.32, 1.45) and 0.98 (0.87, 1.10) compared to 0.98 (0.89, 1.08)mM respectively) whereas C2 was similar to α6 (17.1 (14.9, 19.7) compared to 12.1 (11.4, 12.9)μM). The amino acids responsible for the high sensitivity of furosemide at α6 containing receptors were therefore located between 131 and 279.

Two additional chimeras (C4: α6 between 160 and 279, and C5: α6 between 209 and 279) gave an intermediate furosemide IC_{50} (78.6 (55.8, 110.6) and 56.3 (37.6, 84.3)μM respectively). These results suggest that there may be two amino acids responsible for the high furosemide sensitivity at α6 containing receptors, the first being located within 131 and 160 and the second between 209 and 279. The latter region of 70 amino acids encompasses both TM1 and TM2 and within this region there are 12 amino acids that are different between α1 and α6. Fisher *et al.*, (1997) described a rat α6/α1 chimera with a splice site within TM1 that conferred high furosemide sensitivity and an α1 point mutation (α1L258T) where furosemide sensitivity was unchanged. These results eliminated 5 of the 12 amino acids identified. The remaining 7 amino acids were mutated (in groups of 2 or 3) in α1 to the α6 equivalent. α1V227M,T230I revealed an intermediate furosemide sensitivity (51.4 (44.6, 59.2)μM) similar to that of C4 and C5.

Individual point mutations produced IC_{50}s of 0.7 (0.63, 0.78)mM for α1V227M and 40.9 (34.6, 48.3)μM for α1T230I. Systematic construction of α1/α6 chimeras and α1 point mutations has therefore identified a single amino acid, α6I228 (position equivalent to α1T230), which when mutated from threonine to isoleucine in α1 increases the furosemide sensitivity by approximately 20 fold.

We conclude, that this residue is, in part, responsible for the high furosemide sensitivity at α6 containing receptors. Its location within TM1 leads to speculation that it may be involved in the channel gating mechanism.

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73P MECHANISMS OF LIGAND BINDING AND AGONIST EFFICACY AT THE HUMAN D_{2SHORT} DOPAMINE RECEPTOR

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The aim of this study was to investigate how each of the stages in the Ternary Complex Model (Wreggett & DeLean, 1984) contributes to the efficacy of agonist action. A CHO cell line stably expressing the human D_{2short} receptor was used in competition studies versus [^{3}H]spiperone to define the higher and lower binding affinities (K_h & K_l) of an homologous series of agonists. Functional properties of agonists were also investigated using agonist stimulated [^{35}S]GTPγS binding and agonist inhibition of adenylyl cyclase deriving EC_{50} values (Gardner *et al.*, 1997).

The same rank order of potency (dipropyldopamine > dopamine > *m*-tyramine > β-phenylethylamine > *p*-tyramine) was seen in both ligand binding (K_l, K_h) and functional assays (EC_{50}). Greater maximal effects and amplifications (K_l/EC_{50}) were generally seen in the adenylyl cyclase assays, compared with the [^{35}S]GTPγS binding assays and this is consistent with response amplification along the signal transduction pathway (Table 1). It is thought that the ratio of dissociation constants for the low and high affinity sites (K_l/K_h) provides a measure of the ability of the agonist to promote coupling of receptor to G protein (DeLean *et al.*, 1980) and thus could correlate with maximal agonist effect and the degree of system amplification. However, even using a closely related series of compounds, which may be expected to behave similarly, no correlation was found in this study (Table 1).

From these data, it seems that agonist binding (K_l/K_h) cannot be used to predict agonist efficacy.

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Table 1.Agonist binding and functional parameters.

	K_l/K_h	K_l/EC_{50} [^{35}S]GTPγS binding	K_l/EC_{50} adenylyl cyclase	% Maximal activity ([^{35}S]GTPγS binding) ± s. e. mean	% Maximal activity (adenylyl cyclase) ± s. e. mean
dipropyldopamine	87.2	54.8	1199	98.3 ± 1.6	103 ± 1.6
dopamine	29.4	18.6	2243	100	100
m-tyramine	75.1	6.8	192.5	68.6 ± 6.2	111 ± 3.4
β-phenylethylamine	121.4	16.1	81.6	49.3 ± 2.9	109.1 ± 10.5
p-tyramine	99.8	48.1	53.5	53.1 ± 7.8	99.8 ± 3.6

Maximal activities are measured as % of dopamine response and are from at least 3 experiments.

74P MEN 11420 IS A POTENT ANTAGONIST AT THE NATIVE AND TRANSFECTED HUMAN TACHYKININ NK_2 RECEPTOR

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The aim of this study was to investigate the potency of the tachykinin NK_2 receptor-selective antagonist MEN 11420 (Catalioto *et al.*, 1998) in human smooth muscle preparations and in CHO cells stably transfected with the human NK_2 receptor. MEN 10627 (Maggi *et al.*, 1994) was used as reference compound. Organ bath experiments were performed on circular muscle strips from the human colon (HC), human ileum (HI) and on human urinary bladder detrusor muscle strips (HUB), as described previously (Giuliani *et al.*, 1996; Patacchini *et al.*, 1997). The antagonists under study (15 min incubation period) were tested against cumulative concentration-dependent contractile responses produced by the NK_2 receptor-selective agonist [βAla^8]NKA(4-10) (HC and HI) or by neurokinin A (NKA) (HUB). All the experiments in the HI were performed in the presence of the NK_1 receptor selective antagonist SR 140333 (0.1 μM, 15 min before; Edmonds-Alt *et al.*, 1993).
The transfected CHO cells grown to confluence in 24 well plates were incubated with antagonists or vehicle for 30 min at 37°C and then challenged with increasing concentrations of NKA for further 30 min. The incubation media were then removed and stored at -70°C until assayed for released PGE_2 (EIA, Amersham).
MEN 11420 (0.03-1 μM) competitively antagonized NK_2 receptor-mediated contractions in all preparations, with a potency comparable to MEN 10627, as shown in the table. In transfected CHO cells, NKA (0.1nM-1μM) stimulated the production of PGE_2 in a concentration-dependent manner (ED_{50}=3.0±0.3 nM, n=10). MEN 11420 and MEN 10627 (0.01-1μM) competitively blocked NKA-induced PGE_2 release showing a potency similar to that observed in organ bath experiments (see the table).

Antagonist potency at human NK_2 receptors. pK_B values (mean±s.e.m., n = 9-12) and Schild plot slopes (in parenthesis).

	HUB	HI	HC	CHO
MEN 11420	8.6±0.1 (-0.9)	8.3±0.1 (-0.9)	8.3±0.1 (-1.0)	8.3±0.1 (-1.2)
MEN 10627	8.5±0.1 (-1.0)	8.4±0.1 (-1.1)	8.8±0.1 (-1.0)	8.6±0.1 (-1.3)

The present results show that MEN 11420, a bicyclic peptide antagonist characterized by higher metabolic stability and longer duration of action in animal models as compared to MEN 10627 (Catalioto *et al.*, 1998), blocks with nanomolar affinity and simple competitive kinetics the human NK_2 receptor, either the native type or the transfected form into CHO host cells. Therefore, the latter cells appear to represent a suitable *in vitro* model for the study of the human NK_2 receptor and NK_2 receptor ligands.

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75P GR128107 IS A PARTIAL MELATONIN RECEPTOR AGONIST ON *XENOPUS LAEVIS* MELANOPHORES

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Few specific melatonin receptor antagonists have been identified. GR128107 (3-(1-acetyl-3-methyl-piperidine)-5-methoxy-indole) has been reported to be a potent (pA_2=10.2) competitive melatonin receptor antagonist, blocking melatonin inhibition of [^{3}H]-dopamine release from rabbit retina, a response mediated by the MT_2 receptor subtype (Dubocovich *et al.*, 1997). In the present study, GR128107 was examined in a well-established model of melatonin action, the pigment aggregation response in *Xenopus laevis* melanophores.

Xenopus leavis clonal melanophores were cultured in 96-well plates in conditioned L15 medium (Daniolos *et al.*, 1990) and pigment movement quantitated by measuring the change in cell absorbance (630 nm). Like melatonin (pEC_{50}=10.09±0.03, n=3), GR128107 (pEC_{50}=8.58±0.03, n=3) produced a concentration-related movement of pigment granules toward the centre of melanophores. With both drugs aggregation was maximal by 90 min. GR128107 was a partial agonist (maximal response, E_{max}=0.83±0.02, n=11; melatonin=1). Several melatonin receptor antagonists, from different chemical classes, blocked melatonin and GR128107-induced pigment aggregation with similar potency: estimated pK_B; RJ252 (Garratt *et al.*, 1995) against melatonin 4.60/against GR128107 4.54 < GR135533 (Dubocovich *et al.*, 1997) 6.40/6.14 < Luzindole 6.45/6.49 < S20929 (Ting *et al.*, 1997) 6.58/6.65 < 4-phenyl-2-propionamidotetralin 6.73/6.85. Pigment movement induced by GR128107 and melatonin was also blocked by pretreatment with pertussis toxin (10-1000 ng/ml, 24h), with the response to GR128107 more sensitive to low concentrations of the toxin. Prolonged melatonin treatment (72h, 10^{-9}M) desensitized melanophores to the aggregating action of melatonin and GR128107. The maximal response to melatonin was unchanged but that to GR128107 was reduced to 0.27±0.01 (n=4) while the potency for both agonists was decreased by ~5-fold. Radioligand binding studies using the melatonin agonist, 2-[^{125}I] iodomelatonin and recombinant human receptors expressed in NIH3T3 cells confirmed the MT_2 selectivity of GR128107 (mt_1 K_i=6.85±0.03; MT_2 K_i=8.62±0.12, n=3), and revealed a very high density of melatonin receptors on *Xenopus* melanophores (K_D=63±12 pM, B_{max}=1223±14 fmol/mg protein). Melatonin receptors in rabbit retina have a similar affinity (K_D=47±8 pM) but are expressed at a much lower density (B_{max}=3 fmol/mg protein). It seems likely that GR128107 acts as a partial agonist in melanophores because of the large receptor reserve in these cells, and as an antagonist in rabbit retina because few spare receptors are available.

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76P [^{3}H]CLONIDINE BINDING SITES IN RAT KIDNEY: A MODEL FOR PUTATIVE CENTRAL IMIDAZOLINE-1 SITES

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Imidazoline binding sites (I-sites) have been subclassified by Ernsberger (1992) into those binding sites labelled by [^{3}H]clonidine and [^{3}H]*p*-aminoclonidine (I_1-sites), and those labelled by [^{3}H]idazoxan (I_2-sites). I_1- but not I_2- binding sites are present in the rostral ventrolateral medulla of rats (Ernsberger *et al*, 1987). Peripherally, there is a high density of I_1- and a low density of I_2-binding sites present in the rat kidney (MacKinnon *et al*, 1993). This study now reports a model assay for I_1-binding sites in the rat kidney which may be comparable to those putative I_1-sites found in the rat brain.

Male Wistar rats (250-300 g) were stunned prior to decaptitation and membranes were prepared from whole kidneys based on the method by Hudson *et al* (1992). [^{3}H]Clonidine (3 nM; in the presence of rauwolscine 10 μM, to mask the α_2-adrenoceptor component) was used to label I_1-sites in rat kidneys. Membranes (200 μg; protein), [^{3}H]ligand and competing drugs were incubated in triplicate to equilibrium (30 min. 25°C). Clonidine (10 μM) was used to define non-specific binding. Bound ligand was separated from free by filtration. Table 1 opposite shows mean IC50 values ± s.e.mean (n = 4-5).

Rilmenidine had a high affinity for I-sites labelled by [^{3}H]clonidine in the rat whole kidney, while agmatine and noradrenaline exhibited a very low affinity. Clonidine, BU224 (2-(4,5-dihydroimidazol-2-yl)quinoline), and idazoxan also had reasonable affinity for I-sites labelled by [^{3}H]clonidine in rat kidney membranes. Idazoxan exhibited biphasic inhibition curves, suggesting the presence of another site labelled with [^{3}H]clonidine in the rat whole kidney membranes (* high affinity site).

Table 1

	Rat kidney (IC50; in nM)
Rilmenidine	13.6 ± 1.3
Clonidine	143.7 ± 24.4
BU224	359.5 ± 39.2
Idazoxan	627.7 ± 546.2*
Agmatine	72, 000 ± 48, 000
Noradrenaline	>100, 000

These results show that the I_1-site selective ligands rilmenidine and clonidine show the highest affinity for I-sites labelled by [^{3}H]clonidine in rat kidney membranes, with the I_2-selective ligands BU224 and idazoxan exhibiting a 2.5 and 4.4 times lower affinity than clonidine, respectively, for these sites. Agmatine, the proposed endogenous ligand for I-sites was found to have low affinity. In agreement with MacKinnon *et al* (1993) the kidney contains a high number of I-sites, furthermore we propose it represents a model tissue in which to study the binding characteristics of putative central I_1-binding sites.

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77P IMIPRAMINE AT CLINICALLY RELEVANT CONCENTRATIONS BLOCKS HETEROLOGOUSLY EXPRESSED HERG, A K^+ CHANNEL RESPONSIBLE FOR "LONG QT SYNDROME"

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Tricyclic antidepressants (TCAs) such as imipramine have cardiovascular side effects including orthostatic hypotension, atrioventricular conduction delay, reduced heart rate variability in response to exercise, tachycardia, syncope, and lengthening of the QT interval (which may be associated with arrhythmias), particularly in cases with high dosages and in patients with concurrent cardiovascular disease. Many of these side effects of TCAs have been ascribed to their wide spectrum of actions including alpha-adrenergic, anti-histaminergic, anticholinergic activities, and block of sodium channels (Coupland et al., 1997; Stahl, 1998).

Mutation or pharmacological block of the HERG K+ channel may be responsible for "Long QT Syndrome" (Sanguinetti et al., 1995), a cardiac disorder characterised by the dangerous cardiac arrhythmia *Torsades de pointes*. 1 μM imipramine has been shown to block fully I_{Kr} in guinea pig ventricular myocytes (Valenzuela et al., 1994). The HERG gene product is believed to be the major subunit of the channel mediating I_{Kr}. In this study we investigate a possible role of HERG K+ channels in mediating cardiovascular side effects of TCAs.

HERG channels and the fluorescent indicator protein Green Fluorescent Protein (GFP) were heterologously expressed in CHO cells transiently transfected using lipofectamine. GFP positive cells were voltage-clamped at -80 mV using either the whole-cell or a gramicidin-based perforated patch technique. I_{HERG} was measured as tail current at -40 mV following a 400 ms voltage step from -80 mV to 20 mV. Cells were superfused with Tyrode's solution (NaCl 140, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, Hepes 5, glucose 10, pH 7.4). The electrode filling solution consisted of 130 KCl, $MgCl_2$ 1, EGTA (K+) 5, ATP (Mg) 5, Hepes 10, at pH 7.2. Imipramine (0.1 - 100 μM) was added to the superfusion solution for 3-5 minutes. All experiments were performed at room temperature.

Imipramine produced a concentration-dependent current decrease (IC_{50} 3.4 ± 0.4 μM, n = 5) which was completely reversible (only in perforated patch recordings) at all concentrations except 100 μM. Maximal block of 98% ± 1% of the control current of I_{HERG} could be achieved with 30 μM imipramine. Imipramine (3 μM) reduced the tail current following depolarisation to 0 mV by 34% ± 10%, and after depolarisation to +40 mV by 49% ± 5% (n = 5), but this voltage dependence was not significant ($P > .1$). This suggests that clinically relevant concentrations of imipramine can inhibit the current mediated by HERG, and may potentially play a role in the observed cardiovascular actions of TCAs.

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78P MODULATION OF α7-CONTAINING NICOTINIC RECEPTOR EXPRESSION IN SH-SY5Y CELLS AND PRIMARY HIPPOCAMPAL CULTURES

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α7-Type nicotinic acetylcholine receptors (AChRs), specifically identified with [^{125}I]-α-bungarotoxin (α-bgt) binding, are upregulated by chronic nicotine *in vivo* (Collins *et al.*, 1990) and *in vitro* in primary rat hippocampal cultures (Barrantes *et al.*, 1995) and the SH-SY5Y human neuroblastoma cell line (Peng *et al.*, 1997). In sympathetic neurones, α7-nicotinic AChRs are upregulated by KCl depolarisation through a Ca^{2+} dependent mechanism (De Koninck and Cooper 1995). This study aims to explore the cellular pathways underlying the modulation of α-bgt binding sites in SH-SY5Y cells and in primary hippocampal neurones.

[^{125}I]-α-Bgt binding was measured after four days in the presence or absence of drugs applied to confluent SH-SY5Y cultures. Results demonstrate that the α7 nicotinic AChRs are upregulated by 37 ± 5% and 51 ± 29% by nicotine (10μM; n=18) and KCl (20mM; n=9) respectively. Upregulation by KCl was prevented in the presence of 1-[*N,O*-bis-(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine, KN-62 (5μM), a specific Ca^{2+}-calmodulin-dependent protein kinase II (CaM-Kinase II) inhibitor, and in the presence of the L-type Ca^{2+} channel blocker, verapamil (5μM). KN-62 or verapamil alone produced no significant differences from control. In contrast, upregulation elicited by nicotine was unaffected by these drugs: upregulation of 40 ± 15% in the presence of nicotine and KN-62 (n=5) and 44 ± 16% in the presence of nicotine and verapamil (n=4), Figure1a.

Modulation of [^{125}I]-α-bgt binding was also investigated in E18 primary hippocampal cultures. All drugs were applied to mature cultures for four days before being assayed. Preliminary results parallel those obtained with the SH-SY5Y cells with respect to the action of KN-62 and verapamil on the upregulation elicited by nicotine and KCl. Figure 1b. Another novel nicotinic agonist 3-[(4-dimethylamino) cinnamylidene] anabaseine maleate, DMAC, upregulated [^{125}I]-α-bgt binding in a dose-dependent manner. At 1μM no upregulation was observed, however, when DMAC was applied at 10μM, a significant 25 ± 1% increase from control binding was observed, Figure 1b.

These data suggest that upregulation produced by nicotine and by KCl is the result of different cellular mechanisms which operate in both primary hippocampal neurones and a human neuroblastoma cell line.

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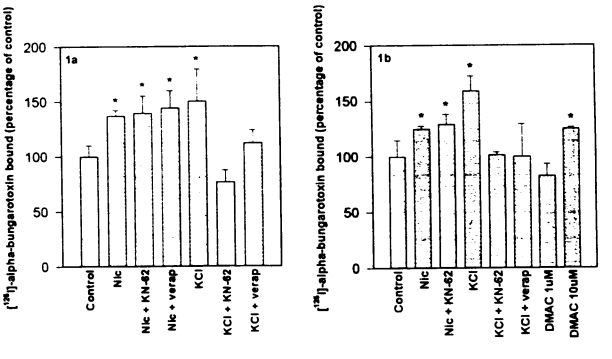

Figure 1a. The effects of KN-62 and verapamil (verap) on nicotine (nic) and KCl mediated upregulation of [^{125}I]-α-bgt binding sites in the SH-SY5Y cell line. The results are expressed as mean ± s.e. mean of at least 4 independent assays. **1b.** Modulation of α7-type nicotinic AChRs by nicotine, KCl and DMAC in primary hippocampal cultures. The results are expressed as mean ± s.e. mean of at least 3 independent results. Significantly different from control, *p<0.05, (one way ANOVA).

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79P PROTEIN SYNTHESIS DEPENDENCE OF NICOTINE MEDIATED UPREGULATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE M10 CELL LINE

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Chronic nicotinic upregulates high affinity nicotinic acetylcholine receptors (nicotinic AChRs) composed of α4 and β2 subunits in mammalian brain. Similar upregulation has been shown *in vitro* using the M10 cell line (Whiteaker *et al.*, 1998) (mouse fibroblasts stably transfected with chick α4 and β2 subunits under the control of a dexamethasone inducible promoter) and the K177 cell line (HEK-293 cells stably transfected with human α4 and β2 subunits – Gopalakrishnan *et al.*, 1997). The mechanism underlying upregulation remains unclear but it is probably post-transcriptional as mRNA levels of constituent subunits are unchanged (Peng *et al.*, 1994). Peng *et al.* ,1994 found in the M10 cell line chronic nicotine was able to stabilise nAChR levels in the presence of the protein synthesis blocker, cycloheximide implying that upregulation is due to a decrease in receptor turnover. Additionally Gopalakrishnan *et al.* suggested that upregulation may in part require *de novo* protein synthesis as cycloheximide blocked upregulation in the K177 cell line. This study addresses nicotine mediated upregulation in the M10 cell line and its dependence on post-transcriptional mechanisms following blockage of protein synthesis by cycloheximide.

M10 cells were cultured in DMEM supplemented with 10% fetal calf serum, 100U/ml penicillin, 100μg/ml streptomycin and 0.5g/L geneticin. Receptor levels were assayed by binding of 500pM [^{3}H]-epibatidine.

The induction of nAChR by 100 nM dexamethasone was followed over an 84 hour timecourse in the presence or absence of 5μM (-)-nicotine. In the presence of dexamethasone alone, receptor levels reached a maximum of 284±61.1 fmol/mg protein (n=3) at 40 hours while chronic nicotine increased receptor levels to a maximum of 581.0 ± 58.2 fmol/mg protein (n=3) at 60 hours.

To establish the dose-response relationship of upregulation M10 cells were treated with nicotine (10nM-300μM) over 48 hours. Following extensive washing to remove nicotine nAChR levels were assayed. The upregulation observed had an EC_{50} of 1.5±0.4 μM and was maximum at 353±18.7 % of control cells treated with dexamethasone alone.

Post-transcriptional effects which may mediate upregulation were examined by utilising cycloheximide and a maximally upregulating concentration of nicotine (50μM). M10 cells were induced for 24 hours with 1μM dexamethasone and a group of cells were assayed for nAChRs. Another group was induced for a further 24 hours with 1μM dexamethasone alone (control) or 1μM dexamethasone supplemented with 50μM (-)-nicotine, 50μM (-)-nicotine + 35μM cycloheximide or 35μM cycloheximide. Following washing to remove added drugs nAChR levels were assayed. Exposure to cycloheximide under these conditions resulted in a small decrease in total protein levels of 22%. Chronic nicotine increased nAChR levels to 192±11% (n=12) of control. Cells treated with nicotine/cycloheximide and cycloheximide alone had values of 100.7±10.8% (n=12) and 84.5±4.7% (n=12) of control, respectively, which were significantly different from the value obtained with the nicotine treated cells (Tukey test following one way ANOVA, $p<0.05$). The level of nAChR expression in the presence of cycloheximide were not significantly different from those measured after 24 hours induction, ie. at the start of the drug treatment. Thus it appears that cycloheximide has prevented most of the nicotine-induced upregulation, suggesting that upregulation requires translation of pre-existing mRNA. This questions the proposed role of metabolic stabilisation in nAChR upregulation (Peng *et al.*, 1994).

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80P EVIDENCE THAT PHENYLALANINE 481 IS A SUBSTRATE-CONTACT RESIDUE IN THE ACTIVE SITE OF CYTOCHROME P450 2D6

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Since cytochrome P450 2D6 (CYP2D6) is an important polymorphic human drug metabolising enzyme, responsible for the oxidation of many commonly prescribed drugs, there is considerable interest within academia and the pharmaceutical industry to develop a predictive model of the active site of the enzyme. Currently, due to the absence of a crystal structure of a eukaryotic cytochrome P450, such predictive models must be based on homology modelling techniques. Such a homology model of CYP2D6 (Ellis *et al.*, 1996) has intimated a role for phenylalanine 481 (Phe^{481}) as a substrate-contact residue in the active site by participating in a π-π interaction with the aromatic ring of substrates.

We have tested this hypothesis by generating a range of CYP2D6 mutants substituting a variety of amino acids (tyrosine, leucine, glycine) at this site. Substitution of Phe^{481} with tyrosine ($Phe^{481}Tyr$), a polar residue retaining an aromatic moiety, had little influence on the kinetics of formation of α-hydroxy (α-OH) and O-demethyl (ODM) S-(-)-metoprolol by microsomes prepared from yeast cells expressing recombinant enzyme (wildtype: α-OH, Km 27 ± 8 μM; Vmax 1.6 ± 0.3 pmol/min/pmol P450; ODM, Km 26 ± 8; Vmax 3.5 ± 0.2; $Phe^{481}Tyr$: α-OH, Km 28 ± 9; Vmax 1.8 ± 0.4; ODM, Km 27 ± 10; Vmax 4.6 ± 0.6 [mean ± SD, n=3]. In contrast, substituting Phe^{481} with leucine, an aliphatic side chain, or glycine, increased significantly the Km of each reaction 5-fold ($Phe^{481}Leu$: α-OH, 135 ± 18 μM; ODM, 120 ± 34; $Phe^{481}Gly$: α-OH, 111 ± 16; ODM, 124 ± 5; $P < 0.001$). However, the Vmax values for each oxidation were not markedly altered compared to wildtype values. Consequently, the respective specificity constants [(Vmax/Km) x 10^{-3}], measures of *in vivo* intrinsic clearance, for the α-hydroxylation and O-demethylation of metoprolol by wild-type (58 and 132) and $Phe^{481}Tyr$ mutant (65 and 172) enzymes were 6- to 9-fold greater compared to $Phe^{481}Leu$ (7 and 24) and $Phe^{481}Gly$ mutant enzymes (9 and 19 μl/min/pmol P450).

The $Phe^{481}Leu$ and $Phe^{481}Gly$ mutations also influenced the enantioselective oxidation of metoprolol. For example, with the wildtype enzyme, the ODM reaction was distinctly R enantioselective (R/S ratio, 1.5) while the $Phe^{481}Leu$ mutant was not enantioselective (R/S, 1.0) and the $Phe^{481}Gly$ mutant showed a preference for the S-enantiomer (R/S, 0.7). Such a shift in enantioselectivity was not observed with the $Phe^{481}Tyr$ mutant (R/S, 1.6). While the αOH reaction was S-enantioselective in all cases, the $Phe^{481}Leu$ and $Phe^{481}Gly$ mutants showed a greater preference for the S-enantiomer (R/S, 0.4) compared with the wildtype and $Phe^{481}Tyr$ mutant (R/S, 0.6). The influence of the Phe^{481} substitutions on the regioselective hydroxylation of R- and S-metoprolol (ODM/αOH ratio) was less marked.

In conclusion, these results are consistent with the proposal that Phe^{481} is a substrate-contact residue within the active site of CYP2D6. Furthermore, the data suggest that the planar, aromatic nature of the residue, as opposed to hydrophobicity, polarity or size, defines the interaction with substrates at this position. This is consistent with the proposed π-π interaction between the aromatic moiety of the residue and that of the substrate.

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Ellis, S.W., Rowland, K., Ackland, M.J. *et al.* (1996) Biochem. J. **316**, 647-654.

