129P STRIATAL DOPAMINE D_2 RECEPTOR BINDING ESTIMATED *IN VIVO* IN OLANZAPINE-TREATED SCHIZOPHRENIC PATIENTS BY ^{123}I IBZM SINGLE PHOTON EMISSION TOMOGRAPHY (SPET)

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Striatal dopamine D2 receptor binding was studied *in vivo* in schizophrenic patients treated with the novel compound olanzapine. It was hypothesized that D2 receptor binding in olanzapine treated patients (n=6) would be similar to that observed in clozapine treated patients (n=10) and higher than typical antipsychotic (n=10), or risperidone treated patients (n=6) from a previously ascertained database. 123I iodobenzamide (IBZM) single photon emission tomography (SPET) estimated striatal D2 receptor binding *in vivo*. Dynamic single slice SPET using a brain dedicated tomographic SME 810 detector (resolution 7-9mm in plane) was performed immediately after intravenous injection of 185MBq IBZM at a slice chosen to include the basal ganglia (BG) and frontal cortex (FC). All subjects were scanned during the plateau portion of the time/activity curve (60-80 minutes post injection). Computerised region of interest analysis was performed on the images obtained. An index approximating to the saturable component of D2 receptor binding was obtained by the mean ratio of BG density (representing total activity)/FC (representing background). Olanzapine treated patients had similar levels of striatal D2 binding *in vivo* (1.41 SE 0.06) as those treated with clozapine (1.49 SE 0.04), reflecting lower levels of D2 receptor occupancy by both drugs. Mean striatal D2 binding was significantly lower in typical antipsychotic (1.25 SE 0.05) and risperidone (1.24 SE 0.04) treated patients (Mann-Whitney U test, $p<0.05$). Symptom severity in olanzapine treated patients was prospectively rated by the Brief Psychiatric Rating Scale (BPRS), blind to scanning data. Mean %BPRS improvement was 49% (SD 44). Thus we provide confirmation for another atypical, clozapine like drug in which therapeutc response is not contingent upon a high degree of striatal D2 occupancy *in vivo*.

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130P THE BINDING PROFILE OF THE NOVEL MUSCARINIC RECEPTOR ANTAGONIST DARIFENACIN AGAINST THE FIVE CLONED HUMAN MUSCARINIC RECEPTORS EXPRESSED IN CHO CELLS

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Darifenacin ((S)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide) is a novel muscarinic antagonist shown to be selective for the M_3 receptor in isolated tissue studies (Wallis *et al.*, 1995). In the present study the affinity of darifenacin for the 5 human cloned muscarinic receptors was investigated together with standard muscarinic antagonists and the clinical agents oxybutynin and dicyclomine.

The binding affinities of test compounds for human muscarinic receptors (m_1-m_5) in stably transfected CHO cells were determined by displacement of 0.1 nM [^{3}H]quinuclidinyl benzilate (QNB) using 12 concentrations of antagonist. Specific binding was defined using atropine (1 μM). The pK_i values are listed in Table 1. Equilibrium binding parameters (K_D, B_{max} (fmol/mg protein)) were m_1 (0.24 ± 0.027, 916 ± 67.92); m_2 (0.21 ± 0.031, 265 ± 45.8); m_3 (0.43 ± 0.058, 1169 ± 69); m_4 (0.26 ± 0.037, 635 ± 50); m_5 (0.73 ± 0.052, 248 ± 29).

The binding affinities and receptor selectivities of standard muscarinic antagonists were comparable to those obtained in previous studies (Dörje *et al.*, 1991; Buckley *et al.*, 1989). Darifenacin showed higher affinity for the m_3 receptor than the other 4 muscarinic receptor subtypes. In contrast, oxybutynin had similar affinity for m_1, m_2, m_3 and m_5 receptors with slightly lower affinity on m_4 receptors whilst dicyclomine had similar affinity for m_1, m_3, m_4 and m_5 receptors with lower affinity on m_2. The unique profile of darifenacin may confer a therapeutic advantage in the treatment of smooth muscle disorders associated with increased cholinergic drive.

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Wallis, R. M., Burges, R.A., Cross, P.E. *et al.* (1995) *Pharmacol. Res.*, 31S, 54

Table 1 pK_i values for muscarinic antagonists for cloned human muscarinic receptor subtypes (mean ± s.e. mean)

COMPOUND	m_1	m_2	m_3	m_4	m_5
Darifenacin (n=8-12)	7.46 ± 0.06	7.38 ± 0.06	8.42 ± 0.10	7.99 ± 0.09	7.93 ± 0.10
Oxybutynin (n=4)	8.03 ± 0.18	7.7 ± 0.17	8.31 ± 0.37	7.43 ± 0.43	7.99 ± 0.05
Dicyclomine (n=6)	8.38 ± 0.04	7.44 ± 0.08	8.45 ± 0.10	8.50 ± 0.167	8.44 ± 0.07
Atropine (n=8-12)	9.01 ± 0.05	9.05 ± 0.08	8.89 ± 0.05	9.16 ± 0.05	8.94 ± 0.12
Pirenzepine (n=7)	7.62 ± 0.06	6.47 ± 0.10	6.56 ± 0.06	7.54 ± 0.08	6.58 ± 0.06
Methoctramine (n=6)	6.17 ± 0.07	7.05 ± 0.26	5.94 ± 0.13	6.5 ± 0.19	6.07 ± 0.06
4-DAMP (n=4)	8.27 ± 0.06	7.6 ± 0.08	8.2 ± 0.27	8.11 ± 0.34	7.55 ± 0.13

131P IS PROTEIN KINASE C INVOLVED IN AGONIST-INDUCED DOWN-REGULATION OF MDCK CELL α_1-ADRENOCEPTORS?

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We have studied regulation of α_{1B}-adrenoceptors in the MDCK cell line by 24 h exposure to the agonist, phenylephrine (in the presence of 10 µM (±)-propranolol to block ß-adrenoceptors), or the protein kinase C (PKC) activator, phorbol-12-myristate-13-acetate (PMA), in the absence and presence of the PKC inhibitor, staurosporine (100 nM). Treatments were performed on confluent cells in the presence of 10% serum unless otherwise indicated. α_1-Adrenoceptor number was determined by [^{3}H]prazosin saturation binding (Michel et al. 1993). Because treatment in some cases altered cell number and/or cell size, α_1-adrenoceptor densities were analyzed both, as fmol/mg protein and sites/cell. MDCK cell α_1-adrenoceptor density under control conditions was 159±10 fmol/mg protein or 12918±999 sites/cell with a K_d of 55±3 pM (n=14). All further data are expressed as % of control values and are mean ± S.E. mean of 3-5 experiments.

Phenylephrine (0.01-100 µM) concentration-dependently down-regulated α_1-adrenoceptors to 51±5% and 51±7% of control fmol/mg protein and sites/cell, respectively, in the presence of 100 µM phenylephrine ($p<0.01$ in ANOVA) without changes in affinity for the radioligand. Similar data were obtained in the presence of 0.5% serum, but data analysis was complicated by a significant increase in cell number in phenylephrine-treated cells (129±7% of control in the presence of 100 µM phenylephrine; $p<0.05$ in ANOVA).

MDCK α_1-adrenoceptors can couple to PKC activation (Slivka et al. 1988). The PKC activator, PMA (1-100 nM), also concentration-dependently down-regulated α_1-adrenoceptors to 20±4% and 23±5% of control fmol/mg protein and sites/cell, respectively, in the presence of 100 nM PMA ($p<0.01$ in ANOVA).

In the presence of 100 nM staurosporine, cell number was smaller (12±2 vs. 44±4 x 10^6 cells/175 cm² flask; $p<0.01$ in paired t-test) and cell size larger (433±63 vs. 133±14 µg protein/10^6 cells; $p<0.05$ in paired t-test) than in its absence. While staurosporine partially prevented α_1-adrenoceptor down-regulation by 100 nM PMA (17±2% vs. 63±2% and 21±4% vs. 55±5% of control fmol/mg protein and sites/cell, respectively; $p<0.001$ in a paired t-test) it did not affect down-regulation by 100 µM phenylephrine (55±5% vs. 54±3% and 60±6% vs. 57±9% of control fmol/mg protein and sites/cell, respectively).

We conclude that the PKC activator, PMA, can imitate agonist-induced down-regulation of MDCK cell α_1-adrenoceptors, but PKC activation may not be of major importance in agonist-induced down-regulation.

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132P HIGH SPECIFIC ACTIVITY [^{3}H]5-CT BINDING: CORRELATION OF GUINEA-PIG CORTEX WITH HUMAN CLONED 5-HT$_7$ RECEPTORS

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A recent addition to the 5-HT receptor family is the 5-HT$_7$ receptor subtype, a G-protein linked receptor that is positively coupled to adenyl cyclase (Bard et al, 1993). Binding studies in native tissue have been hampered by the small signals involved and the lack of an appropriate radioligand. We have evaluated the binding profile of commercially available [^{3}H]5-CT (83 Ci/mmol; Amersham International plc.) to endogenous 5-HT$_7$ receptors in guinea pig and rat whole cortex and human cloned 5-HT$_7$ receptors obtained by low homology screening of a human brain cDNA library. The 5-HT$_7$ receptor clone used for binding studies was expressed in HEK293 cells at a level of ~100,000 receptors / cell. Tissue and cell membrane preparations were labelled with 0.5nM [^{3}H]5-CT and were incubated at 37ºC for 1h. Studies using whole cortex preparations were performed in the presence of 1µM cyanopindolol and sumatriptan to prevent binding to native 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (as detailed by To et al, 1995). Nonspecific binding was defined using 10µM 5-HT.

Studies using rat whole cortex revealed a small and complex signal. It is possible that despite 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ blockade, [^{3}H]5-CT continues to label a heterogeneous receptor population. Results obtained using both guinea pig whole cortex and human cloned 5-HT$_7$ receptors revealed binding to be high affinity, reversible, saturable and homogenous. Scatchard analysis yielded pK_d values of 9.08 +/- 0.04 (n=3) and 9.38 +/- 0.18 (n=3) for guinea pig cortex and human clones respectively. Competition studies using ligands of different affinities (see Table 1) produced monophasic displacement curves and confirmed affinity and rank order of potencies previously published (Bard et al, 1993; To et al, 1995). Correlation of the pharmacological specificity between the two tissues was very high (r =0.9) suggesting that guinea pig cortex is a suitable alternative for the study of human 5-HT$_7$ receptors.

Table 1: pK_i values vs [^{3}H]5-CT radioligand binding

Tissue	5-CT	5-HT	Mesulergine	Ritanserin	Spiperone	8-OH-DPAT	Ketanserin
Guinea pig cortex	9.02±0.02	8.09±0.05	7.15±0.03	6.98±0.03	6.76±0.06	6.55±0.04	5.92±0.02
Human 5-HT$_7$ receptor	9.18±0.05	8.28±0.03	7.54±0.003	7.33±0.10	7.08±0.01	6.71±0.03	6.16±0.08

mean ± SEM, n=3

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133P THE NOVEL 5-$HT_{2C/2B}$ RECEPTOR ANTAGONIST SB206553 IS A POTENT AND SURMOUNTABLE ANTAGONIST AT CLONED HUMAN RECEPTORS

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SB 200646A has previously been reported as the first compound with significant selectivity for the 5-HT_{2C} receptor compared to the 5-HT_{2A} receptor (Forbes etal, 1993). Subsequent cloning and identification of the 5-HT_{2B} receptor has shown it not to discriminate 5-$HT_{2C/2B}$ receptors (Bonhaus et al, 1995). We now report on SB 206553 (Forbes et al, 1995) which has improved selectivity and potency at the human 5-HT_{2C} and 5-HT_{2B} receptors.

Receptor binding studies were carried out on the cloned human receptor expressed in HEK 293 cells using [^{3}H]-mesulergine and [^{3}H]-ketanserin to label 5-HT_{2C} and 5-HT_{2A} receptors respectively (Wood et al, 1995) and [^{3}H]-5-HT to label 5-HT_{2B} receptors. Functional studies (phosphoinositide, PI hydrolysis) were performed by pre-labelling of cells with [^{3}H]-myo-inositol and incubation with antagonist prior to twenty minute agonist and lithium exposure. Labelled inositol phosphates were isolated as described previously (Wood et al, 1995).

Data are summarised in Table 1. SB 206553 was more potent than SB 200646A at 5-HT_{2C} receptors and showed slight and marked selectivity over the human 5HT_{2B} and 5-HT_{2A} receptors respectively. In PI functional studies it displayed no agonist activity and antagonised the effects of 5-hydroxytryptamine (5-HT) in a competitive manner at all three receptors.

In conclusion, SB 206553 is a potent and selective novel 5-$HT_{2C/2B}$ receptor antagonist and should be a useful tool in elucidating the roles of these receptors.

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Table 1: Inhibitory potencies at cloned human 5-HT_2 receptor subtypes

Compound	5-HT_{2A}		5-HT_{2B}		5-HT_{2C}	
	Binding pK_I	PI pEC_{50}* or pK_B	Binding pK_I	PI pEC_{50}* or pK_B	Binding pK_I	PI pEC_{50}* or pK_B
5-HT	6.54 ± 0.06 (3)	6.87 ± 0.04 (22)*	7.87 ± 0.07 (3)	7.90 ± 0.20 (3)*	8.67 ± 0.09 (3)	8.50 ± 0.10 (3)*
SB 200646	< 5.2	<5.2	6.30 ± 0.08(3)		6.96 ± 0.10 (3)	7.10 ± 0.20 (3)
SB 206553	5.77 ± 0.03 (7)	5.97 ± 0.30 (3)	7.59 ± 0.11(4)	8.77 ± 0.10 (3)	8.02 ± 0.03 (8)	9.00 ± 0.10 (3)

134P EFFECT OF ANTIDEPRESSANT DRUGS ON 5-HT_{2A} RECEPTORS IN CULTURED RAT GLIOMA C_6 CELLS

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Rat glioma C_6 cells have been shown to express 5-HT_{2A} receptors which are functionally coupled to phosphoinositide (PI) hydrolysis (Ananth *et al.*, 1987). In this study, we have compared the potency of a range of compounds to inhibit [^{3}H]-ketanserin binding to membranes prepared from C_6 cells and rat frontal cortex. In addition, we have examined the inhibition of 5-HT stimulated PI hydrolysis in C_6 cells by the 5-HT_{2A} antagonist, ketanserin. We have also investigated the effects of 24 hour exposure to mianserin, desipramine and fluoxetine on maximal 5-HT-stimulated PI hydrolysis in these cells.

Cells were cultured in Dulbecco's Modification of Eagles Medium supplemented with 5mM glutamine, penicillin (10 IU ml^{-1}), streptomycin (10µg ml^{-1}) and 5% dialysed foetal calf serum. Binding assays were carried out using [^{3}H]-ketanserin (0.5 nM), non-specific binding defined by 5 µM methysergide. PI hydrolysis experiments were performed essentially as described by Newton *et al.* (1995).

[^{3}H]-Ketanserin binding to C_6 cell membranes was of high affinity and fitted well to a single site binding model. Although in general ligands had higher affinity for 5-HT_{2A} receptors on C_6 cells, there was an excellent correlation between the potency of compounds to inhibit [^{3}H]-ketanserin binding to C_6 cells and rat frontal cortex (r=0.99, P<0.001, Table 1). Ketanserin dose-dependently inhibited maximal 5-HT-stimulated PI hydrolysis (mean ± s.e.mean; control 332 ± 23%; 0.5nM 224 ± 34%; 5nM 87 ± 7%, P<0.01; William's test, n=3). Exposure of C_6 cells to desipramine and fluoxetine (10, 100, 1000nM) produced no significant changes in PI hydrolysis. However, mianserin (1, 10, 100nM) dose-dependently reduced maximal PI hydrolysis compared with controls (mean ± s.e.mean; control 228 ± 15%; 1nM 168 ± 13% P<0.05; 10nM 123 ± 16% P<0.01; 100 nM 69 ± 10% P<0.01; William's test, n=3).