81P ACTIONS OF DOPAMINE AND A68930 ON BHK CELLS CO-TRANSFECTED WITH D_1 AND D_2 RECEPTORS

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D_1-Like and D_2-Like dopamine receptors are G-protein coupled receptors known to stimulate and inhibit adenylyl cyclase respectively. D_1-Like receptors mediate these effects through activation of G_s G-protein while D_2-Like receptors activate G_i G-protein. A68930 (Kebabian et al., 1992) is classified as a full efficacy D_1-selective agonist and as such is widely used in behavioural experiments to elucidate D_1 receptor function. This study examined the activity of A68930 in baby hamster kidney (BHK) cells transfected singly with the D_1 dopamine receptor or co-transfected with both D_1 and D_{2S} (short form) dopamine receptors.

Membranes were prepared from D_1/BHK and D_1/D_2/BHK cells grown in culture. $D_1(D_2)$ receptor characteristics were studied in radioligand binding experiments using ^{3}H-SCH23390 (^{3}H-spiperone) and 10μM flupenthixol (domperidone) to determine non-specific binding. G-protein activation was investigated by measuring the binding of ^{35}S-GTPγS (Lawlor et al., 1995). Changes in cAMP levels were measured using a cAMP protein binding method.

The D_1 and D_2 receptor density was 1340 ± 136 and 2830 ± 304 fmol/mg protein respectively in D_1/D_2/BHK cell membranes. In D_1/BHK cell membranes the D1 receptor density was 523 ± 27 fmol/mg protein. Dopamine (10nM-0.1mM) and A68930 (0.1nM-10μM) caused dose-dependent increases in the binding of ^{35}S-GTPγS in D_1/BHK and D_1/D_2/BHK membranes. In D_1/BHK cells 1μM A68930 increased binding by 26.3 ± 3.0 % (n=5) over basal binding levels. This increase was blocked by the D_1-selective antagonist SCH23390 (5.3 ± 1.1 %, n=3, $p<0.01$, t-test) but was unaffected by the D_2-selective antagonist eticlopride (27.6 ± 3.8 %, n=3). In D_1/D_2/BHK cells the responses to dopamine and A68930 were partially antagonised by SCH23390 and eticlopride (Table 1).

		1μM dopamine	1μM A68930
GTPγS	Control	119.7 ± 4.2	118.3 ± 5.4
% increase	+1μM SCH23390	38.6 ± 1.6***	43.1 ± 2.5***
over basal	+1μM eticlopride	40.1 ± 3.2***	48.1 ± 5.3***
cAMP	Control	1081 ± 95	1678 ± 95
pmoles/	+1μM SCH23390	396 ± 62**	393 ± 21**
mg protein	+1μM eticlopride	1650 ± 116*	1695 ± 289

mean±sem, n=3, *p<0.05, **p<0.01, ***p<0.0001, unpaired t-test

In intact cells, dopamine (1nM-10μM) and A68930 (0.1nM-10μM) increased production of cAMP from a basal level of 85 ± 6 fmol/mg protein in a dose-dependent manner. The response to 1μM dopamine was antagonised by SCH23390 and increased by eticlopride. The response to 1μM A68930 was blocked by SCH23390 but unaffected by eticlopride (Table 1).

These results indicate a potent D_2 component in the activation of G-proteins by A68930 in D_1/D_2/BHK co-transfected cells. The D_2 action of A68930 was not associated with inhibition of adenylyl cyclase.

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82P VALIDATION OF A HIGH THROUGHPUT SCREEN TO DETECT POTENTIAL MODULATORS OF Ca^{2+}-RELEASE-ACTIVATED Ca^{2+} CHANNELS IN JURKAT T-CELLS

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Although the biophysics of Ca^{2+}-release-activated Ca^{2+} (CRAC) channels in T-cells have been well characterised (see Fanger *et al.*, 1997), their pharmacology is poorly defined. In the absence of potent, selective ligands in this area, we set out to develop and validate a high throughput screen capable of detecting potential CRAC channel modulators. This involved a comparison of thapsigargin (Tg)-induced increases in intracellular calcium ($[Ca^{2+}]_i$) in two different fluorimeters: a low throughput, cuvette-based FluoroMax™ system (SPEX), and a high throughput, 96 well plate-based FLIPR® (Molecular Devices).

Changes in $[Ca^{2+}]_i$ were measured at 37°C in the FluoroMax™ assay using FURA-2 loaded Jurkat T-cells (clone E6.1) in suspension. In the FLIPR® assay, changes in $[Ca^{2+}]_i$ were measured at 22°C using FLUO-3 loaded Jurkat T-cells adhered by centrifugation to the bottom of wells in a 96 well plate. A standard, Na^+-rich, HEPES-based buffer (pH 7.3) with 0.9 mM Ca^{2+} and 0.5 mM Mg^{2+} was used in both assays. Tg and econazole were dissolved in DMSO, but the maximum final DMSO concentration in either assay was 0.2%, a concentration which alone had no effect on Tg responses. All additions were made 30-60 s before Tg. Data sets (mean ± s.e.m. (n)) were compared using one-way ANOVA, if appropriate, followed by an unpaired, two-tailed, Student's *t*-test (* $p<0.05$ versus corresponding Tg control).

Non-cumulative concentration-response curves to Tg produced concentration-related increases in $[Ca^{2+}]_i$ with a $p[A]_{50}$ of 8.2 ± 0.1 (5) in the FluoroMax™ and 6.5 ± 0.1 (5) in the FLIPR®. On the basis of these data, 100 nM Tg and 3 μM Tg was used as the standard stimulus in the FluoroMax™ and FLIPR®, respectively. As shown in tables 1 and 2, the standard Tg concentration evoked an increase in $[Ca^{2+}]_i$ which could be significantly inhibited by extracellular Ca^{2+} chelation (1 mM EGTA) in both systems. Further evidence of the influx component was obtained by depolarising the T-cell membrane potential with K^+-rich buffer (equimolar substitution of K^+ for Na^+). This significantly inhibited the Tg response in both assays. Tg responses in high K^+ buffer could, however, be returned to control levels by increasing the extracellular calcium concentration from 0.9 mM to 10.9 mM.

It was important that the FLIPR® could detect the inhibitory activity of known CRAC channel modulators like econazole and La^{3+}. Each agent (10 μM) significantly inhibited the Ca^{2+} influx component of the Tg response in both fluorimeters.

The results suggest that despite the differences between these assays, the high throughput, FLIPR®-based assay is a viable way to detect and quantify CRAC channel modulators.

Table 1. FluoroMax™ Data (Increase in $[Ca^{2+}]_i$ (nM))

CONTROL		TREATMENT	
Tg	677 ± 25 (6)	Tg + EGTA	78 ± 6 (6)*
Tg	594 ± 101 (4)	Tg in K^+-rich	169 ± 15 (4)*
Tg	594 ± 101 (4)	Tg in K^+-rich+ high Ca^{2+}	607 ± 87 (4)
Tg	658 ± 59 (5)	Tg + econazole	144 ± 17 (5)*
Tg	891 ± 169 (4)	Tg + La^{3+}	261 ± 20 (4)*

Table 2. FLIPR® Data (Increase in $[Ca^{2+}]_i$ (fluorescence units))

CONTROL		TREATMENT	
Tg	30047 ± 1596(5)	Tg + EGTA	12241± 906(5)*
Tg	13036 ± 180 (5)	Tg in K^+-rich	6875 ± 325 (5)*
Tg	13036 ± 180 (5)	Tg in K^+-rich+ high Ca^{2+}	16074 ± 729 (5)
Tg	17806 ± 952 (5)	Tg + econazole	11966±1310(5)*
Tg	17806 ± 952 (5)	Tg + La^{3+}	3169 ±1102(5)*

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83P EFFECTS OF KN-62, Ro-31-8220, Mo^{2+}, Ni^{2+} AND Co^{2+} ON ATP^{4-}-STIMULATED RESPONSES IN RAT PAROTID SALIVARY GLANDS *IN VITRO*

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ATP^{4-} elicits amylase secretion in rat parotid acini via stimulation of $P2X_7$ receptors and elevation of intracellular free Ca^{2+} ($[Ca^{2+}]_i$). We have investigated the effects of protein kinase inhibitors and divalent cations on these responses. The kinase inhibitors employed were KN-62 which, in addition to its inhibition of Ca^{2+}-calmodulin-dependent protein kinase II (PKII) at micromolar concentrations, has previously been reported to act in lymphocytes as a selective $P2X_7$ receptor antagonist at nanomolar concentrations (Gargett & Wiley, 1997) and the protein kinase C inhibitor 3-[1-[3-(amidinothio)propyl-1H-indoyl-3-yl]-3-(1-methyl-1H-indoyl-3-yl) maleimide methane sulphonate (R0-31-8220). The divalent cations used were Mn^{2+}, Ni^{2+} and Co^{2+} which have previously been proposed as allosteric modulators of ATP^{4-} binding to a recombinant $P2X_7$ receptor (Virginio *et al.*, 1997).

$[Ca^{2+}]_i$ was measured in suspensions of collagenase-dispersed rat parotid acini by fura-2 fluorescence and amylase release measured from parotid pieces incubated in the presence of 30-100μM ATP^{4-} or 100μM acetylcholine iodide (ACh). Preincubation of acini for 30 min. with either 170nM or 10μM KN-62 had no significant effect on basal $[Ca^{2+}]_i$ (control, 282±22nM (4); 170nM KN-62, 312±59nM (4); 10μM KN-62, 308±33nM (4)) or on 30μM ATP^{4-} (control increase in $[Ca^{2+}]_i$, 536±56nM (4); 170nM KN-62 plus ATP^{4-}, 626±53nM (4); 10μM KN-62 plus ATP^{4-}, 554±39nM (4)) or 100μM ACh-stimulated (control increase in $[Ca^{2+}]_i$, 674±110nM (5); 170nM KN-62 plus ACh, 703±147nM (4); 10μM KN-62 plus ACh, 682±96nM (4)) (mean±S.E.M., (n)) elevation of $[Ca^{2+}]_i$. but 10μM KN-62 significantly decreased ATP^{4-}-stimulated amylase release by 59±12% (n=5, $P<0.05$). In similarity, ATP^{4-}-stimulated amylase release was marginally, but significantly reduced by 29±10% (n=5, $P<0.05$) in parotid pieces preincubated for 30 min. with 500nM Ro-31-8220.

Preincubation of acini with 500μM $MnCl_2$ for 30 s. prior to application of 30μM ATP^{4} reversed the ATP^{4-}-stimulated increase in fura-2 fluorescence in 5 of 6 preparations such that application of ATP^{4-} was accompanied by decreased fluorescence instead of the usual increase. Preincubation with 1mM $NiCl_2$ decreased the response to ATP^{4-} by 72±12% (n=4, $P<0.05$) but did not reverse it and preincubation with 250μM $CoCl_2$ had no effect on the stimulated fluorescence change (121±25% of control) (n=4).

We conclude from these data that, unlike its effect in human lymphocytes, KN-62 is not a $P2X_7$ antagonist at nanomolar concentrations in rat parotid acini although, at higher concentrations, it may inhibit ATP^{4-}-stimulated secretion at a site downstream of Ca^{2+} mobilisation (presumably through its effect at PKII). The inhibition of secretion by Ro-31-8220 supports an involvement of protein kinase C in the secretory pathway. Our data from the effects of divalent cations on fura-2 fluorescence support modulation of the $P2X_7$ receptor by Mn^{2+} and Ni^{2+} but not by Cd^{2+}.

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84P PROTEIN KINASE ACTIVITIES IN RAT BRAIN FOLLOWING EXPOSURE TO ANTIDEPRESSANT DRUGS OF DIFFERENT CLASSES

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The delay in the onset of the clinical action of antidepressant drugs may be due to a beneficial neuroadaptive process provoked by drug exposure. Among several candidates for this role, the serine-threonine protein kinases have been suggested (Shelton *et al.* 1996), as they are intimately involved in synaptic transmission. Investigations of protein kinase activities after antidepressant treatment have yielded conflicting results and have been restricted in scope. We have investigated the effects on protein kinase activites in rat brain of continuous chronic exposure to a member of each class of antidepressant drugs.

Under methoxyflurane anaesthesia, male Sprague-Dawley rats (250-300g, n = 6-10 in each group) were implanted in the subcutaneous dorsum with Alzet® osmotic mini-pumps set to deliver 5μl/h for a 14 day period. Drugs used were tranylcypromine (TCP; 1mg/kg/day), imipramine (IMI; 10mg/kg/day), both dissolved in water, and fluoxetine (FLU; 10mg/kg/day) dissolved in 25% DMSO. Controls received pumps containing saline or 25% DMSO in saline. Animals were sacrificed 24-48 h after the end of the infusion period and the brains rapidly removed for further dissection into cortex and hippocampus. The cyclic AMP dependent protein kinase (PKA) activity of the hippocampus was determined by the method of Nestler and Tallman (1988), and that of protein kinase C (PKC) in the cerebral cortex by the method of Mann *et al.* (1995).

As shown in Table 1, PKC activity was significantly decreased in the soluble fraction after exposure to both TCP and IMI ($P<0.001$, ANOVA) but was slightly increased after FLU ($P<0.005$, Student's t). In contrast, the PKC activity of the particulate fraction was significantly increased by TCP ($P<0.01$) and IMI ($P<0.05$), but was not significantly affected by FLU. PKA activity of the soluble fraction of homogenate was significantly increased after TCP and IMI treatment ($P<0.01$) but unaffected by FLU. PKA activity in the particulate fraction was not significantly affected by any drug.

PKA and PKC activities appear to be modified independently of each other, and are primarily affected by drugs that are not selective for serotonergic synapses.

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Table 1 Protein kinase activities of cortex and hippocampus (Results are expressed as mean ± s.e.mean)

	PKC Activity (pmol phosphate/mg protein)				PKA Activity (pmol phosphate/mg protein)			
Fraction	Control	TCP	IMI	FLU	Control	TCP	IMI	FLU
Soluble	4.2±0.4	2.6±0.2	2.4±0.2		0.28±0.03	0.68±0.05	0.66±0.06	
	3.2±0.2			4.2±0.3	0.46±0.06			0.57±0.08
Particulate	0.49±0.03	0.89±0.1	0.87±0.09		0.35±0.05	0.32±0.04	0.41±0.03	
	0.22±0.06			0.18±0.02	0.48±0.05			0.33±0.02

85P INSULIN-INDUCED MODULATION OF AGONIST ACTION AT THE HUMAN ADENOSINE A_1 RECEPTOR IN CHO CELLS EXPRESSING BOTH INSULIN AND HUMAN ADENOSINE A_1 RECEPTORS

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It has been known for some time that intracellular crosstalk occurs between the human adenosine A_1 receptor and G_q-coupled G protein coupled receptors in cultured cells (Dickenson and Hill, 1993; Dickenson and Hill, 1996). It is also known that the insulin receptor tyrosine kinase can phosphorylate G protein coupled receptors (Baltensperger *et al.*, 1996). In this study we have examined the effect of insulin on the dose-dependent N^6-cyclopentyladenosine (CPA)-induced accumulation of [^{3}H]-phosphoinositides in CHO cells expressing both insulin- and human adenosine A_1 receptors.

CHO cells, stably expressing the human insulin receptor (gift from J. Tavare) were transfected with the expression vector pZEOSV (Invitrogen) containing the human adenosine A_1 receptor cDNA. Recombinant clones were isolated and screened for using CPA-dependent [^{35}S]GTPγS binding. A single clone was selected for further study. Cells were grown in 24-well plates in DMEM/F-12 medium containing 10 % (v/v) FCS, 2 mM L-glutamine, 100 μg/ml zeocin and 500 μg/ml geneticin. Cells were labelled with [^{3}H]-*myo*-inositol for 24 hours prior to the measurement of total [^{3}H]-inositol phosphate (IP) accumulation. This was performed using the method of Megson *et al.*, (1995). All data are expressed as mean ± s.e.mean, $n \geq 3$. Statistical analyses were performed by paired t-test.

A concentration-dependent increase in the accumulation of IP was produced by CPA. This was antagonised by pre-incubation with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an adenosine A_1 receptor antagonist, resulting in a decrease of the -log EC_{50} from 8.02 ± 0.20 (n=4) in the absence of DPCPX, to 6.62 ± 0.18 in the presence of DPCPX. CPA concentration-response experiments were also performed in the absence and presence of the tyrosine kinase inhibitors genistein and tyrphostin A47 (TA47), as well as porcine insulin (50 μM, 50 μM and 100 nM respectively). TA47 had no effect on the CPA-dependent accumulation of IP. However, in the presence of genistein the maximal response was significantly increased when compared to CPA alone (Table 1). Conversely, in the presence of insulin the maximal response was significantly reduced without significantly altering the EC_{50} (Table 1). In addition, insulin had no direct effect on IP accumulation.

Table 1. Effect of genistein, tyrphostin A47 and insulin on CPA-induced IP accumulation

	genistein (n=7)	TA47 (n=5)	insulin (n=5)
		-log EC_{50}	
CPA only	8.25 ± 0.17	7.44 ± 0.26	7.94 ± 0.45
% response*	70.2 ± 11.2	78.0 ± 8.9	54.0 ± 12.0
CPA + drug	7.85 ± 0.09$^+$	7.82 ± 0.11	8.22 ± 0.29
% response	124.0 ± 25.0$^+$	72.1 ± 11.8	39.5 ± 4.4$^+$

(*% response compared to 100 μM ATP; $^+$ statistically significant difference ($p<0.05$) when compared to CPA only).

In summary, the data presented demonstrate that the tyrosine kinase inhibitor genistein potentiates the CPA response through the adenosine A_1 receptor, while tyrphostin A47 had no effect. This may be because tyrphostin A47 is unable to inhibit the insulin receptor tyrosine kinase (Yaish *et al.*, 1988). Furthermore, insulin receptor stimulation attenuates IP accumulation induced by CPA via the adenosine A_1 receptor.

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86P Zn^{2+} MODULATION OF THE 5-HT_3 RECEPTOR

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Zn^{2+} is known to be present in nerve terminals at concentrations up to 300μM (Frederickson *et al.*, 1983) and can be released from these terminals as a result of stimulation (Howell *et al.*, 1984). It therefore has the potential to modulate neurotransmitter receptor function. 5-HT_3 receptors, like several other ligand-gated ion channels, can be modulated by a variety of divalent metal cations, and there are reports that Zn^{2+} can both enhance and/or inhibit 5-HT_3 receptor function (Gill *et al.*, 1995; Lovinger, 1991). Here we have used both electrophysiology and ligand binding to examine the effects of Zn^{2+} on recombinant 5-HT_3 receptors.

HEK 293 cells were stably transfected with 5-HT_3 receptor cDNA cloned from N1E 115 neuroblastoma cells, and 5-HT_3 receptor currents were examined using whole cell voltage clamp and radioligand binding as previously described (Hargreaves *et al.*, 1996), except that in the whole cell voltage clamp experiments Ca^{2+} and Mg^{2+} were nominally absent from extra-cellular solutions.

Zn^{2+} had multiple effects on the 5-HT_3 receptor function. 1,10 and 100μM Zn^{2+} caused an increase in maximum current (I_{max}) of 8.9 ± 2.2% (n=4), 22.5 ± 3.6% (n=4) and 22.1 ± 9.1% (n=3) respectively. 1 and 10μM Zn^{2+} changed the EC_{50} from 1.96 ± 0.08μM (n=4) to 1.06 ± 0.27μM (n=4) and 0.96 ± 0.05μM (n=4) respectively. The pEC_{50} in 100μM Zn^{2+} was not significantly different to control (2.97 ± 1.67μM, n=3). 1 and 10μM Zn^{2+} also caused a decrease in the rate of desensitisation, $t_{1/2}$ values were 145.5 ± 15% (n=12) and 175.9 ± 29% (n=12) of control respectively. Radioligand binding studies in the presence and absence of 10μM Zn^{2+} revealed no change in the affinity of either agonist ([^{3}H]meta-chlorophenylbiguanide; mCPBG) or antagonist ([^{3}H]-granisetron) binding sites, but did show an increase in the maximum number of [^{3}H]mCPBG binding sites (131± 14.5%, n=6, $p<0.05$, paired t-test) in the presence of Zn^{2+}; no change in B_{max} was observed for [^{3}H]granisetron binding sites.

The results suggest that Zn^{2+} can modulate a number of characteristics of 5-HT_3 receptor function, with a site of action that is probably distinct from, but allosterically linked to, the ligand binding site.

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87P EFFECTS OF PDE INHIBITION ON *IN VITRO* DESENSITIZATION OF TRACHEAL β-ADRENOCEPTOR FUNCTION

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β_2-adrenoceptor-mediated relaxation responses of guinea-pig trachea undergo desensitization when incubated with isoprenaline (Fernandes *et al.*, 1988; Matran *et al.*, 1989). However, airways smooth muscle β_2-adrenoceptors appear to be more resistant to desensitization than those on mast cells, which explains why tolerance does not develop to the bronchodilator actions of β-agonists (Barnes, 1995). One reason for this differential desensitization is suggested to involve phosphodiesterase (PDE), the activity of which may increase during chronic exposure and thus explain the reduced sensitivity to β-agonists (Giembycz, 1996). In this study we examine this hypothesis by determining desensitization of tracheal responses in the absence and presence of PDE inhibitors.

Guinea-pig isolated tracheal spirals were set up in Krebs-bicarbonate solution at 37°C gassed with 5% CO_2 in oxygen. Atenolol (10μM) was present throughout to block β_1-adrenoceptors. Isometric tension was recorded and after equilibration, tone was induced with carbachol (16μM). A cumulative concentration-response curve (CRC) to (-)-isoprenaline was then 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 washed over 1 h and, after contracting with carbachol, a second CRC for isoprenaline was obtained. In control experiments, the 1 h washout followed the first CRC and the tissues were sham incubated for 4 h. PDE inhibitors were added at the first curve maximum and were present with isoprenaline until the 1 h washout. Responses were measured as the fall in tension, pre-incubation responses being corrected from the controls. Relaxation responses were plotted as a percentage of the carbachol-induced contraction. 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 0.14(0.09-0.21) to 0.22(0.17-0.27)μM (n=8). In the presence of the non-selective PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX, 1μM), there was still a significant ($P<0.05$) shift of the CRC by isoprenaline incubation. The EC50 was increased from 0.18(0.08-0.34) to 0.32(0.15-0.41)μM (n=6). In the presence of the PDE3-selective inhibitor, SKF95654 (1μM), isoprenaline incubation also significantly ($P<0.05$) shifted the CRC to the right (EC50 values, 0.17(0.08-0.34) and 0.32(0.16-0.66)μM) (n=7). Similarly, in the presence of the selective PDE4 inhibitor, rolipram (0.1μM), there was a rightwards shift of the curve and the EC50 value was significantly ($P<0.05$) increased from 0.08(0.06-0.11) to 0.23(0.17-0.31)μM (n=8). As a measure of the effectiveness of rolipram in inhibiting PDE in this tissue, isoprenaline was examined in its presence. The CRC was significantly shifted to the left indicating the expected potentiation of the responses.

These results indicate that incubation with isoprenaline causes a small but significant functional desensitization of the smooth muscle β_2-adrenoceptors of guinea-pig trachea. Inhibition of PDE types 3 and 4 does not appear to modify the desensitization. Thus, since PDE was inhibited throughout the incubation, an increase in PDE activity cannot explain the reduced sensitivity to isoprenaline in this tissue.

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88P FUNCTIONAL SIGNIFICANCE OF $I_{K(N)}$ IN RAT PULMONARY ARTERY

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In pulmonary artery myocytes freshly isolated from male Sprague-Dawley rats (200 - 300g), a delayed rectifier K-current ($I_{K(V)}$) was generated at test potentials (range −80 to +50 mV) from a holding potential of -90 mV under Ca^{2+}-free, whole-cell patch-clamp conditions. After inactivation of $I_{K(V)}$ by holding at 0 mV for at least 10 min, an outward $I_{K(N)}$-like current (previously described in rabbit pulmonary myocytes; Evans *et al.*, 1996) was generated from a holding potential of 0 mV using a voltage ramp from +60 mV to −100 mV over 1.2s. This current remained in the presence of glibenclamide (10 μM) and iberiotoxin (250 nM) which were used to block K_{ATP} and BK_{Ca}, respectively. At the +50mV test potential, $I_{K(V)}$ was inhibited by 4-aminopyridine (4-AP, 1 mM; 78 ± 9%, n=6), astemizole (10 μM; 63 ± 2%, n=6), terfenadine (500 nM; 75 ± 4%, n=10), ciclazindol (10 μM; 48 ± 2%, n=6) and quinidine (100 μM; 86 ± 3%, n=4) (Walker *et al.*, 1996). $I_{K(N)}$ was sensitive to 4-AP (1 mM) and to quinidine (100 μM) which produced reductions of 43 ± 7% and 64 ± 5%, respectively in current amplitude at +60 mV (n=5). $I_{K(N)}$ was, however, insensitive to astemizole and terfenadine. Hypoxia inhibited both $I_{K(V)}$ (40 ± 6% reduction at +50 m V, n=8) and $I_{K(N)}$ (57 ± 5% reduction of the current at +60 m V, n=8). Hypoxia and 4-AP each inhibited $I_{K(N)}$ whereas when 4-AP was administered under hypoxic conditions, the amplitude of $I_{K(N)}$ returned to control levels.

4-AP (1 - 20 mM) produced an increase in tone of rat intact pulmonary artery rings whereas neither astemizole nor terfenadine (each 0.5 - 10 μM) had any functional effect. Hypoxic conditions induced a biphasic contraction in intact pulmonary artery which was insensitive to modulators of K_{ATP} and BK_{Ca} and to astemizole and terfenadine. However, 4-AP reversed the hypoxia-induced increase in tone. Collectively, these observations suggest that inhibition of $I_{K(N)}$ is a more critical factor than reduction of $I_{K(V)}$ in the phenomenon of hypoxic vasoconstriction.

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89P EFFECT OF THE P38 KINASE INHIBITOR, SB 203580, IN A MODEL OF AIRWAY INFLAMMATION

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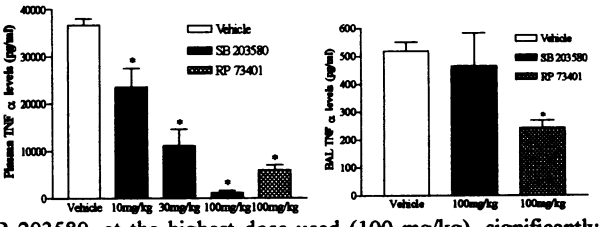
Cytokines such as IL-1ß and TNFα play a predominant role in inflammatory responses. The pyridinyl imidazoles, such as the p38 inhibitor SB 203580, have potent inhibitory effects on the production of these cytokines *in vitro* and *in vivo*, and also show anti-inflammatory activity in animal models of arthritis (Badger *et al.*, 1996). The aim of this study was to examine the activity of SB 203580 in a model of Lipopolysaccharide (LPS)-induced airway inflammation in the rat.