The results of this study show that [^{3}H]-ketanserin labels 5-HT_{2A} receptors which are functionally coupled to PI hydrolysis in C_6 cells. Maximal PI hydrolysis was not altered by 24 hour exposure to desipramine or fluoxetine, but was significantly reduced by mianserin. Mianserin has high affinity for 5-HT_{2A} receptors, therefore this down-regulation is probably due to a direct drug action on the receptor.

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Table 1. Inhibition of [^{3}H]-ketanserin binding to membranes prepared from rat C_6 glioma cells and rat frontal cortex.

Compound	C_6 cells	Rat cortex		C_6 cells	Rat cortex		C_6 cel ls	Rat cortex
Ketanserin	0.73 ± 0.2	0.58 ± 0.05	GR 127935	25 ± 3	74 ± 3	Fluoxetine	286 ± 52	925 ± 169
Mianserin	2.1 ± 0.5	2.4 ± 0.3	Imipramine	68 ± 8	100 ± 8	RU 24969	130 ± 14	1346 ± 128
Methysergide	2.6 ± 0.6	2.1 ± 0.2	Desipramine	95 ± 12	282 ± 25	8-OH-DPAT	1911 ± 66	8546 ± 392
Doxepin	17 ± 2	17 ± 1						

Ki values (nM) are mean ± s.e.mean, n = 3 - 4. Hill slopes ranged from 0.9 - 1.20. GR 127935 = N-[4-methoxy-3-(4-methyl-p-piperazinyl)-phenyl]-2'-methyl-4'-5-methyl-1,2,4-oxadiazol-3-yl[1,1'-biphenyl]-4-carboxamide, RU 24969 = 5-methoxy-3-(1,2,3,6 tetrahydropyridin-4-yl)-1H-indole succinate, 8-OH-DPAT = ±8-hydroxy-dipropylaminotetralin HBr.

135P A STUDY OF THE EFFECT OF PROPOFOL ON NEURONAL $GABA_A$, $5\text{-}HT_3$, P_{2X} AND nACh RECEPTORS

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The intravenous general anaesthetic agent, propofol (2,6-di-isopropylphenol), potentiates the effects of GABA at the $GABA_A$ receptor, an effect that probably contributes to its central depressant actions (Franks & Leib, 1994). Recent studies have also shown that at higher concentrations propofol inhibits NMDA receptor gated cation channels (Orser *et al.*, 1995). The effects of propofol on other neuronal ligand-gated ion channels, which may contribute to its clinical actions including its sedative, hypnotic, anticonvulsant and antiemetic effects are not fully known (Borgeat *et al.*, 1994). In the present study, therefore, the effects of propofol were determined on the $GABA_A$, $5\text{-}HT_3$, P_{2x} purinoreceptor and nicotinic acetylcholine (nACh) receptors of the rat isolated vagus nerve using an extracellular recording technique.

Agonist-evoked responses were recorded from vagus nerves excised from male Sprague Dawley rats (200-250g) using a grease gap technique. Agonists and antagonists were perfused on to the nerve dissolved in a physiologically balanced salt solution, with recordings being made at ambient room temperature (20-23°C). Contact times for agonists were pre-determined on each nerve and were normally 2 mins. Antagonists were perfused onto the nerve for at least 15 mins before agonist-evoked responses were repeated in the continued presence of such drugs. All responses were measured at their peak. Responses in the presence of drugs are expressed as a percentage of those in their absence (control).

Application of GABA (3-3000μM), 5-hydroxytryptamine (5-HT, 0.1-10μM) α,β-methylene adenosine 5'-triphosphate (α,β-MeATP, 1-300μM) or 1,1-dimethyl 1-4 phenyl piperazinium (DMPP, 1-300μM) evoked concentration-dependent depolarizations of the vagus nerve with EC_{50} values (geometric mean and 95% C.I.) of 34μM [25-43μM, n=5], 0.8μM [0.5-1.0μM, n=6], 48μM [34-62μM, n=5] and 32μM [3-62μM, n=9], respectively. Submaximal GABA responses were inhibited by picrotoxin (1μM) and potentiated by sodium pentobarbitone (100μM) to (mean ± s.e.m) 55±7% (n=5) and 366±50% (n=9) of control, respectively. 5-HT (0.5μM) responses were 17±4% (n=11) of control in the presence of 3-tropanyl-3,5-dichlorobenzoate (MDL 72222, 0.1μM); α,β-MeATP (30μM) responses were 37±4% (n=5) of control in the presence of suramin (30μM) and DMPP (30μM) responses were 29±13% (n=6) of control in the presence of hexamethonium (300μM). Propofol (1-100μM) concentration-dependently potentiated GABA-evoked responses with a bell-shaped concentration-effect curve. The maximal potentiation of the GABA (10μM) response to 360±46% (n=15) was observed in the presence of 10μM propofol. By contrast, 5-HT (0.5μM), α,β-MeATP (30μM) and DMPP (30μM) responses were little or unaffected in the presence of propofol up to 10μM. However, in the presence of 100μM propofol, the 5-HT, α,β-MeATP and DMPP-evoked responses were reduced to 60±8% (n=6), 40±9% (n=5) and 57±16% (n=5) of control, respectively.

In conclusion, these data demonstrate that therapeutic concentrations of propofol, (Vuyk *et al.*, 1992) have little or no effect on rat vagus $5\text{-}HT_3$, P_{2x}, or nACh receptors but markedly potentiate $GABA_A$ receptor mediated responses.

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136P CHARACTERISATION OF THE RECEPTORS MEDIATING 5-HT-INDUCED FLUID SECRETION IN HUMAN COLONIC MUCOSA

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We have previously shown that application of 5-HT evokes electrogenic fluid secretion in the small and large intestine of man. In human terminal ileum, the response is mediated by a receptor of the $5\text{-}HT_4$ sub-type (Borman & Burleigh, 1993), whereas the response in sigmoid colon was abolished by application of ketanserin (1μM; Borman & Burleigh, 1994). The present investigation aims to confirm the preliminary characterisation of the receptor in human sigmoid colon, and to identify the receptor responsible for 5-HT-induced secretion in human ascending colon. Sections of human sigmoid or ascending colonic mucosa were stripped of muscle layers and voltage-clamped in Ussing chambers, where changes in short-circuit current were monitored. 5-HT was applied to the serosal surface of preparations, either cumulatively (for ascending colon) or, because of rapid desensitisation, only a single concentration of 5-HT was applied to any one tissue (for sigmoid colon), either in the absence or presence of antagonist. Statistical comparisons used the Mann-Whitney U-test, with $p<0.05$ being taken to indicate a significant difference.

Application of 5-HT led to a concentration-dependent increase in short-circuit current of both ascending and sigmoid colonic mucosa. In control experiments, the maximum response to 5-HT was $32.4\pm2.5\mu Acm^{-2}$ for ascending colon and $35.0\pm3.0\mu Acm^{-2}$ for sigmoid colon, with an EC_{50} (with 95% confidence limits) for 5-HT of 3.7μM (3.0-4.7, n=7) and 5.3μM (2.8-9.1, n=4) respectively. For sigmoid colonic mucosa, prior application of 30nM ketanserin was shown to cause a rightward displacement of the concentration-response curve to 5-HT, with no significant alteration of the maximum response to 5-HT. This led to a single-concentration pA_2 estimate for ketanserin of 8.5 ± 1.1 (n=4).

In human ascending colonic mucosa, application of either methysergide (10μM) or ondansetron (10μM) had no significant effect on the secretory response to 5-HT (both n=6). Application of DAU 6285 (10μM; a $5\text{-}HT_4$ receptor antagonist; Schiavone *et al.*, 1992), however, caused a rightward displacement of the concentration- response curve to 5-HT in 4 out of 6 specimens, with no significant alteration of the maximum response to 5-HT, yielding a single-concentration pA_2 estimate in those 4 tissues of 5.78 ± 0.20. In the remaining 2 specimens, the response to 5-HT was shown to be insensitive to prior application of DAU 6285, however application of ketanserin (1μM) was shown to abolish the secretory response to 5-HT. This concentration of ketanserin had no significant effect on the secretory response to 5-HT in the 4 specimens which were shown to be sensitive to DAU 6285.

In summary, 5-HT induces electrogenic fluid secretion in both proximal (ascending) and distal (sigmoid) colonic mucosa, however the receptor responsible for these effects differs between regions. We have shown that 5-HT induces secretion in sigmoid colon via a $5\text{-}HT_{2A}$ receptor, whereas the response in ascending colon cannot be definitively classified at this time, but may reflect the presence of a heterogenous receptor population.

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137P SB 204741 IS A SELECTIVE ANTAGONIST AT THE CLONED HUMAN 5-HT$_{2B}$ RECEPTOR STABLY EXPRESSED IN HEK 293 CELLS

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SB 204741 has been reported to be a selective antagonist of the 5-HT$_{2B}$ receptor in rat stomach fundus (Baxter et al., 1995) and to show selectivity for the human 5-HT$_{2B}$ receptor when transiently expressed in COS cells (Bonhaus et al., 1995). In the present study we have investigated the affinity and selectivity of SB 204741 for the human 5-HT$_{2B}$ receptor stably expressed in HEK 293 cells using both receptor binding and phosphoinositide (PI) functional assays.

[^{3}H]-5-HT binding to 5-HT$_{2B}$ receptors was carried out according to Wainscott et al. (1993) and 5-HT$_{2A}$ and 5-HT$_{2C}$ binding according to Wood et al. (1995a). PI hydrolysis was measured in stably transfected SH-SY5Y (5-HT$_{2A}$) or HEK 293 (5-HT$_{2B}$, 5-HT$_{2C}$) cells pre-incubated with [^{3}H]-myo-inositol (Wood et al., 1995b).

Table 1 shows the interaction of SB 204741, mianserin and ketanserin with the human 5-HT$_2$ receptor subtypes. The binding affinity and selectivity of SB 204741 for the human 5-HT$_{2B}$ receptor was similar to that reported by Bonhaus et al. (1995). SB 204741 produced surmountable antagonism of 5-HT$_{2B}$ receptor stimulated PI hydrolysis by 5-HT and showed selectivity for this subtype versus the 5-HT$_{2A}$ and 5-HT$_{2C}$ subtypes with an antagonist potency similar to that at the native rat 5-HT$_{2B}$ receptor (Baxter et al., 1995). Although functional selectivity for the 5-HT$_{2B}$ receptor was similar to that from receptor binding, the apparent pK$_B$ for SB 204741 at 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors was higher than the corresponding pK$_I$. The reason for this difference is unclear.

In conclusion, SB 204741 is a selective and surmountable antagonist at the cloned human 5-HT$_{2B}$ receptor. Whether SB 204741 shows selectivity for the native human 5-HT$_{2B}$ receptor remains to be determined.

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Table 1. Interaction of drugs with cloned human 5-HT$_2$ receptor subtypes (pK$_I$ from receptor binding, pEC$_{50}$ or apparent pK$_B$ from PI hydrolysis; data represent the mean ± s.e.mean, n≥3)

Compound	5-HT$_{2A}$		5-HT$_{2B}$		5-HT$_{2C}$	
	pK$_I$	pEC$_{50}$* or pK$_B$	pK$_I$	pEC$_{50}$* or pK$_B$	pK$_I$	pEC$_{50}$* or pK$_B$
5-HT	6.5±0.1	6.9±0.1*	7.9±0.1	7.9±0.2*	8.7±0.2	8.5±0.1*
Mianserin	8.2±0.1	8.4±0.1	7.8±0.1	7.8±0.1	8.6±0.1	8.2±0.1
Ketanserin	8.8±0.2	8.6±0.2	5.4±0.1	6.1±0.1	7.4±0.1	6.8±0.2
SB 204741	<5.2	4.7±0.3	6.7±0.1	7.5±0.2	5.8±0.1	6.4±0.2

138P FUNCTIONAL, ENDOGENOUSLY EXPRESSED 5-HT$_7$ RECEPTORS IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

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5-Hydroxytryptamine (5-HT) 5-ht$_7$ receptors represent a structurally and pharmacologically distinct 5-HT receptor class, coupling positively to adenylyl cyclase. However, as indicated by the lowercase appellation, they still await full operational and transductional characterization in intact (i.e., not genetically engineered) tissues or cells (see Hoyer et al., 1994). We now show that human uterine artery smooth muscle cells constitutively express both 5-ht$_7$ receptor mRNA and 5-ht$_7$-like receptors functionally linked to cyclic AMP formation.

Smooth muscle cells were prepared from human uterine artery by conventional techniques and propagated in Dulbecco's modified Eagle Medium supplemented with 10 % foetal calf serum. Cells passaged up to 9 times were grown to confluency in 24-well plates and deprived of serum 24 h before the experiments. Cyclic AMP accumulation was measured as previously described (Schoeffter et al., 1995). Results are given as mean ± s.e.mean. Reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed using oligonucleotide primers specific for the various human 5-HT receptors (Ullmer et al., 1995).

Cyclic AMP experiments were conducted in the presence of forskolin (10 μM), which by itself increased cyclic AMP accumulation by about 9-fold. 5-Carboxamidotryptamine (5-CT) and 5-HT induced further, concentration-dependent increases in cyclic AMP formation. Maximal effects amounted to 110-130 % above forskolin-stimulated effects. 5-CT was more potent than 5-HT (pEC$_{50}$ value 7.12 ± 0.22, n=3, versus 6.25 ± 0.27, n=4). Sumatriptan had a weak effect (37 ± 6 % stimulation at 0.1 mM, n=4). 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) was inactive up to 0.1 mM. In antagonist studies, methiothepin (0.1 μM) and clozapine (1 μM) were able to shift the concentration-response curve of 5-CT to the right, without loss in the E$_{max}$ (respective pA$_2$ values, 8.30 ± 0.18 and 7.54 ± 0.12, n=3 for both). The 5-HT$_4$ receptor antagonist, 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester (SDZ 205-557, 10 μM) did not significantly alter the concentration-response of 5-CT (n=3).

In RT-PCR studies, the mRNA's for 5-HT$_{2A}$ and 5-ht$_7$ receptors were detected in homogenates from human uterine artery smooth muscle cells, with about the same signal density. mRNA's for other 5-HT receptors, in particular the cyclic AMP-positively coupled 5-HT$_4$ and 5-ht$_6$ receptors, were not detected.

These results confirm that 5-ht$_7$ receptor mRNA is expressed in human vascular smooth muscle cells (Bard et al., 1993; Ullmer et al., 1995). In addition, they show that 5-HT receptors with a 5-ht$_7$-like pharmacology mediate stimulation of adenylyl cyclase in human uterine artery smooth muscle cells. This constitutes further evidence for 5-ht$_7$ receptors being real, functional receptors, possibly involved in the regulation of vascular tone.

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139P FUNCTIONAL POTENCIES OF NOVEL HUMAN 5-HT$_{2C/2B}$ RECEPTOR ANTAGONISTS DETERMINED IN THE CYTOSENSOR MICROPHYSIOMETER

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SB206553 (Forbes et al, 1995) and SB200646A (Forbes et al, 1993) are reported to be selective 5-HT$_{2C/2B}$ receptor antagonists compared to the 5-HT$_{2A}$ receptor. However, for SB206553, there is a discrepancy between binding affinity and functional potency at the 5-HT$_{2C}$ receptor (Gager et al, 1995). We have therefore examined these compounds in a different functional assay system - the ability of cloned cells to acidify extracellular medium, determined by microphysiometry - and compared the results with those for the non-selective antagonist mianserin. The acidification rates of cloned human 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors, expressed in HEK293 cells, were determined as in Boyfield et al (1996). All drugs were diluted in medium. Cells were exposed (53 sec) to six increasing concentrations of 5-HT before addition of the antagonist. After 30 min, the agonist concentration-response curve was repeated. The peak acidification rate to each agonist concentration was determined and concentration-response curves fitted using Robofit (Tilford et al 1995).

5-HT caused a large (>40 uV/sec), rapid, transient increase in acidification rate in both cell lines, with a transient alkalinisation upon agonist withdrawal. Increases in acidification rates were of similar magnitude in the two clones. Functional data are summarised in Table 1. For both clones, 5-HT potency was higher than binding affinity. Mianserin and the SB compounds lacked agonist activity and showed surmountable antagonism. SB206553 was more potent than SB200646A at 5-HT$_{2C}$ receptors, but both showed marked selectivity over the human 5-HT$_{2A}$ receptor. For all 3 compounds there were differences between receptor binding affinities and functional potencies, which reduced 5-HT$_{2C}$ selectivity. These data confirm that there are discrepancies between binding affinities and functional potencies of antagonists in 5-HT$_{2C}$ and 5-HT$_{2A}$ clones.