Male Wistar rats (250g) were orally dosed with vehicle (1% CMC, 2ml/kg) or compound 30 min prior to LPS administration (either 1.5 mg/kg, i.p. or aerosolised at 0.3mg/ml for 30 min). TNFα and IL-1ß levels in bronchoalveolar lavage (BAL) and/or plasma were assessed by ELISA 90 min (peak levels of TNFα) and 4h (peak levels of IL-1ß) post LPS. Inflammatory cell recruitment into the BAL was evaluated at 90 min and 4 h (n= 8 in each group). Supernatant from BAL samples (from vehicle and SB 203580 treated rats) was assessed for its inhibitory activity on TNFα release from cultured human monocytes that had been stimulated with LPS (2.5ng/well) for 18 h (BAL from n= 4 rats was assessed in quadruplicate). TNFα levels were determined by ELISA. p38 kinase protein expression was detected by standard Western blotting techniques. Statistical analysis was carried out using one way ANOVA with a correction for Dunnett's multiple t test (•P<0.05, •• P< 0.01).

p38 protein was present in lung homogenates but the level of expression was not affected by LPS inhalation. SB 203580 significantly reduced plasma levels of TNFα in rats pre-treated with LPS (figure). However, SB 203580 had no effect on TNFα levels (figure) or on cell recruitment in the BAL in rats 90 min post aerosolised LPS exposure. The type 4 phosphodiesterase inhibitor, RP 73401, was included as a positive control and significantly reduced TNFα levels in BAL and plasma.

However, BAL fluid from rats dosed with SB 203580 or RP 73401 significantly inhibited LPS-induced TNFα release from human monocytes compared to BAL from vehicle treated rats (83.6 %, P<0.01 and 44.5 %, P<0.05 inhibition, respectively) suggesting the presence of SB 203580 in the BAL after oral dosing.



SB 203580, at the highest dose used (100 mg/kg), significantly reduced IL-1β levels and eosinophil/ neutrophil recruitment in the BAL 4 h post aerosolised LPS exposure (table). This reduction was only seen at a high dose and so it is not clear whether the effect is due to inhibition of p38 kinase or a non-selective action of the compound.

GROUP	Eosinophil ($x10^6$/ml)	Neutrophil ($x10^6$/ml)	IL-1ß (pg/ml)
LPS alone	0.11 ± 0.02	1.34 ± 0.13	720.8 ± 146
LPS + SB 203580	0.05 ± 0.011••	0.72 ± 0.07••	234.1 ± 36.2••

In conclusion, the lack of effect of SB 203580 on TNFα levels in BAL compared to plasma is not clear but may reflect differential tissue expression of p38 kinase isoforms and their sensitivity to inhibition by SB 203580 (Kumar *et al.*,1997). Alternatively, p38 kinase may not be involved in TNFα release from the cells which generate/release TNFα in the lung.

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90P CARDIAC REACTIVITY TO ENDOTHELIN-1 (ET-1) AND U46619 IS DIFFERENTIALLY ALTERED IN ISOLATED PERFUSED HEARTS FROM CHRONICALLY-HYPOXIC (CH) RATS

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CH-induced right ventricular hypertrophy is associated with increased plasma levels of ET-1 (Li *et al.*, 1994). Cardiac reactivity to vasoactive agents such as ET-1 may be altered in CH. Therefore, we have studied the actions of ET-1 and U46619 (thromboxane A_2 receptor agonist) in isolated perfused hearts from CH rats.

CH male Wistar rats (250-270g) were kept in a chamber at PO_2 of 10% for 3 weeks. CH or control rats were anaesthetised with Sagatal (60 mg kg^{-1}, i.p.) heparinised (500 units; i.v) and hearts were isolated and perfused with Krebs' solution (gassed with 95% O_2, 5% CO_2) via the aorta using a dual-perfusion cannula at a constant pressure of 75-80 mmHg (Avkiran and Curtis, 1991). Right coronary flow (RCF) and left coronary flow (LCF) were recorded using in-line flow probes. Cling-film balloons were inserted into each ventricle to record right ventricular developed pressure (RVDP) and left ventricular developed pressure (LVDP). Hearts were allowed to stabilise for 15 min after which cumulative concentration response curves for ET-1 or U46619 were determined. Data were analysed statistically with the students' t-test and are expressed as mean ± s.e.mean).

CH significantly increased the ratios of the right ventricular to total ventricular weight when compared with control rats (0.36 ± 0.01 vs. 0.21 ± 0.01, p< 0.01, n=7). Basal RCF in CH hearts (7 ± 0.76 ml min^{-1}, p< 0.05, n=9) was significantly higher than in control hearts (4.5 ± 0.3 ml min^{-1}, n=9). Whereas LCF in CH hearts (6.4 ± 0.4 ml min^{-1}) was not different to control hearts (5.0 ± 0.33 ml min^{-1}).

In CH hearts basal RVDP (79 ± 4 mmHg, n=9, p< 0.01) was also significantly higher than that in control hearts (40 ± 4 mmHg, n=9). While, LVDP in hearts from control (110 ± 6 mmHg) or CH (125 ± 9 mmHg) animals were not different (p> 0.05).

ET-1 (0.01-3 nM) caused decreases in coronary flow in control and CH hearts. At a concentration of 1 nM ET-1 caused a similar decrease in RCF in control vs. CH hearts (52 ± 11% vs. 45 ± 7%, p> 0.05, n=4-5). It also caused LCF to decrease in control vs. CH hearts to a similar extent (79 ± 5 % vs. 70 ± 4 %, p> 0.05).

ET-1 (1nM) reduced RVDP, in CH (22 ± 6%, n=5) which was significantly less as compared to control hearts (60 ± 10%, p< 0.05, n=4). Furthermore, ET-1 (1 nM) induced a decrease in LVDP, in CH hearts (31 ± 6%, n=5) was also less than in control hearts (64 ± 7%, n=4, p< 0.05).

U46619 (1-100 nM) also produced a concentration-dependent decrease in coronary flow. At a concentration of 100 nM it produced a decrease in RCF in CH (28 ± 4%, n=4, p< 0.001) which was lower than in control animals (59 ± 2%, n=5). There was also a decrease in LCF in CH (47 ± 19%, n=4) and control (84 ± 7%, n=5), however, this difference failed to achieve significance (p=0.17).

U46619 (100 nM) caused a decrease in RVDP, in control vs. CH hearts (38 ± 6% vs. 36 ± 3%), and LVDP in control vs. CH hearts (52 ±7% vs. 35 ± 9%) these changes were not significantly different (n=4-5).

In summary, the results of this study have shown that in CH hearts basal RCF but not LCF is significantly higher when compared with control hearts. Furthermore, in CH hearts basal RVDP is significantly higher than that in control hearts, whereas, LVDP in CH is not altered. The depressant effects of ET-1 on RVDP and LVDP are significantly reduced in CH heart. In contrast the depressant effects of U46619 is not altered. However, U46619-induced effects on coronary flows were significantly reduced in CH hearts, whereas, ET-1-induced effects are not altered. These data suggest that cardiac actions of ET-1 and U46619 are altered differentially in isolated hearts from CH rats. The underlying mechanisms require further investigation.

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91P RECOMBINANT HUMAN, BUT NOT MURINE, LEPTIN CAUSES HINDQUARTERS VASOCONSTRICTION IN CONSCIOUS RATS

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In anaesthetised rats, leptin (unspecified type) given i.c.v. increases sympathetic nerve activity (SNA) and blood pressure (BP) (Dunbar *et al.*, 1997), whereas recombinant murine leptin given i.v. has no effect on BP, in spite of increasing SNA (Haynes *et al.*, 1997). Haynes *et al.* (1997) raised two unresolved possibilities that could explain the lack of a pressor effect of i.v. murine leptin:- 1) the increased SNA does not alter vascular tone, and 2) murine leptin causes diuresis, thereby opposing its vasoconstrictor effects. To distinguish between these possibilities we assessed the regional haemodynamic effects of recombinant murine leptin (R & D Systems) in conscious, male, Long Evans rats (350-450g); for comparison we studied the effects of recombinant human leptin in a separate group of animals. Animals were chronically instrumented with intravascular catheters and pulsed Doppler probes to monitor changes in renal, superior mesenteric, and hindquarters flows (all surgery was carried out under sodium methohexitone anaesthesia, 40-60 mg kg^{-1} i.p., supplemented as required). On the first experimental day, one group (n = 6) was given a primed infusion of murine leptin, and the other (n = 6) was given human leptin (both at 500 μg kg^{-1} bolus, 167 μg kg^{-1} h^{-1} infusion) for 3 h. On the second experimental day, both groups were given an infusion of saline (0.1 ml, 0.4 ml h^{-1}) for 3 h. Table 1 summarises the results. Neither murine nor human leptin affected BP; indeed, murine leptin had no significant cardiovascular effects, whereas human leptin caused hindquarters vasoconstriction. Since i.v. murine leptin causes an increase in adrenal and hindlimb SNA (Haynes *et al.*, 1997) it is feasible that its failure to affect hindquarters vascular conductance was due to adrenal adrenaline release causing β_2-adrenoceptor-mediated vasodilatation, thereby opposing SNA-mediated vasoconstriction. We tested this hypothesis by giving murine leptin in the presence of the β_2-adrenoceptor antagonist, ICI 118551 (0.2 mg kg^{-1}, 0.1 mg kg^{-1} h^{-1}), on the third experimental day (to those animals given murine leptin on the first day). Since the integrated change in hindquarters vascular conductance (-10 ± 3% h) was not different from that in the absence of ICI 118551, it appears β_2-adrenoceptor activation does not mask an hindquarters vasoconstrictor effect of murine leptin. Our observation that i.v. murine leptin does not increase BP corroborates the findings of Haynes *et al.* (1997), and is consistent with its failure to influence regional haemodynamics. However, this latter observation is puzzling against the background that i.v. murine leptin (at the dose used here) causes widespread increases in SNA (Haynes *et al.*, 1997), and human leptin causes hindquarters vasoconstriction.

Table 1. Cardiovascular changes during infusion of saline (control) or murine (m) leptin, or saline or human (h) leptin in the same conscious rats. Values show mean integrated responses (areas over curves during 3 h infusion) ± s.e. mean. HR = heart rate (beats); MBP = mean blood pressure (mmHg h); RVC, MVC, HVC = renal, mesenteric and hindquarters vascular conductance respectively (% h). * $P < 0.5$ versus corresponding control value; (Wilcoxon test) † $P < 0.05$ versus corresponding murine leptin value (Mann-Whitney U test).

Δ HR	control	-30 ± 12	control	-5 ± 3
	m leptin	-15 ± 7	h leptin	-4 ± 2
Δ MBP	control	-6 ± 3	control	-4 ± 3
	m leptin	-2 ± 1	h leptin	-3 ± 1
Δ RVC	control	-13 ± 4	control	-14 ± 6
	m leptin	-12 ± 4	h leptin	-8 ± 6
Δ MVC	control	-29 ± 9	control	-30 ± 8
	m leptin	-35 ± 8	h leptin	-34 ± 5
Δ HVC	control	-13 ± 5	control	-5 ± 3
	m leptin	-18 ± 5	h leptin	-34 ± 4*†

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92P EFFECTS OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR-1 (rhIGF-1) ON REGIONAL HAEMODYNAMICS IN CONSCIOUS RATS

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The possibility that endogenous IGF-1 may be involved in cardiovascular regulation is indicated by the occurrence of IGF-1 and its binding proteins in cardiovascular tissues (see Delafontaine, 1995, for review), and the reports that exogenous IGF-1 causes hypotension and vasodilatation in anaesthetised rats (e.g., Haylor *et al.*, 1991; Pete *et al.*, 1996; Gillespie *et al.*, 1997). Although Pete *et al.* (1996) considered such effects of IGF-1 were consistent with the previously reported hypotensive effects of insulin, we have not seen a fall in mean arterial blood pressure in response to insulin in conscious rats (Bennett *et al.*, 1989). Therefore, we investigated the cardiovascular effects of rhIGF-1 (Genentech, USA), in conscious, male, Long Evans, rats (350-450g).

Animals (anaesthetised with sodium methohexitone 40-60 mg kg^{-1} i.p.) were instrumented with intravascular catheters and pulsed Doppler flow probes to monitor changes in renal, mesenteric and hindquarters flow. Following a 30 min baseline period, conscious rats (n = 9) were given a primed, i.v. infusion of rhIGF-1 (100 μg kg^{-1} bolus in 0.1 ml, 100 μg kg^{-1} h^{-1} infusion for 60 min at 0.4 ml h^{-1}); control rats (n = 9) were given vehicle (sterile isotonic NaCl). Recordings were made for the following 6h.

Resting cardiovascular variables are shown in Table 1, as are the integrated changes. During the 60 min infusion of IGF-1 there was a significantly greater rise in mean blood pressure and significantly greater reductions in renal mesenteric and hindquarters vascular conductances than seen during saline infusion. These results are the opposite of those obtained by Gillespie *et al.* (1997), who gave a primed infusion of IGF-1 at a similar dose, but in anaesthetised rats. Thus, it is feasible that the presence of anaesthesia modifies cardiovascular responses to rhIGF-1.

Table 1. Resting cardiovascular variables before, and integrated changes (areas under or over curves 0-60 min), during infusion of saline (control) or rhIGF-1 in conscious, Long Evans rats. Values are mean ± s.e. mean; * $P < 0.05$ versus control value (Mann-Whitney U test). Units: heart rate, resting = beats min^{-1}, change = beats; mean blood pressure, resting = mmHg, change = mmHg min; vascular conductance, resting = (kHz $mmHg^{-1}$)10^3, change = % min.

		Resting	Integrated changes
Heart rate	Control	313 ± 4	-222 ± 143
	IGF-1	337 ± 9*	-521 ± 217
Mean blood pressure	Control	100 ± 1	+64 ± 27
	IGF-1	102 ± 2	+115 ± 25*
Renal vascular conductance	Control	80 ± 6	-75 ± 35
	IGF-1	92 ± 5*	-142 ± 28*
Mesenteric vascular conductance	Control	72 ± 4	-253 ± 74
	IGF-1	83 ± 6	-628 ± 122*
Hindquarters vascular conductance	Control	33 ± 3	-239 ± 148
	IGF-1	39 ± 6	-598 ± 174*

Alternatively, it could be that the IGF-1 used in previous studies contained sufficient bacterial lipopolysaccharide (derived from the *E.coli* expression system) to cause endotoxaemia, and the haemodynamic changes observed were due to that, rather than to IGF-1

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93P NO ROLE FOR ANGIOTENSIN II (AII) OR ENDOTHELIN (ET) IN THE PRESSOR RESPONSE INDUCED BY NITRIC OXIDE SYNTHASE (NOS) INHIBITION IN VASOPRESSIN-DEFICIENT, HYPERTENSIVE (DI/H) RATS

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In transgenic (m(Ren-2)27) rats, in which hypertension is dependent on both AII and ET, the pressor response to NOS inhibition with N^G-nitro-L-arginine methyl ester (L-NAME) is attenuated by AT_1-, and ET_A- and ET_B-receptor antagonism, with losartan (L) and SB 209670 (SB) (Ishak *et al.*, 1998), respectively. In the present study, we examined the effects of L and SB on the pressor response to L-NAME in vasopressin-deficient, hypertensive (DI/H) rats (Ashton and Balment, 1989), in which the effects of L or SB on mean arterial pressure (MAP) are no greater than in the normotensive control (DI/N) rats (Gardiner *et al.*, 1996).

Under sodium methohexitone anaesthesia (40-60 mg kg^{-1}, i.p., supplemented as required), male DI/H and DI/N rats (400-600 g), had i.v. and i.a. catheters implanted. The following day i.v. saline (0.1 ml) or L (10 mg kg^{-1}) and SB (bolus: 300 μg kg^{-1}; infusion: 5 μg kg^{-1} min^{-1}) were given 360 min before L-NAME (10 mg kg^{-1}, i.v.); MAP and heart rate (HR) were recorded over the subsequent 40 min.

Baseline MAP in DI/N rats (125 ± 1 mm Hg; n = 15; mean ± s.e. mean) was lower than that of DI/H rats (150 ± 3 mm Hg; n = 13; $P < 0.05$; Mann-Whitney U test), but HR was higher (DI/N, 356 ± 9 beats min^{-1}; DI/H, 296 ± 3 beats min^{-1}). Baseline MAP and HR in the 2 groups of DI/N and the 2 groups of DI/H rats were not different (Table 1). During treatment with L and SB, the fall in MAP and increase in HR in DI/H and DI/N rats were similar (Table 1). The pressor response to L-NAME was also similar in DI/H and DI/N rats, and this was unaffected by treatment with L and SB (Table 2). The bradycardic effect of L-NAME was less in DI/H than in DI/N rats in the presence of saline or L and SB, possibly due to abnormal cardiac baroreflex sensitivity.

These results extend our previous observations by showing that L and SB together have no greater hypotensive effect in DI/H than in DI/N rats. Furthermore, in contrast to our findings in hypertensive, transgenic (m(Ren-2)27) rats (Ishak *et al.*, 1998), L and SB treatment does not affect the pressor response to L-NAME in DI/H rats.

Table 1. Heart rate (HR) and mean arterial pressure (MAP) under resting conditions (control) and 360 min after administration of saline or losartan and SB 209670 (L + SB) in conscious DI/N and DI/H rats. Values are mean ± s.e. mean. *P < 0.05 vs control (Friedman's test).

	DI/N		DI/H	
	Saline (n = 8)	L + SB (n = 7)	Saline (n = 6)	L + SB (n = 7)
Heart rate (beats min^{-1})				
Control	345 ± 12	369 ± 13	295 ± 3	297 ± 6
360 min	335 ± 10	416 ± 14*	299 ± 6	328 ± 10*
MAP (mm Hg)		DI/N		DI/H
Control	125 ± 2	125 ± 2	151 ± 5	150 ± 5
360 min	127 ± 2	109 ± 3*	154 ± 4	132 ± 3*

Table 2. Integrated (area under (for MAP) or over (for HR) $curves_{0\text{-}40min}$) responses to L-NAME (10 mg kg^{-1}, i.v.) in conscious DI/H and DI/N rats, 360 min after treatment with saline or L + SB. For abbreviations and treatment groups, see Table 1. *P < 0.05 vs DI/N (Mann-Whitney U test).

	Saline	
	DI/N (n = 8)	DI/H (n = 6)
HR (beats)	2054 ± 207	1266 ± 256*
MAP (mm Hg min)	1967 ± 42	1859 ± 154
	L + SB	
	DI/N (n = 7)	DI/H (n = 7)
HR (beats)	2570 ± 317	1694 ± 165*
MAP (mm Hg min)	1666 ± 191	1906 ± 123

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94P ATP-INDUCED VASODILATATION IN THE RAT ISOLATED MESENTERIC BED EXHIBITS TWO APPARENT PHASES

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In the rat isolated mesenteric bed, ATP produces vasodilatation which is endothelium-dependent and via P_{2y}-purinoceptor activation (Burnstock & Kennedy, 1986; Raleric & Burnstock, 1988). More recently, the dilator actions of ATP, at doses of up to $1x10^{-8}$ moles, were shown to be inhibited by the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) (Rubino *et al.*, 1995). Here, using the rat isolated mesenteric bed, we have identified two components of the dilator response to ATP, which become apparent at concentrations of more than $1x10^{-8}$ moles.

Male Wistar rats (250-300g) were anaesthetised with sodium pentobarbitone (100mg.kg^{-1}; ip) and killed by cervical dislocation. The mesenteric artery was cannulated and the mesentery excised. The bed was perfused at a constant rate with Krebs' buffer, warmed to 37°C and gassed (95% O_2: 5% CO_2). Perfusion pressure, recorded via an arterial cannula, was raised to approximately 120mmHg by titration of methoxamine ($1x10^{-6}$ to $1.2x10^{-5}$M) added to the perfusate. The effects of 1-3μl volume injections of ATP ($1x10^{-11}$ to $3x10^{-7}$ moles) were recorded. In some experiments L-NAME ($1x10^{-4}$M) or the guanylyl cyclase inhibitor ODQ (1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one; $3x10^{-6}$M) were added to the perfusate. All data is shown as mean±s.e.m.

ATP induced reductions in perfusion pressure which were transient at doses of $1x10^{-11}$ to $1x10^{-8}$ moles. At doses of $1x10^{-8}$ to $3x10^{-7}$ moles a second, and more prolonged, phase in the relaxant response to ATP was observed (Figure 1). L-NAME inhibited the first phase of the ATP induced relaxant response at most concentrations (representative data at $3x10^{-10}$ moles given), (control 16.8±4.0%; n=7: + L-NAME, 8.4±1.8%; n=7). Similarly, ODQ inhibited first phase responses to ATP (control 16.8±4.0%; n=7: + ODQ 3.6±1.8%; n=4). D-NAME had no effect on first phase of ATP-induced responses (control 16.8±4.0; n=7: + D-NAME 15.3±3.4%; n=7). In contrast the second phase of the relaxant response to ATP was unaffected by ODQ (representative dose of $3x10^{-7}$ moles given), (control 42.7±8.1%; n=7: ODQ 43.9±6.3%; n=4), L-NAME (control 42.7±8.1%; n=7: + L-NAME 60.9±10.5%; n=7) or D-NAME (control 42.7±8.1%; n=7: + D-NAME 52.6±3.7%; n=7).

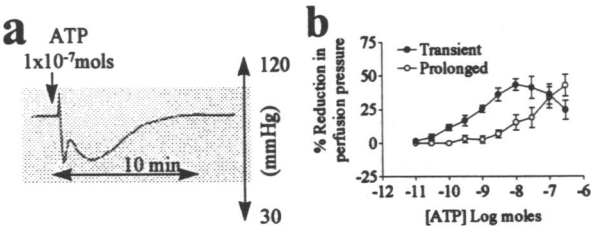

Figure 1. ATP-induced biphasic reduction in perfusion pressure in the rat isolated mesenteric bed illustrated by a) a representative trace and b) a dose-response curve (n=7).

In the rat isolated mesenteric bed ATP induces a biphasic reduction in perfusion pressure. The transient first phase is inhibited by both L-NAME and ODQ suggesting that NO is the mediator involved. However, the second phase was not affected by either of these agents indicating a dual mechanism of action for ATP in this bed.

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95P CANNABINOID (CB_1) RECEPTOR EXPRESSION IS ASSOCIATED WITH MESENTERIC RESISTANCE VESSELS BUT NOT THORACIC AORTA IN THE RAT

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Studies in mesenteric arterial beds suggest that an endocannabinoid may represent an endothelium derived hyperpolarising factor (EDHF) (Randall *et al*, 1996). The present study investigates the relationship between CB_1 receptor expression and cannabinoid induced vasorelaxations in rat mesenteric resistance arterioles and rat thoracic aorta, determined by reverse transcriptase polymerase chain reaction (RT-PCR), antibody staining and functional responses.

RT-PCR: Primary, secondary and tertiary branches of mesenteric resistance arterioles and mesenteric fat excluding arterioles were taken from male hooded Lister (n=2) and Wistar (n=10) rats. Total RNA was extracted from the samples and reverse transcribed. The resulting cDNA was amplified by polymerase chain reaction with Taq DNA polymerase using two runs of 35 amplification cycles. Each run used a different primer set. The first run utilised primers based on the rat CB_1 transcript (Genbank accession number: x55812 (Matsuda *et al*, 1990)) designed to amplify a 170 base pair (bp) sequence. The second run utilised primers designed to amplify a 140 bp sequence, nested inside the first primer set. PCR products were analysed by agarose gel electrophoresis and visualised with ethidium bromide under ultra violet light. Antibody staining: Frozen sections of rat mesenteric vascular bed and thoracic aorta were cut, slide mounted and fixed with 3.8% formaldehyde followed by acetone. The primary CB_1 cannabinoid receptor antibody (rabbit anti-human) was incubated on the slide overnight at 4°C (6 μg/ml). Visualisation of the bound CB_1 antibody was achieved using the the Vectastain Elite ABC Kit, which was used to perform the rest of the staining procedure. Functional responses: The mechanical response of rat thoracic aorta rings (n=5), preconstricted with methoxamine (50μM), to anandamide (30μM) and of isolated mesenteric vascular beds (n=6), preconstricted with methoxamine (30μM), to anandamide (10μM), was determined in the presence of indomethacin (10μM) and L-NAME (100μM).

The RT-PCR yielded the 140 bp product characteristic of the CB_1 receptor from rat mesenteric resistance arterioles. Rat mesenteric fat excluding resistance arterioles did not yield this PCR product. This suggests the CB_1 mRNA transcript was only present in mesenteric resistance arterioles. Antibody staining associated with the endothelium and also smooth muscle was observed in mesenteric vessels (50 - 500 μm diameter). Staining was not observed in the rat mesenteric fat or thoracic aorta. Anandamide failed to relax methoxamine preconstricted rat thoracic aorta (11.6% ± 10.2% reduction in tone), however anandamide did relax isolated rat mesenteric vascular beds (98.31% ± 3.2% reduction in tone). (Values are mean ± s.e. mean).

CB_1-like mRNA transcripts and CB_1-like immuno-reactivity are associated with mesenteric resistance vessels. CB_1-like immuno-reactivity is not present in rat thoracic aorta. Additionally, anandamide caused significant relaxations of rat mesenteric resistance vessels but not of rat thoracic aorta. The identification of the CB_1 receptor in resistance vasculature but not in conduit vessels correlates with anandamide's ability to induce significant vasorelaxation only in resistance vasculature. These findings suggest endocannabinoids have a role in controlling local blood flow and implicate the CB_1 receptor in this role.

Thanks to David Harris (contact address, as first author) for data on the mechanical responses of rat mesenteric vascular beds. Supported by Pharmagene Laboratories Ltd.

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96P CHARACTERIZATION OF CANNABINOID RECEPTORS MEDIATING EDHF RESPONSES IN THE RAT ISOLATED MESENTERY

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It has recently been proposed that an endogenous cannabinoid may be an endothelium-derived hyperpolarising factor (EDHF) in the rat isolated mesenteric vasculature (Randall *et al.*, 1996). We have now examined the effects of the relatively selective CB_1 cannabinoid receptor antagonist LY320135 ((6-methoxy-2-(4-methoxyphenyl)benzo[*b*]thien-3-yl)(4-cyanophenyl) methanone) (Pertwee, 1997) and the selective CB_2 cannabinoid receptor antagonist SR144528 (N-([1*s*]-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) (Rinaldi-Carmona *et al.*, 1998) on EDHF-mediated relaxations.