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Table 1: Functional (microphysiometer) and radioligand binding potencies at cloned human 5-HT$_2$ receptor subtypes

Compound	5HT$_{2A}$		5HT$_{2C}$	
	Binding pK$_I$[+]	pEC$_{50}$* or pK$_B$	Binding pK$_I$[+]	pEC$_{50}$* or pK$_B$
5-HT	6.5 ± 0.1 (3)	8.4 ± 0.1 (8)*	8.7 ± 0.1 (3)	9.3 ± 0.1 (9)*
Mianserin	8.2 ± 0.1 (3)	7.7 ± 0.4 (4)	8.6 ± 0.1 (5)	7.0 ± 0.1 (4)
SB200646A	< 5.2	5.2 ± 0.2 (3)	7.0 ± 0.1 (3)	6.2 ± 0.1 (4)
SB206553	5.8 ± 0.1 (7)	6.7 ± 0.1 (3)	8.0 ± 0.03 (8)	7.9 ± 0.1 (3)

[+] Data from Gager et al 1995. Results are mean ± sem from (n) experiments

140P BINDING OF ZOTEPINE, CLOZAPINE AND HALOPERIDOL TO 5-HT RECEPTOR SUBTYPES

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The antipsychotic drug, zotepine, has an atypical profile in animal models (Needham et al., 1995) and man (Barnas et al., 1992). To provide a greater insight into zotepine's atypical actions, we have examined its binding to eight 5-HT receptor subtypes (5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_4$, 5-ht$_6$ and 5-ht$_7$) and compared this with the atypical drug, clozapine and the typical neuroleptic, haloperidol.

Competition binding experiments used membrane fragments from animal tissues or commercially available rat receptor clones, as follows: receptor (tissue), radioligand, non-specific; 5-HT$_{1A}$ (rat hippocampus) 1 nM [^{3}H]-8-OH-DPAT, 10 µM 5-HT; 5-HT$_{1D}$ (pig caudate) 4 nM [^{3}H]-sumatriptan, 10 µM 5-HT; 5-HT$_{2A}$ (rat frontal cortex) 5 nM [^{3}H]-ketanserin, 5 µM methysergide; 5-HT$_{2C}$ (pig choroid plexus) 1 nM [^{3}H]-mesulergine, 10 µM 5-HT; 5-HT$_3$ (rat entorhinal cortex) 0.2 nM [^{3}H]-GR 65630, 30 µM metoclopramide; 5-HT$_4$ (pig hippocampus) 0.1 nM [^{3}H]-GR 113808, 30 µM 5-HT; 5-ht$_6$ and 5-ht$_7$ (rat clones; Biosignal Inc. Canada) 3 nM [^{3}H]-LSD, 10 µM methiothepin.

The atypical drugs zotepine and clozapine share a profile of selective binding to four of the 5-HT subtypes tested, viz. 5-HT$_{2A}$, 5-HT$_{2C}$, 5-ht$_6$ and 5-ht$_7$ (table 1). This profile is quite different from that of the typical neuroleptic, haloperidol, which has only moderate affinity for 5-HT$_{2A}$ and no relevant affinity for 5-HT$_{2C}$, 5-ht$_6$ and 5-ht$_7$. Affinity for 5-ht$_6$ and 5-ht$_7$ has been suggested to be an important property of atypical antipsychotics (Roth et al., 1994). These data suggest that zotepine and clozapine may derive their atypical activity in man, at least in part, from their relative affinity for the 5-HT$_{2A}$, 5-HT$_{2C}$ 5-ht$_6$ and 5-ht$_7$ receptors. By contrast, 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_3$, and 5-HT$_4$ receptor affinity appears to have little relevance to the efficacy or side-effect profile of these drugs.

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Table 1. Affinity of zotepine and comparator antipsychotics for eight 5-HT receptor subtypes

	5-HT$_{1A}$	5-HT$_{1D}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_3$	5-HT$_4$	5-ht$_6$	5-ht$_7$
zotepine	>1000	220 ± 37	2.5 ± 0.3	1.2 ± 0.3	>1000	>1000	6.8 ± 0.5	7.0 ± 0.7
clozapine	415 ± 33	460 ± 16	8.3 ± 2.3	2.1 ± 0.4	300 ± 55	>1000	13 ± 2.0	37 ± 3.0
haloperidol	>1000	>1000	51 ± 12	>1000	>1000	>1000	>1000	556 ± 63

Data are mean ki's (nM) ± s.e.mean; n = 3

141P REGIONAL DIFFERENCES IN FUNCTIONAL 5-HT$_7$ RECEPTOR EXPRESSION BY CULTURED ASTROCYTES

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Primary astrocyte cultures have been shown to express neurotransmitter receptors for amines, purines, amino acids and peptides which are coupled to second messenger systems. (Kimelberg, 1995). The expression of these receptors varies considerably between different regions of the CNS (Wilkin *et al.*, 1990). We have shown that astrocytes *in vitro* express functional 5-ht$_7$ receptors (Hirst *et al.*, 1995). In the present study we have investigated the regional heterogeneity of mRNA and functional receptor expression by astrocytes cultured from several brain regions.

Astrocytes were cultured from the thalamic/hypothalamic(Thal.) area, brainstem (BS.), cerebral cortex, colliculus (Col.) and cerebellum of 2 day old Sprague-Dawley rat pups and maintained in serum containing DMEM media until 24 hours prior to the cAMP assays, when the media was changed to serum-free. cAMP assays were performed as previously described (Hirst *et al.*, 1995). Total RNA was extracted as described by Too and Maggio (1995) and reverse transcribed using oligodT primers. PCR amplification was performed for 35 cycles (1 min 95°C, 1 min 56°C and 1.5 min 72°C) followed by a 10 minute extension at 72°C. Amplified products were subjected to agarose gel electrophoresis (1%) and visualised by UV illumination in the presence of ethidium bromide.

Astrocytes cultured from cortex and cerebellum were highly variable in their cAMP response to 5-HT agonists, the reason for which is unclear. Astrocytes from thalamus/hypothalamus, brainstem and colliculus showed consistent, concentration dependent increases in cAMP to 5-HT and 5-CT (5-Carboxyamidotryptamine) stimulation, consistent with 5-ht$_7$ receptor activation (see Table 1.). EC_{50} values for both 5-HT and 5-CT showed no regional variation whereas the Emax in the thalamus/ hypothalamus was significantly greater than other regions. This suggests that the density of 5-ht$_7$ sites is greater in astrocytes from this region although there are other possible explanations. Messenger RNA for the 5-ht$_7$ receptor was detected by reverse transcriptase PCR in astrocytes from all regions and in hippocampus and hypothalamus dissected from adult rat brains, which was used as a positive controls for the reaction. However, in agreement with the pharmacological data some inconsistencies were observed in the PCR results using mRNA from cortical and cerebellar astrocytes. In conclusion, we have shown that astrocytes cultured from different brain regions express mRNA and functional 5-ht$_7$ receptors in differing amounts.

Table 1.

	pEC_{50} 5-HT	pEC_{50} 5-CT	E_{max} (%) 5-HT	E_{max}(%) 5-CT
Tha	6.63 ± 0.08	7.80 ± 0.12	625 ± 110**	789 ± 256**
BS	6.48 ± 0.22	7.65 ± 0.19	318 ± 46	279 ± 55
Col	6.56 ± 0.12	7.23 ± 0.21	382 ± 91	336 ± 106

Values shown are mean ± S.E.M. ** $p < 0.05$ Kruskall-Wallis test with post-hoc Mann-Whitney U-test.

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142P EVIDENCE WHICH INDICATES THAT THE ACTIVATION OF CENTRAL 5-HT$_{1D\alpha}$ RECEPTORS CAN CAUSE HYPOTENSION IN ANAESTHETIZED RATS

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In the presence of the 5-HT$_{1A}$ receptor antagonist WAY-100635 (i.c.v.; Forster et al., 1995) i.c.v. sumatriptan caused a fall in blood pressure in anaesthetized rats (Gallacher & Ramage, 1995) which was blocked by additional pretreatment i.v. with GR127935, a 5-HT$_{1B/1D}$ receptors antagonist (Skingle et al., 1993). In the rat brain 5-HT$_{1D\alpha}$ and 5-HT$_{1B}$ receptors have been shown to have different pharmacological profiles, in that mianserin is highly selective for the 5-HT$_{1D\alpha}$ receptor subtype (Hamblin et al, 1992). The present study was carried out to determine if mianserin could block this depressor action of sumatriptan and also if this depressor effect could be mimicked by the 5-HT$_{1B}$ agonist CP-93129 (Macor et al, 1990) given i.c.v.

In male Sprague-Dawley rats (250-350g) anaesthesia was induced with halothane and maintained with α-chloralose. The carotid artery and vein were cannulated for the recording of mean blood pressure (MAP) and heart rate (HR) and the i.v. administration of drugs. Rats were artificially ventilated following neuromuscular blockade with decamethonium (3 mg kg^{-1}) and placed in a stereotaxic frame for i.c.v. injections (5 µl over 20s). Using retroperitoneal approach pulse Doppler flow probes were placed around the mesenteric (M), renal (R) arteries and the abdominal aorta (H) below the ileocaecal artery (a measurement of hindquarters flow) from which changes (%) in conductance (C) were calculated. Drug induced changes were compared by two-way ANOVA with time-matched saline controls. All changes presented were considered significant as $P < 0.05$.

Sumatriptan (10 nmol kg^{-1};i.c.v.; n=5) in the presence of WAY-100635 (200 nmol kg^{-1};i.c.v.) caused a significant decrease in MAP (9 ± 2 mmHg) and HR (32 ± 15 beats min^{-1}), with an increase in RC (7 ± 3%) and HC (17 ± 5%). Pretreatment with both mianserin (600 nmol kg^{-1};i.v.; n=6) and WAY-100635 (200 nmol kg^{-1};i.c.v.) blocked the effect of sumatriptan on MAP (2 ± 2 mmHg) for up to 15 min. However, the tachycardia was now reversed to a bradycardia (-23 ± 3 beats min^{-1}). There was no change in regional haemodynamics in the 3 beds (MC 1 ± 6; RC 3 ± 7; HC 3 ± 5%). In the presence of WAY-100635 (200 nmol kg^{-1};i.c.v.; n=5) CP-93129 (10 nmol kg^{-1};i.c.v;) caused a significant increase in both MAP (7 ± 1 mmHg by 10 min.) and HR (20 ± 4 beats min^{-1} by 20 min.) and a decrease in HC (-8 ± 3%). CP-93129 alone (10 nmol kg^{-1};i.c.v; n=9) had no effect on MAP (2 ± 2 mmHg), although there was a small but significant bradycardia (-10 ± 4 beats min^{-1}) by 5 min. There were no changes in regional haemodynamics.

These results demonstrate that i.v. mianserin can block the depressor effect of i.c.v. sumatriptan in the presence of WAY-100635. Further, CP-93129 (i.c.v) in the presence of WAY-100635 causes a pressor effect. These combined data suggest that the sumatriptan depressor effect is mediated by 5HT$_{1D\alpha}$ receptors, while blockade of central 5-HT$_{1A}$ receptors also unmasks a pressor response mediated by activation of 5-HT$_{1B}$ receptors.

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143P EFFECTS OF PHORBOL ESTER VERSUS AGONIST-MEDIATED PROTEIN KINASE C ACTIVATION ON MUSCARINIC RECEPTOR-STIMULATED PHOSPHOINOSITIDE TURNOVER IN SH-SY5Y CELLS

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M_1, M_3 and M_5 muscarinic receptors couple preferentially to phosphoinositidase C (PIC) and recent studies suggest that this is acutely regulated (Wojcikiewicz *et al.*, 1993). This includes a rapid, partial desensitization of PIC within seconds of agonist exposure, resulting in a peak and lower sustained Ins(1,4,5)P_3 accumulation. Studies with phorbol esters have suggested that protein kinase C (PKC) attenuates PIC activation by muscarinic receptors. It is presently unclear (i) if this underlies rapid desensitization, (ii) if sensitized or partially desensitized receptors are affected (iii) where the site of PKC action is and (iv) if agonist- rather than phorbol ester -activation of PKC is sufficient. These issues have been addressed in the human neuroblastoma, SH-SY5Y, which has muscarinic receptors of predominantly the M_3 subtype. All experiments were performed at 37°C. Statistical comparisons were by Student's t-test unless stated.

Phorbol 12,13-dibutyrate (PDBu) (5min, 1μM throughout) preferentially attenuated peak Ins(1,4,5)P_3 responses (measured by radioreceptor assay) to 1mM carbachol (179±34 vs. 85±21 at 10s, 53% inhibition, $p<0.05$, Ins(1,4,5)P_3 data in pmol. mg protein^{-1}, data are mean±sem, n=3 unless stated) compared to the sustained phase (68±5 vs. 49±4 at 300s, 28% inhibition, $p<0.05$, basal 21±6). Ins(1,4,5)P_3 responses remained biphasic indicating PKC alone does not mediate the rapid partial desensitization. In fura-2 loaded (5μM fura-2-AM, 40min, 20°C) populations, intracellular [Ca^{2+}] ([Ca^{2+}]$_i$) elevations during peak but not sustained phases were also attenuated by PDBu (peak change 590±18 vs.424±19 nM, <0.005; sustained change 103±4 vs. 109±2 nM, control basal 77±6). The PKC inhibitor, Ro-318220 (compound 3, Davis *et al.*, 1989) (10min, 10μM), prevented PDBu's effects. PDBu reduced Ins(1,4,5)P_3 responses (81±9 vs. 15±1, $p<0.01$, basal 11±10) in the absence of a [Ca^{2+}]$_i$ response (5min 2μM thapsigargin to deplete Ca^{2+} stores, 50-100nM extracellular [Ca^{2+}] to prevent Ca^{2+} entry). Thus, the primary effect of PDBu was on the Ins(1,4,5)P_3 response. This was not due to increased metabolism of Ins(1,4,5)P_3 as carbachol-mediated [^{3}H]InsP_x accumulation was reduced over a 15min experiment in Li^+-blocked cells ($p=0.0009$, two-way ANOVA) . Furthermore, the carbachol-stimulated decrease in PtdInsP_2 was less over 15min ($p=1.38\times10^{-6}$) after PDBu treatment (eg. at 15s, 63±4% vs. 46±1%, n=4).

In electropermeabilized cells (3 discharges, 3μF capacitor, 3.75KV/cm) PDBu inhibited ($p<0.01$) peak Ins(1,4,5)P_3 responses to carbachol (147±11 vs. 93±10, n=7) and GTPγS (100μM) (90±6 vs. 55±4, n=4-7) (basal 26±3). Thus, PDBu acts at least partially at a post-receptor site. Ro-318220 did not affect Ins(1,4,5)P_3 (eg. peak, 152±20 vs. 182±29 control, basal 19±3) or [Ca^{2+}]$_i$ (eg. peak, 559±43 vs. 575±85 control, basal 84±9) responses to carbachol in intact cells nor Ins(1,4,5)P_3 responses to GTPγS in permeabilized cells (81±7 vs. 90±6 control, basal 26±3). These data indicate a lack of PKC feedback unless directly activated with PDBu. Use of Ro-318220 at sub-maximal [carbachol] was precluded by its antagonism of muscarinic receptors, indicated by inhibition of N-methyl-[^{3}H]scopolamine binding to human muscarinic M_3 receptors in CHO cells (log K_d Ro-318220, -4.89±0.05 M (13μM)).

Thus, whilst activation of PKC by phorbol esters inhibits muscarinic-receptor-mediated hydrolysis of PtdInsP_2, at least in part at a post-receptor site, there is no evidence that muscarinic receptors activate this feedback in SH-SY5Y cells.