Male Wistar rats (300-550g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and the mesenteric arterial bed was isolated (Randall *et al.*, 1997) and perfused with oxygenated Krebs-Henseleit containing indomethacin (3μM) and L-NAME (300μM) to inhibit prostanoid and nitric oxide synthesis respectively. Following a 20min equilibrium period, methoxamine (1-2μM) was added to increase perfusion pressure by 80-100mmHg. The vasorelaxant effects of carbachol (acting via EDHF) were assessed in the absence and presence of LY320135 (2μM or 10μM) or SR144528 (100nM or 1μM). Each antagonist was added to the buffer 20min prior to construction of the dose-response curves. All data were compared with ANOVA with Bonferroni's *post hoc* test.

Carbachol induced dose-related vasorelaxations (ED_{50}=3.26± 0.57nmol, mean±s.e.mean; maximum relaxation (R_{max})= 87.0±2.5%, n=16). Addition of 2μM LY320135 (n=9) caused significant reduction of carbachol induced EDHF relaxations, with an ED_{50} value of 10.4±2.6nmol, ($P<0.01$) and an R_{max} value of 66.9±6.2% ($P<0.01$), whilst 10μM (n=7) gave further inhibition (ED_{50}=15.9±4.0nmol, $P=<0.001$, R_{max}= 34.0±4.3%, $P=<0.001$). However, SR144528 at either 100nM (n=5) or 1μM (n=5) had no effect on EDHF-mediated relaxations with respective ED_{50} values of 3.62±1.67nmol and 1.61±0.70nmol and R_{max} values of 86.8±2.5% and 89.6±3.6%. Neither LY320135 nor SR144528 had any significant effects against vasorelaxation induced by the K^+-channel activator, levcromakalim or the nitric oxide donor, sodium nitroprusside.

The results of the present study indicate that the relatively selective CB_1 antagonist, LY320135, significantly opposes EDHF-mediated relaxations whereas the CB_2 antagonist, SR144528, has no effect. This suggests that vasorelaxations mediated by EDHF are via activation of a CB_1-like receptor.

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97P EVIDENCE THAT ENDOTHELIUM-DERIVED NITRIC OXIDE PRODUCTION IS INCREASED FOLLOWING ORAL PROPYLTHIOURACIL TREATMENT IN THE RAT

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Propylthiouracil (PTU) has been used routinely in the treatment of hyperthyroidism for many years (Klein et al., 1994). However, as with other treatments, although it is effective in lowering thyroid hormone production, it also causes side effects such as hepatotoxicity and immune-mediated reactions (Baker et al., 1989). PTU-induced hypothyroidism has also been shown to cause reduced nitric oxide (NO) synthase expression in the rat hypothalamus (Ueta et al., 1995). However, as far as we are aware, the actions of PTU on vascular NO synthase expression have not been investigated. The aim of this study was to determine whether oral PTU treatment causes modulation of NO production in the rat aorta and to investigate the underlying mechanisms involved.

Male Wistar rats (300-350 g) were fed a low fat pellet diet (control) or the same diet supplemented with 0.1 % 6-n-propyl-2-thiouracil for 2 or 4 weeks. After this period the animals were killed and 3 mm rings of aorta prepared for isometric tension recording. Vessel segments were contracted with depolarising Krebs solution (118 mM KCl) to ensure their viability, then washed with Krebs solution. Cumulative concentration response curves (CRCs) were then constructed to phenylephrine (PE; 1 nM to 10 μM) in both endothelium intact and denuded rings, and in endothelium intact rings incubated with 0.1 mM N^{ω}-nitro-L-arginine (L-NOARG) for 30 min. In addition, immunohistochemical analyses were performed on cryostat sections from these vessels, using antibodies to both inducible and endothelial NO synthase. Blood samples were taken before and after PTU treatment for measurement of serum thyroxine and cholesterol.

EC_{50} and maximum response values derived by non-linear curve fitting of CRCs to PE are shown in Table 1. PTU treatment significantly reduced both maximum response and vessel sensitivity to PE, as compared to controls. Both incubation with L-NOARG and endothelial removal restored the maximum response after 2 but not 4 weeks. In addition, vessel sensitivity was restored by endothelial removal after 2, but not 4 weeks. Immunohistochemical studies suggested that the reduced responses caused by PTU were due to NO production by endothelial NO synthase. PTU also caused a slight, but significant increase ($P<0.05$, unpaired Student,s *t* test) in serum [cholesterol] after 2 (control, 1.74 ± 0.03; treated, 1.99 ± 0.08 mM) and 4 weeks (control, 2.00 ± 0.14; treated 2.47 ± 0.09 mM). Radioimmunoassay confirmed that PTU completely inhibited thyroxine production (serum [thyroxine]: control, 48.1 ± 1.9; treated, ≤ 3.2 nM).

The hyporesponsiveness of aortic rings to PE which resulted from 2 weeks of PTU treatment appeared to be endothelium-dependent. Enhanced endothelial production of NO seemed to be responsible, in part, since the effect was partially reversed by L-NOARG and immunohistochemistry showed increased expression of NO synthase in the endothelium of PTU-treated animals. After 4 weeks of PTU treatment, endothelium-independent mechanisms contribute to the resulting hyporesponsiveness. Further work is necessary to determine whether this effect is mediated via the hypothyroid state which PTU induces.

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Table 1

	Endothelium Intact			Endothelium Denuded			L-NOARG Treated		
	Control	2 wk PTU	4 wk PTU	Control	2 wk PTU	4 wk PTU	Control	2 wk PTU	4 wk PTU
EC_{50} (x 10^{-7} M)	1.00 ± 0.17	7.45 ± 1.15*	9.73 ± 0.45*	0.50 ± 0.16	3.08 ± 0.58	7.85 ± 1.42*†	0.64 ± 0.09	4.25 ± 1.22*	7.44 ± 0.79*†
Max.Response (g/mg)	0.40 ± 0.05	0.20 ± 0.07*	0.07 ± 0.02*	0.60 ± 0.03	0.50 ± 0.05	0.28 ± 0.07*†	0.65 ± 0.08	0.69 ± 0.09	0.37 ± 0.07*†

Data expressed as mean ± s.e.m. ($n = 4$). * = significant vs control; † = significant vs 2 wk PTU ($P<0.05$, ANOVA with Fisher's comparison)

98P A PRELIMINARY STUDY OF THE TP-RECEPTOR POPULATION ALONG THE LENGTH OF THE HUMAN ISOLATED UMBILICAL ARTERY

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Umbilical arteries are unique vessels in the sense that they must remain patent *in utero* but must close quickly and completely after delivery. Previous studies in this laboratory have suggested the presence of TP-receptors on human umbilical artery taken from the placental end of the umbilical cord (Amin *et al.*, 1995), which mediate constriction. It is possible that thromboxane is involved in the closure of this artery at birth. In the present study an attempt has been made to examine the arterial TP-receptor population along the length of the umbilical cord.

Human umbilical cords were obtained from full term pregnancies (all women gave written consent), at either vaginal delivery or Caesarean section, and were placed immediately into Krebs' solution at room temperature. The cords were then transported to the laboratory and set up within 60 minutes. Three segments of the cord were used: placental end; mid portion and foetal portion. The artery was carefully dissected from the surrounding Wharton's Jelly and cut into 5 mm rings. Rings of umbilical artery were set up under 2 g tension in 10 ml organ baths containing Krebs' solution at 37°C, and aerated with 2.5% O_2/8% CO_2/balance N_2. Indomethacin (1μM) was included in the Krebs' solution at all times. Agonist was added directly into the organ bath in a cumulative fashion. Cumulative concentration-effect curves were constructed to the stable TP-mimetic U46619 with and without the TP-receptor antagonist, Bay u3405 (McKenniff *et al.*, 1989). Bay u3405 was added to the Krebs' physiological solution at a concentration of 10^{-6}M, 30 minutes before addition of agonist. In all cases $n \geq 6$.

In samples taken from each of the three cord sections U46619 (10^{-8}-10^{-6}M) evoked a concentration dependent constrictor response. The EC_{50} obtained with U46619 on the placental portion rings of 3 x 10^{-7}M was similar to that obtained by Amin *et al.*, 1995. However, the maximum response (mean ± s.e.m.) evoked by U46619 varied between the different cord sections: placental portion = 0.72 ± 0.09g; middle portion = 0.39 ± 0.05g; foetal portion = 0.38 ± 0.08g. The response to U46619 in the presence of BAY u3405 was also influenced by the cord segment as no significant antagonism of the response was seen in rings from the foetal and middle portions, but the concentration effect curve was significantly depressed ($P<0.05$-0.01) on tissue taken from the placental end of the cord (results analysed using ANOVA plus a post-hoc Dunnett's t-test).

These results may be indicative of variations in the TP-receptor population along the length of the umbilical artery and lends support to the work of Abbas *et al.* (1997) with regards to TP heterogeneity. This study is currently being extended using selective TP-receptor mimetics and antagonists as well as tissue samples taken from deliveries at different gestational times as it has been shown that umbilical arteries from premature deliveries may have different sensitivities compared with vessels from term pregnancies (White, 1989).

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99P DETECTION OF mRNA FOR 5-HT_{1B} AND 5-HT_{1D} RECEPTORS IN PORCINE CORONARY ARTERIES BY *IN SITU* RT-PCR

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Activation of 5-hydroxytryptamine (5-HT) receptors causes constriction in porcine coronary arteries (Cushing & Cohen, 1992). Endothelium-dependent relaxation in this vessel has also been demonstrated in response to activation of 5-HT_{1B} and 5-HT_{1D} receptors (Schoeffter & Hoyer, 1990). Solution-based reverse transcription-polymerase chain reaction (RT-PCR) has been used to demonstrate the presence of mRNAs for 5-HT receptors in the porcine coronary artery (Ullmer *et al.*, 1995), however, this approach does not allow cellular localisation of the mRNA. In this study we used *in situ* RT-PCR to detect mRNA for 5-HT_{1B} and 5-HT_{1D} receptors in tissue sections of porcine coronary arteries.

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 free and fixed in 4% paraformaldehyde for 4 hours at 4°C. Tissue processing and sectioning was by standard histological methods (Dury & Wallington, 1967). Following proteinase K digestion 10 mg/ml, 30 min at 37°C the tissues sections were incubated in DNase 10u/slide overnight at room temperature. DNase digestion was stopped by the addition of 5µl of 20mM EDTA and incubated at 65°C for 10 min and washed in 50mM Tris-HCl. Oligonucleotide primers for the 5-HT_{1B} and 5-HT_{1D} receptor (Ullmer *et al.*, 1995) were fluorescently labelled at the 5'-terminal. The RT-PCR reaction mixture was overlaid onto the slide and placed in a PTC-200 thermal cycler (MJ Research) fitted with Twin Towers blocks. Following reverse transcription at 50°C for 30 min and 20 cycles of PCR (94°C for 30 sec, 45°C for 30 sec and 68°C for 45 sec) the sections were washed in standard saline citrate (SSC) and mounted. Negative controls consisted of RNase pretreatment (60 min at 37°C), omission of the reverse transcription step and omission of the primers. The fluorescent images were captured on a Leica TCS-NT confocal laser scanning microscope.

The tissue sections of the porcine coronary arteries showed a large amount of fluorescent amplified product in the tunica intima and the tunica adventitia for both the 5-HT_{1B} and the 5-HT_{1D} receptor mRNA. The tunica media contained only a small amount of the fluorescent RT-PCR products, this may suggest that the mRNAs are of low abundance in the tunica media compared to that in the tunica intima and adventitia. Overlaying of the images indicates that the 5-HT_{1B} and the 5-HT_{1D} receptor mRNAs are expressed in the same, or similar cell types. In conclusion, these data demonstrate the presence of mRNAs for the 5-HT_{1B} and 5-HT_{1D} receptors in the tunica intima, media and adventitia of porcine coronary arteries by *in situ* RT-PCR.

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100P RECEPTOR SUBTYPES FOR 5-HYDROXYTRYPTAMINE IN BOVINE PULMONARY CONVENTIONAL AND SUPERNUMERARY ARTERIES

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The pulmonary circulation consists of two populations of arteries: conventional arteries (CA) which divide with and accompany the airway and supernumerary arteries (SNA) which leave the conventional artery at 90° and are unaccompanied by an airway (Elliott & Reid, 1965). Because the SNA are small muscular arteries accounting for 40% of the total cross sectional area of all CA branches, they are likely to play a key role in regulating pulmonary vascular resistance. Pathological studies have implicated SNA in the development of pulmonary hypertension (PH) (Wagenvoort, 1994). Platelet-derived 5-hydroxytryptamine (5-HT) has been implicated in various forms of PH (Herve, 1990, 1995). The present study compared the receptor types mediating 5-HT-induced constriction in CA and SNA.

Bovine lungs were obtained fresh from the abattoir. Segments of conventional (diameter 4 - 5 mm) and supernumerary arteries (diameter 0.5 - 1 mm) were dissected from the lung and freed of surrounding connective tissue. The vessels were then weighed and suspended between stainless steel hooks in Krebs buffer (37°C) under a tension of 1 g and gassed with a mixture of O_2 : CO_2 (95%/5% v/v). The tissues were allowed to equilibrate for 1 hour before carrying out concentration response curves (CRCs) to 5-HT. Second CRCs to 5-HT or 5-HT in the presence of a selective 5-$HT_{1B/1D}$ or 5-HT_2 receptor antagonist (GR127935T and ritanserin respectively) were then conducted. Results are means ± s.e. mean. The significance of differences was determined using Student's t-test.

5-HT (1 nM - 1 mM), produced concentration-dependent contractile responses in SNA and CA. 5-HT was a significantly more potent agonist in SNA than CA (pD_2 values: SNA, 6.3 ± 0.1, n = 15; CA, 5.05 ± 0.14, n = 7, $p < 0.05$). The 5-HT_2 receptor antagonist ritanserin (0.1 nM - 100 nM) produced a concentration-dependent reduction in the magnitude of the maximum response to 5-HT in CA and SNA. Ritanserin unsurmountably inhibited responses to 5-HT in both SNA and CA but there was a ritanserin-resistant component in SNA (40 ± 10% of 5-HT maximum). The 5-$HT_{1B/1D}$ receptor antagonist GR127935T (Skingle *et al.*, 1993) produced rightward shifts of the CRC to 5-HT in both SNA and CA. GR127935T produced a greater shift in SNA than CA so that in the presence of 1 µM GR127935T the CRCs to 5-HT in CA and SNA were similar (pD_2 values: SNA, 5.22 ± 0.17; CA, 4.98 ± 0.11).

The present results suggest that in CA contractile responses to 5-HT are mediated predominantly by the 5-HT_2 receptor class. In SNA, both 5-$HT_{1B/1D}$ and 5-HT_2 receptors appear to be important and the greater sensitivity of SNA to 5-HT may be related to the presence of a greater density of 5-$HT_{1B/1D}$ receptors in SNA than CA.

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101P THAPSIGARGIN INHIBITS MICROANGIOGENESIS IN RAT ISOLATED AORTA, *IN VITRO*

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Microangiogenesis (MA) plays a role in wound healing, inflammation and cancer and involves the proliferation and migration of endothelial (EC) and vascular smooth muscle cells (VSMC). We recently demontrated that low concentrations (<10 nM) of thapsigargin (TG) inhibits both these latter processes, an effect mediated by the depeletion of intracellular Ca^{2+} pools ($[Ca^{2+}]_i$) (Birkett *et al.*, 1997; George *et al.*, 1997). Thus, this intracellular pool may constitute a therapeutic target for the inhibition of MA. In order to test this possibility, the effect of TG on *in vitro* MA by rat aortic rings was investigated (Nicosia and Ottinetti, 1990).

Male Sprague Dawley rats (250g) were killed by decapitation. Segments of aorta (2 mm in length) were embedded in fibrin discs and cultured for 15 days in serum-free medium (Nicosia & Ottinetti, 1990). TG was present in the medium at all stages of culture. Microvessels were counted daily using a microscope. Viability was examined histologically and biochemically by measurement of cGMP and cAMP (Jeremy *et al.*, 1997). As an internal control, rings were also treated with hydrocortisone (HC) since this had previously been shown to inhibit MA in this model (Nicosia and Ottinetti, 1990).

TG significantly inhibited microvessel formation in a dose-dependent manner (IC_{50}; 2 nM; figure 1). At these concentrations tissues were viable when appraised histologically. cAMP (stimulated with 10 μM forskolin) and cGMP (stimulated with 10 μM nitroprusside-stimulated) were unaffected by the inhibitory concentrations of TG. HC at 100 nM and 1 μM also inhibited MA without affecting viability.

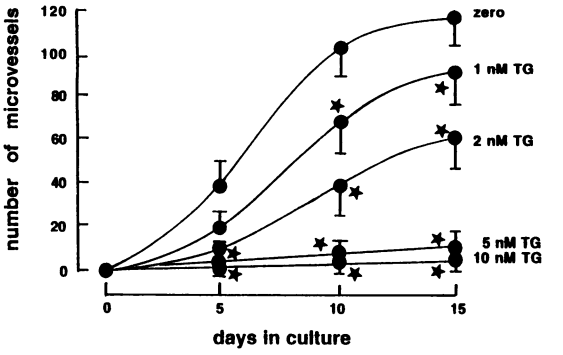

figure 1. Effect of TG on microvessel formation by rat cultured aortae. Each point = mean ± SEM; n = 6.
*p < 0.05 compared to controls (ANOVA)

The IC_{50} of TG on MA is similar to that which inhibits mobilisation of $[Ca^{2+}]_i$, migration and proliferation of ECs and VSMCs (Birkett *et al.*, 1997; George *et al.*.., 1997). Thus, TG probably inhibits MA through depletion of $[Ca^{2+}]_i$ pools. It is suggested that these pools constitute a potential therapeutic target for diseases associated with MA, in particular cancer.

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102P V_1-RECEPTORS MEDIATE THE CONTRACTILE RESPONSE TO ARGININE VASOPRESSIN IN HEPATIC ARTERIES FROM PATIENTS WITH AND WITHOUT CIRRHOSIS

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The impaired pressor response in cirrhosis of the liver (Hadoke & Hayes, 1997) is a condition which necessitates effective pharmacological intervention. Variceal haemorrhage, a life threatening complication of cirrhosis, is treated with the vasoconstrictor arginine vasopressin (AVP) or its analogues (Freeman et al., 1998). These may act by contracting splanchnic arteries and thus reducing pressure in the portal system (Barr et al., 1975) but the precise mechanism has not been identified. This study aimed to identify the receptor responsible for AVP-induced contraction in hepatic arteries from patients with and without cirrhosis.

Hepatic arteries were obtained from 9 liver donors (non-cirrhotic; 5M, 4F) and 7 recipients (cirrhotic; 4M, 3F), of similar age (45.22±5.67yrs. and 53.14±4.13yrs; P=0.32) during transplantation. Four rings (2mm long) from each artery were suspended in Krebs'-Henseleit solution (containing 10^{-5}M indomethacin) at 37°C, perfused with 95% O_2; 5% CO_2, for measurement of isometric force. The endothelium was removed by rubbing the luminal surface of each ring. The vessels were equilibrated at their optimum resting force (4g; Hadoke et al., 1998) and sensitised with potassium chloride (KCl; 100mM). Three rings were incubated with the V_1 receptor antagonist $d(CH_2)_5$Tyr(Me)AVP (10^{-8}, 10^{-7} and 10^{-6}M, for 15 min) whilst the fourth acted as a control. Cumulative concentration-response curves were obtained to AVP (10^{-11}-$3x10^{-6}$M), the V_2-agonist desmopressin (DDAVP; 10^{-11}-$3x10^{-6}$M) and KCl (2.5-120mM). Results are mean ± s.e.mean for n patients and were compared using Student's unpaired t-test.

The maximum contraction to AVP, but not KCl, was significantly smaller (P=0.002) in arteries from patients with cirrhosis (Table 1) whilst DDAVP did not contract vessels from either group. In both sets of arteries, incubation with the V_1-receptor antagonist produced a concentration-dependent rightward shift in the response to AVP but did not significantly alter the maximum contraction. Responses to KCl were not effected by treatment with the V_1-antagonist (Table 1).

We have previously reported impaired AVP-mediated contraction in hepatic arteries from patients with cirrhosis (Islam et al., 1998). The lack of response to DDAVP, and the competitive antagonism of the response to AVP by the V_1-receptor antagonist, indicate that AVP acts on the V_1 receptor in the human hepatic artery. This compares with results obtained using the human gastric artery (Calo et al., 1997). These data demonstrate that V_1-mediated contraction is impaired in hepatic arteries from patients with cirrhosis.

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Table 1. Contractile responses of hepatic arteries to AVP and KCl in the presence and absence of the V_1 antagonist $d(CH_2)_5$Tyr(Me)AVP.

	Donor Hepatic Artery				Recipient Hepatic Artery			
AVP	Control(9)	10^{-8}MAnt.(7)	10^{-7}M Ant(8)	10^{-6}M Ant(7).	Control (7)	10^{-8}MAnt.(6)	10^{-7}M Ant.(4)	10^{-6}M Ant.(4)
Max.Con.(g)	7.46±0.80	6.33±0.73	5.97±1.07	5.84±1.00	3.85±0.59	3.23±0.61	3.68±0.83	3.78±0.28
Max.Con.(%)	81.32±10.60	71.15±13.14	63.83±13.05	60.39±13.67	33.68±5.01	37.19±9.80	29.98±5.05	33.77±1.24
pD_2	7.95±0.71	7.39±0.19*	6.82±0.14**	6.14±0.13***	8.07±0.15	7.33±0.19**	6.39±0.16**	5.92±0.07***
KCl								
Max.Con.(g)	10.81±1.82	10.77±2.14	10.82±1.61	12.28±2.22	11.69±1.02	10.77±0.71	12.30±1.36	11.31±1.14
pD_2	1.63±0.08	1.53±0.10	1.58±0.07	1.62±0.09	1.64±0.08	1.68±0.07	1.60±0.09	1.59±0.13

Values are mean±s.e.mean for (n) patients. *P<0.05, **P<0.001 and ***P<0.0001 when compared with control using students unpaired t-test.

103P HEME OXYGENASE-1 INDUCTION HAS NO SIGNIFICANT EFFECT ON THE VASCULAR REACTIVITY OF RAT AORTIC RINGS

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Nitric oxide (NO) is a powerful vasodilator that is continuously released by endothelial cells to regulate blood flow and pressure. NO activates soluble guanylate cyclase (sGC) increasing cyclic 3:5 guanosine monophosphate (cGMP). Heme metabolism by heme oxygenase (HO) releases carbon monoxide (CO) which also activates sGC (Christodoulides *et al.*, 1995). Heme-derived CO has a peripheral vasodilatory action (Kozma, *et al.*, 1997) and plays a role in the central regulation of arterial pressure (Johnston *et al.*, 1996). HO exists in constitutive, HO-2 and inducible HO-1 isoforms. HO-1 is readily induced by pro-inflammatory stimuli and may be important in the resolution of inflammation (Willis *et al.*). Therefore, increased CO release during inflammatory episodes has the potential to influence vascular tone and reactivity. Here we have investigated whether induction of HO-1, and hence increased CO release, has a significant effect on the vascular tone of rat aortic rings.

Male wistar rats (220-250g) were administered either hemoglobin (hb; 300 mg kg^{-1}; iv) or vehicle (saline) and then 18 h later, killed and their aortae removed. De-endothelialised aortic rings (5 mm) were then mounted in organ baths containing warmed (37°C) and gassed (5% CO_2/95% O_2) Krebs' buffer, plus L-NAME (100 μM). Following equilibration for 15 min at 2g tension, the rings were incubated with tin protoporphyrin (SnPP; 100 μM) or vehicle. Cumulative concentration response curves were constructed to endothelin-1 (ET-1) and phenylephrine (PE). Hematin (100 μM), HO substrate, was incubated with some tissues. Using aortae homogenates from vehicle- and hb-treated rats, HO-1 proteien was analysed by western blot.

HO-1 protein was upregulated (35%) in aortic rings from Hb-treated compared to vehicle-treated rats, as confirmed by western blot analysis. Contractile responses to ET-1 in *ex vivo* rings from rats pre-treated with Hb were not significantly different (ANOVA) to those from vehicle-treated animals, in the presence or absence of inhibitors (Table 1). Vascular reactivity was also unaffected by the presence of hematin (Table 1). Similarly, responses to PE were also not significantly different in Hb- and vehicle-treated animals (Table 1).

In conclusion, HO-1 induction does not affect the contractile responses to either the potent vasoconstrictor ET-1 or to the α_1 receptor agonist, phenylephrine. Therefore, we have found no evidence that increased HO-1 expression affects the vascular reactivity of rat aortic rings.

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Table 1 EC_{50} (nM) and apparent E_{max} values (% response KCl, 60 mM) for ET-1 and PE in rat aortic rings

	Aortic rings from vehicle-treated rats				Aortic rings from Hb-treated rats			
Inhibitors	L-NAME (100 μM)		L-NAME + SnPP (both 100 μM)		L-NAME (100 μM)		L-NAME + SnPP (both 100 μM)	
Parameter	EC_{50} nM	E_{max}	EC_{50} nM	E_{max}	EC_{50} nM	E_{max}	EC_{50} nM	E_{max}
ET-1 (n=6)	1.5	112 ± 8	1.3	103 ± 9	1.4	121 ± 7	1.8	112 ± 37
ET-1 + hematin (n=6)	1.2	95 ± 15	1.1	52 ± 31	1.7	97 ± 20	2.6	103 ± 47
PE (n=8)	4.1	112 ± 3	5	113 ± 3	8.2	123 ± 5	10	136

104P THE EFFECT OF EXTERNAL ACIDOSIS ON THE ACTION OF CALCIUM ENTRY BLOCKERS IN RAT AORTA

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Ischaemia results in a decrease in blood pH due to the build up of metabolites and increase in CO_2 concentration (Levine, 1993). This external acidosis may compromise the effect of vasodilators used in the treatment of this condition. To investigate this we have studied the effect of changes in pH on the action of two calcium entry blockers (CEBs), nicardipine (NIC) and verapamil (VER) in rat aorta.

Male Wistar rats (200-300g) were stunned and killed by cervical dislocation. The thoracic aorta was removed, sectioned into ring segments (5 mm in length) and the rings, devoid of endothelium, suspended under 2g resting tension in 10 ml of physiological saline (PS) of the following composition (10^{-3}M): NaCl 137; KCl 5.9; $MgCl_2$ 1.25; $CaCl_2$ 2.5; dextrose 11; BIS-TRIS 10. The PS was buffered to pH 7.4 or 6.4 with HCl, supplemented with propranolol (10^{-6}M), ascorbic acid ($5x10^{-5}$M) and EDTA (10^{-5}M) maintained at 37°C and gassed with oxygen. Isometric contractures were recorded. After 1h equilibration, the tissues were contracted with 10^{-6}M noradrenaline (NA), the sensitising dose, and at the peak of the contraction (SR) 10^{-6}M ACh was added to test for release of EDRF. Non-cumulative concentration-response curves were then constructed to NA prior to and 30 min after 10^{-6}M NIC or VER.