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144P THE LACK OF EFFECT OF NITRIC OXIDE ON RAT PERITONEAL NEUTROPHIL AGGREGATION

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Elicited, rather than circulating, rat neutrophils are thought to produce nitric oxide (NO) (Stewart *et al.*, 1993). However whether neutrophil NO acts in an autocrine or a paracrine fasion is still unclear. In the present study, rat peritoneal neutrophils were harvested 19 h following injection of thioglycollate (0.78 g i.p.) and purified over a Ficoll-paque density gradient. Neutrophils were incubated for 30 min at 37°C in HEPES-buffered saline plus $CaCl_2$ (1 mM), glucose (10 mM) and test compound. Neutrophil aggregation (5 x 10^6 cells/ml) was then measured with a Chrono-log platelet aggregometer as the response to an EC_{50} concentration of formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP 16 nM) added after a further 3 min. Results are expressed as % control response to FMLP alone (100 ± 10%, n = 5), mean ± s.e. mean.

None of the nitric oxide synthase (NOS) inhibitors tested (100 μM) had any significant effect on the ability of FMLP to aggregate rat peritoneal neutrophils: N^G-nitro-L-arginine-methyl-ester (L-NAME) 109 ± 7; N^G-monomethyl-L-arginine (L-NMMA) 93 ± 6; N^G-nitro-L-arginine (L-NNA) 111 ± 7; N^G-iminoethyl-L-ornithine (L-NIO) 124 ± 10; L-canavanine 91 ± 6% control, n = 5. Similarly the nitric oxide donors sodium nitroprusside (SNP) and 3-morpholinosydnonimine HCl (SIN-1) were also without effect at 100 μM (95 ± 9 and 88 + 6% control respectively, n = 4).

Superoxide dismutase (SOD 20U/ml) had a slight potentiating effect on FMLP-stimulated neutrophil aggregation (132 ± 11% control, n = 4), suggesting that FMLP-stimulated superoxide anion production normally acts to decrease the aggregation response. The use of SOD to remove superoxide anions failed to reveal any effect of nitric oxide on neutrophil aggregation (SNP plus SOD, 138 ± 6; SIN-1 plus SOD, 137 ± 15% control respectively, n = 4).

Although NO appears to have no role in the regulation of rat peritoneal neutrophil aggregation, we observed a marked inhibitory effect of dibutyryl cyclic GMP when added 2 min before FMLP (Bt_2cGMP; 51 ± 6% control at 1 mM, n = 10). To investigate the mechanism of action of Bt_2cGMP, we preincubated the cells at 37°C for 30 min with a cyclic GMP-specific protein kinase inhibitor KT5823 (Kase *et al.*, 1987). Surprisingly KT5823 itself produced a concentration-dependent inhibition of FMLP-stimulated neutrophil aggregation at 1 and 10 μM (52 ± 6 and 39 ± 1% control, n ≥ 4).

The results of this study suggest that (1) NO plays no role in the regulation of FMLP-stimulated rat peritoneal neutrophil aggregation, (2) superoxide anions and Bt_2cGMP are inhibitory, and (3) that KT5823 might not be an inhibitor of cyclic GMP-dependent protein kinase in rat neutrophils.

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Stewart, A.G., Dusting, G.J., Giarracca, R.G. *et al.* (1993) *Mediators of Inflammation* 2, 349-356.

145P EFFECTS OF 1-AMINO-2-HYDROXY-GUANIDINE, A POTENT INHIBITOR OF INDUCIBLE NITRIC OXIDE SYNTHASE, ON MULTIPLE ORGAN DYSFUNCTION CAUSED BY ENDOTOXIN

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An enhanced formation of nitric oxide (NO) by the inducible NO synthase (iNOS) has been implicated in the pathogenesis of circulatory shock (Szabo & Thiemermann, 1995). We have investigated the effects of (i) several guanidines on the activity of iNOS in cultured macrophages, and (ii) 1-amino-2-hydroxy-guanidine, the most potent inhibitor of iNOS activity discovered, on haemodynamics, multiple organ (liver, pancreas and lung) failure and iNOS activity (liver homogenates) in rats with endotoxic shock.

Murine macrophages (J774.2) were cultured in DMEM containing L-glutamine (3.5 mM) and 10% foetal calf serum. To induce iNOS, fresh culture medium containing *E. coli* lipopolysaccharide (LPS, 1 $\mu g\ ml^{-1}$, serotyp: 0127:B8) was added. Nitrite accumulation in the cell culture medium was measured after 24 h. Male Wistar rats were anaesthetised with thiopentone sodium (12 $mg\ kg^{-1}$, i.p.). The carotid artery was cannulated for the measurement of mean arterial blood pressure (MAP) and the femoral vein for the administration of drugs. At time 0, rats received vehicle (saline, n=7) or LPS (10 $mg\ kg^{-1}$ i.v., n=20). The pressor response to noradrenaline (NA; 1 $\mu g\ kg^{-1}$ i.v.) was assessed 10 min prior to and every h after LPS . At 2 h, a continuous infusion of vehicle (0.6 $ml\ kg^{-1}\ h^{-1}$ saline, i.v., n=10) or 1-amino-2-hydroxy-guanidine (10 $mg\ kg^{-1}\ h^{-1}$, i.v., n=10) was started. At 6 h, serum samples were taken and analysed for alanine aminotransferase (ALT) and bilirubin (liver function) and lipase (pancreatic function) as well as for blood gas analysis (PCO_2 and HCO_3). In addition, iNOS activity was measured in liver homogenates by determing the conversion of [^{3}H] L-arginine to [^{3}H] L-citrulline.

The guanidine analogues caused concentration-dependent inhibitions of the increase in nitrite elicited by LPS in macrophages with the following rank order of potency: 1-amino-2-hydroxy-guanidine > 1-amino-2-methyl-g. > 1-amino-1-methyl-g. > 1-amino-1,2-dimethyl-g. (n=9). In the anaethetised rat, LPS caused hypotension, vascular hyporeactivity, pancreatic and liver dysfunction, as well as a metabolic acidosis (falls in pH, PCO_2 and HCO_3)($p<0.05$, Table 1). Treatment of LPS-rats with 1-amino-2-hydroxy-guanidine attenuated (i) the delayed hypotension and vascular hyporeactivity, (ii) the increase in the serum ALT, bilirubin, lipase, (v) metabolic acidosis, (vi) as well as iNOS activity of the liver. (Table 1)

Thus, 1-amino-2-hydroxy-guanidine is a potent inhibitor of iNOS activity in cultured macrophages and in rats with endotoxic shock. Inhibition of iNOS activity with 1-amino-2-hydroxy-guanidine prevents the delayed circulatory failure and attenuates the dysfunction of liver and pancreas, as well as the metabolic acidosis caused by endotoxaemia.

HR is a fellow of the Deutsche Forschungsgemeinschaft (Ru595/1-1)

Szabo, C. & Thiemermann, C. (1995). Adv. in Pharmacol. 34, 113-159.

Table 1.

Treatment	MAP (mmHg)	Hyporeactivity (% time 0)	ALT ($iu\ l^{-1}$)	Bilirubin (μM)	lipase ($iu\ l^{-1}$)	iNOS activity liver (pmol/mg)	PCO_2 (mmHg)	HCO_3 (mmol/l)
Sham	109±1.6	26±3	106±14	2.5±0.3	18±4	0.1±0.04	42±2.5	26±1.1
LPS + saline	71±4.8	12±3	575±119	6.7±0.9	97±9	1,9±0.5	32±1.8	17±1.1
LPS + HG	101±4.6*	21±3*	232±93*	3.4±0.3*	65±8*	0.3±0.1*	35±1.1*	21±0.7*

mean ± s.e.mean. *$p<0.05$ vs. LPS + saline, unpaired Student's *t* test.

146P INHIBITION BY TOXIN B OF G PROTEIN-COUPLED AND TYROSINE KINASE RECEPTOR-MEDIATED PHOSPHOLIPASE C STIMULATION

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A large number of hormone and neurotransmitter receptors activate phospholipase C (PLC), catalyzing hydrolysis of phosphatidyl-inositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate and diacylglycerol. Receptors coupled to heterotrimeric GTP-binding proteins (G proteins) activate PLC-β isoenzymes via Gα or Gβγ subunits of the G proteins, whereas receptors possessing intrinsic tyrosine kinase activity activate PLC-γ by phosphorylation of the enzyme on tyrosine residues. To study whether small molecular weight G proteins of the Rho family are involved in PLC activation by G protein-coupled and/or tyrosine kinase receptors, we examined the effect of *Clostridium difficile* toxin B, which specifically inactivates Rho proteins (Just et al. 1995), on PLC stimulation by various receptor agonists in N1E-115 neuroblastoma cells. Phosphoinositides were pre-labeled with *myo*-[^{3}H]inositol for 3 days, and production of [^{3}H]inositol phosphates (IP) was measured by ion chromatography. Toxin B (100 pg/ml) was applied to cells for 24 h before stimulation with agonists.

Treatment with toxin B significantly reduced IP formation induced by the G protein-coupled receptor agonists, bradykinin and lyso-phosphatidic acid (LPA), and the tyrosine kinase receptor agonist, platelet-derived growth factor (PDGF). In untreated cells, increase (over basal) in IP production induced by bradykinin (1 μM), LPA (1 μM) and PDGF (25 ng/ml) was 82 ± 8%, 93 ± 22% and 184 ± 45%, respectively (mean ± SEM from 3-6 experiments). In toxin B-treated cells, the corresponding numbers were 22 ± 7%, 16 ± 6% and 9 ± 3%, respectively. In contrast, cytochalasin B treatment (5 μg/ml for 15 min) had essentially no effect on IP production, although it produced similar morphological changes, i.e. rounding-up of cells, as toxin B. Neither toxin B nor cytochalasin B changed basal IP production.

To test whether the intrinsic enzymatic activity of PLC was altered by toxin B, PLC assays were performed on cell lysates with exogenous [^{3}H]PIP_2. Cell lysates from toxin B-treated and untreated cells showed similar PLC activities upon stimulation with Ca^{2+}. At 10 nM Ca^{2+}, PLC activity was 0.46 ± 0.11 and 0.42 ± 0.05 nmol/min/mg protein in untreated and toxin B-treated cells, respectively, and at 100 nM Ca^{2+}, the corresponding values were 20.5 ± 1.0 and 23.0 ± 2.3 nmol/min/mg protein, respectively. Stimulation of PLC activity by GTPγS (100 μM) in lysates of toxin B-treated cells was also not reduced. These data indicate that the reduced IP production observed in intact cells did not result from decreased enzymatic activity of PLC.

It is concluded from the present study that toxin B efficiently inhibits PLC stimulation in intact cells by both G protein-coupled and tyrosine kinase receptors. Based on the recent observation that Rho proteins are apparently involved in PIP_2 synthesis (Chong et al. 1994), it is suggested that the inhibitory effect of toxin B on IP production is caused by inactivation of Rho proteins resulting in depletion of cellular PIP_2, the PLC substrate.

Just, I. *et al.* (1995) *Nature* 375: 500-503.
Chong, L.D. *et al.* (1994) *Cell* 79: 507-513.

147P INVESTIGATION OF SIGNAL TRANSDUCTION PROCESSES INVOLVED IN AGONIST-INDUCED LEUKOTRIENE C_4 GENERATION IN HUMAN EOSINOPHILS

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We have previously shown that C5a- and fMLP-induced leukotriene (LT) C_4 release in human eosinophils is dependent upon activation of phospholipase C and receptor-mediated Ca^{2+} influx (Grix et al, 1994). In this study, we have further characterised the signal transduction processes involved in these responses using a range of known modulators (table 1). LTC_4 release from agonist-stimulated human eosinophils, in the presence of cytochalasin B (5.5μM), was measured by radioimmunoassay.

Table 1		% Inhibition of LTC_4 release mean ± s.e.m. (n=3-6)	
Inhibitor	Conc (M)	vs C5a (30nM)	vs fMLP (1μM)
Wortmannin	$1x10^{-8}$	86.6 ± 2.2#	42.2 ± 6.2#
Forskolin	$3x10^{-5}$	15.1 ± 30.1	-12.2 ± 15.0
Rolipram	$1x10^{-7}$	91.0 ± 7.0*	nt
PMA	$1x10^{-8}$	79.8 ± 10.3**	78.3 ± 7.5**
Bis-indolymaleimide	$1x10^{-7}$	-26.4 ± 37.5	-49.4 ± 29.3
Erbstatin ana	$1x10^{-5}$	71.6 ± 21.0*	96.4 ± 0.8**
Calyculin A	$1x10^{-7}$	89.6 ± 6.5**	80.5 ± 9.9**
Okadaic acid	$1x10^{-6}$	88.7 ± 5.0#	86.3 ± 2.2#

 # denotes mean ± s.d. (n=2). * denotes p<0.05, ** denotes p<0.005 (students paired t-test).

Stimulation of adenylyl cyclase by forskolin had no significant effect on either C5a- or fMLP-induced LTC_4. In contrast, inhibition of the breakdown of cAMP by the phosphodiesterase enzyme (PDE) IV inhibitor, rolipram (100nM) abolished C5a-induced LTC_4 generation.

Modulation of protein kinase C (PKC) activity also affected the responses to both stimuli, with activation by PMA having an inhibitory effect and PKC inhibition by bis-indolymaleimide causing a trend towards enhancement of LTC_4 generation.

Activation of tyrosine kinase appeared to be a common requirement for both C5a- and fMLP-induced LTC_4 release, as the tyrosine kinase inhibitor erbstatin analogue (methyl 2,5-dihydroxycinnamate) inhibited both stimuli.

Wortmannin (10nM) caused marked inhibition of LTC_4 release. The effect of wortmannin, at this low concentration, is thought to be due to inhibition of PI 3-kinase (Thelen et al., 1994).

Investigation of the effect of protein phosphatase (PP) inhibitors showed calyculin A and okadaic acid to inhibit LTC_4 release, with mean (95% CL) IC_{50} values of 17.8 (1.8 - 176.0) nM and 0.3 (0.1 - 0.8) μM vs C5a and 63.4 (44.7 - 89.9) nM and 0.2 (0.2 - 0.3) μM vs fMLP, respectively. The low potency of okadaic acid compared with calyculin suggested that PP1 rather than PP2A was involved in the responses.

In conclusion, C5a- and fMLP-induced LTC_4 generation appear to be mediated via similar signal transduction pathways, involving activation of tyrosine and PI 3-kinases. These responses are also inhibited by activation of PKC, inhibition of PDE IV and PP1.

Grix, S. *et al.*, 1994, Br. J. Pharmacol., 112, 85P.

Thelen, M. *et al.*, 1991, Proc. Nat. Acad. Sci, 91, 4960-4964.

148P NON-DEPOLARIZING MUSCLE RELAXANTS DIFFERENTIATE BETWEEN MOUSE MUSCLE NICOTINIC ACETYLCHOLINE RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES AND QUAIL FIBROBLASTS

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The muscle relaxants pancuronium and d-tubocurarine (d-TC) act on the rat phrenic nerve-diaphragm preparation with IC_{50} values of 2.0 and 0.72μM respectively (Aziz *et al.*, 1994). In contrast, they act on fetal mouse muscle nicotinic receptors (AChRs) expressed in *Xenopus* oocytes with IC_{50} values of 5nM for pancuronium, and 20nM for d-tubocurarine (Filatov *et al.*, 1993). It is unclear whether these differences are due to the expression system used, or due to differences between fetal and adult type receptors.

We have examined the actions of these compounds on fetal mouse muscle nicotinic AChRs stably expressed in the quail fibroblast cell line QF18 (Phillips *et al.*, 1991), using ratiometric confocal microscopy with the calcium-sensitive fluorescent dye Indo-1 to measure intracellular calcium ion (Ca^{2+}i) activity (Cross *et al.*, 1995a,b). Experiments were performed at room temperature. Drugs were added to the medium bathing the cells, and for blocking experiments, the cells were incubated for three minutes in the appropriate dose of the drug, before addition of suberyldicholine (SDC). Mean Ca^{2+}i was calculated for a 140μm^2 area within each cell. Results are expressed as mean±s.e.mean, with P-values from the students paired t-test for the difference of the means. n values are the number of cells analyzed from a minimum of 5 replicates.

Application of SDC (10μM) to the medium bathing QF18 cells gave rise to an increase in Ca^{2+}i from 148±11nM at rest, to a peak of 677±63nM (n=48). Application of 10μM d-TC alone was found to give rise to a small, but detectable increase in Ca^{2+}i in QF18 cells. The resting calcium concentration was 146±20nM, rising to 157±15nM after addition of 10μM d-TC (n=15; $p<0.001$). d-TC was also found to block the response of the QF18 cells to 10μM SDC in a dose-dependent manner, acting with an IC_{50} of around 500nM (n=15). Pancuronium alone did not alter Ca^{2+}i, but blocked the response to SDC (10μM), again in a dose-dependent manner, with an IC_{50} value of 300nM (n=13-15).

Our IC_{50} values for the effects of d-TC and pancuronium are comparable to those found in adult rat diaphragm. However, these drugs are substantially more potent at blocking nicotinic AChRs expressed in oocytes. This may reflect differences in processing and post-translational modifications between the two systems.

Aziz, L., Ono, K., Morita, K., *et al.* (1991) *Science* 251, 568-570.

Cross, K.M.L., Jane, S.D., Wild, A.E. *et al.*, (1995) *Br. J. Pharmacol.* in press.

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Filatov, G.N., Aylwin, M.L., and White, M.M. (1993) *Mol. Pharmacol.* 44, 237-241.

Phillips, W.D., Kopta, C., Blount, P., *et al.* (1991) *Science* 251, 568-570.

149P THE EFFECT OF PANCURONIUM BROMIDE ON MUSCLE AND NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) EXPRESSED IN *XENOPUS* OOCYTES

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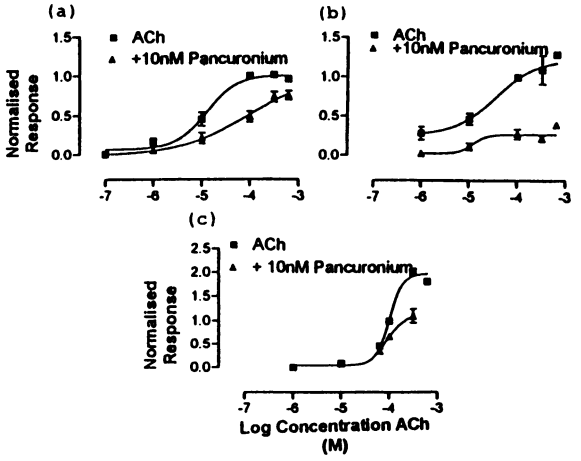
It has been shown clinically that pancuronium is a more effective muscle relaxant than (+)-tubocurarine (Kreig *et al.*, 1980). We are investigating the molecular basis for the action of these compounds at nAChRs using *Xenopus* oocytes as an expression system. *Xenopus* oocytes were injected with either mouse muscle nAChR subunits' ($\alpha\beta\gamma\delta$) mRNA, α_7 (chick) mRNA, or $\alpha_4\beta_2$ (rat) mRNA and used for two electrode voltage-clamp recordings, at -80mV. Acetylcholine (ACh) elicited concentration-dependent inward currents for each of the 3 nAChR subunit combinations. The currents were blocked by pancuronium in all 3 receptor subtypes (Figure 1). The % inhibition (mean±s.e.m) of the response to 100μM ACh by 10nM pancuronium was as follows: muscle 39.3±2.4% (n=9), $\alpha_4\beta_2$ 73.3±11.0% (n=4), α_7 43.3±3.1% (n=3). By comparison, in a similar set of experiments, the % inhibition by 10nM (+)-tubocurarine was found to be 28.7±5.7% in muscle, and 20.7±6.1% for the α_7 receptor.

The muscle relaxant pancuronium is therefore significantly more effective ($p<0.05$, Students' t-test) at blocking the $\alpha_4\beta_2$ subunit combination than $\alpha\beta\gamma\delta$.

cDNA clones were kindly donated by Dr. S. Heinneman, Salk Institute, California.
C.M.G. is supported by Organon Teknika, Belgium.
Kreig, N., Crue, J.F. & Booij,L.H.D.J. (1980) *Br. J. Anaesth.* 52, 783-787.

Figure 1. Concentration-response curves to ACh in the presence (▲) and absence (■) of 10nM pancuronium in oocytes expressing (a)$\alpha\beta\gamma\delta$ (n=9), (b)$\alpha_4\beta_2$ (n=4) and (c)α_7 (n=3), voltage clamped at -80mV. Data points are mean ±s.e.mean.



150P SPECIES COMPARISON OF THE *IN VITRO* HEPATIC METABOLISM OF RETINOIC ACID

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Retinoic acid (RA) is a physiologically important metabolite of retinol and is considered to be the most active naturally occurring retinoid for Vitamin A-dependent functions. Increase of biological potency by inhibiting its oxidative metabolism (Williams & Napoli, 1985) is consistent with this. As part of a study to develop novel compounds as selective inhibitors of hepatic 4-hydroxylation of RA that could have potential value as anticancer agents, we have compared the in vitro metabolism of ^{3}H-RA by liver microsomes from several common laboratory animal species and have examined the inhibitory action of the P450 3A4 ligand, ketoconazole on this process.

The microsomal fractions (sedimenting between 10 and 100S) from the male and female albino rat, male albino mouse and Syrian hamster (10μl; 5, 10, 2.1 and 10mg protein ml^{-1}, respectively) and male New Zealand rabbit (15μl; 10mg protein ml^{-1}) were incubated with 0.1-0.8μM (11,12-^{3}H)-RA at pH 7.4 and 37°C in the presence of NADPH (Ahmad et al., 1994) for optimal periods (male rat and hamster 15 min; mouse 20 min; female rat 35 min; rabbit 40 min). Metabolism of ^{3}H-RA was determined by HPLC after extraction of the incubation mixture (Ahmad et al., 1994).

From the results, analysed by Lineweaver Burk plots, it may be observed that both K_m and V_{max} were species (and sex)-dependent. Of the male animals, rat liver appeared to be the most active in metabolising RA, although it was not significantly more so than that of the mouse. Metabolism was less variable in the remaining cases examined. For the metabolism of RA by the rat, there was a marked sex difference, liver from the male being about 5.5 times more active than that from the female. This is consistent with the well recognised sex differences in P450-based drug metabolising activity in this species (Kato & Gillette, 1965). Inhibition of ^{3}H-RA metabolism by ketoconazole (100μM) was very similar in the liver microsomes of all the species examined (87.4±0.4, 85.0±5.2, 70.5±2.4, 89.7±3.2 and 88.9±2.5 % inhibition with the male and female rat, mouse, hamster and rabbit liver respectively). Overall the results indicate that male rat hepatic microsomes represent a useful enzyme source for screening novel compounds as inhibitors of RA metabolism.

Table 1 In vitro metabolism of ^{3}H-RA by hepatic microsomes from several animal species

Species	K_m* (μM)	V_{max}* (p mole mg protein^{-1}min^{-1})
Rat (male)	0.51±0.10	102±39
Rat (female)	0.19±0.04	18± 1
Mouse (male)	0.96±0.13	67±21
Hamster (male)	0.90±0.08	56±11
Rabbit (male)	1.51±0.94	17± 1

*mean ± sd, n=3

This work is sponsored by the Tenovus Cancer Charity.

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151P *IN VITRO* METABOLISM OF RETINOIC ACID BY VARIOUS RAT TISSUES: IMPLICATIONS FOR INHIBITORS

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The ability of retinoic acid (RA) to control growth and differentiation of epithelial tissue, to support growth in vitamin A (VA)-deficient animals and to suppress neoplastic development has resulted in interest in its metabolism (Sporn & Newton, 1979). While the liver is recognised as an important site for the P450-mediated oxidation of RA (Ahmad et al., 1995), it is possible that other sites in the body may contribute to the metabolic clearance of RA. A current strategy of potential anticancer drug development is the design of selective inhibitors of RA oxidation to enhance its beneficial effects (Ahmad et al., 1995). However, significant variation between tissues in respect of RA metabolism may be an important determinant of the effectiveness at a particular tissue site of a systemically administered inhibitor. The present study compares the in vitro metabolism of RA in liver, kidney, intestinal mucosa and lung of adult male albino rats.

Microsomes were prepared from the tissues (Roberts et al., 1979) and incubations with ^{3}H-RA were performed under optimal conditions. The incubation mixture contained (11, 12-^{3}H)-RA (0.1-0.8μM; 10μl), NADPH (2mM; 50μl), solvent (ethanol; 10μl), microsomes [liver and kidney (protein 5mgml^{-1}; 10μl), intestinal mucosa (protein 0.2mgml^{-1}; 150μl), lung (protein 1.6mgml^{-1}; 150μl)] and PBS pH7.4 to a final volume of 400μl. Incubation was at 37°C for either 15 min (liver and kidney) or 60 min (intestine and lung). Metabolism of ^{3}H-RA in extracts of the incubates was assessed by HPLC as previously described (Ahmad et al., 1994).

The results (Table 1) demonstrate that RA metabolism occurred in microsomes from all tissue sites. Analysis by Lineweaver Burk plots revealed that, while K_m values did not differ significantly, there were major differences between the selected sites in the V_{max} for oxidative metabolism of RA, with liver being the most and lung being the least active, a ranking order not likely to be altered by enzyme activity being based on total tissue mass. This suggests that clearance of RA by kidney, lung and intestinal mucosa is unlikely to exert a controlling influence on the total clearance of this substance. It is thus probable that administration of inhibitors of RA metabolism selectively targeted to these tissue sites would fail to confer therapeutic advantage. However, it remains to be tested whether this is the case for other tissues and organs, e.g. the skin. An additional factor to be considered is the varying susceptibility of the metabolism at these selected sites to ketoconazole, an established inhibitor of hepatic oxidation of RA (Ahmad et al., 1995); the present study demonstrated that ketoconazole (100μM) inhibited RA metabolism by 87.5±0.4, 80.5±2.3, 63.7±2.7 and 52.8±4.0% (mean±sd, n=3) in liver, kidney, lung and intestinal mucosal microsomes respectively.

Table 1 In vitro metabolism of ^{3}H-RA by microsomes from various rat tissues

Microsomal source	K_m* (μM)	V_{max}* (p mole mg protein^{-1}min^{-1}
Liver	0.51±0.10	102±39
Kidney	0.48±0.05	17± 2
Lung	0.50±0.05	1±0.03
Intestinal mucosa	0.49+0.07	4±0.3

*(mean ± sd, n=3)

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152P BLOCK OF MIN K CURRENT BY PROPOFOL AND THIOPENTONE IN *XENOPUS* OOCYTES

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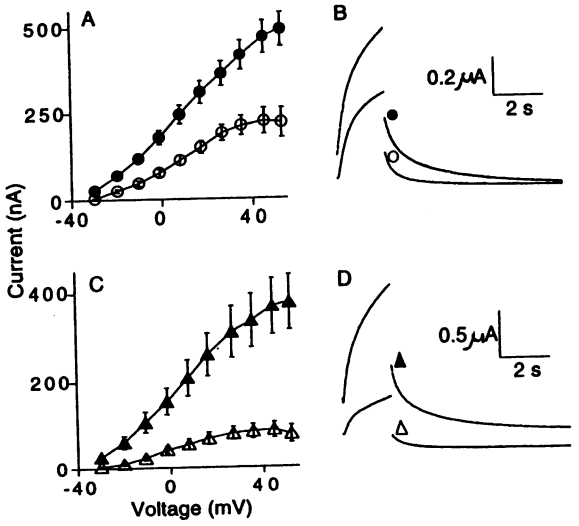
Propofol and thiopentone suppress the slowly activating component of the cardiac delayed rectifier potassium current (I_{Ks}) with no effect on the rapidly activating component of I_K (Takahashi and Terrar, 1995). The potassium current induced by the expression of the min K protein (I_{SK}; Takumi, et. al., 1988) in *Xenopus* oocytes has similar characteristics to cardiac I_{Ks} and therefore the possible effect of propofol and thiopentone on I_{SK} were investigated.

The synthetic min K gene (Hausdorff et. al., 1991; kindly supplied by S. Goldstein and C. Miller) was linearized using *Not I* and the corresponding mRNA (50 ng *in vitro* transcribed) was injected into defolliculated stage V or VI *Xenopus* oocytes. Currents were recorded at room temperature using two electrode voltage-clamp with electrodes containing 3 M KCl (resistance 1-5 MΩ). Currents were activated by 2 s step depolarizations from -40 to positive potentials and recorded as deactivating tail currents upon repolarization to -40 mV.

Both propofol and thiopentone suppressed I_{SK}. Propofol (300 μM) significantly reduced the amplitude of I_{SK} tail currents at all test potentials ($p<0.01$; n=8; Figure 1A). Figure 1B shows a typical current recorded during a step to +52 mV before and after propofol. The mean reduction in tail current amplitude at this voltage was 56 ± 6 % after propofol. Thiopentone (100 μM) also significantly reduced I_{SK} at all test potentials ($p<0.01$; n=9; Figure 1C). At +53 mV, thiopentone reduced the tail current amplitude by 80 ± 4% and figure 1D shows a typical current recorded at this voltage before and after thiopentone. Thiopentone also reduced the holding current (Figure1D).

These observations are consistent with block of I_{SK} by propofol and thiopentone and therefore support the hypothesis that the potassium current induced by min K is related to cardiac I_{Ks}.

Figure 1: filled symbols = control data, open circles = propofol and open triangles = thiopentone. Bars are s. e. mean.



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153P EFFECTS OF SB 206553, A 5-$HT_{2C/2B}$ RECEPTOR ANTAGONIST, IN A MARMOSET CONFLICT MODEL OF ANXIETY

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We have previously observed that the 5-$HT_{2C/2B}$ receptor antagonist, SB 200646A, has an anxiolytic-like effect in the rat social interaction and Geller-Seifter tests, and in a marmoset conflict model (see Kennett et al., 1995). We now report the effects of a more potent and selective 5-$HT_{2C/2B}$ receptor antagonist, SB 206553, as well as mianserin (MIAN) and diazepam (DZP) in the marmoset conflict test.

Male and female marmosets (bred in-house, 400-500g) fed a normal diet were trained to lever press for a liquid food reward. The schedule consisted of 4 x 2 minute periods where responding was rewarded following a fixed number of lever presses; unsuppressed responding (USR). These were alternated with 4 x 2 minute periods where responding was rewarded following a fixed number of lever presses and each reward was associated with an aversive air puff to the face; suppressed responding (SR).

SB 206553, MIAN and DZP were administered as suspensions in 1% methyl cellulose (dose volume 1ml per animal) by oral gavage 1h pretest (4-6 animals per dose). Results are cited as mean ± s.e.m. and analysed by 2-way ANOVA (treatment x subjects). Comparisons were made with levels of responding on two preceding vehicle treated days for each animal and expressed as % change. The number of lever presses on the two days prior to challenge with test compound was 157.3 ± 17.9 during unsuppressed and 9.0 ± 1.2 during suppressed trials respectively (mean from all tests).

SB 206553 increased SR, an index of anxiolytic efficacy, (15mg/kg +320.2 ± 197.9%, $p<0.05$; 20 mg/kg +134.9 ± 79.9%, $p<0.05$) and reduced USR (15 mg/kg -45.3 ± 9.8%, $p<0.05$; 20 mg/kg -42.3 ± 10.8%, $p<0.01$). DZP increased both USR and SR at 2 mg/kg (USR +51.6 ± 19.9%, $p<0.01$; SR +439.5% ± 250.2, $p<0.01$). MIAN increased SR at 2.5 mg/kg (+234.9 ± 125.5%, $p<0.01$) with no effect on USR but at 5 mg/kg, all responding was reduced in the two marmosets tested such that no more animals were tested at this dose.

The benzodiazepine, DZP, increased SR consistent with its known anxiolytic efficacy. MIAN, a non-selective 5-HT_2 receptor antagonist, also showed anxiolytic-like efficacy, as reported clinically (Murphy, 1978), albeit over a restricted dose range, perhaps due to its high affinity for the α_2-adrenoceptor (Leysen 1981). The reduction in USR with SB 206553 is unlikely to be due to 5-$HT_{2C/2B}$ receptor blockade since SB 200646A did not have this effect (Kennett et al., 1995). In conclusion, the activity of SB 206553 in the present study and of SB 200646A previously, suggests that blockade of 5-$HT_{2C/2B}$ receptors has anxiolytic-like consequences in marmosets, as in rats (Kennett et al., this meeting), an action that is also likely to explain the anxiolytic-like effect of MIAN.