NA contractures were reduced by decreasing the pH of the PS from 7.4 to 6.4. A rightward shift of the concentration-response curve was seen, as indicated by the PD_2 values of the control responses to NA shown in table 1, and the maximum response (Emax) was reduced (pH 7.4 =111.4±17.0 % of SR; pH 6.4 = 67.4±10.6 % of SR at pH 7.4, n=8, $P<0.05$). At pH 7.4 both NIC and VER moved the control concentration-response curves to NA to the right and with NIC there was also a decrease in E_{max} (see Table 1). In contrast, at pH 6.4 neither NIC nor VER caused any further shift of the control concentration-response curves but both CEBs reduced E_{max} (Table 1).

	pH 7.4		pH 6.4	
	PD_2	E_{max} (% SR)	PD_2	E_{max} (% SR)
Control	7.4±0.08	111.4±17.0	6.3±0.09[2]	141.0±19.1
NIC	6.8±0.07[1]	37.0±6.0[1]	6.3±0.19[2]	36.5±3.9[1]
VER	6.8±0.06[1]	117.2±22.5	6.7±0.18	38.4±9.8[1,2]

Table 1. Values are mean±s.e.m. from 8 tissues.[1]indicates significant difference from control. [2]significant difference from pH 7.4. $P<0.01$, Students unpaired t-test.

The results show that external acidosis increases the action of VER on E_{max}, an effect not seen with NIC. It has been reported that decreasing extracellular pH induces depolarisation in aortic cells (Furukawa et al., 1996) and as VER, unlike NIC, binds preferentially to the calcium channel in the open-state, which is favoured by depolarisation, we can conclude that acidosis increases the binding of this CEB.

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105P INTERACTION OF HYPOGLYCAEMIC IMIDAZOLINES WITH K_{ATP}-CHANNEL-MEDIATED RELAXATION IN RAT AORTA

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Agents which increase the open probability of ATP-dependent potassium channels (K_{ATP}) cause inhibition of insulin release from pancreatic β-cells and arterial vasodilatation, whereas drugs which inhibit K_{ATP} stimulate insulin secretion and inhibit vasodilatation to activators of K_{ATP} (Longman & Hamilton, 1992). Imidazoline compounds have been shown to stimulate insulin secretion, possibly via an interaction with pancreatic K_{ATP} (Rustenbeck *et al.*, 1997). Thus, the aim of the present study was to investigate whether the glycaemic effects of the imidazoline compounds efaroxan, S21663 (PMS 812; 1-(2,4-dichlorobenzyl)-2-(4,5-dihydro-1H-2-imidazol)-4-methyl piperazine), S22068 ((1,4-di-isopropyl-2-(4,5-dihydro-1H-imidazol-2-yl) piperazine) and S22954 (4-methyl-2-(4,5-dihydro-1H-imidazol-2-yl)benzo(β)morpholine) may be mediated by an interaction with K_{ATP}.

Male Wistar-Kyoto rats (250-350g) were killed by decapitation. The descending aorta was removed, cleaned and cut into segments (2mm in length). Endothelium-denuded segments were mounted in organ baths for recording of isometric tension as previously described (Plane *et al.*, 1997). All data are expressed as mean ± s.e. mean and differences between mean values were calculated using the Students t-test.

Levcromakalim (0.01-10 μM) and diazoxide (0.01-10 μM), activators of K_{ATP}, each evoked concentration-dependent relaxation of isolated aortic rings pre-contracted with phenylephrine (3 μM). The maximal relaxations to levcromakalim and diazoxide were 89.0 ± 3.7 % (n=8) and 57.5 ± 5.0 % (n=6), respectively. In contrast, efaroxan, S22068, S22954 and S21663 (all 0.01-10 μM), each failed to alter the tone of pre-contracted arterial segments (n=4-6 in each case).

Pre-incubation with either efaroxan or S22068 (100 μM; 10 mins) did not significantly alter relaxation to levcromakalim (n=4 in each case). In contrast, both glibenclamide (0.1, 1.0 and 10 μM; 10 mins) and S21663 (1-100 μM; 10 mins) each caused concentration-dependent inhibition of levcromakalim-evoked relaxation. In the presence of 0.1, 1.0 and 10 μM glibenclamide, the maximal relaxation to levcromakalim was reduced to 73.5 ± 2.1 %, 32.3 ± 5.0 % and 7.5 ± 1.7 % (n=5-7; P<0.01), respectively. Similarly, in the presence of 1.0, 10 and 100 μM S21663, the maximal relaxation to levcromakalim was reduced to 82.5 ± 3.0 %, 49.1 ± 3.0 % and 4.3 ± 1.2 % (n=4-5; P<0.01), respectively. Pre-incubation with S22954 (100 μM; 10 mins) significantly potentiated relaxation to both levcromakalim and diazoxide (n=4 in each case). However, this compound did not prevent the inhibition of levcromakalim-evoked relaxation caused by pre-incubation with glibenclamide (10 μM; 10 mins; n=4).

These data indicate that the imidazoline compounds efaroxan and S22068 do not interact with K_{ATP} in rat isolated aortic rings. In contrast, S21663 inhibited vasorelaxation to levcromakalim, indicating that inhibition of K_{ATP} may contribute to the reported insulinotropic effects of this compound (Wang *et al.*, 1996). Although S22954 did not cause relaxation of pre-contracted aortic rings, this compound did significantly potentiate vasorelaxation to levcromakalim and diazoxide, indicating that it may cause an enhancement of K_{ATP} activation via other mechanisms.

S21663, S22068 and S22954 were donated by Servier and levcromakalim was a gift from SKB. F. Plane is a Wellcome Trust Career Development Fellow.

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106P COMPARISON OF EFFECTS OF 1-EBIO AND NS1619 ON K^+ CURRENTS IN VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS

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NS1619 (1-(2'-hydroxy-5'trifluoromethylphenyl)-5-trifluoromethyl-2(3*H*)benzimidazolone), an opener of the large-conductance calcium-sensitive K-channel (BK_{Ca}; Olesen *et al.*, 1994), induces a voltage-sensitive K^+ current ($I_{BK(Ca)}$) in vascular smooth muscle cells (Edwards et al. 1994; Walker et al., 1996). A structurally-related compound, 1-EBIO (1-ethyl-2-benzimidazolinone), was recently shown to induce a charybdotoxin-sensitive K-current in epithelial cells (Devor et al., 1996), although the underlying channel was not fully characterised. In the present study, we have compared the effects of these two compounds on K^+ currents in cultured bovine aortic endothelial cells (BAE1) and in smooth muscle cells freshly-isolated from rat pulmonary and hepatic arteries. Myocytes were dispersed from vessels obtained from male Sprague-Dawley rats (150-200 g; Edwards et al., 1994).

Using whole-cell, patch-clamp recording techniques, and with a quasi-physiological K^+ gradient (intracellular [K^+] 125 mM, intracellular [K^+] 6 mM; Edwards et al., 1994), a large inwardly-rectifying K^+ current ($I_{K(IR)}$) was found in BAE1 cells. In the presence of 1-EBIO (600 μM) an additional, voltage-insensitive K^+ current was induced (current at +50 mV after stepping from −10 mV : control 15 ± 14 pA, 10 min 1-EBIO 406 ± 69 pA, n=5). Charybdotoxin (100 nM), which alone was without effect on $I_{K(IR)}$, abolished the 1-EBIO-induced current (current at +50 mV after stepping from −10 mV: 25 ± 11 pA) and revealed that the benzimidazolone had inhibited $I_{K(IR)}$ (current at -140 mV : control -298 ± 61 pA, 10 min 1-EBIO -262 ± 46 pA, 1-EBIO + 100 nM charybdotoxin -143 ± 31 pA, n=5). The charybdotoxin-sensitive current was not modified by exposure to iberiotoxin (100 nM) or to apamin (100 nM). In contrast, 1-EBIO did not induce a K^+ current in smooth muscle cells from either the rat pulmonary artery (current at +50 mV after stepping from −10 mV: control 91 ± 18 pA, 10 min 1-EBIO 73 ± 19 pA, n=5) or the rat hepatic artery (control 148 ± 34 pA, 10 min 1-EBIO 119 ± 18 pA, n=9), although it did inhibit $I_{K(IR)}$ in the latter. Furthermore, the current induced by NS1619 in the rat pulmonary artery was inhibited by iberiotoxin (Walker et al., 1996). Both NS1619 and 1-EBIO inhibited the delayed-rectifier K^+ current ($I_{K(V)}$) in pulmonary artery myocytes. In the BAE1 cells, NS1619 did not induce a K^+ current, although it did inhibit $I_{K(IR)}$.

Endothelial cells possess intermediate-conductance (IK_{Ca}) and small-conductance (SK_{Ca}) calcium-sensitive K-channels which are inhibited by charybdotoxin and apamin, respectively (Marchenko & Sage, 1996). We conclude that, of the three calcium-sensitive K^+ channels, 1-EBIO is a selective opener of IK_{Ca} whereas NS1619 selectively opens BK_{Ca}. The mechanism by which the benzimidazolones inhibited $I_{K(V)}$ and $I_{K(IR)}$ is presently unknown and is the subject of further study.

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107P SELECTIVE ATTENUATION OF ADRENOCEPTOR-MEDIATED CONTRACTION BY ENDOTHELIUM-DERIVED NITRIC OXIDE IN ISOLATED MOUSE AORTAE

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Dilator compounds from the endothelium attenuate vasoconstriction in a variety of arteries (Woodman, 1995). Endothelium-derived nitric oxide (NO) significantly inhibits noradrenaline (NA)-mediated contraction in isolated mouse aortae (Kamata & Kojima, 1997) but its effect on other vasoconstrictors is not known. This study aimed to determine whether the inhibitory effect of NO is specific to NA-induced contraction in the mouse aorta.

Male MF1 mice (weight 35-53g, age 86-131 days) were killed by cervical dislocation. 2mm rings of thoracic aorta were mounted in a myograph, containing physiological salt solution at 37°C gassed with 95%O_2;5%CO_2, for measurement of isometric force. The endothelium was removed from some rings by rubbing the luminal surface. Rings were equilibrated at their optimum resting force. Cumulative concentration-response curves were produced for the adrenoceptor agonists NA (non-specific adrenoceptor agonist; 10^{-9}-3x10^{-5}M), phenylephrine (PE; α_1-agonist; 10^{-9}-3x10^{-5}M) and clonidine (α_2-agonist; 10^{-11}-3x10^{-5}M) and also for 5-hydroxytryptamine (5-HT, 10^{-9}-3x10^{-5}M), KCl (2.5-120mM), angiotensin II (AII; 10^{-10}-3x10^{-6}M) and endothelin-1 (ET-1; 10^{-9}-3x10^{-5}M). Following contraction with 5-HT (10^{-7}-3x10^{-7}M), cumulative concentration-response curves were produced for the endothelium-dependent vasodilator acetylcholine (ACh; 10^{-9}-3x10^{-5}M) and the NO donor 3'-morpholinosydnonimine (SIN-1; 10^{-9}-3x10^{-5}M). In some rings, concentration-response curves to NA and ACh were repeated in the presence of the NO synthase inhibitor L-N^G-nitro-arginine (L-NNA; 10^{-4}M). Results are mean ± s.e.mean and were compared using Student's paired or unpaired t-test, as appropriate.

Maximum responses, but not sensitivity, to NA, PE and clonidine were increased following removal of the endothelium (Table 1). In contrast, sensitivity to 5-HT was increased in denuded arteries but maximum contraction was not enhanced significantly (Table 1). KCl-induced contraction was similar in intact and denuded vessels (Table 1) whilst ET-1 and AII produced only very small contractions. In denuded arteries, ACh-mediated relaxation (maximum 77.4±5.1% of precontraction; n=14) was abolished (4.3±6.0%; n=13; P<0.0001), whereas maximum relaxation to SIN-1 (95.6±1.7 %; n=13) was increased (108.4±3.5%; n=12; P<0.05). Incubation with L-NNA had no effect on tone in quiescent vessels but enhanced NA-mediated contraction (3.0±0.6mN/mm, n=7; P<0.002) and abolished ACh-mediated relaxation (9.2±5.6%; n=8; P<0.0001)

Table 1. Maximum contraction and sensitivity (pD_2) values.

	Max.Cont. (mN/ mm)		pD_2	
	Intact	Denuded	Intact	Denuded
NA	1.0±0.2 (15)	3.1±0.5**(14)	7.80±0.14 (13)	7.55±0.17 (14)
Clon.	0.1±0.1 (4)	1.3±0.4**(4)	not calculable	6.73±0.11 (4)
PE	0.9±0.3 (5)	3.7±0.5**(5)	6.80±0.06 (5)	6.69±0.15 (5)
5-HT	3.8±0.6 (9)	4.5±0.5 (7)	6.78±0.07 (9)	7.28±0.13* (7)
KCl	1.8±0.3 (13)	1.9±0.3 (12)	1.49±0.06 (13)	1.53±0.07 (12)

n values in brackets. *P<0.005 and **P<0.001 compared with intact rings.

Thus, an endothelium-derived factor opposes vasoconstriction in mouse aortae. This effect is marked for adrenoceptor agonists (5-HT is a weak α-agonist) but not specific to α1 or α2 subtypes. It is mediated by NO since it is abolished by L-NNA. However, since basal NO production in these vessels is low as judged by lack of vasoconstriction to L-NNA alone, it appears that α-adrenoceptor agonists also stimulate NO release and thereby indirectly oppose their own direct vasoconstriction.

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108P LOW MOLECULAR MASS COMPLEXES OF TRANSITION METALS INHIBIT VASOCONSTRICTION IN RAT ISOLATED AORTIC RINGS

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Low molecular mass complexes of essential metalloelements such as $Cu(II)_2$(3,5-Diisopropylsalicyclate)$_4$ (CuDIPS), have been suggested to have therapeutic potential for the treament of a number of inflammatory disorders (Sorenson, 1989). Cu-DIPS and $Mn(II)_2$(3,5-Diisopropylsalicyclate)$_4$ (MnDIPS) have been shown to down-regulate the activity of nitric oxide synthase (NOS) *in vitro* (Baquail & Sorenson, 1995), but the effect of these metallocomplexes on vasoconstriction has not been examined.

Male Wistar rats (250-300 g) were stunned and then killed by cervical dislocation. Segments of aorta (2 mm wide) were mounted in organ baths for recording of isometric tension as previously described (Plane *et al.*, 1997). In some experiments, the endothelial cell layer was removed mechanically by gently rubbing the intima with a wire. Tissues were maintained at 37°C in oxygenated Krebs buffer, containing indomethacin (2.8 μM). All data are expressed as mean ± s.e. mean and differences between mean values were calculated using the Students t-test.

Phenylephrine (PE; 0.01-10 μM) and potassium chloride (KCl; 20-120 mM) each evoked concentration-dependent contraction of endothelium-denuded aortic rings (maximum contraction 20 ± 1 mN (n=10) and 18 ± 1 mN (n=4), respectively). Pre-incubation with CuDIPS (100 μM; 30 mins) reversibly inhibited contractions to both PE and KCl, significantly reducing the maximal response to both agents (Table 1). Exposure to either MnDIPS or $Zn(II)_2$(3,5-Diisopropylsalicyclate)$_4$ (ZnDIPS; both 100 μM; 30 mins) inhibited contractions to lower concentrations of PE but did not significantly reduce the maximum response (Table 1). However, these complexes each significantly depressed contractions to all concentrations of KCl (n=5). The metal-free carrier molecule 3,5-Diisopropylsalicyclate$_4$ (DIPS; 100 μM; 30 mins), also reversibly reduced the maximum response to both PE and KCl (Table 1).

Table 1 : % Inhibition of maximum contraction (n=4-5; *P<0.01)

	CuDIPS	MnDIPS	ZnDIPS	DIPS
PE	55 ± 2 %*	16 ± 10 %	24 ± 13 %	35 ± 7%*
KCl	75 ± 9 %*	40 ± 9 %*	39 ± 7 %*	65 ± 12%*

Pre-incubation with nifedipine (10 μM; 10 mins), an inhibitor of voltage-sensitive calcium channels, significantly inhibited contraction to PE and KCl and reduced the maximal responses by 47 ± 7 % and 54 ± 6 % (both n=7; P<0.01), respectively.

Application of CuDIPS (0.1-100 μM) to endothelium-intact arterial segments pre-constricted with PE (3 μM) caused concentration-dependent relaxation (maximum relaxation 90 ± 7 %; n=7) which was significantly reduced following pre-incubation with the NOS inhibitor L-N^G-nitroarginine (100 μM; 30 mins; maximum relaxation 33 ± 11 %; n=5; P<0.01). In endothelium-denuded tissues relaxation to CuDIPS was also significantly smaller (maximum relaxation 54 ± 13 %; n=4; P<0.01).

These data indicate that metalloelement complexes can inhibit vasoconstriction to both PE and KCl in rat isolated aortic rings. In addition, application of CuDIPS to pre-constricted arterial segments causes concentration-dependent relaxation which is partially due to the release of endothelium-derived NO. However, the attenuation of contraction caused by the complexes was observed in endothelium-denuded tissues and was of a similar magnitude to the inhibition caused by nifedipine. Thus, the inhibition of smooth muscle activation by these complexes may be due to a direct action on the vascular smooth muscle.

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109P EXCITATORY EFFECTS OF ISOPROSTANES IN THE HUMAN UMBILICAL ARTERY *IN VITRO*

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The 8-isoprostanes are a series of prostaglandin-like compounds in which the side chains are oriented *cis* rather than *trans* (Morrow & Roberts, 1996). Most evidence indicates that vasoconstrictor effects of the 8-isoprostanes are mediated by the TP receptor (John & Valentin, 1997). Previously, we have shown that the only excitatory prostanoid receptor present in the human umbilical artery (HUA) is the TP receptor (Boersma *et al.*, 1997). In this study we have investigated the vasoconstrictor actions of 6 structurally different isoprostanes and the TP receptor agonist U46619 on the HUA.

Sections of umbilical cords within 20 cm of the placenta were collected from full-term vaginal or Caesarean section births. HUA were dissected free of Wharton's Jelly, cut into rings, and mechanically denuded of endothelium. Tissues were mounted in organ baths containing oxygenated (95% O_2, 5% CO_2) physiological salt solution with 30 μM indomethacin, and maintained at 37°C under a resting tension of 30 mN. Concentration-effect curves to the isoprostanes and U46619 were obtained by cumulative addition in the presence and absence of the TP receptor selective antagonist GR32191 (0.1 μM) (Lumley *et al.*, 1989). Responses were normalised to the contraction produced by hypotonic shock.

All compounds except 8-isoPGF$_{3\alpha}$ produced concentration-dependent contractions. The contractile response to each cumulative addition of isoprostane increased up to a point, after which subsequent addition reduced the contraction below the previous level. Such a downturn in the concentration-effect curve did not occur with U46619. The potencies of the compounds tested were as follows (pEC_{50} ± s.e.mean): 8-isoPGE$_2$, 6.5 ± 0.06; U46619, 6.4 ± 0.1; 8-isoPGF$_{2\alpha}$, 5.8 ± 0.2; 8-isoPGE$_1$, 5.4 ± 0.1; 8-isoPGF$_{1\alpha}$, 5.0 ± 0.06; 8-isoPGF$_{2\beta}$, > 3.9; 8-isoPGF$_{3\alpha}$, >> 4.8 (n = 4 to 17).

GR32191 caused rightward shifts in the responses to all the active compounds, however, sufficient bath concentrations to obtain complete concentration-effect curves could not be achieved in all cases. Apparent pA_2 values for GR32191 against U46619, 8-isoPGE$_2$, 8-isoPGF$_{2\alpha}$, 8-isoPGE$_1$ and were 7.6 ± 0.2, 9 ± 1, 8.2 ± 0.3 and 7.7 ± 0.3 , respectively (n = 4).

An equipotency of 8-isoPGE$_2$ and U46619 has not been reported previously. Among the isoprostanes tested, E-ring is more potent than F-ring, α configuration is more potent than β, and in the side-chain, a double unsaturation is most potent. Constrictor actions of isoprostanes in HUA are mediated by the TP receptor.

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110P AN INVESTIGATION INTO THE DOWNTURN IN RESPONSE TO 8-ISOPROSTAGLANDIN E$_2$ IN HUMAN UMBILICAL ARTERY *IN VITRO*

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Isoprostanes constrict the human isolated umbilical artery (HUA) by action at TP receptors but a downturn in the concentration-effect curve, suggestive of inhibition, occurs at high concentrations (Oliveira & Crankshaw, 1998). Inhibitory actions of isoprostanes were previously shown to involve release of NO (Jourdan *et al.*, 1997), or direct effects at unidentified receptors (Leitinger *et al.*, 1997). In the present study, we have attempted to identify the mechanism by which 8-isoPGE$_2$ appears to relax HUA.

Sections of umbilical cords within 20 cm of the placenta were collected from full-term vaginal or Caesarean section births. HUA were dissected free of Wharton's Jelly, cut into rings, and mechanically denuded of endothelium. Tissues were mounted in organ baths containing oxygenated (95% O_2, 5% CO_2) physiological salt solution with 30 μM indomethacin, and maintained at 37°C under a resting tension of 30 mN. Concentration-effect curves to 8-isoPGE$_2$ were obtained by cumulative addition in the presence and absence of N^G-nitro-L-arginine methyl ester (L-NAME) (100 μM) or the DP receptor selective antagonist BW A868C (50 nM) (Giles *et al.*, 1989). Bell-shaped concentration-effect curves were analysed assuming simultaneous activation of excitatory and inhibitory receptors (Szabadi, 1977). In other experiments, stable contractions were obtained with 1-3 μM U46619 and attempts were made to mimic the effects of high concentrations of 8-isoPGE$_2$ by addition of cicaprost to 42 μM, PGE$_2$ to 0.3 μM, or anandamide to 60 μM.

Neither L-NAME nor BW A868C significantly altered any parameter of the complex concentration-effect curve to 8-isoPGE$_2$ (Student's t-test, n=3). Under the conditions of our experiments, cicaprost, PGE$_2$ and anandamide were all incapable of reversing the U46619-induced contraction at the concentrations tested (n=3).

The absence of endothelium and lack of effect of L-NAME argues against involvement of NO in the inhibitory phase of the response to 8-isoPGE$_2$. Similarly, the lack of effect of BW A868C does not support DP receptor involvement. Failures of cicaprost, PGE$_2$ and anandamide to reverse TP receptor-mediated contraction of HUA suggest that neither IP, EP$_2$, EP$_4$ nor cannabinoid receptors mediate the downturn. The inhibition by high concentrations of 8-isoPGE$_2$ may be mediated by the TP receptor, since GR32191 appeared to shift both the excitatory and inhibitory phases of the response (Oliveira & Crankshaw, 1998). However, the mechanism is unlikely to be a simple agonist-induced desensitization since no downturn was observed with U46619 (Oliveira & Crankshaw, 1998).

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111P PATHWAYS FOR ARACHIDONIC ACID-EVOKED RELAXATION IN THE RABBIT ISOLATED FEMORAL ARTERY

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Endothelium dependent relaxation of the rabbit isolated femoral artery appears to be mediated by the release of a number of factors including nitric oxide (NO) and the putative endothelium-derived hyperpolarizing factor (EDHF; Plane *et.al.*, 1995; Rowe *et al.*, 1998). A number of studies have indicated that EDHF may be a metabolite of arachidonic acid (Bauersachs *et al.*, 1994; Campbell *et al.*, 1996). In the present study, we have investigated the mechanisms underlying the possible role played by arachidonic acid in the endothelium-dependent relaxation of the rabbit isolated femoral artery.

Female New Zealand White rabbits (2.0-2.5 kg) were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.v.) and killed by rapid exsanguination. Segments of femoral artery were mounted in a Mulvany-Halpern myograph under a previously determined optimal preload of 1g for isometric recording of tension changes. The tissues were maintained at 37°C in oxygenated Krebs buffer. All data are expressed as mean ± s.e. mean. Differences between mean values were calculated using Students t-test.

Arachidonic acid (0.01 μM-1 mM) evoked *transient*, concentration-dependent relaxation of endothelium-intact arterial segments pre-contracted with phenylephrine (3 μM). The maximum response to arachidonic acid was 59.0 ± 4.5 % (n=4). In contrast, in endothelium-denuded arterial segments, the application of arachidonic acid failed to evoke any relaxation (n=4).

Pre-incubation of endothelium-intact arterial segments with either the cyclooxygenase inhibitor indomethacin (10 μM; 10 mins), the cytochrome P450 inhibitor 17-octydecynoic acid (17-ODYA; 5 μM; 10 mins), or the inhibitor of ATP-dependent potassium channels glibenclamide (5 μM; 10 mins) did not significantly attenuate relaxation to arachidonic acid (n=4; P>0.05). In contrast, the lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA, 10 μM; 30 minutes) significantly inhibited relaxation to arachidonic acid by reducing the maximum response from 59.0 ± 4.5 % to 16.6 ± 4.9 % (n=4; P<0.01). Incubation with the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (100 μM; 30 mins), abolished relaxation to arachidonic acid (n=4). Furthermore, following pre-incubation of arterial segments with thimerosal (100 μM; 10 mins), an inhibitor of acyltransferases, relaxation to arachidonic acid was both prolonged and potentiated, and the maximum relaxation increased to 94.0 ± 6.0 % (n=4; P<0.01).

These data indicate that in the rabbit isolated femoral artery, arachidonic acid evokes endothelium-dependent relaxation which seems to be explained entirely by the release of NO. However, the relaxation to arachidonic acid was also significantly inhibited by NDGA, indicating that a lipoxygenase-derived metabolite of arachidonic acid may be involved in the synthesis/ release of NO in response to arachidonic acid. In contrast, it appears that activation of the cyclooxygenase and cytochrome P450 pathways do not contribute to arachidonic acid-evoked relaxation in this artery.

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112P INTERLEUKIN-6 KNOCK OUT MICE EXHIBIT A RESISTANCE TO SPLANCHNIC ARTERY OCCLUSION SHOCK

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In the present study, we used IL-6 knock out mice (IL-6KO, kindly supplied by IRBM, Pomezia, Italy) to evaluate a possible role of IL-6 on the pathogenesis of splanchnic artery occlusion shock (SAO). SAO shock was induced in mice, anaesthetised with sodium uretane (45 mg kg^{-1}, i.p.), by clamping both the superior mesenteric artery and the celiac trunk for 45 min, followed thereafter by release of the clamp (reperfusion). There was a marked increase in the oxidation of dihydrorhodamine 123 to rhodamine (a marker of peroxynitriteinduced oxidative processes) in the plasma of the SAO shocked IL-6WT mice after reperfusion, but not during ischemia alone. Immunohistochemical examination demonstrated a marked increase in the immunoreactivity to nitrotyrosine, a specific "footprint" of peroxynitrite, in the necrotic ileum in shocked IL-6WT mice. At 60 minutes after reperfusion, animals were sacrificed for histological examination and biochemical studies. SAO shocked WT mice developed a significant increase of tissue myeloperoxidase and malonaldehyde activity, and marked histological injury to the distal ileum. SAO shock was also associated with a significant mortality (0% survival at 2 h after reperfusion). Reperfused ileum tissue sections from SAO-shocked WT mice showed positive staining for P-selectin, which was mainly localized in the vascular endothelial cells. Little specific staining was observed in sham-WT mice.