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154P BLOCKADE OF BOTH GLUTAMATE RELEASE AND SEIZURE ACTIVITY BY THE PRESYNAPTIC GLUTAMATE RECEPTOR AGONIST (1S,3S)-ACPD

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The presynaptic mechanisms involved in seizure generation have recently become a focus of interest. There are two classes of metabotropic glutamate receptors (mGluR), one negatively linked to the cAMP cascade and the other positively linked to phospholipase C (Pin & Duvoisin, 1995). These are suggested to be mainly active at presynaptic and postsynaptic sites respectively (Watkins & Collingridge, 1994). The purpose of the present study was to investigate the involvement of presynaptic mGluRs in the development of kindled epilepsy and in full seizure expression. The (1S,3S) isomer of 1-aminocyclopentane-1,3-dicarboxylate ((1S,3S)-ACPD) was used for this study as it has been shown to have relatively selective agonist activity at presynaptic receptors that mediate synaptic depression (Pook *et al.*, 1992). The action of this compound on synaptic release of glutamate from isolated synaptosomes was also studied.

Male Sprague-Dawley rats (290-330g) were anaesthetised and implanted with guide cannula/bipolar electrode units in the vicinity of the right basolateral amygdala. At least one week after surgery, after discharge thresholds (ADT) were determined and animals 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 threshold (GST) was determined. The effect of the drugs on both the development of kindled epilepsy and on the fully kindled seizure itself were studied by injecting the drugs (in 0.5µl buffer) close to the kindling site 20min prior to applying the kindling stimulus or, in fully kindled animals, prior to redetermining the GST. Synaptosomes were prepared from rat cerebral cortex and the effect of drug treatment on veratridine (50µM)-evoked [^{3}H]L-glutamate and [^{3}H]D-aspartate release was studied using superfusion (Hughes *et al.*, 1993).

(1S,3S)-ACPD (10nmol) markedly inhibited the development of amygdaloid kindling. When drug treatment was withdrawn (day 10) the animals proceeded to fully kindled seizures. The total number of stimulations required to achieve full kindling in control animals was 5.83±0.60 compared to 13.67±1.31 in drug treated animals (mean±s.e.mean, n=6; $P<0.001$, Student's t-test), the latter only being achieved after cessation of drug administration. (1S,3S)-ACPD also dose dependently increased the GST in fully kindled animals. The estimated GST_{100} (dose required to increase GST by 100%) was 0.6nmol. Superfusion of synaptosomes with Krebs-buffer containing (1S,3S)-ACPD inhibited the veratridine evoked release of [^{3}H]L-glutamate (≈55700 dpm/mg protein) by a maximum of 44% ($IC50=63\mu M$). With [^{3}H]D-aspartate the maximum inhibition achieved was 96% ($IC50=50\mu M$).

The above findings suggest that presynaptic mGluR activation decreases epileptiform activity, probably by reducing synaptic glutamate release. Targeting these receptors may be a useful new approach for the development of novel anticonvulsant drugs.

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155P FURTHER STUDIES ON THE LINK BETWEEN THE LOSS OF NEUROPROTECTIVE EFFECTS OF LIFARIZINE AT A HIGH DOSE AND ITS HYPOTENSIVE EFFECTS, USING RATS IMPLANTED WITH TELEMETRY TRANSDUCERS

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Lifarizine (RS-87476) is an ion channel modulator that is neuroprotective in the rose Bengal model of focal ischaemia in the rat, when administered at a dose of 0.1 mg/kg, i.v., 5 min post-insult, with follow-up doses of 0.5 mg/kg i.p. given twice-daily during the 72h recovery period (McBean *et al.*, 1995a). However, there is loss of efficacy when the initial dose is increased to 1 mg/kg, i.v., associated with a hypotensive effect (McBean *et al.*, 1995b). In these studies we were only able to monitor blood pressure for the first 10 min post-insult, before discontinuing the anaesthesia. The aim of the present study was to investigate, using telemetry, whether the hypotension persisted beyond these first 10 minutes, and whether there were further hypotensive episodes during the 72h recovery period.

Male Sprague-Dawley rats (230-310g) were anaesthetized with halothane and implanted with aortic telemetry transducers (Data Sciences, type TA11PA-C40), using strict aseptic techniques. Following recovery, rats were housed singly in standard cages placed on flat-pad receivers, to enable 24h monitoring of blood pressure and heart rate (5 min intervals) and activity (30 min bins). 14-17 Days later, the rats were anaesthetized with halothane, and subjected to the rose Bengal procedure as described previously (McBean *et al.*, 1995a, b). At 5 min post-illumination, the telemetry sampling interval was reduced to 30s, and the rat was injected intravenously with either lifarizine (1 mg/kg; n = 6) or its vehicle (sorbitol/tartaric acid, 1 ml/kg; n = 8). Potentially lethal hypotension was averted by reducing the level of halothane. Ten minutes later, the halothane anaesthesia was discontinued, and the telemetry sampling interval was returned to 5 min. Supplementary doses of lifarizine (0.5 mg/kg, i.p.) were administered twice-daily during the 72h recovery period. After 72h the rats were killed by an overdose of halothane and decapitated. The brains were removed rapidly and processed for image analysis of cresyl violet-stained sections. Infarct volume was calculated for each brain.

Throughout the two weeks of recording prior to the rose Bengal procedure, the rats showed a circadian rhythm, with blood pressure and heart rate higher during the active dark phase. The circadian rhythm was flattened for 24h after surgery, but then returned to normal. The maintenance doses of 0.5 mg/kg i.p. had no effect on blood pressure or heart rate, and there were no hypotensive episodes during the 72h recovery period in any of the rats. From 15-30 min after the initial 1 mg/kg i.v. dose, there was a significant bradycardia ($P < 0.05$; *t*-test following repeated measures ANOVA) relative to the vehicle-treated rats (e.g. at 15 min post-dose, vehicle = 308.8 ± 16.2 b.p.m.; lifarizine = 254.7 ± 12.0 b.p.m.), associated with a trend towards a hypotensive effect which was not significant (repeated measures ANOVA). There was no significant effect on infarct volume (vehicle: 36.0 ± 4.8 mm^3; lifarizine: 27.9 ± 7.7 mm^3). Pooling the data from this and our previous study with this dose (McBean *et al.*, 1995b) revealed a significant inverse correlation between infarct volume and mean arterial blood pressure at 3 min post injection of 1 mg/kg i.v lifarizine ($r = 0.583$; $n = 21$; $P < 0.01$).

In conclusion, hypotension following injection of a large dose of lifarizine (1 mg/kg, i.v.), occurring in the critical early phase of cerebral ischaemia, may contribute to the loss of efficacy of this drug in the rose Bengal model of focal ischaemia in the rat. This phenomenon has been encountered with other classes of neuroprotective agents in other models of focal cerebral ischaemia (e.g. Gill *et al.*, 1991).

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156P NEUROPROTECTIVE AND CARDIOVASCULAR EFFECTS OF SB 206284A IN RATS

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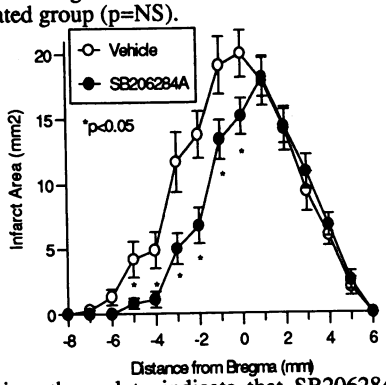
SB206284A, a novel calcium channel blocker, has displayed neuroprotective activity in photothrombotic models of cerebral ischaemia in rats and marmosets [Wood, *et al*, 1995]. The aims of the present investigations were to identify the effects of SB206284A following mechanical induction of permanent focal cerebral ischaemia and to ascertain whether the compound produced any cardiovascular effects in conscious normotensive rats.

In all experiments 10% hydroxypropyl-β-cyclodextrin in saline was used as vehicle. Under isoflurane anaesthesia, right middle cerebral and common carotid artery permanent occlusions were made in male Fisher F-344 rats (290-340g). A 1 hour intravenous infusion of 10mg/kg SB206284A (n=18) or vehicle (1ml/h; n=18) was initiated 30 minutes post-occlusion. Cerebral infarct size was measured 48 hours post-occlusion using triphenyltetrazolium staining. For cardiovascular studies, arterial and venous cannulae were chronically implanted in male Hooded-Lister rats (350-450g) under anaesthesia using aseptic techniques. At least 7 days later, SB206284A (10mg/kg; n=7) or vehicle (1.5ml; n=8) was intravenously infused over 15 minutes and mean arterial pressure (MAP) and heart rate (HR) were monitored for 4h.

Following cerebral ischaemia, SB206284A significantly reduced the hemispheric swelling (9.4±0.7 vs 7.1±0.7%, $p<0.05$) and significantly ($p<0.05$) reduced the infarct size in some areas of the posterior forebrain (see figure) but did not significantly reduce total infarct size compared to vehicle treated rats. In the cardiovascular study in conscious rats, at time 0 min the MAP and HR of the SB206284A treated group were 108±2 mmHg and 401±3 beats/min, respectively, and were 109±3 mmHg and 398±6 beats/min, respectively, in the vehicle treated group (p=NS). There were no significant differences between the groups throughout the observation period and at four hours following the start of the 15 min infusion, the MAP and HR of the SB206284A treated group were 106±3 mmHg and 377±3 beats/min, respectively, and were 110±3 mmHg and 379±4 beats/min, respectively, in the vehicle treated group (p=NS).



In conclusion, these data indicate that SB206284A displays some neuroprotective efficacy in a model of permanent middle cerebral artery occlusion, and does not cause any significant haemodynamic disturbance in rats at neuroprotective doses. Thus, in contrast to dihydropyridine calcium channel blockers which are hypotensive at neuroprotective doses SB206284A has the advantage of not affecting the cardiovascular system.

Wood NI, Benham CD, Brown TH, *et al.* J Cereb Blood Flow Metab (1995) 15 suppl 1, S384.

157P EFFECT OF LESIONS OF THE 5-HYDROXYTRYPTAMINERGIC PATHWAYS ON PERFORMANCE IN AN OPERANT TIMING TASK

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The ascending 5-hydroxytryptaminergic (5HTergic) pathways are believed to play a role in the inhibitory regulation of operant behaviour controlled by both aversive and positively reinforcing stimuli (see Ho *et al*, 1995). Recently we have found that destruction of these pathways alters performance in various operant behaviour paradigms which entail temporal discrimination or the temporal regulation of behaviour (Morrissey *et al.*, 1994; Ho *et al.*, 1995). We report here the effect of lesions of these pathways on performance maintained under a new timing procedure (Bizo & White, 1994).

Under halothane anaesthesia, 18 female Wistar rats (250–300 g) received injections of 5,7-dihydroxytryptamine (4 μg base in 2 μl vehicle) into the dorsal and median raphe nuclei; 18 rats received sham lesions (method: Ho *et al*, 1995). They were then trained in operant conditioning chambers to press levers for a sucrose reinforcer (50 μl, 0.6 M). Daily training sessions consisted of 40 50-s trials in which reinforcers were available on a variable-interval 25-s schedule; in the first 25 s of each trial, reinforcers were only available for responses on lever A, whereas in the last 25 s reinforcers were only available for responses on lever B. Data were collected from 'probe' trials (4 per session) in which no reinforcers were delivered, during the last 10 of 50 sessions. At the end of the experiment, the rats were killed and their brains dissected for measurement of 5HT, 5-hydroxyindoleacetic acid (5HIAA), noradrenaline and dopamine by high-performance liquid chromatography with electrochemical detection (method: Ho *et al.*, 1995).

Both groups showed decreasing response rates on lever A and increasing response rates on lever B as a function of time from the onset of the trial (t). Responding on lever B, expressed as a percentage of overall response rate (%B), could be described by the logistic function $\%B=100/(1+[\ln(t)/\ln(T_{50})]^{\varepsilon})$ (goodness of fit, p^2: *control (sham-lesioned) group*, 0.907; *lesioned group*, 0.909), from which the 'indifference point', T_{50} (time corresponding to 50% responding on lever B) and the slope parameter ε were derived. Neither parameter differed significantly between the groups (mean ± s.e. mean: T_{50}, *control* 22.4 ± 2.4 s, *lesioned* 19.4 ± 2.7 s; ε, *control* −5.08 ± 0.74, *lesioned* −4.37 ± 0.72; t-test, $P>0.1$ in each case). In both groups the rate of switching between levers increased towards the middle of the trial and then declined; the rate of switching was higher in the lesioned group (maximum rate ± s.e.mean: 16.3 ± 1.6 switches minute^{-1}) than in the control group (10.3 ± 1.2) ($P<0.01$). The levels of 5HT and 5HIAA in the parietal cortex, hippocampus, amygdala, nucleus accumbens and hypothalamus of the lesioned group were less than 8% of those of the control group (t-test, $P<0.01$ in each case), but the levels of noradrenaline and dopamine did not differ significantly between the groups ($P>0.1$ in each case).

The results confirm that performance in timing schedules is sensitive to destruction of the 5HTergic pathways, and suggest that these pathways may contribute to the inhibitory regulation of switching between behavioural states (see Morrissey *et al.*, 1994).

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158P THE EFFECT OF DOPAMINE RECEPTOR ANTAGONISTS ON RESPONDING FOR A CONDITIONED REINFORCER IN THE RAT

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Previous research has demonstrated that administration of dopamine (DA) D_1 and D_2 receptor antagonists results in a decline in responding for food reward (Beninger *et al.*, 1987). However, recent evidence suggests that administration of D_2 specific DA antagonists can also enhance the incentive value of food (Guyon *et al.*, 1993). The incentive properties of a primary reinforcer can be examined by evaluating the control over behaviour by a conditioned stimulus (CS) in the absence of the primary reinforcer, thus the present investigation was carried out to determine the effect of DA antagonists on responding for a CS.

30 Female Lister Hooded rats (250-300g) maintained at 90% of their free feeding weight were trained to associate a CS (houselight off, stimulus lights on, pellet feeder activated) with the delivery of a 45mg Noyes food pellet. Over 12 days the stimulus was presented for 1s every 30s for a training period of 20 min. In the test phase two levers were introduced into the chamber. One lever was randomly assigned the conditioned reinforcement lever (CR), and the other the non-conditioned reinforcement lever (NCR). Responding on NCR had no programmed consequence, whilst responding on CR resulted in the presentation of the CS, but with no accompanying pellet delivery. Test sessions lasted 30 min and were separated by at least two drug free days. Drugs were administered i.p. 30 min (or 2h s.c. for SCH23390) prior to testing. Data was subjected to square root transformation before subsequent analysis by 2-way ANOVA followed by Dunnett's t-test.

Responding on the CR lever was significantly increased following injection of low doses of sulpiride, raclopride, amphetamine, and haloperidol (Table 1). Administration of low doses of the D1 antagonist SCH23390 (0.001-0.005mg/kg) did not significantly alter CR responding. Treatment with higher doses of amphetamine, haloperidol, SCH23390, sulpiride and raclopride significantly reduced responding on CR (Table 1). Responding on NCR was only influenced following administration of haloperidol which significantly potentiated NCR responding from 8.43±0.96 to 17.54±1.94 ($F(4,25) = 3.25$ $P<0.05$), and amphetamine which significantly reduced NCR responding from 10.5±0.88 to 1.16±0.36 ($F(4,25) = 2.32$ $P<0.01$).

Results of the present study indicate that responding on CR is potentiated following administration of low doses of D_2, but not D_1 receptor antagonists, and reduced by higher doses of both D_1 and D_2 receptor antagonists. These results, in agreement with Guyon *et al.* (1993), suggest that administration of a D_2 specific antagonist enhances the incentive properties of a primary reinforcer.

Guyon A. *et al.*, (1993) Psychopharmacology 110, 460-466.
Beninger R.J. *et al.*, (1987) Psychopharmacology 92, 343-349.