Staining of ileum tissue section obtained from sham operated WT mice with anti-ICAM-1 antibody showed a weak but diffuse staining demonstrating that ICAM-I is constitutively expressed. However, after SAO shock the staining intensity increased substantially in the ileum section from WT mice. The intensity and degree of P-selectin and ICAM-1 were markedly reduced in tissue sections from SAO-shocked IL-6KO mice. SAO-shocked IL-6KO mice also showed a significant reduction of neutrophil infiltration into the reperfused intestine, as evidenced by reduced myeloperoxidase activity; improved histological status of the reperfused tissues, reduced peroxynitrite formation, reduction of the malonaldehyde levels and improved survival. Taken together, our results clearly demonstrate that IL-6KO mice are resistant to SAO shock and part of this effect may be due to inhibition of the expression of adhesion molecules, and subsequent reduction of neutrophil-mediated cellular injury.

Table 1. Myeloperoxidase and malondialdehyde levels in the reperfused intestine of SAO-shocked mice.

Group	Myeloperoxidase (U/g wet tissue)	Malonaldehyde (μU/100 ng wet tissue)
Sham IL-6WT	12.3 ± 4	89 ± 4
Sham IL-6KP	11.9 ± 3	93 ± 3.2
SAO/R IL-6WT	69.9 ± 2*	289 ± 2*
SAO/R IL-6KO	35 ± 3.8°	135 ± 4.2°

*P<0.01 versus Sham. °P<0.01 versus IL-6W. Data sets were examined by one- and two-way analysis of variance and individual group means were then compared with Student's unpaired t-test. Non-parametric data were analyzed with the Fisher's exact test. A *p* value less than 0.05 was considered significant.

113P THE POLY(ADP-RIBOSE) SYNTHETASE INHIBITOR 3-AMINOBENZAMIDE PROTECTS RAT RENAL PROXIMAL TUBULAR CELLS AGAINST OXIDANT STRESS-MEDIATED CELLULAR INJURY AND DEATH

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Reactive oxygen species (ROS)-mediated cellular injury and death has been implicated in the pathogenesis of renal ischaemia-reperfusion injury and associated acute renal failure (Weight *et al.*, 1996). Within the kidney, the proximal tubule (PT) appears to be particularly susceptible to reperfusion-injury (Weight *et al.*, 1996). ROS produce strand breaks in DNA triggering energy-consuming DNA repair mechanisms including the activation of the nuclear enzyme poly(ADP-ribose) synthetase (PARS) (Schraufstatter *et al.*, 1986). However, excessive activation of PARS can result in significant depletion of intracellular levels of its substrate NAD and of ATP, resulting in cellular injury and ultimately, cell death (Schraufstatter *et al.*, 1986).

The aim of this study was to investigate the effect of the PARS inhibitor 3-aminobenzamide (3-AB) on cellular injury and death in cultures of rat PT cells exposed to oxidant stress in the form of hydrogen peroxide (H_2O_2).

Kidneys were obtained from male Wistar rats (250-350 g) anaesthetised with thiopentone sodium (120 mg/kg i.p.) and heparinised (1400 U/kg i.p.). Rat PT cells were isolated from kidney cortex using collagenase digestion, differential sieving and Percoll density centrifugation and cultured on 24 well plates ($1x10^5$ cells/well) in Minimum Essential Medium containing 10% (v/v) fetal calf serum. On reaching confluence, monolayers were incubated with H_2O_2 (1 mM) for up to 4 hours in the presence or absence of increasing concentrations of 3-AB (0.1-30 mM). Cellular injury and death were then assessed spectrophotometrically by measurement of the mitochondrial-dependent conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into formazan or by the measurement of intracellular lactate dehydrogenase (LDH) released into incubation medium respectively. PARS activation was determined by measurement of the incorporation of [^{3}H]NAD into nuclear proteins. Data are expressed as mean±s.e.m. from triplicate measurements from 4 separate isolations and were analysed using one-way ANOVA followed by Bonferoni's post significance testing. A p value less than 0.05 was considered to be significant.

Treatment		Mitochondrial respiration (% control)	LDH release (% Triton-X100 control)
$[H_2O_2]$ (mM)	[3-AB] (mM)		
0 (control)	0	100	100
1	0	17±2*	60±2*
1	0.1	24±2	49±5
1	0.3	42±3$^+$	46±6
1	1	43±2$^+$	43±5
1	3	41±3$^+$	37±4$^+$
1	10	32±4$^+$	47±2
1	30	29±2$^+$	48±6

Table 1: Effect of increasing concentrations of 3-AB on H_2O_2-mediated inhibition of mitochondrial respiration and increase in LDH release respectively (*$p<0.05$ vs. control, $^+p<0.05$ vs. 1 mM H_2O_2 only, n=4).

Incubation of rat PT cell cultures with 1 mM H_2O_2 for 4 hours significantly inhibited mitochondrial respiration and increased LDH release respectively as shown above in Table 1. 3-AB significantly attenuated H_2O_2-mediated decrease in mitochondrial respiration and at 3 mM, attenuated H_2O_2-mediated LDH release. Incubation with 1 mM H_2O_2 produced a maximal increase in PARS activity after 1 hour (7± 2 (control) vs. 35±4 (1 mM H_2O_2) pmol NAD incorporated/well/min, $p<0.05$, n=4). H_2O_2-mediated PARS activation was significantly inhibited by 3 mM 3-AB (35±4 (1 mM H_2O_2) vs. 14±6 (3 mM 3-AB) pmol NAD incorporated/well/min, $p<0.05$, n=4).

These results demonstrate that H_2O_2 produces significant cellular injury and death in primary cultures of rat PT cells. Furthermore, H_2O_2-mediated oxidant stress activates PARS. The ability of the PARS inhibitor 3-AB to significantly reduce H_2O_2-mediated PT cell injury and death suggests that the activation of PARS by ROS may contribute to renal reperfusion-injury.

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114P DIETARY VITAMIN E REDUCES OXIDANT STRESS AND IMPROVES INSULIN ACTION IN THE OBESE ZUCKER RAT *IN VIVO*

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The obese Zucker rat exhibits features of metabolic Syndrome X, including impaired glucose tolerance and insulin resistance. Oxidant stress contributes to poor insulin action in non-insulin dependent diabetes mellitus (NIDDM) (Paolisso *et al.*, 1994) and in this respect the obese Zucker rat may provide a paradigm for studying the role of oxidative damage in insulin resistance. Indeed, the antioxidant lipoic acid enhances insulin action in skeletal muscle of the obese Zucker rat *in vitro* (Jacob *et al.*, 1996). However, concurrent determinations of oxidant stress and insulin action are lacking. We therefore assessed the effects of the lipophilic antioxidant vitamin E on i.v. glucose tolerance and insulinaemia in the obese Zucker rat, in addition to determining plasma levels of the lipid peroxidation product 8-epi-$PGF_{2\alpha}$, a sensitive, isoprostane marker of oxidant stress *in vivo* (Gopaul *et al.*, 1995).

Male, 9-week old obese Zucker rats and age-matched, lean littermates were maintained for 4 weeks on control diet or diet supplemented with vitamin E ((±)-α-tocopherol acetate, 0.5 % w w^{-1} added to chow). Rats were then fasted overnight, anaesthetised with thiopentone sodium (120 mg kg^{-1} i.p.) and a basal venous blood sample collected in EDTA. D-glucose (0.5 g kg^{-1} i.v.) was then injected at t=0 and venous blood samples collected at t=1, 3, 6, 12 and 24 min. Data are mean±s.e. mean and analysed by Student's unpaired t test. Glucose tolerance and glucose-stimulated insulinaemia were assessed by area under the curve (AUC) for plasma glucose or insulin values between t=0 and t=24 min.

Body weight was greater in obese (control diet: 435.7±18.4 g; vitamin E: 453±7.1 g) relative to lean animals (control diet: 268.2±11.9 g; vitamin E: 294.7±4.0 g; n=4-6) ($P<0.01$). Obese plasma 8-epi-$PGF_{2\alpha}$ (10.4±1.1 nM, n=5) was elevated relative to the lean value (2.0±0.6 nM, n=5) ($P<0.01$) and reduced by dietary vitamin E (1.4±0.3 nM, n=5) ($P<0.01$) to become comparable with the lean+vitamin E value (1.1±0.3 nM, n=4). Obese basal plasma glucose (7.7±0.7 mM, n=4) and insulin (4.1±1.1 nM, n=4) levels, which were significantly elevated relative to lean levels (glucose: 4.1±0.5 mM; insulin: 0.1±0.03 nM, n=5) ($P<0.05$), tended to be reduced after dietary vitamin E (glucose: 6.1±0.9 mM; insulin: 2.8±1.3 nM, n=6) ($P>0.05$). In contrast, the basal lean plasma glucose level was elevated by dietary vitamin E (7.0±0.9 mM, n=5) ($P<0.05$) while the basal insulin level was not significantly affected (0.3±0.2 nM, n=5) ($P>0.05$). Both the plasma glucose and insulin response following an i.v. glucose load were greater in obese animals (Table 1). Dietary vitamin E in obese animals significantly reduced insulin AUC without a significant effect on glucose AUC. In contrast, dietary vitamin E appeared to elevate glucose and insulin AUC in lean animals, although statistical significance was not attained.

Table 1. Glucose and insulin AUC in lean and obese Zucker rats. *$P<0.05$ obese vs lean; †$P<0.05$ vitamin E vs control diet, n=4-6.

LEAN	glucose(AUC, mM.min)	insulin(AUC,nM.min)
control diet	140.8±17.9	4.9±0.4
vitamin E	169.9±20.5	7.0±2.0
OBESE		
control diet	221.6±15.3*	19.6±5.3*
vitamin E	183.7±21.3	5.2±0.6†

The elevation in plasma 8-epi-$PGF_{2\alpha}$ in the obese Zucker rat, agrees well with a report of raised levels of this isoprostane in NIDDM patients, indicating oxidant stress (Gopaul *et al.*, 1995). Vitamin E markedly reduced both plasma 8-epi-$PGF_{2\alpha}$ and glucose-stimulated hyperinsulinaemia without compromising glucose tolerance in the obese Zucker rat, which probably reflects improved insulin sensitivity (see Jacob *et al.*, 1996). In conclusion, our data establishes a clear link between oxidant stress and insulin resistance in this animal model of Syndrome X.

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115P REDUCTION IN PLASMA LEVELS OF 8-EPI-PROSTAGLANDIN $F_{2\alpha}$ AND REVERSAL OF ENDOTHELIAL DYSFUNCTION BY VITAMIN E IN THE WATENABE HERITABLE HYPERLIPIDAEMIC RABBIT

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Oxidant injury contributes to endothelial dysfunction in a variety of pathological conditions. This injury leads to lipid peroxidation during which F_2-isoprostanes are produced which can then be used as markers of oxidative stress (Morrow *et al*, 1996). We investigated the effect of the antioxidant vitamin E (Vit E) on the levels of the isoprostane, 8-epi-prostaglandin-$F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$), endothelial dysfunction and lesion development in the Watenabe heritable hyperlipidaemic (WHHL) rabbit.

Two groups of 45 weeks old male WHHL rabbits (3.9-4.1 kg) were maintained on standard chow diet or diet containing 0.5% Vit E for 12 weeks. At 0, 1, 3, 8 and 12 weeks, the animals were sedated with hypnorm (0.1ml.kg^{-1}, i.m.) and a marginal ear vein was cannulated for drug infusions. Endothelial function was assessed non-invasively using an infra-red photoplethysmography probe placed on the ear artery and subsequently analysing the percentage change in the relative height of the dicrotic notch (ΔRhDN), on arterial pulse waves, caused by acetylcholine (Ach, 0.5-2.0 μg^{-1}.kg^{-1}.min for 5 min, i.v.) (Klemsdal *et al*, 1994). Plasma levels of esterified 8-epi-$PGF_{2\alpha}$ were measured by gas-chromatography mass-spectrometry after hydrolysation (with potassium hydroxide) and immunoaffinity purification of plasma samples (Nourooz-Zadeh *et al*, 1995). Plasma lipid (cholesterol and tryglyceride), Vit E levels and the fatty streak formation in the thoracic aorta was assessed by standard methods. Data are expressed as mean±s.e. and statistical significant difference between means tested by unpaired *Students'* t –test.

Vit E treatment significantly reduced plasma levels of esterified 8-epi-$PGF_{2\alpha}$ (in ng.ml^{-1}; control, 0.179±0.017; Vit E, 0.124±0.010, n=5-7, $p<0.05$). These control levels were significantly higher than those observed in the New Zealand white rabbit given either cholesterol diet (1% for 8 weeks, 0.120±0.04 ng.ml^{-1}, n=6) or chow diet (0.03±0.01 ng.ml^{-1}, n=6) (Kengatharan *et al*, 1997). Vit E restored the reduced ability of Ach to cause a dose-dependent ΔRhDN (response to 2.0μg.kg^{-1}min^{-1} Ach at 8 weeks; control, -4.0±7.3; Vit E, -43.7±5.4, n=8-9, $p<0.05$). Fatty streak formation in the thoracic aorta and plasma lipid levels were not affected by Vit E.

Importantly, improvement in endothelial function by Vit E was not associated with reduced lesion formation, but with lower plasma 8-epi-$PGF_{2\alpha}$ levels in the WHHL rabbit. This suggests that endothelial dysfunction can be attenuated by reducing oxidative stress without necessarily reducing lesion formation in the thoracic aorta. Our data also confirms that, in this rabbit model of atherosclerosis, oxidative stress contributes to endothelial dysfunction.

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116P EVIDENCE FOR ENHANCED ENDOTHELIAL FUNCTION IN THE OBESE ZUCKER RAT WHICH IS REVERSIBLE BY DIETARY ANTIOXIDANTS

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The obese Zucker rat is a widely used model of insulin resistance. However, while exhibiting features of metabolic Syndrome X, including oxidant stress (Keen *et al.*, 1992), there is little evidence of endothelial dysfunction in this animal. Indeed, endothelium-dependent vasodilation has been reported to be enhanced in obese relative to lean Zucker rat isolated aorta (Auguet *et al.*, 1989). This may reflect some form of protective compensation in obese animals, possibly for oxidant stress. Our aim was therefore to investigate the relationship between oxidant stress and endothelial function in the perfused hindlimb *in situ* of the obese/lean Zucker rat.

Male 9-week old obese and lean Zucker rats were maintained for 4 weeks on control diet or diet supplemented with the antioxidants vitamin E ((±)-α tocopherol acetate, 0.5 % w/w added to chow) or vitamin C (L-ascorbic acid, 0.1 % w/v added to drinking water). Animals were anaesthetised with pentobarbitone sodium (60 mg kg^{-1} i.p.) and euthanised after heparinisation (200 u kg^{-1} i.v.). The hindlimbs were perfused *in situ* at constant flow (7 ml min^{-1}) via the abdominal aorta with physiological salt solution, gassed with carbogen, at 37 °C and pH 7.4. Following preconstriction with a constant infusion of phenylephrine (PEP, 100 nmol min^{-1}), endothelium-dependent vasodilation to acetylcholine (ACh) was assessed and standardised with respect to the maximal vasodilator response to the NO donor sodium nitroprusside (SNP, 100 nmol), as a relative measure of endothelial function. Data are mean±s.e. mean.

Body weight was greater in obese (control: 413.6±15.2 g; vitamin E: 428.2±8.6 g; vitamin C: 440.8±21.1; n=5-6) relative to lean animals (control: 280.2±4.2 g; vitamin E: 280.6±6.3 g; vitamin C: 285.5±6.3 g; n=4-5) ($P<0.01$). PEP-elevated perfusion pressure (PP) was comparable in obese (144.2±20.5 mmHg, n=5) and lean (129.6±6.0 mmHg, n=5) hindlimbs and remained similar after vitamin E (obese: 144.8±13.0 mmHg, n=6; lean: 141.6±13.3 mmHg, n=5) and vitamin C (obese: 119.6±10.1 mmHg, n=5; lean: 124.0±17.4 mmHg, n=4). Vasodilation to ACh was augmented in obese (pD_2=11.03±0.19) relative to lean (pD_2=10.53±0.13) ($P=0.06$, n=5) animals after control diet ($P<0.01$, 2 way ANOVA) but became similar after dietary vitamin E or C (Figure 1). This normalisation by antioxidants was associated with a reduction in obese ($P<0.01$, 2 way ANOVA) but little change in lean, endothelial function.

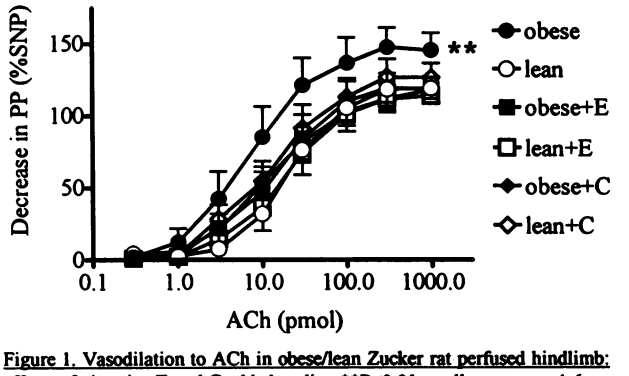

Figure 1. Vasodilation to ACh in obese/lean Zucker rat perfused hindlimb: effects of vitamins E and C added to diet. **P<0.01 vs all groups, n=4-6.

In summary, endothelial function was enhanced in insulin-sensitive tissue of the 13-week old obese Zucker rat, in an antioxidant-reversible manner. This suggests that obese endothelial hyperfunction is a response to oxidant stress, conceivably via an improvement in agonist-stimulated Ca^{2+}/NO signalling by reactive oxygen species (see Graier *et al.*, 1997), which can be normalised by antioxidants.

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117P PHARMACOLOGICAL ACTION OF INSULIN TO INHIBIT ENDOTHELIUM-DEPENDENT VASODILATION IN THE OBESE ZUCKER RAT PERFUSED HINDLIMB *IN SITU*

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Hypertension associated with insulin resistant states such as metabolic Syndrome X, may partly reflect a deficient action of insulin to attenuate vasoconstriction (Walker *et al.*, 1995) and/or enhance agonist-stimulated endothelial function (Gros *et al.*, 1994; Laight *et al.*, 1997), due to vascular insulin resistance. Our aim was to observe the effect of insulin, at a pharmacological concentration, on endothelium-dependent vasodilation in the perfused hindlimb *in situ* of the obese Zucker rat, a model of metabolic Syndrome X which exhibits hyperinsulinaemia and hypertension as early as 6 weeks of age (Ambrozy *et al.*, 1991; Zemel *et al.*, 1992).

Male 8-week old obese (294.4±8.8* g, n=21) and lean (215.6±3.2 g, n=20) (*P<0.01) Zucker rats were anaesthetised with pentobarbitone sodium (60 mg kg^{-1} i.p.) and killed after heparinisation (200 u kg^{-1} i.v.). The hindlimbs were perfused *in situ* at constant flow (7 ml min^{-1}) via the abdominal aorta with physiological salt solution, gassed with carbogen, at 37 °C and pH 7.4. Hindlimbs received a continuous infusion of human insulin (100 nM, Laight *et al.*, 1997) or its vehicle (0.1 % bovine serum albumin in phosphate buffered saline, pH 7.4) commencing 20 min before preconstriction (Laight *et al.*, 1997) with a continuous infusion of phenylephrine (PEP, 100 nmol min^{-1}). Vasodilation to bolus dose acetylcholine (ACh, 0.001-10 nmol) and sodium nitroprusside (SNP, 0.001-100 nmol)) was then assessed. Data are mean±s.e. mean and dose-response curves were analysed by 2 way ANOVA.

Insulin had no significant effect on basal perfusion pressure (PP) in obese (vehicle: 30.3±2.7, n=9; insulin: 27.5±3.2 mm Hg; n=12, P>0.05) or lean animals (vehicle: 33.3±1.5 mm Hg, n=10; insulin: 31.4±3.6 mm Hg; n=10, P>0.05). Similarly, insulin had no significant effect on PEP infusion-elevated PP in obese (vehicle: 129±19.8 mm Hg, n=9; insulin: 157.6±14.7 mm Hg; n=12, P>0.05) or lean animals (vehicle: 160.0±11.6 mm Hg, n=10; insulin: 163.7±19.2 mm Hg; n=10, P>0.05). Vasodilation to ACh was inhibited by insulin in obese animals (P<0.01, 2 way ANOVA) (Figure 1a) (vehicle: pD_2=10.68±0.18, n=5; insulin: pD_2=10.11±0.22, n=7; P=0.05). In contrast, obese vasodilation to SNP was unaffected by insulin (P>0.05, 2 way ANOVA; vehicle: pD_2=8.74±0.19, n=4; insulin: pD_2=8.81±0.30, n=5; P>0.05). Neither lean vasodilation to ACh (pD_2=10.85±0.22, n=6) (Figure 1b) nor SNP (pD_2=8.67±0.12, n=4) was significantly altered by insulin (ACh: pD_2=10.47±0.26, n=6; SNP: pD_2=8.85±0.19, n=4) (both P>0.05, 2 way ANOVA).

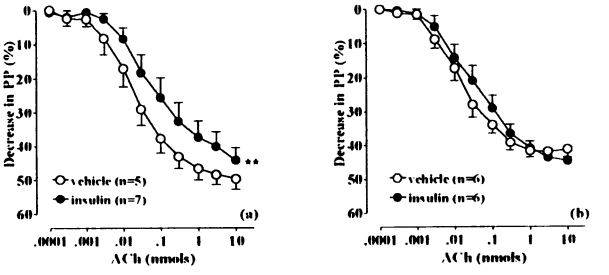

Figure 1. Effect of insulin on vasodilation to ACh in 8-week old obese (a) and lean (b) Zucker rat perfused hindlimb *in situ*. **P<0.01, 2 way ANOVA.

In summary, insulin even at 100 nM failed to modulate hindlimb vascular reactivity in the lean Zucker rat. In contrast, we demonstrate a pharmacological action of insulin to inhibit endothelial function in a vascular resistance bed of the obese Zucker rat. Our data therefore support the notion that hyperinsulinaemia may be an adverse influence on endothelial function in this animal.

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118P ENDOTHELIAL DYSFUNCTION FOLLOWING EXPERIMENTAL OXIDANT STRESS IN THE RAT *IN VIVO*

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Oxidant stress is associated with several diseases, including atherosclerosis and diabetes mellitus where it contributes to endothelial dysfunction (Halliwell, 1991). In particular, reactive oxygen species (ROS) such as superoxide anion can inactivate endothelium-derived nitric oxide (NO) (Gryglewski *et al.*, 1986). In this study, we evaluated vascular endothelial function *in vitro* following experimental oxidant stress generated *in vivo* by the redox cycling agent hydroquinone (HQ), the Cu/Zn superoxide dismutase inhibitor triethylene tetramine (TETA) and the glutathione (GSH) depleting agent L-buthionine-[S,R]-sulfoximine (BSO). The ability of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) to enhance contraction to an α_1-adrenoceptor agonist in rat isolated aorta was taken as an indicator of basal NO function while plasma total antioxidant status (TAOS) provided a marker of oxidant stress.

Male Wistar rats (250-300 g) were treated daily for 1 week with HQ (50 mg kg^{-1} i.p.), TETA (100 mg kg^{-1} i.p.) or BSO (50 mg kg^{-1} i.p.) either alone or in combination while a temporal control group received normal saline only (1 ml kg^{-1} i.p.). Animals were subsequently euthanised and thoracic aortic rings prepared for organ bath studies in physiological salt solution (pH 7.4) maintained at 37 °C and gassed with carbogen. Contraction to phenylephrine (PEP, 1 nM-3 μM) was then assessed with and without a 10 min preincubation with L-NAME (0.3 mM). Plasma TAOS was assessed at 37 °C and pH 7.4 in an inhibition assay by measuring the peroxidase-catalysed accumulation of the radical cation 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS$^+$, absorbance read at 405 nm) after 6 min and related to the ascorbate equivalent antioxidant concentration (AEAC) (see Arnao *et al.* (1996)). Data are mean±s.e. mean and were compared by 1 way ANOVA and Dunnett's test.

L-NAME elicited a leftward shift in contraction to PEP (control: ΔpD_2=0.79±0.09, n=6), which was significantly attenuated by HQ (ΔpD_2=0.44±0.03, n=5, P<0.05) and HQ+BSO (ΔpD_2=0.30±0.08, n=4, P<0.01) and also reduced by TETA (ΔpD_2=0.57±0.08, n=4) and TETA+BSO (ΔpD_2=0.52±0.08, n=5), although statistical significance was not attained. In contrast, BSO alone had no effect (ΔpD_2=0.76±0.07, n=6, P>0.05). In addition, oxidants *in vivo* significantly reduced plasma TAOS (Figure 1).

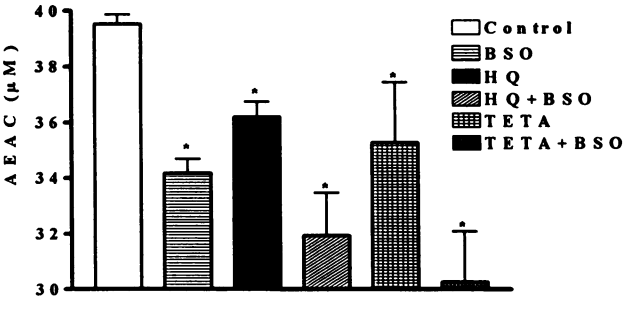

Figure 1. Plasma TAOS in rats after oxidant treatments. *P<0.05, n=4-6.

In conclusion, two dissimilar oxidants, HQ and TETA, elicited endothelial dysfunction in the rat which was associated with evidence of oxidant stress, consistent with a role for ROS in the inactivation of NO. Although these effects may be aggravated by GSH depletion, the failure of BSO *per se* to impair endothelial function despite a fall in TAOS in plasma, suggests an additional site of oxidant action for HQ and TETA, eg. a lipophilic compartment, which does not involve thiol-dependent antioxidant status.

This work was supported by Lipha s.a., Lyon, France.

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119P ELEVATED PLASMA TOTAL ANTIOXIDANT STATUS IN THE OBESE ZUCKER RAT ASSESSED BY A PHYSIOLOGICAL MICROASSAY

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The inhibition by antioxidants of the generation of the radical cation 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) ($ABTS^{\cdot}$), is a well established principle in the spectrophotometric determination of total antioxidant status (TAOS) in plasma (Rice-Evans & Miller, 1994; Romay *et al.*, 1996). We have now developed a rapid, platereader-based microassay of plasma TAOS based on the suppression of $ABTS^{\cdot}$ formation catalysed by peroxidase (Arnao *et al.*, 1996) at physiological temperature and pH. We then assessed plasma TAOS of the obese Zucker rat, a model of metabolic Syndrome X and insulin resistance, which has been reported to exhibit a higher antioxidant defence than its lean, insulin-sensitive littermate (Keen *et al.*, 1992), possibly as a response to obesity-related oxidant stress.