Table 1 The effect of DA antagonists on CR reponding

Drug	F value	Vehicle	Dose (mg/kg)		Dose (mg/kg)	
Amphetamine	F(4,25) = 16.97 ***	6.93±0.27	0.5	12.50±0.99 **	1.0	1.39±0.42 **
Haloperidol	F(4,25) = 12.58 ***	6.51±0.53	0.01	9.24±0.83 *	0.25	3.16±0.48 *
Raclopride	F(4,25) = 9.67 **	7.16±0.91	0.05	11.60±1.00 **	5.0	3.87±0.46 **
SCH23390	F(4,25) = 7.98 *	7.82±0.87	0.005	8.17±1.01	0.01	4.83±0.34 *
Sulpiride	F(4,25) = 9.30 **	6.96±0.86	4.0	10.76±1.13 **	32.0	3.05±0.27 **

Data are expressed as mean±s.e.m. square root of lever press responses (n = 6 per group). Significant effect of drug compared to vehicle treatment: *P<0.05, **P<0.01, ***P<0.001 (Dunnett's t-test)

159P A COMPARISON OF ACQUISITION AND EXPRESSION OF THE ETHANOL DISCRIMINATIVE CUE IN FEMALE RATS OF SPRAGUE DAWLEY AND LISTER-HOODED STRAIN

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A drug discrimination paradigm has been employed to study the interoceptive cue produced by ethanol. Alcohol-accepting (AA) and alcohol-non accepting (ANA) rat strains selectively bred for different sensitivity to ethanol have been differentiated by their ability to discriminate an ethanol cue from saline, demonstrating a genetic involvement in drug discrimination (York 1981). It remains unclear, however, whether differences also exist in the ability of heterogeneous rat strains to discriminate ethanol from saline. The aim of the present study was to compare the ability of Lister-Hooded (LH) and Sprague Dawley (SD) rats to discriminate ethanol from saline in a two lever drug discrimination paradigm. 15 Adult SD and 12 LH group housed female rats (275-325g) were food deprived to 85% of their free-feeding body weight. All animals were trained to press one of two available levers on an FR10 schedule of reinforcement in an operant chamber while under the influence of either 0.75 g/kg ethanol or saline, according to a previously described protocol (Sanger 1993). Ethanol or saline was administered (i.p.) 30 min prior to training. Animals were considered to have reached criterion if they correctly selected the appropriate lever at the onset of each session for 8 out of 10 consecutive training days. Correct responding was defined as no more than 15 responses being made before delivery of the first pellet, and 90% correct responding over the duration of the session. Following acquisition of criterion, a dose-response relationship for ethanol (0.125-1.5 g/kg) was determined for the two rat strains. Data were analysed by a one-way ANOVA followed by Dunnett's t-test.

The mean number of sessions for rats to reach criterion was 154.3± 9.2 for the SD strain and 62.9±6.7 for the HL strain. However, this was mainly due to differences in acquisition of responding for food as rats of the SD strain acquired the ethanol cue in 31.0±2.0 days and rats of the LH strain in 27.0±3.0 days, once responding for food had been acquired. 75% of the SD strain and 92% of the LH strain reached criterion. The dose-response relationship for both strains of rat showed a similar pattern, with approximately 50% of animals choosing the ethanol-appropriate lever at 0.5 g/kg of ethanol and 100% of animals at 0.75 g/kg and 1.0 g/kg of ethanol. Rate of responding was not significantly affected by any dose of ethanol for SD ($F(11,55)=1.10$, N.S.) and LH rats ($F(10,50)=2.40$, N.S.).

Table 1. Ethanol dose-response relationship in SD and LH rats.

	SD rats			LH rats		
Ethanol g/kg	a	a1	% control rate	a	a1	% control rate
0	0/12	0	100	0/11	0	100
0.125	0/12	0	96.3±10.0	0/11	0	88.6±7.8
0.25	2/12	17	93.2±19.8	1/11	9	107.6±14.7
0.5	5/12	42	105.8±11.6	5/11	46	108.3±10.7
0.75	12/12	100	101.8±15.7	11/11	100	114±17.2
1.0	12/12	100	106.0±13.4	11/11	100	91.6±12.8
1.5	9/12	75	75.8±10.0	10/11	91	90.5±11.1

a = n ethanol responding/n responding, a1 = % ethanol responding.

In summary, the present results show that, although LH rats acquired responding for food quicker than the SD strain, both strains acquired the ethanol cue over an equivalent training period. No strain differences in sensitivity to ethanol were shown when an ethanol dose-response relationship was examined. These data show that there is no difference in the ability of LH and SD strains of rat to acquire and express the interoceptive ethanol cue under the present experimental conditions.

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160P THE EFFECT OF RENZAPRIDE AND ITS ENANTIOMERS ON LEARNING IN THE COMMON MARMOSET

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Renzapride (BRL 24924) and its enantiomers, BRL 44277 (+) and BRL 44278 (-), have both 5-HT_3 receptor antagonist and 5-HT_4 receptor agonist properties (King *et al.*, 1993). Cognitive enhancing effects of 5-HT_3 receptor antagonists in the marmoset have been documented (Samson *et al.*, 1994). 5-HT_4 receptor agonists, zacopride and renzapride increase electroencephalogram (EEG) energy in the rat consequent to an increase of acetylcholine release (Boddeke & Kalkman, 1990). A positive effect of the 5-HT_4 agonists, BIMU-1 and BIMU-8, on social learning in rats has also been reported (Ghelardini *et al.*, 1994). The present study compares the effects of renzapride and its enantiomers in a visual discrimination with reversal task in the common marmoset.

Seven fully trained adult marmosets (3 male, 4 female), weighing between 350-450g were used. Testing was carried out on a daily basis in a scaled down version of the Wisconsin General Test Apparatus (WGTA). The animals were presented with a pair of junk stimuli covering two food wells at each trial. In order to learn the task, the animal had to place a value on the positive object, to retrieve a food reward. Testing was carried out to a criterion of 27 correct out of 30 successive trials before reversal learning was implemented. Reversal learning involved placing the positive value on the previously unrewarded object. The left and right position of the positive rewarded object was determined by a pseudorandom schedule (Gellerman, 1933). The inter-trial interval was approximately 14s with each trial lasting until the marmoset made a response Animals were orally dosed with either vehicle or drug 30 min prior to testing. Renzapride was dissolved in water whilst its enantiomers were dissolved in 0.03% w/v tartaric acid in water. All animals received all treatments in a random manner and thus acted as their own controls. Different pairs of junk objects were used for each comparison in a random manner.

Renzapride at 0.06 mg/kg gave a significantly positive effect on learning in both the initial and reversal stages of the task ($p=0.0008$). The mean number (± s.e.m.) of trials taken to reach criterion was reduced from 97.7 ± 15.4 after vehicle to 31.3 ± 9.2 after renzapride in the initial stage. In the reversal stage, the mean reduction in the number of trials was from 69.1 ± 13.2 under control conditions to 33.1 ± 10.9 with renzapride. BRL 44278 at 0.06mg/kg had a significant effect ($p=0.024$) on the initial stage of the task only, reducing the mean number of trials from 193.3 ± 21.0 under control conditions to 60.0 ± 11.2 with BRL 44278. BRL 44277 had no effect on learning and memory. This difference in activity between the two enantiomers has not been reported in any other pharmacological tests and further study is needed to determine the reasons for this difference. However, this study does show that a drug such as renzapride could be useful in the treatment of learning and memory disorders.

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161P INHIBITION BY GALANIN OF 5-HT$_2$ RECEPTOR-MEDIATED PI HYDROLYSIS AND HEAD TWITCH BEHAVIOUR

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Previous stusies have demonstrated that the neuropeptide galanin can block the head twitch behaviour induced by the 5HT$_2$ receptor agonist (±)2,5-dimethoxy-4-bromoamphetamine (DOB) in rat (Ogren and Fuxe, 1989). The 5HT$_2$ receptor is known to be coupled to the phosphatidylinositol (PI) cycle, thus in the present study we have examined the interaction between galanin and 5HT$_2$ receptor agonist induced PI turnover in the mouse.
Male BKTO mice (20-30g) were housed singly in observation boxes. Thirty minutes later mice were administered with either saline (4ml/kg s.c.) or the 5HT$_2$ receptor agonists mescaline (1-30mg/kg s.c.), DOB (0.3-30mg/kg s.c.) or its iodinated form DOI (0.3-30mg/kg s.c.) and the number of head twitches counted in 10min. Consistent with Ogren and Fuxe (1989), DOB dose dependently stimulated head twitch frequency in the mouse (EC50 = 5mg/kg s.c.). Mescaline and DOI (EC50 = 10mg/kg, 10mg/kg s.c. respectively) also dose dependently stimulated head twitch frequency. Pretreating animals with ketanserin (0.01-1mg/kg s.c.) for 30min blocked head twitch behaviour to mescaline (minimum effective dose, M.E.D. = 0.1mg/kg s.c., n = 5) and DOB (M.E.D. = 0.03mg/kg s.c., n = 5), and attenuated that to DOI (M.E.D. = 0.3mg/kg s.c., n = 5). Galanin, administered under metofane anaesthesia into the cerebral ventricle, 5min prior to the 5HT$_2$ receptor agonist, dose dependently blocked the head twitches induced by 30mg/kg mescaline (M.E.D. = 1nmol) and 3mg/kg DOB (M.E.D. = 1nmol, n = 5). The galanin receptor antagonist galantide ((3nmol/5μl) administered under metofane anaesthesia immediately prior to galanin and into the cisterna magna blocked the inhibition by galanin of mescaline and DOB induced behaviour, but did not affect the inhibition by galanin of the response to DOI.

To investigate the effects of galanin on PI turnover, tissue slices of mouse cortex were preincubated (30min) with 2μCi [^{3}H]myo-2-inositol and 10mM lithium. They were subsequently incubated in the presence of 5HT$_2$ receptor agonists for 45min before terminating the incubation using a chloroform:methanol (1:2) mixture to allow brain lipid and water soluble inositol monophosphate extraction. These monophosphates were then isolated by ion-exchange chromotography (Brown *et al*, 1884). 5HT (0.01-1000μM) concentration dependently stimulated PI hydrolysis in mouse cortex (60% over basal at concentrations ≥ 10μM 5HT). DOB, DOI and mescaline stimulated PI hydrolysis to 150±28% (n=4) and 130±22, (n=4) and 54±13% (n=4) respectively over that produced by 10μM 5HT.
Preincubationwith the 5HT$_2$ receptor antagonist ketanserin (0.1-1000μM) concentration dependently blocked the stimulation to 1μM DOB and mescaline, and attenuated the response to 1μM DOI (6.2±6%, 5.4±6% and 42±22% , n=3 respectively over 10μM 5HT response at 10μM antagonist). Preincubation with galanin (0.1-10μM) at doses which had no effect on PI hydrolysis alone, blocked the response to 1μM DOB and mescaline, and attenuated the response to 1μM DOI (25±9%, 8±7% and 82±12%, n=3 respectively of 10μM 5HT response at 10μM galanin).

These results confirm and extend the previous findings and show that galanin may block 5HT receptor agonist mediated behaviour by inhibiting 5HT2 agonist induced PI hydrolysis.

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162P MUTUAL INTERACTION BETWEEN DEXTROMETHORPHAN AND PAROXETINE IN RAT BRAIN

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Dextromethorphan (DM) is a widely used non-opioid antitussive agent which is present in many "over the counter" cough remedies. It also exhibits anticonvulsant and neuroprotective properties (Prince *et al.*, 1988; Löscher *et al.*, 1993). Its mechanism of action has yet to be fully elucidated, but high affinity binding sites for this substance have been demonstrated in guinea-pig and rat brain (Craviso *et al.*, 1983). A major component of DM binding, in most areas of rat brain, is sodium dependent (Meoni *et al.*, 1995). Since the distribution of this sodium-dependent binding resembles that of 5-HT uptake inhibitors we have looked for a possible identity between DM and paroxetine (PX), the selective 5HT uptake inhibitor (Caley *et al.*, 1993), using receptor autoradiography.

Brain sections (10μm), obtained from Wistar male rats (250g), were cut at -16°C and thaw-mounted on to gelatin coated glass slides. ^{3}H-DM (10nM, 81Ci/mmol) binding was performed by incubation (60 min, 4°C) in tris HCl buffer (50mM, pH 7.4) containing NaCl 120mM. Sections were subsequently washed for 2.5 min in buffer containing 0.1M choline chloride and 0.1% triton X-100. Sections for PX binding were incubated for 90 min at 4°C with ^{3}H-PX (1nM, 15 Ci/mmol) in tris HCl (50mM, pH 7.4) containing NaCl 300mM. Sections were subsequently washed for 30 min in incubation buffer, dried and apposed to Hyperfilm (Amersham) for 3 weeks. Non-specific binding was determined in the presence of DM (100μM) or fluoxetine (100μM).

Binding of DM in the presence of sodium was high (>100 fmoles/mg protein) in the medial mammillary nucleus (MM), dorsal raphe (DR), ventral tegmental area (VT), medial pretectal nucleus (MPT), superior culliculi (SC) and dorsal tegmental area (DTG). Medium levels of binding (50-100 fmoles/mg of protein) were detected in the anterior hypothalamus (AH) and frontal cortex (FR), and low levels (0-50 fmoles/mg protein) in the CA3 field of the hippocampus (CA3), dentate gyrus (DG), medio dorsal thalamus (MD), pons (P), occipital cortex (OC) and cerebellum (CER). The distribution of PX followed a similar pattern, with high levels of binding (>80 fmoles/mg protein) in MM and VTA, medium levels (40-80 fmoles/mg protein) in DR, MPT, SC, DTG, and AH, and low levels in CG, P, FR, MD, CA3, DG, OC and CER. There was significant correlation between the two distributions ($r=0.85$, $p<0.001$). Displacement studies showed a significant ($p<0.05$, ANOVA) concentration-dependent inhibition of ^{3}H-DM binding in all areas by PX (>10nM). In the presence of 100nM of PX, ^{3}H-DM binding was reduced to 30% of control. In comparison, PX binding to MM, SC, CG, and P was dose-dependently reduced by DM ($p<0.05$, ANOVA). DM reduced PX binding to these nuclei by 30% at 40nM.

These results show that DM in the presence of sodium binds to a recognition site that is common with PX. Since PX is a highly selective inhibitor of 5-HT uptake with little or no affinity for other recognition sites (Habert *et al.*, 1985), these results suggest that DM in the presence of sodium may bind to the 5-HT transport system. It might be predicted therefore that DM could possibly share some of the therapeutic properties of 5-HT uptake inhibitors.

Paroxetine was a gift from SB Pharmaceuticals.

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163P A COMPARISON OF THE CHOLINESTERASE INHIBITORS TACRINE AND E-2020

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The present study compared the in vitro and in vivo pharmacology of the cholinesterase inhibitors tacrine and E2020 (Rogers *et al.*, 1991), in order to identify differences of potential clinical relevance.

Analysis of the mechanism of inhibition of purified acetylcholinesterase (Type VI from electric eel), revealed that both tacrine and E2020 show "mixed type" inhibition, with both competitive and non-competitive components. The inhibition shown by tacrine and E2020 was mutually exclusive, suggesting that both compounds act on the same site. E2020 (IC_{50} = 33 nM) was a more potent inhibitor of rat brain AChE than tacrine (IC_{50} = 125 nM), and showed less activity at BuChE purified from horse serum (IC_{50} = 988 and 7.2 nM, respectively).

Both tacrine and E2020 bound to nicotinic receptors, (IC_{50} for displacement of [^{3}H] nicotine binding (8 nM) = 50 µM and 75 µM respectively). Both compounds showed muscarinic activity, displacing [^{3}H] pirenzipine binding (1 nM) (IC_{50} tacrine = 0.7 µM and E2020 = 0.5 µM). Rat whole brain was used for binding studies. Both tacrine and E2020 inhibited carbachol-induced calcium influx into SHSY5Y cells, (IC_{50} = 15 µM and 4 µM, respectively), suggesting muscarinic antagonism. E2020 was more potent than tacrine in inhibiting 5-HT uptake into synaptosomes from rat cortex (IC_{50} 1.8 µM and 17 µM respectively. IC_{50} imipramine = 0.12 µM).