The generation and detection of $ABTS^{\cdot}$ was a modification of the method described by Arnao *et al.* (1996) in the assessment of TAOS of food. The reaction mixture consisted of (final concentration): 20 µl ABTS (2 mM); 10 µl horseradish peroxidase (30 mU/ml); 20 µl H_2O_2 (0.1 mM); and 10 mM phosphate-buffered saline (pH 7.4) to make a total volume of 100 µl in a 96 well plate in a platereader. The reaction was initiated by the addition of peroxide and conducted at 37 °C. Data are mean±s.e. mean and were compared by Student's unpaired t test.

The increase in absorbance at 405 nm, reflecting the generation of $ABTS^{\cdot}$, was linear with time over at least the first 8 min ($r=0.981$, $n=3$, $P<0.001$). Rat plasma derived from blood collected in EDTA, reduced the rate of $ABTS^{\cdot}$ accumulation, as has been described earlier for human serum (Romay *et al.*, 1996). The addition of 6 µl rat plasma resulted in an optimal, stable reduction in $ABTS^{\cdot}$ accumulation after 6 min and therefore these parameters were adopted in future determinations of TAOS in an inhibition microassay with a fixed time point. L-ascorbic acid (10-60 µM) inhibited $ABTS^{\cdot}$ accumulation measured at 6 min in a linear manner ($r=0.996$, $n=3$, $P<0.001$), allowing plasma TAOS to be expressed as an ascorbate equivalent antioxidant concentration (AEAC, µM). Plasma TAOS was subsequently determined to be significantly higher in 13-week old obese relative to age-matched lean, male Zucker rats (Figure 1). This difference was preserved in 13-week old Zucker rats which had been maintained for 4 weeks on diet supplemented with vitamin E ((±)-α tocopherol acetate, 0.5 % w/w), which improved plasma TAOS in both obese and lean animals (Figure 1).

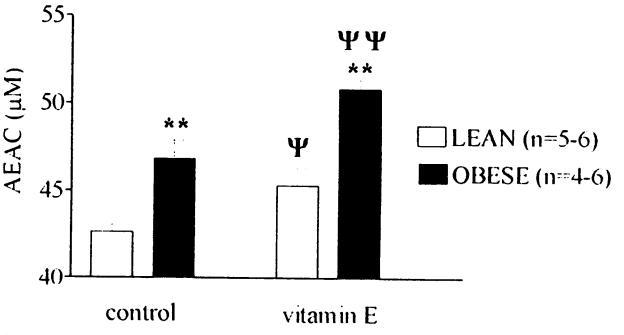

Figure 1. Plasma TAOS in obese and lean Zucker rats: effects of dietary vitamin E. **P<0.01 lean vs obese; ΨP<0.05, ΨΨP<0.01 control vs vitamin E.

In conclusion, our physiological microassay of plasma TAOS *in vitro* supports the report by Keen *et al.* (1992) that the obese Zucker rat has a superior antioxidant defence system, which may be further improved by dietary vitamin E.

This work was supported by Lipha s.a., Lyon, France.

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120P ROLE OF REACTIVE OXYGEN SPECIES ON TRANS-ENDOTHELIAL INSULIN TRANSPORT

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Uptake of insulin into insulin sensitive tissues may, in part, be dependent on active insulin transport across the endothelium, possibly mediated by insulin receptors (Jansson *et al.*, 1993; King & Johnson, 1985). Defects in this process associated with endothelial dysfunction could therefore delay the onset of insulin action and contribute to the reduced peripheral insulin sensitivity observed in Non-Insulin-Dependent Diabetes Mellitus (NIDDM). As oxidative stress has been linked to NIDDM and a beneficial effect of antioxidant treatment has been documented (Gopaul *et al.*, 1995; Paolisso *et al.*, 1993), we investigated the effect of reactive oxygen species (ROS) on insulin receptor mRNA expression in Ea.Hy926 cells (a human endothelial cell line) and insulin transport across a confluent Ea.Hy926 cell monolayer.

Ea.Hy926 cells were incubated with the ROS generating system xanthine oxidase/hypoxanthine (XO/HX; 20mU/ml/1mM). A 49±6% decrease in insulin receptor mRNA levels was observed after 2h, using RT-PCR with subsequent densitometry scanning of ethidium bromide stained agarose gels ($P<0.05$; $n=3$). This decrease was prevented with catalase (117±11%; 160U/ml; $n=3$). The superoxide ($O_2^{\cdot}$) generator LY83583 (10µM) also reduced insulin receptor mRNA levels after 2h (56±4%; $P<0.01$; $n=3$). Since LY83583 only generates $O_2^{\cdot}$ when metabolized intracellularly, a polyethylene glycol derivative of superoxide dismutase (PEG-SOD; 200U/ml) was utilized to facilitate its cellular uptake. PEG-SOD reversed the effect of LY83583 to 91±4% of control levels ($n=3$).

Insulin transport across a confluent monolayer of Ea.Hy926 cells was assessed by adding human insulin (100nM) to the upper chamber of a dual chambered culture plate. [^{14}C]-inulin (0.36µCi/0.29mg/ml) was used as a marker of passive diffusion and quantitated by liquid scintillation spectrometry. Insulin in the lower chamber was measured using a sandwich ELISA assay (DAKO Insulin, DAKO, UK). In cells exposed to XO/HX (20mU/ml/1mM) for 2h, transport was reduced to 50±6% of control levels ($P<0.01$; $n=4$). Concomitant catalase (160U/ml) exposure restored insulin transport back to control levels (97±24%; $n=4$). Following 2h incubation with LY83583 (10µM), insulin transport was reduced to 52±6% of control ($P<0.001$; $n=4$). Incubation with the RNA synthesis inhibitor Actinomycin D (15µM) for 2h reduced insulin receptor mRNA levels by 40%, but had no effect on insulin transport ($n=4$). Data are expressed as mean±s.e.m. and were analyzed using one-sample t-test.

The above data demonstrate the capacity of both hydrogen peroxide and $O_2^{\cdot}$ to reduce insulin receptor mRNA levels and decrease transendothelial insulin transport. Since Actinomycin D had no effect on insulin transport, this suggests that the primary effect of ROS is on insulin transport and not on the reduction of insulin receptor mRNA levels.

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121P CHARACTERISATION OF GLUCOCORTICOID-INDUCED p155 EXPRESSION BY HUMAN MONOCYTES

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Glucocorticoids are potent anti-inflammatory drugs which exert profound suppressive effects on blood monocyte and tissue macrophage functions. A novel 155kDa mononuclear phagocyte surface antigen (p155) has been identified which is upregulated by glucocorticoids but whose function has yet to be determined (1).

The aim of this study was to examine the dynamics and specificity of p155 induction by dexamethasone (DEX) *in vitro* by human peripheral blood mononuclear cells (MNC).

Citrated blood from healthy subjects was incubated at 37°C in the presence of DEX (0 - 1μM) for 24h. A time course of 6-24h was also performed. Following incubation, the cell pellet was washed twice with phosphate-buffered saline and reacted with monoclonal anti-p155 mAb Mac1.58 in the presence of human IgG to block non-specific sites (1h 4°C). After further washing, cells were stained with a FITC-goat anti-mouse $F(ab')_2$ IgG for 30 min. at 4°C. Finally, erythrocytes were lysed (Coulter Clone blood lysing reagent, Coulter, Miami,FL) and leukocytes fixed in 2% paraformaldehyde. For the confocal experiments, MNC were isolated by density centrifugation over histopaque prior to staining for p155. Saponin (0.02%) was added to all staining reagents to permeabilise the cells for intracellular staining. MNC p155 expression was analysed by confocal microscopy (Bio-Rad MRC 1024) or by flow cytometry (FACScan, Becton Dickinson). In the latter case, monocytes and lymphocytes were identified by the forward and side scatter profiles and p155 expression reported as FITC-equivalent molecules per cell (FEMC), standardised against FITC-coated beads.

DEX treatment of MNC *in vitro* resulted in a concentration-dependent increase in p155 expression by monocytes, rising from a basal value of 50,600±1,500 to 197,000±2,700 FEMC at 1μM DEX ($p<0.001$, ANOVA, n=5) with a mean ED_{50} of 2×10^{-8}M. Lymphocytes did not express p155 at any concentration of DEX. Maximum expression was observed after 24h incubation. Co-incubation of DEX (0.1μM) with the glucocorticoid receptor (GR) antagonist RU38486 (10μM) completely blocked p155 induction demonstrating that the effect is via a GR-mediated event. Untreated cells 16,500±2,300; 0.1μM DEX 101,700±12,100; DEX (0.1μM) + RU38486 (10μM) 18,000±7,200 ($p<0.05$, paired T-test, n=3). Saponin-permeabilisation of control or 0.1μM DEX-treated MNC revealed that monocyte intracellular p155 expression increased 50-fold from 3,000±400 to 152,000±8,200 FEMC whereas lymphocyte staining was not detected. (1 expt. performed in quadruplicate). Confocal microscopy of cell surface p155 distribution on 1μM DEX treated-MNC demonstrated intense, punctate peripheral staining by monocytes which was absent in lymphocytes and in untreated monocytes. Intracellularly, monocytes incubated with 1μM DEX showed a higher intensity of cytoplasmic p155 staining than control cells.

In conclusion, p155 expression is exquisitely upregulated by glucocorticoids in human monocytes and is expressed both on the cell surface and within the cytoplasm. Expression appears to be a GR-mediated process with a time-course of 24h required for maximal expression. Whilst the identity and function of the p155 antigen is as yet unknown, it is an interesting and rare example of a phagocyte surface and intracellular antigen whose expression is dramatically increased by glucocorticoids.

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122P A MODEL OF PERSISTENT, INFLAMMATION-INDUCED MECHANICAL HYPERALGESIA IN THE MOUSE

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In chronic inflammatory pain conditions noxious stimuli induce the release of inflammatory mediators including prostaglandins, bradykinin and neurokinins resulting in the lowering of nociceptive thresholds. Most behavioural models that aid our understanding of the role of mediators and mechanisms of persistent pain come from nociceptive assays in the rat. The aim of this study was to develop a model of chronic inflammatory pain in the mouse. Here we show the effect of standard analgesic drugs and compounds with activity against hyperalgesic mediators on inflammation-induced mechanical hyperalgesia.

Female C57BL/6 mice (20-25g n=6/group) were injected with 15μl of Freunds complete adjuvant (FCA) intraplantar into the left hind paw. The paw withdrawal threshold to a mechanical stimulus was measured in both the left and right hind paws using an Ugo Basile Analgesymeter with a blunt, dome-shaped probe (cut-off threshold of 180g). The effects of diclofenac (a non-selective cyclooxyganase inhibitor), morphine, RP67580 (NK_1 receptor antagonist), desArg^{10}Hoe140 and Hoe140 (bradykinin B_1 and B_2 receptor antagonists, respectively) were assessed 48h following FCA injection. Drugs were administered either orally (p.o.) in a suspension of 1% (w/v) tragacanth or subcutaneously (s.c.), dissolved in sterile saline except for RP67580 which had 10% DMSO + Tween as vehicle. Paw withdrawal thresholds were taken prior to and then at 1, 3, 6h following drug administration. The percentage reversal of hyperalgesia was calculated and presented as mean ± s.e.mean. Drug effects are expressed as the D_{50} values (dose at which a 50% reversal of hyperalgesia was achieved).

FCA produced a marked reduction of paw withdrawal threshold that reached a maximum within 30 h (control 98 ± 2g; FCA 59 ± 2g) and was maintained for at least 96 h. No hyperalgesia was observed in the contralateral paw. Diclofenac, morphine, RP67580 and desArg^{10}Hoe140 all produced a dose-dependent reduction of the mechanical hyperalgesia with D_{50} values shown in Table 1. In contrast Hoe 140 was only weakly active at reversing the FCA induced hyperalgesia. The maximum effect of all drugs was observed at 1 h following drug administration. The results show that standard analgesics and specific inhibitors of inflammatory mediators are efficacious in this mouse model of inflammatory mechanical hyperalgesia. As the observed profile of drug activity is similar to that seen in the rat, we propose that this mouse model could be a useful alternative technique to the rat for assessing potential analgesic drugs.

Table 1: D_{50} values and % reversal of mechanical hyperalgesia 1 h following drug administration in the mouse paw pressure model.

compound	dose (mg/kg)	D_{50} (mg/kg)	maximum % reversal	compound	dose (mg/kg)	D_{50} (mg/kg)	maximum % reversal
Diclofenac (p.o.)	3-300	39.3	59.9±5.2*	DesArg^{10}Hoe140(s.c.)	0.012-0.6	0.26	56.6±6.2*
Morphine (s.c.)	0.3-10	1.2	157.1±5.8*	Hoe140 (s.c.)	0.0016-0.08	>0.08	32.3±6.1*
RP67580 (s.c.)	3-30	>30	49.0±7.8*				

* $p< 0.05$ compared to relevant control group (ANOVA followed by post hoc analysis of means using Tukeys HSD). All statistics was done on the raw data.

123P POLY-L-LYSINE (PLL)-INDUCED NEUTROPHIL (PMN) ACCUMULATION AND PLASMA EXUDATION (PE) IN RABBIT SKIN: ROLE OF SULPHATION IN INHIBITORY ACTION OF HEPARIN

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Heparin has long been used as an anticoagulant and anti-thrombotic. More recently, there has been much interest in this drug as a potential anti-inflammatory treatment. For example, heparin inhibits eosinophil accumulation in guinea-pig skin (Teixeira & Hellewell, 1993). Intradermal injection of unfractionated heparin (UH), prior to the polycation, PLL, caused significant reductions in PMN accumulation and plasma exudation (PE), whereas poly-glutamic acid (PGA; 100 μg site^{-1}) had no significant effect, suggesting that the action of UH was not a simple charge related effect (Jones *et al*, 1997). However, heparin has been shown to interact with PLL in solution *via* sulphate groups (Mulloy *et al.*, 1996). Here, we have further investigated the effects of UH, N-desulphated heparin (NDH) and PGA against PMN accumulation and PE induced by PLL in rabbit skin.

Rabbit (NZW) PMNs, separated from arterial blood by sedimentation and Ficoll centrifugation, were labelled with ^{111}Indium (In) chloride/mercaptopyridine, washed and injected (10^7 kg^{-1}) into pentobarbitone anaesthetised recipient rabbits (2.5 - 3.1 kg). To measure PE, ^{125}I albumin (0.1 MBq kg^{-1}) was injected intravenously 1 h after the labelled cells. Intradermal injections (0.1 ml) were made in the shaved dorsal skin of these rabbits. Responses were measured over a 3 h period. ^{111}In-PMN accumulation in excised skin sites was expressed as cells site^{-1} and PE in the same sites as μl plasma. Mean ± s.e.m. values (n = 5 or 6) were calculated and the significance of differences assessed by ANOVA followed by a modified t-test.

To measure interaction, 100 μl volumes of PLL (1mg ml^{-1}) were mixed in 96 well plates with 100 μl volumes of saline, UH (250-1000 iu ml^{-1}), NDH or PGA (50-5000 μg ml^{-1}) and light absorbance measured at 450 nm. Peak absorbance values (n=4) occurred at 250 iu ml^{-1} (UH, 0.365 ± 0.002), 5 mg ml^{-1} (NDH, 0.899 ± 0.026) and 1 mg ml^{-1} (PGA, 0.497 ± 0.078). Absorbance of PLL + saline was 0.09 ± 0.002.

Injection of NDH (5-100 μg site^{-1}), 10 min prior to PLL (100 μg site^{-1}), had no significant effect on PMN accumulation or PE whereas UH (100 iu site^{-1}) produced significant ($P<0.01$) inhibition of 72% and 62%, respectively. In a further experiment, control PLL-induced PMN accumulation and PE responses were 43997 ± 9520 cells and 174 ± 14 μl. These responses were significantly reduced by UH (100 iu site^{-1}; 11134 ± 2711, 53 ± 10 μl; $P<0.001$, $P<0.01$), NDH (500 μg site^{-1}; 23924 ± 6537, 92 ± 28 μl; $P<0.01$, $P<0.01$) and PGA (500 μg site^{-1}; 13157 ± 2697, 85 ± 16 μl; $P<0.001$, $P<0.01$).

From these results, it appears that the inhibitory effect of intradermal UH on polycation-induced cutaneous inflammatory responses may not be determined solely by sulphation. However, inhibition does appear to be related to ability to interact with PLL.

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124P EFFECT OF 7-NITROINDAZOLE AND RELATED INDAZOLES ON INDUCIBLE NITRIC OXIDE SYNTHASE *IN VITRO* AND *IN VIVO*

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7-nitroindazole (7-NI) is a potent inhibitor of neuronal nitric oxide synthase (nNOS) and, as such, has been extensively used by researchers to study the biological roles of nitric oxide (NO) in the central nervous system (reviewed by Moore & Handy, 1997). In contrast, little attention has been paid to the effect of 7-NI and related indazoles on the inducible isoform of NOS (iNOS).

Purified mouse macrophage iNOS (9.5U ml^{-1}, Caymen Ltd) was incubated (15min, 37°C) with 0.5μCi L-H^3 arginine (120nM), NADPH (0.5mM) and tetrahydrobiopterin (BH_4, 9.1μM) in the presence of different concentrations of the test drug (final volume of 105μl). NOS activity was determined as the conversion of L-arginine to citrulline (Babbedge et al., 1993). IC_{50} values (mean of 6 experiments) were, 7-NI (1.9 μM), 3-bromo 7-NI (0.11 μM), 2,7 dinitro 7-NI (2.1 μM), L-N^G nitroarginine (L-NOARG, 1.02 μM) and L-N-iminoethyllysine (L-NIL, 0.41 μM). Inhibition of iNOS by 7-NI was dependent on BH_4 concentration (e.g. 10μM 7-NI; inhibition was 67.8±0.6%, 50.3±3.9% and 0%, all n=6, at BH_4 concentrations of 0.91μM, 9.1μM and 45.6 μM respectively).

Incubation (37°C, 95%O2:5% CO_2, 1-24h) of cultured J774.2 macrophages (500,000 ml^{-1}) with E.Coli lipopolysaccharide (LPS, 10μg ml^{-1}) and interferon-γ (IFNγ, 10U ml^{-1}) resulted in a time dependent appearence of nitrite in the culture medium (e.g. 19.0±1.34μM, n=6, at 18h, measured by Greiss reaction). Compared with studies using purified iNOS, 7-NI and 3-bromo 7-NI were 70x less potent as iNOS inhibitors in J774.2 cells. IC_{50} values (mean of 6 experiments) were 7-NI (133.6 μM), 3-bromo 7-NI (77.2 μM) and L-NIL (4.33 μM). The IC_{30} for L-NOARG was 165.7 μM. None of the drugs (up to 500 μM) affected cell viability (reduction of MTT to formazan).

LPS (10mg kg^{-1}, i.p.) administered to urethane (5g kg^{-1}, i.p.) anaesthetised rats reduced MAP (76.4±5.7mm Hg before c.f. 43.0±4.2mm Hg, 5h after LPS, n=5, $P<0.05$) and increased plasma nitrate/nitrite (32.2±5.3μM to 100.7±25.7μM, n=5, $P<0.05$), creatinine and bilirubin without affecting lipase concentration. In control experiments, 7-NI and 3-bromo 7-NI (25mg kg^{-1}, i.p.) caused a gradual fall (<10 mm Hg over 5h) in MAP which was similar to that observed in vehicle-treated animals. In contrast, animals administered 7-NI (25mg kg^{-1}, i.p.) followed 15 min thereafter by LPS (10mg kg^{-1}, i.p.) experienced a rapid fall in blood pressure and died 175±15min (n=8) after 7-NI injection. Accordingly, further experiments were conducted in animals killed 2h after LPS injection. LPS did not reduce MAP or influence plasma creatinine, bilirubin, lipase or nitrate/nitrite concentration at 2h. Furthermore, administration of 7-NI or 3-bromo 7-NI (25mg kg^{-1}, i.p.) to LPS-injected animals was without effect on any of these parameters.

These data confirm the iNOS inhibitory activity of indazoles. 3-bromo 7-NI is 3.7x less potent (purified enzyme) or 18x less potent (J774.2 cells) than L-NIL as an iNOS inhibitor. Intriguingly, both 7-NI and 3-bromo 7-NI promote (not inhibit) LPS-induced hypotension in the rat by a mechanism which appears not to be associated with changes in plasma markers of organ failure or nitrate/nitrite concentration.

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125P THE EFFECT OF N$^{\omega}$-NITRO-L-ARGININE METHYL ESTER ON BONE MARROW EOSINOPHIL NUMBER AND NITRIC OXIDE SYNTHASE ISOFORM EXPRESSION IN OVALBUMIN-SENSITIZED RATS

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Nitric oxide (NO) plays a major role in eosinophil locomotion (Zanardo et al., 1997). Chronic N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) treatment in rats induced a marked reduction in the *in vivo* and *ex vivo* migration of eosinophils (Ferreira et al., 1997) and an inhibiton of eosinophil infiltration into the airways of ovalbumin (OVA)-sensitized animals (Ferreira et al., 1998). In addition, it was observed that rat peritoneal eosinophils do express both type II and type III NO synthase (Zanardo et al, .1997). We have now investigated whether chronic NO inhibition influences both the number and expression of NO synthase isoforms of eosinophils (EOs) obtained from the bone marrow .

Male Wistar rats (180-200 g) were treated with L-NAME (75 μmol/rat/day) for 4 weeks. Active sensitization against OVA was performed by s.c. injection of 200 μg OVA + 8 mg $Al(OH)_3$. On day 14, OVA (1 mg) was injected into the airways. At 48 h after antigen challenge, the rats were killed and the femoral bone marrow aspirated. Differential cell counts were performed using cytospin preparation stained with Leishman. The NO synthase expression was detected by immunohistochemistry method using polyclonal anti-type I, monoclonal anti-type II or monoclonal anti-type III antibodies. Our results showed that following OVA challenge, the total content of bone marrow EOs was significantly higher ($p<0.05$) in L-NAME-treated animals animals. The increase of EOs by L-NAME was due to the mature forms of EOs (see table below).

	Bone Marrow Eosinophils (%)		
Treatment	Total	Myelocyte	Metamye/Mature
Control	3.9±0.5	1.3±0.2	2.6±0.4
L-NAME	7.1±1.3*	1.3±0.4	5.7±1.3*

* $p<0.05$

Type III NOS immunoreactivity was detected in 19±2.6% and 17±0.6% of bone marrow EOs from control- and L-NAME-treated rats, respectively. Type II NO was detected in 41±1.4% and 25±1.5% of bone marrow EOs from control and L-NAME-treated rats, respectively. Type I NOS was not detected in either group.

Whether the increase of eosinophils in bone marrow reflects impaired emigration due to NO synthesis blockade is under investigation.

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126P DETECTION AND PRELIMINARY CHARACTERISATION OF A UNIQUE PROTEIN CONSTITUENT OF BASOPHIL SECRETORY GRANULES

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Basophils have long been implicated in the pathogenesis of allergic disease, but investigation of their role has been frustrated by the lack of reliable means for detecting this cell type in tissues. We have prepared a monoclonal antibody, designated BB1, specific for human basophils, by immunising mice with purified peripheral blood basophils. We have found that this antibody reacts specifically in immunocytochemistry with basophils, but not with eosinophils, neutrophils, monocytes, lymphocytes, mast cells, or any other cell type or tissue structure. However, BB1 did react with a proportion of KU812F cells, a leukaemic cell line which possesses some basophil-like properties.

The relative amounts of BB1 antigen in various cell types was assessed by dot blotting of serial dilutions of cell lysates. KU812F cells contained approximately twice as much antigen per cell as did basophils, whilst neither eosinophils nor neutrophils had any detectable antigen by this technique. As less than 10% of the KU812F cells expressed the antigen, these results suggest that the cells that do express it do so at levels 20-30 times that found in basophils.

The granular appearance of basophils stained with BB1 suggested that the antigen is present primarily in the secretory granules. This was confirmed when basophils were challenged with anti-IgE antibody or with the calcium ionophore A23187. Analysis of the supernatants by serial dilution and dot blotting revealed the release of 19% and 18% of the total antigen content was provoked by anti-IgE and A23187, respectively, compared to a spontaneous release of 6%. Following experimental activation, fewer cells stained with BB1 on immunocytochemistry, and in those that did, fewer granules were identified. FACS analysis allowed detection of a proportion of nonpermeabilised cells, suggesting that some of the antigen is present on the cell surface, as well as in the granules.

BB1 antibody failed to react on immunoblots following SDS-PAGE, but when gel electrophoresis was performed in the absence of SDS, positive reactions were achieved on the subsequent Western blots. Under these conditions, the molecular mass of the BB1 antigen expressed in KU812F cells was determined by the method of Hedrick and Smith (1967) to be 124 ± 11 kDa. The antigen expressed in peripheral blood basophils differed from that expressed in KU812F in that it did not enter the gel under the conditions employed, which suggests that it is a more basic protein.

The antigen identified by antibody BB1 should prove valuable as a biochemical marker for human basophils and may represent a new mediator of allergic reactions.

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127P CARDIOTOXIC EFFECTS OF LEVOBUPIVACAINE, BUPIVACAINE AND ROPIVACAINE: AN *IN VITRO* STUDY IN GUINEA-PIG AND HUMAN CARDIAC MUSCLE

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The cardiotoxicity of local anaesthetics results from a depressant action on both mechanical function and excitability of the heart[1-3]. We have conducted *in vitro* studies to compare the effects of levobupivacaine HCl (L), bupivacaine HCl (B) and ropivacaine HCl (R) on both contractility and electrophysiology of cardiac muscle.

Papillary muscles and myocytes were isolated from guinea-pig (♂ Dunkin-Hartley, ~300g) heart, and pectinate muscles isolated from human atrial appendage. Tissue was placed in Krebs-Henseleit buffer at 30 or 37°C, stimulated at 1Hz and isometric tension or cell shortening recorded. Action potential parameters were recorded in papillary muscles: Vmax (maximum rate of rise of membrane potential), APA (action potential amplitude) and APD90 (action potential duration to 90% repolarisation). Data represent mean ± sem (analysed by unpaired t-test or ANOVA where appropriate), for L, B & R, respectively. L (Chiroscience Ltd), B (Marcain™) & R (Naropin™) were added directly to the buffer.

In myocytes, at 5μM, only B had a significant negative inotropic effect (peak shortening amplitude decreased to 74.6 ± 3% control, n=7). The drug concentration producing a 50% reduction in either cell shortening or developed tension (DT) was obtained from both cumulative dose-response curves in myocytes (12.9±2.3, 10.3±0.6 & 18.0±2.6μM; n=7) and from non-cumulative dose-response curves in papillary muscle (9.7±2.6, 8.8±2.2, & 12.3±3.6μM; n=5-8), respectively. In pectinate muscle, there was a similar decrease in DT for L, B & R at both 3μM (65.5±11.9, 62.8±16.1 & 59.9±9.6%; n=8) and 30μM (20.6±5.8, 24.6±6.6 & 30.6±6.9%; n=6-7). The rate of recovery of contraction following washout of L was significantly ($P<0.05$) greater than that for B in both myocytes (by 24%) and papillary muscle (by 27%). In papillary muscle, at 3μM, only B produced a significant decrease in Vmax (by 11%) compared to time-matched control ($P<0.01$). At 30μM, the decrease in Vmax with L & R was significantly less (to 64.1±4.0% & 65.6±6.2% of control; n=6-8) than with B (to 44.8±3.3% control; n=6, $P<0.01$). At 30μM, only R produced a significant increase in APD90 from time-matched control (by 14.6±3.7%; n=6, $P<0.01$). After 40min washout (from 30μM) Vmax fully recovered for L & R, but not B ($P<0.001$ compared to time-matched control).

Considering L & B, these results suggest that at comparable anaesthetic potency, L would be less likely to induce arrhythmias than B and in addition, recovery of cardiac function following accidental overdose would be more rapid for L than B.

We are grateful to Mr M Galiñanes, (Glenfield Hospital NHS Trust, Leicester) for supply of human tissue.

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128P EFFECTS OF THE ANTILEUKEMIC AGENT, AMSACRINE, ON THE CARDIAC ACTION POTENTIAL RECORDED IN SHEEP ISOLATED PURKINJE FIBRES

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Amsacrine is a widely used DNA topoisomerase inhibitor whose adverse effects include prolongation of the QT interval of the ECG (Shinar & Hasin, 1984). In the present experiments we have attempted to provide an *in vitro* correlate to this clinical observation.

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 amsacrine (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). Amsacrine was formulated as a 1 mM stock solution in water and diluted directly in the perfusant. Appropriate amounts of water were added to the perfusant of vehicle-treated fibres. Data are presented as arithmetic means ± s.e. mean. Amsacrine- and vehicle-related changes are relative to the baseline values.

Baseline values. In vehicle-treated fibres ($n = 4$) the pretreatment values for APD_{90}, MRD, UA and E_m were 279 ± 26 ms, 566 ± 51 V/s, 119 ± 4 mV and -79 ± 1 mV, respectively. The respective values in amsacrine-treated fibres ($n = 4$) were: 280 ± 28 ms, 479 ± 69 V/s, 118 ± 2 mV and -83 ± 4 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 amsacrine. The table *(above right)* shows the mean differences (± s.e. mean difference) in the various parameters between amsacrine- and vehicle-treated fibres (differences are for amsacrine-treated relative to the vehicle-treated group):

	Amsacrine/vehicle concentration		
	0.1 μM/0.01%	1 μM/0.1%	10 μM/1%
APD_{90} (%)	+4.4 ± 1.6*	+16.3 ± 1.9**	+13.9 ± 4.5*
MRD (%)	-5.8 ± 3.8	+0.8 ± 10.6	-15.4 ± 12.8
UA (mV)	+1.3 ± 1.5	+0.3 ± 1.1	-4.0 ± 2.1
E_m (mV)	-2.0 ± 1.4	-1.5 ± 1.2	+0.5 ± 1.6

*$P < 0.05$; **$P < 0.0002$ (amsacrine *vs.* vehicle; unpaired 2-tailed *t*-test; $n = 4$ per group)

These experiments indicate that action potential prolonging effects of amsacrine may be detected in the sheep Purkinje fibre preparation *in vitro*, and that this activity is observed over a therapeutically relevant concentration range (Linssen *et al.*, 1993). It seems likely that such an action underlies the QT interval prolongation observed clinically. Although the present study was not designed to address the molecular basis of the amsacrine-induced action potential prolongation, this compound is structurally related to tacrine (an acridine derivative) and the methanesulphonamide class III antiarrhythmics (e.g., dofetilide), both of which exert potent effects on cardiac action potential duration by block of K^+ channels (Osterrieder, 1987; Cross *et al.*, 1990).

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129P ACTIONS OF CERAMIDE ON INSULIN STIMULATED GLYCOGEN SYNTHESIS IN THE ISOLATED PERFUSED RAT HEART

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We have shown that tumour necrosis factor-α (TNFα) disrupts insulin stimulated glycogen synthesis in the rat heart (Edmunds & Woodward, 1997). One of the major signalling mechanisms involved in the actions of TNFα is the sphingomyelinase (SMase) pathway (Dressler *et al.*, 1992). Activation of SMase causes breakdown of sphingomyelin to ceramide, which may then be converted to sphingosine via the actions of ceramidase. Others have shown that ceramide can disrupt insulin signalling in cultured cell lines, and that ceramide is probably involved in TNFα-induced insulin resistance (Begum & Ragolia, 1996). Therefore we have investigated the actions of ceramide on insulin stimulated glycogen synthesis and glucose uptake in the isolated rat heart.

Hearts from male Wistar rats (280-310g) were perfused, using the Langendorff technique, with recirculating (50ml) Krebs-Henseleit solution (NaCl 118mM, $NaHCO_3$ 25mM, KCl 4.7mM, KH_2PO_4 1.2mM, $MgSO_4$ 1.2mM, CaCl 1.23mM and D-glucose 11.6mM, 95% O_2, 5% CO_2, pH 7.4, 37°C and a flow rate of 10ml/min). When added insulin, 10 $U.l^{-1}$, was present throughout the experiment. After 10 min the glucose concentration in the Krebs buffer was changed to 2 mM. Recirculation commenced at 30 min, and the cell permeable form of ceramide, C_2–ceramide (C_2-Cer), 1, 5 and 10 μM, was added to the perfusate 5 min prior to this. 100 μl aliquots were taken from the recirculating perfusate, and glucose concentrations assayed and used as a measure of glucose uptake. After 105 min, hearts were freeze clamped and assayed for glycogen by alkaline extraction followed by acid hydrolysis to glucose.

Figure 1 shows the actions of insulin on end point glycogen levels, and how this was altered by each concentration of ceramide. It can be seen that both 5 μM and 10 μM ceramide blocked insulin stimulated glycogen synthesis, but 1 μM C_2-Cer did not alter it. It can be seen from table 1 that insulin stimulated glucose uptake from the Krebs buffer, and that C_2-Cer did not significantly alter this action.

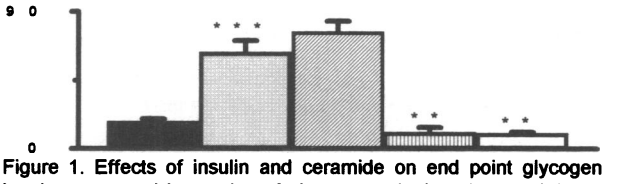

Figure 1. Effects of insulin and ceramide on end point glycogen levels, expressed in μmoles of glucose equivalents/gram of tissue dry weight, where control, black (n=8); insulin, 10 $U.l^{-1}$ grey (n=8); C_2-Cer, 1μM, diagonal hatch (n=6), 5μM, vertical lines, (n=6) and 10μM, white (n=6). *** P<0.001, insulin *vs* control. ** P<0.01, ceramide *vs* insulin. (ANOVA coupled to Tukey's post hoc test).

Time (min)	Control	Insulin, 10 $U.l^{-1}$	Insulin + C_2-Cer, 1μM	Insulin + C_2-Cer, 5μM	Insulin + C_2-Cer, 10μM
0	1.95±0.04	2.04±0.04	1.96±0.03	1.94±0.14	1.95±0.12
15	1.89±0.08	1.68±0.13	1.73±0.05	1.79±0.14	1.81±0.08
30	1.85±0.05	1.51±0.16	1.48±0.07	1.55±0.15	1.58±0.08
45	1.82±0.05	1.35±0.15*	1.31±0.07*	1.49±0.17	1.36±0.12*
60	1.75±0.08	1.24±0.11*	1.12±0.05*	1.39±0.17	1.29±0.11*
75	1.63±0.08	1.13±0.09*	1.02±0.08*	1.32±0.22	1.05±0.16*

Table 1. Effects of insulin and C_2-Cer on glucose concentrations (mM) in recirculating Krebs solution after recirculation. Expressed as glucose concentration ± S.E.M for n=5-6.* P < 0.05, *vs* control.

The data presented here agree with previous work from other laboratories, showing that ceramide can disrupt the actions of insulin. To our knowledge, this is the first time that ceramide has been shown to alter insulin action in cardiac tissue.

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130P THE EP_3-RECEPTOR AGONISTS M&B 28767 AND GR 63799X REDUCE THE CELL INJURY AND DEATH CAUSED BY HYDROGEN PEROXIDE IN RAT CARDIOMYOBLASTS.

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The prostanoid $EP_{3\alpha}$-receptor agonist ONO-AE-248 reduces myocardial infarct size and protects cardiomyoblasts against oxidant stress (Zacharowski *et al.*, 1998). Here we investigate whether two selective EP_3-receptor agonists (M&B 28767 or GR 63799X, Coleman *et al.*, 1994) affect the cell injury/death caused by hydrogen peroxide (H_2O_2) in rat cardiomyoblasts *in vitro*.

Rat cardiomyoblasts (H9c2 (2-1) cells passage > 23) were seeded onto 96-well plates and cultured to confluence in Dulbecco's Modified Eagle Medium (DMEM) containing glutamine (2 mM) and 10% fetal calf serum (FCS). Cell injury (i. e. reduction in mitochondrial respiration) was determined via the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide) to formazan. Cell death (i. e. cytotoxicity), was assessed by measuring the release of lactate dehydrogenase (LDH) into the supernatant. To evaluate the degree of cell injury/death induced by H_2O_2, the cardiomyoblasts were exposed to various concentrations of H_2O_2 (0.1, 0.3, 1 or 3 mM, n=6). In the subsequent intervention studies, the cells were challenged with a submaximal concentration of H_2O_2 (1 mM) for 4 h. To elucidate the effects of M&B 28767 or GR 63799X on the cell injury/death caused by H_2O_2 in H9c2 (2-1) cells, the cells were preincubated (10 min prior to H_2O_2) in media (1% FCS) containing vehicle (saline/ethanol) or 0.1, 1, 10 or 100 nM (n=6) of the EP_3-receptor agonists, respectively. To evaluate whether activation of ATP-sensitive potassium (K_{ATP}) channels contributes to the cytoprotective effects of the EP_3-receptor agonists used, cells were pretreated with 5-hydroxy-decanoate (5-HD, 0.07 mg/ml, 10 min prior to incubation with M&B 28767 or GR 63799X, 0.1, 1, 10 or 100 nM, n=6), an inhibitor of mitochondrial K_{ATP} channels (Garlid *et al.*, 1997). Adequate controls (without exposure to H_2O_2) were done for all groups (n=6). All data are expressed as mean±s.e.mean. Statistical differences between groups were analysed by ANOVA followed by a Bonferroni's test (P<0.05; * : versus H_2O_2, ⋆ : versus M&B/GR).

Exposure of rat cardiomyoblasts to H_2O_2 for 4 h caused a reduction in mitochondrial respiration (control = 100%, 1 mM H_2O_2 = 16.1±3.7% •) and an increase in cell death (0.1 % Triton X-100 = 100%, 1 mM H_2O_2 = 107.2± 5.2% •). The EP_3-receptor agonists M&B 287676 and GR 63799X caused a concentration-dependent attenuation of the cytotoxic effects of H_2O_2 (see Table).

Conc. EP_3	MTT		LDH		MTT		LDH	
	M&B	+5-HD	M&B	+5-HD	GR	+5-HD	GR	+5-HD
0.1 nM	15±1	12±2	72±6•	78±7•	20±6	13±4	59±9•	65±7•
1 nM	22±1	15±3	58±6•	73±6•	24±4	15±3	49±8•	60±6•
10 nM	24±1•	17±1*	57±6•	65±5•	26±1	20±2*	45±8•	62±3**
100 nM	30±2•	19±2*	46±2•	62±5**	46±3•	28±4**	35±2•	50±5**

Pretreatment of the cells with 5-HD attenuated the cytoprotective effects of both M&B 28767 and GR 63799X (see Table). Incubation of the cells (in absence of H_2O_2) with the two EP_3-receptor agonists, with vehicle or with 5-HD did not affect cell viability.

Thus, this study demonstrates that two selective, chemically distinct agonists of the prostanoid EP_3-receptor protect cultured rat cardiomyoblasts against the cell injury caused by oxidant stress inflicted by H_2O_2. The finding that pretreatment with 5-HD attenuated the cytoprotective effects afforded by M&B 28767 and GR 63799X suggests that these cytoprotective effects are - at least in part - mediated by the opening of mitochondrial K_{ATP} channels.

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131P THE EFFECT OF PARS INHIBITORS ON CHANGES IN NAD AND HIGH ENERGY PHOSPHATES CAUSED BY MYOCARDIAL ISCHAEMIA AND REPERFUSION IN THE RABBIT.

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Reactive oxygen species (ROS) contribute to the pathophysiology of ischaemia-reperfusion (I/R) injury. In cultured cells ROS cause strand breaks in DNA, which activate the nuclear enzyme poly (ADP ribose) synthetase (PARS). Excessive activation of PARS leads to NAD and ATP depletion and cell death (Schraufstatter *et al.*, 1986). We have recently discovered that inhibitiors of the activity of PARS reduce the degree of necrosis caused by I/R of the heart (Thiemermann *et al.*, 1997). The exact mechanism of the cardioprotective effect is unclear, but may be related to the preservation of NAD, or of high energy phosphates (HEP; ATP and PCr). Here we investigate the effects of the PARS inhibitors 3-aminobenzamide (3-AB) or 1,5-dihydroxyisoquinoline (ISO) (Banasik *et al.*, 1992), on the changes in NAD, ATP and PCr caused by regional myocardial ischaemia and reperfusion in the anaesthetised rabbit.

Male New Zealand white rabbits (2.5-3.0 kg) were premedicated with Hypnorm (0.1 $ml \cdot kg^{-1}$, i.m.). General anaesthesia was induced (20 $mg \cdot kg^{-1}$, i.v.) and maintained with sodium pentobarbitone. The animals were ventilated with room air. A left intercostal thoracotomy was performed, and a ligature was placed around the first antero-lateral branch of the left coronary artery (LAL). The LAL was occluded for 45 min and reperfused for 120 min. The PARS inhibitors, 3-AB (10 $mg \cdot kg^{-1}$, n=4) or ISO (1 $mg \cdot kg^{-1}$, n=4) or vehicle (saline [controls] or 10% DMSO, respectively, n=3-4) were injected into the left ventricular chamber 1 min prior to reperfusion. At the end of the experiment, the area at risk was freeze-clamped and the metabolites (NAD, ATP and CrP) were extracted using perchloric acid (9% v/v). Extracts were analysed by Capillary Zone Electrophoresis (Perrett *et al.*, 1998). Values are expressed as mean ± s.e.mean (*$p < 0.05$ compared to sham or control, ANOVA and Bonferroni test).

In control animals, regional myocardial ischaemia caused a fall in ATP (sham; 23.9±3.5, 45 min ischaemia; 6.2±1.7* μmoles/g dry wt) which did not recover during reperfusion (120 min Rep; 8.5±2.8* μmoles/g dry wt). Administration of the PARS inhibitors 3-AB or ISO did not have a significant effect on the ATP levels at the end of Rep (120 min Rep, 3-AB; 12.6±1.5, ISO; 13.4±2.1 μmoles/g dry wt) when compared with controls. Similarly, ischaemia caused a fall in the levels of PCr (sham: 46.0±5.2, 45 min ischaemia: 7.8±1.5* μmoles/g dry wt) which recovered in controls during reperfusion to 38.0±2.6 μmoles/g dry wt. 3-AB (38.4±3.7 μmoles/g dry wt) or ISO (28.5±0.4 μmoles/g dry wt) did not have any effect on the levels of PCr when compared with controls. Ischaemia also caused a fall in NAD (sham: 2.3±0.3, 45 min ischaemia: 0.72±1.5 μmoles/g dry wt) which was further decreased during reperfusion (0.1±0.02 μmoles/g dry wt). 3-AB (0.90±0.2* μmoles/g dry wt) or ISO (0.81±0.2* μmoles/g dry wt) significantly increased the intracellular levels of NAD when compared with controls. Administration of DMSO (10%) did not have any effect (data not shown).

In conclusion, administration of the PARS inhibitors, 3-AB or ISO did not affect the fall in ATP or PCr, but attenuated the fall in NAD caused by I/R of the rabbit heart *in vivo*. Thus, the cardioprotective mechanism of PARS inhibitors in the rabbit heart is not mediated by the preservation of HEP, but is related to preservation of NAD.

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132P THE PARS INHIBITOR 3-AMINOBENZAMIDE REDUCES INFARCT SIZE IN A PIG MODEL OF MYOCARDIAL ISCHAEMIA AND REPERFUSION.

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Reactive oxygen species (ROS) contribute to the pathophysiology of ischaemia-reperfusion (I/R) injury (Kukreja and Hess, 1992). In cultured cells ROS cause strand breaks in DNA, which trigger an energy-consuming repair process by activating the nuclear enzyme poly (ADP ribose) synthetase (PARS). Excessive activation of PARS leads to NAD and ATP depletion and cell death (Schraufstatter *et al.*, 1986). We have recently discovered that inhibitiors of the activity of PARS reduce the degree of necrosis caused by myocardial I/R in the anaesthetised rabbit (Thiemermann *et al.*, 1997). Here we investigate the effects of the PARS inhibitor 3-aminobenzamide (3-AB), on the infarct size and post-ischaemic contractile dysfunction caused by regional myocardial ischaemia and reperfusion in the anaesthetised, open-chest pig.

Male pigs (23-30 kg) were sedated with ketamine/xylazine (40 $mg \cdot kg^{1}$ and 0.8 $mg \cdot kg^{-1}$, respectively, i.m.). General anaesthesia was induced (25 $mg \cdot kg^{-1}$, bolus i.v.) and maintained with sodium pentobarbitone (20 $mg \cdot kg \cdot h^{-1}$, i.v.). The animals were ventilated with air supplemented with oxygen as required. A left intercostal thoracotomy was performed, and a ligature was placed around the left anterior descending coronary artery (LAD). Mean arterial pressure (MAP), heart rate (HR), left ventricular pressure (LVP) and myocardial contractility (dP/dt_{max}) were continuously recorded. The LAD was occluded for 60 min and reperfused for 3 h. Vehicle (saline, n=6), 3-AB (10 $mg \cdot kg^{-1}$, n=6) or 3-aminobenzoic acid (3-ABA, 10 $mg \cdot kg^{-1}$, n=4) were injected into the left ventricular chamber 1 min prior to reperfusion. At the end of the experiment, the LAD was reoccluded, the heart excised and perfused retrogradely (100 mmHg, 37°C) with firstly Evans blue dye (0.5%) for determination of area at risk (AAR), and secondly, with triphenyltetrazolium chloride (1.5%) for determination of infarct size. Planimetry of the stained (viable) and unstained (infarct) regions allowed calculation of AAR and infarct size. Values are expressed as mean ± s.e.mean (*$p < 0.05$ compared to control, ANOVA and Dunnett's test).

AAR (range 39±2% to 43±3% of left ventricle), and haemodynamic parameters (MAP, HR, LVP) were not significantly different between groups ($p > 0.05$). The PARS inhibitor, 3-AB caused a significant reduction in infarct size (expressed as a % of the AAR) from 66±3% (control n=6) to 44±2%* (n=6). In contrast, 3-ABA, which does not inhibit PARS activity, did not reduce myocardial infarct size (66±5%, n=4). Reperfusion was associated with a decrease in myocardial contractility (dP/dt_{max}). Administration of 3-AB, but not 3-ABA, improved contractility during the reperfusion period (change in dP/dt_{max}: control, -367±30, 3-AB, -124±21*, 3-ABA, -455±122 $mmHg \cdot s^{-1}$).

Thus, administration prior to reperfusion, of 3-AB, but not 3-ABA, causes a reduction in infarct size and improves myocardial contractility in a porcine model of regional myocardial ischaemia and reperfusion. The precise mechanism of the cardioprotective effect of 3-AB is unclear, but may involve inhibition of PARS activity and preservation of intracellular levels of NAD and ATP during reperfusion.

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133P THE STABLE NITROXIDE RADICAL TEMPOL REDUCES THE INFARCT SIZE CAUSED BY REGIONAL MYOCARDIAL ISCHAEMIA AND REPERFUSION IN THE ANAESTHETISED RAT

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Reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and hydrogen peroxide contribute to reperfusion injury of the previously ischaemic myocardium (Bolli *et al.*, 1988). 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 (IS) caused by regional myocardial ischaemia and reperfusion in the isolated perfused heart of the rat (McDonald *et al.*, 1998). This study investigates the effects of Tempol on the infarct size caused by regional myocardial ischaemia and reperfusion in the anaesthetised rat.

Twenty-four male Wistar rats (240-350 g) were anaesthetised with thiopentone sodium (120 mg·kg^{-1} i.p.). All animals were tracheotomised and ventilated with room air (tidal volume: 8-10 ml·kg^{-1}; respiration rate: 70 strokes·min^{-1}). Subdermal platinum electrodes were placed to allow the determination of a lead II electrocardiogram (ECG). The carotid artery was cannulated to measure mean arterial blood pressure (MAP), the jugular vein for the administration of drugs. The chest was opened (left sided thoracotomy) a needle was placed around the left anterior descending coronary artery (LAD). The animals were allowed to recover for 30 min and subsequently the LAD was occluded for 25 min and then reperfused (for 2 h). Administration of either high dose (100 mg·kg^{-1} i.v. bolus followed by a continuous infusion of 30 mg·kg^{-1}·h^{-1}, n=6) or low dose of Tempol (30 mg·kg^{-1} i.v. bolus followed by infusion of 10 mg·kg^{-1}·h^{-1}, n=4) was commenced 5 min prior to reperfusion. At the end of the experiment, the LAD was re-occluded, and 1 ml of Evans Blue dye (2% w/v) was injected into the jugular vein to determine the non-perfused (area at risk, AR). Infarct size was determined by incubation of the slices with nitro-blue tetrazolium (NBT, 0.5 mg·ml^{-1} at 37°C for 20 min). All data are expressed as mean±s.e.mean. Statistical differences between groups were analysed by ANOVA followed by a Bonferroni's test ($p<0.05$).

Tempol caused a dose-related reduction in infarct size from 60±3% (vehicle-control, n=8) to 24±5% (high dose, $p<0.05$) and 35±4% (low dose) of the AR, $p<0.05$). The AR was similar in all groups studied (Control: 46±2%, high dose Tempol: 50±3%, low dose Tempol: 54±8%; $p>0.05$). Administration of Tempol caused an immediate, transient (5 min) fall in MAP (high dose: from 113±10 to 65±2 mmHg; $p<0.05$; low dose: from 116±10 to 80±4 mmHg, $p<0.05$). The mean values for MAP in rats treated with either high dose or low dose of Tempol recovered throughout the subsequent reperfusion period ($p>0.05$ vs control). In rats which were not subjected to LAD-occlusion (sham-operated animals), the high dose of Tempol (n=3) also caused a significant reduction in MAP, while in sham-control animals (n=3) no significant changes in MAP were obeserved.

Thus, administration upon reperfusion of the membrane-permeable, radical scavenger Tempol reduces blood pressure as well as the infarct size caused by regional myocardial ischaemia and reperfusion in the rat. The mechanism(s) of the cardioprotective effect of Tempol warrants further investigation, but is likely related to the ability of Tempol to scavenge ROS in the early reperfusion period.

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134P THE STABLE NITROXIDE RADICAL TEMPOL ATTENUATES THE MULTIPLE ORGAN DYSFUNCTION CAUSED BY ENDOTOXIN IN THE RAT

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There is good evidence that 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). Here we investigate the effects of the spin trap agent, Tempol, on the circulatory failure and multiple organ injury/dysfunction (MODS) caused by endotoxin in the rat. Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-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).

Male Wistar rats (240 - 320 g) were anaesthetised with sodium thiopentone (120 mg·kg^{-1}, i. p.). The trachea was cannulated to facilitate respiration, and rectal temperature maintained at 37 °C. The left carotid artery and the femoral vein were cannulated for the measurement of mean arterial blood pressure (MAP) and drug administration, respectively. At completion of the surgical procedure, animals were allowed to equilibrate for 15 min after which they received Tempol (100 mg·kg^{-1} i.v. over 15 min, n = 9) or its vehicle (saline, 0.3 ml i.v., n = 11). The animals then received *E. coli* lipopolysaccharide (LPS, 6 mg·kg^{-1} i.v. for 15 min) followed by an infusion for 6 h of Tempol (30 mg·kg·h^{-1} i.v., n=9) or saline (1.5ml·h^{-1} i.v., n=11). All values are expressed as mean ± s. e. mean.

Endotoxaemia for 6 h resulted in significant rises in the serum levels of urea, creatinine (Creat, an indicator of renal dysfunction/failure), alanine aminotransferase (ALT, an indicator of liver injury), aspartate aminotransferase (AST, a non-specific marker for hepatic injury), γ-glutamyl transferase (γ-GT) and bilirubin (Bili, indicator of liver dysfunction, see Table 1). Tempol caused a transient fall in MAP (not shown) and significantly attenuated the renal dysfunction as well as the liver injury/dysfunction caused by LPS (Table 1). In rats not receiving LPS (control), Tempol did not affect any of the parameters measured (data not shown).

Table 1: Effects of Tempol on MAP (mmHg) and organ injury/dysfunction (Urea: mmol L^{-1}, Creat, Bili: μmol L^{-1}, ALT, AST, γGT: iu L^{-1}) caused by LPS (*: $p < 0.05$ vs. LPS + saline. ANOVA followed by Bonferroni's test).

	Urea	Creat	ALT	AST	γGT	Bili	MAP
Control (n=9)	7 ± 0.4 *	33 ± 2 *	83 ± 4 *	194 ± 20 *	0.4 ± 0.1 *	1 ± 0.2 *	107 ± 5 *
LPS + saline (n=11)	21 ± 1	73 ± 5	272 ± 48	522 ± 50	7 ± 1	3 ± 0.8	82 ± 5
LPS + Tempol (n=9)	12 ± 1 *	47 ± 3 *	171 ± 18	303 ± 35 *	0.2 ± 0.1 *	0.7 ± 0.4 *	94 ± 4

Thus, pretreatment of rats with the superoxide anion scavenger Tempol attenuates the renal and liver dysfunction and/or injury caused by endotoxin in the rat. These results imply that enhanced formation of reactive radical species (including superoxide anions) contributes to the MODS associated with endotoxic shock.

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