Tremor and salivation, in vivo measures of central and peripheral cholinergic activation were also examined in male Lister Hooded rats (weight 250 - 350 g.) (Hunter et al., 1989). After i.p. administration (20 min. prior to test, n = 6 per dose), both compounds elicited dose dependent effects. E2020 was more potent in eliciting both tremor and salivation (Table 1). However, following equipotent tremorigenic doses of the two drugs, effects of tacrine, but not E2020 were apparent at eight hours.

In conclusion E2020 is both a more potent inhibitor of acetylcholinesterase than tacrine, both in vitro and in vivo, but with a shorter duration of action.

Table 1: Effects of tacrine and E2020 in inducing tremor and salivation.

	Tacrine	E2020
ED_{50} tremor µmol/kg I.P.	15	6
ED_{50} salivation µmol/kg I.P.	47	18

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164P LOSIGAMONE REDUCES GLUTAMATE AND ASPARTATE RELEASE FROM MOUSE CORTEX

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Losigamone (AO-33) is a new anticonvulsant undergoing clinical trials in patients with partial seizures and is effective against maximal electro-shock, pentylenetetrazol, bicuculline and 4-aminopyridine seizures in rats (Stein, 1995). This study looked at the effect of losigamone on potassium- and veratridine-stimulated release of amino acids from mouse cortical slices.

Coronal slices (400 µm) were prepared from the cortex of adult BALB/c mice (25-30g) and perfused with gassed (95% O_2 / 5% CO_2) artificial cerebrospinal fluid at 37°C. Two-minute aliquots of perfusate were collected and assayed for amino acids by HPLC with fluorometric detection. Transmitter release was elicited with two two-minute pulses of potassium (60 mM) or two one-minute pulses of veratridine (20µM) at samples 4 and 14. Losigamone was perfused between samples 7 and 17. The results are presented as the mean stimulated release per two-minute sample as a percentage of basal release.

The basal release of glutamate was between 2-5 pmol mg^{-1} 2 min^{-1}. Potassium (60 mM) and veratridine (20 µM) both produced significant increases in the release of glutamate (800% and 700% respectively). The second pulse of potassium resulted in an average glutamate release of 88±6% (n=7) of the first pulse, while the second pulse of veratridine resulted in an average glutamate release of 68±6% (n=9) of the first pulse. Potassium-stimulated release of glutamate was inhibited significantly by losigamone at 200 µM (44.8±5.9%, P <0.01, n=4), whilst veratridine-stimulated release was inhibited significantly by 100 µM (36.5±6.6%, P <0.01, n=6) and 200 µM (24.1±7.2%, P <0.01, n=4). The basal release of aspartate was 1.5-4.5 pmol mg^{-1} 2 min^{-1}. Potassium (60 mM) and veratridine (20 µM) both increased the release of aspartate significantly (400% and 300% respectively). The second pulse of potassium resulted in an average aspartate release of 100±14.4% (n=7) of the first pulse, while the second pulse of veratridine resulted in an average aspartate release of 60±5%, (n=9) of the first pulse. Potassium- and veratridine-stimulated release of aspartate was inhibited significantly by 200 µM losigamone (28.8±4.2%, P <0.01, n=4) and (33±2.9%, P <0.01, n=4) respectively. Losigamone, in concentrations up to 200 µM, had no effect on the stimulated release of glycine, serine, taurine or GABA.

The mechanism of action of losigamone is unknown. It has been shown to reduce epileptiform activity induced by picrotoxin, low Ca^{2+} or Mg^{2+} in hippocampal slices; and potentiate the effect of GABA and increase chloride uptake in cultured neurons (Stein, 1995). However, it does not bind to any site on the $GABA_A$ receptor. The reduction of glutamate and aspartate release shown here could be contributing to the anticonvulsant action of losigamone.

Acknowledgement We should like to thank Dr. S.S.Chatterjee of Willmar Schwabe Arzneimittel, for the gift of losigamone.

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165P METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) GROUP 1 AGONISTS INDUCE CATALEPSY IN MICE

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The behavioural effects of the mGluRs agonists (S)-3-Hydroxyphenylglycine ((S)-3-HPG) and (S)-3,5-Dihydroxyphenylglycine ((S)-DHPG) were evaluated following intracerebroventricular (i.c.v.) administration to mice. These compounds have been shown to have some selectivity for mGluRs Group I with respect to Groups II and III (Pin & Duvoisin, 1995). The ability of the mGluR antagonist α-Methyl-4-carboxyphenylglycine ((+)-MCPG) to inhibit the observed catalepsy was also studied.

Male MORO mice (25g) were used for all experiments which were performed on a 'blind' basis. Doses were expressed in nmol/2µl/mouse, i.c.v. for each compound. Behavioural observation was done (Irwin, 1968) with the mice (N = 6/group) placed in clear perpex cages (40 x 20 x 15 cm). The intensity of the catalepsy induced by the agonists was measured in 2 tests (N = 8/group). Bar test: the forepaws of the mice were placed on a horizontal metal bar at a height of 3 cm. Grid test: mice were placed on a stainless steel grid which was inclined at 60° to the horizontal. The time taken to step down from the bar or to move any paw from the grid was recorded (cut-off time = 4 min). Catalepsy was measured 30 min after treatment with (S)-3-HPG (3-300 nmol) and (S)-DHPG (1-30 nmol).

In antagonism studies, (+)-MCPG (30-300 nmol) was given 10 min prior to injection of the agonists.
Catalepsy was the major common behaviour observed after i.c.v. injections of the agonists. (S)-3-HPG and (S)-DHPG induced catalepsy in both tests in a dose-dependent manner (bar test (ED_{50}) = 35.30 and 4.41 nmol, respectively; grid test (ED_{50}) = 42.06 and 7.57 nmol, respectively). (S)-3-HPG at its maximally effective dose of 100 nmol induced catalepsy for up to 1h whilst an equipotent dose of (S)-DHPG (30 nmol) induced catalepsy for more than 2h. (+)-MCPG inhibited (S)-HPG (100 nmol)- and (S)-DHPG (10 nmol)-induced catalepsy (bar test (IC_{50}) = 52.44 and 61.26 nmol, respectively; grid test (IC_{50}) = 47.90 and 50.28 nmol, respectively).

These results indicate that the mGluRs agonists (S)-3-HPG and (S)-DHPG induce catalepsy in mice. This catalepsy could be reversed by (+)-MCPG. It has been suggested that they have selectivity for Group I receptors and therefore, it could be tentatively proposed that this response is mediated by these Group I receptors. The antagonism of the catalepsy by (+)-MCPG, an antagonist at Group I and II receptors, would support this hypothesis.

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166P NEUROPROTECTIVE PROPERTIES OF THE NMDA RECEPTOR OPEN CHANNEL BLOCKER Ro 24-6173 IN RATS

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The aryl-morphinan derivative Ro 24-6173, ((+)-17-methyl-3-[6-methyl-2-(pyridinyl)oxy]morphinan), is a high affinity and use-dependent NMDA receptor open channel blocker. Here we show that Ro 24-6173 is a potent anticonvulsant in mice and has neuroprotective properties in the middle cerebral artey occlusion (MCAO) model in rat.

Seizures were induced by intracerebroventricular administration of NMDA (0.15µg/2µl), electroshock (0.5 mA, 0.2s) in male MORO mice (20-22g) and by sound (110db for 60s) in male DBA/2J mice (21 days old). Animals not exhibiting tonic seizures following application of electroshock or sound and tonic and clonic seizures after NMDA administration were considered protected. Ro 24-6173 was administered i.p. 30 min before the tests. Groups of 8 mice were used per dose. The dose protecting 50% of the mice (ED_{50}) was calculated by probit analysis. All experiments were done on a blind basis. MCAO was performed in young adult male Fischer 344 rats (215 to 240 g with n=12 per dosing group) by diathermy under isoflurane anaesthesia which lasted until 5 to 10 min after MCAO. The rectal temperature was monitored for 8 hours and kept within physiological limits by heating if necessary. The cerebrocortical and neostriatal infarct volumes, 48h after MCAO, were measured in serial paraffin sections stained with toluidine blue.

Ro 24-6173 was active against seizures induced by sound (ED_{50}=5.48 mg/kg). It also dose-dependently antagonised NMDA-induced seizures (ED_{50}=14.82 mg/kg) and electroshock-induced convulsions (ED_{50}=3.21 mg/kg). In MCAO, Ro 24-6173 showed consistent dose-dependent protection and 51 ± 4.7 % (mean ± SEM) reduction of the cortical infarct volume using 2.7 mg/kg i.v. bolus (given from 5 to 7 min after occlusion) and 2 mg/kg/h maintenance infusion for 6 h. About half maximal protection with a reduction of the cortical infarct volume by 25 ± 6 % was obtained with 0.9 mg/kg bolus and 0.7 mg/kg/h maintenance infusion. This treatment produced only slight effects on motor behaviour (mild ataxia) and neither cardiovascular effects nor neuronal vacuoles in the cingulate-retrosplenial cortex were seen.

Therefore, Ro 24-6173 shows anticonvulsant and neuroprotective properties and presents a promising profile as a potential treatment for ischemic brain injury in man.

167P EFFECT OF VARIOUS MONOAMINE RECEPTOR ANTAGONISTS ON THE DECREASE IN FOOD INTAKE INDUCED BY SIBUTRAMINE IN THE RAT

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Sibutramine is a novel 5-hydroxytryptamine (5-HT) and noradrenaline (NA) reuptake inhibitor (Buckett *et al.*, 1988) which decreases food intake in rats (Fantino *et al.*, 1994). This study investigates the effects of various monoamine receptor antagonists on sibutramine-induced hypophagia.

Individually-housed male Long-Evans rats (260±4g; n=16) were maintained on a 12 h light-dark cycle (lights off at 17.00 h) with free access to tap water and a paste made from standard rat diet (see Stricker-Krongrad *et al.*, 1994). Animals were given sibutramine (3 mg/kg p.o.) 15 min before the onset of the dark period. Antagonists were injected i.p. 30 min later and food intake was monitored over the following 12 h dark period.

The decrease in food intake induced by sibutramine was significantly attenuated by the β1-adrenoceptor antagonist, metoprolol, and a low dose of the 5-HT2A/2C receptor antagonist, ritanserin, but not inhibited by the β2-adrenoceptor antagonist, ICI 118,551 (1-[2,3-dihydro-(7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol HCl), or the dopamine D2-receptor antagonist, remoxipride (Table 1). Metoprolol, ICI 118,551, ritanserin and remoxipride had no effect on food intake when given alone.

These results are consistent with the hypothesis that sibutramine reduces food intake by inhibiting 5-HT and NA reuptake and that the enhanced function of these neurotransmitters is mediated, at least in part, via activation of 5-HT2A/2C receptors and β1-adrenoceptors. In addition, the lack of effect of remoxipride on sibutramine-induced hypophagia provides further evidence that dopamine is not a pharmacological target of sibutramine (Heal *et al.*, 1992).

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Table 1. Effect of i.p. administration of metoprolol, ICI 118, 551, ritanserin and remoxipride on the hypophagia induced by sibutramine (3 mg/kg p.o.) in the rat

Antagonist	Dose	Vehicle	Sibutramine	Antagonist	Dose	Vehicle	Sibutramine
-	Vehicle	14.2 ± 0.6	7.8 ± 0.7 *	-	Vehicle	14.2 ± 0.6	7.8 ± 0.7 *
Metoprolol	2 mg/kg	10.9 ± 1.1	4.5 ± 0.8 *	Ritanserin	0.1 mg/kg	16.2 ± 0.7	11.8 ± 0.7 †
Metoprolol	10 mg/kg	12.3 ± 1.2	12.0 ± 0.4 †	Ritanserin	1 mg/kg	15.2 ± 0.8	4.3 ± 1.1 *
ICI 118,551	1 mg/kg	11.9 ± 0.9	9.1 ± 1.3 *	Remoxipride	1 mg/kg	12.3 ± 0.7	7.7 ± 1.0 *
ICI 118,551	5 mg/kg	13.6 ± 0.7	8.1 ± 1.4 *	Remoxipride	5 mg/kg	13.3 ±1.2	7.2 ± 1.1 *

Results are mean food intakes (g)±s.e.mean; n=16; *P<0.01 vs vehicle, †P<0.05 vs sibutramine; 2-way ANOVA; Student's paired t-test.

168P INVESTIGATION OF THE MECHANISMS UNDERLYING THE HYPOPHAGIC EFFECTS OF THE 5-HT AND NA REUPTAKE INHIBITOR SIBUTRAMINE IN THE RAT

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The 5-HT2A/2C receptor antagonist, ritanserin, and the β1-adrenoceptor antagonist, metoprolol, inhibit the hypophagic effects of the 5-HT and NA reuptake inhibitor, sibutramine, in rats (Stricker-Krongrad *et al.*, 1995). This study confirms these findings and extends them by exploring the effects of additional 5-HT receptor antagonists, and also of α1- and α2-adrenoceptor antagonists, on the decrease in food intake induced by sibutramine.

Individually-housed male Sprague-Dawley rats (350-500g; n=6-8) were maintained on reversed phase lighting (lights off 09.00-17.00h) with free access to powdered diet. Antagonists and sibutramine were injected at 09.00h and food intake was assessed over 8 h.

The decrease in food intake induced by sibutramine was significantly inhibited by the following antagonists: prazosin (α1); metoprolol (β1), low dose metergoline (5-HT), ritanserin (5-HT2A/2C) and SB200646 (N-(1-methyl-5-indolyl)-*N'*-(3-pyridyl urea; 5-HT2B/2C; Kennett *et al.*, 1994) as shown in Table 1. The α2-receptor antagonist RX821002 (2 - methoxy idazoxan) and the β2-antagonist ICI 118,551 (1-[2,3-dihydro-(7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol HCl) did not attenuate sibutramine-induced hypophagia (Veh 51.5±3.1, Sib 17.8±3.6*, Sib+RX821002 0.3 mg/kg 14.6±7.0*, Sib+RX821002 1 mg/kg 18.5±3.3*; Veh 46.5±7.4, Sib 10.0±2.5*, Sib+ICI 118,551 3 mg/kg 12.8±2.2*, Sib+ICI 118,551 10 mg/kg 14.5±3.1*; units as in Table 1). The antagonists had no effect on food intake when given alone.

These findings confirm that β1-adrenoceptors and 5-HT-receptors are involved in sibutramine-induced hypophagia and suggest that α1-adrenoceptors also contribute to this response. The data with ritanserin and SB200646 implicate 5-HT2C receptors in sibutramine's effects on food intake. However, activity at 5-HT2A (and possibly 5-HT2B) receptors cannot be excluded without studies with more selective antagonists. These results support the hypothesis that the hypophagic effects of sibutramine are due to its ability to inhibit both 5-HT and NA reuptake with the subsequent activation of a variety of 5-HT and NA receptor systems.

Kennett, G.A., Wood, M.D., Glen, A. *et al.* (1994) *Brit. J. Pharmacol.* 111, 797-802.
Stricker-Krongrad, A., Souquet, A.-M., Jackson, H.C. *et al.* (1995) This meeting.

Table 1 Effect of monoamine receptor antagonists on the decrease in food intake induced by sibutramine (10 mg/kg p.o.) in the rat

Treatment	Prazosin	Metergoline	Treatment	Metoprolol	Treatment	Ritanserin	Treatment	SB200646
Veh: Veh	39.0±2.6	46.6±1.5	Veh: Veh	38.1±3.8	Veh: Veh	50.3±4.2	Veh: Veh	34.0±3.4
Sib: Veh	7.3±1.8*	13.5±2.1*	Sib: Veh	2.8±0.4*	Sib: Veh	9.5±2.8*	Sib: Veh	0.9±0.3*
Sib: 0.3	24.4±2.9*†	25.0±3.3*†	Sib: 3	15.1±2.2*†	Sib: 0.1	22.5±3.0*†	Sib: 20	10.1±4.1*†
Sib: 1	34.4±2.7†	17.5±2.9*	Sib: 10	15.4±3.1*†	Sib: 0.5	19.5±2.6*†	Sib: 40	10.4±1.6*†
Veh	43.2±3.1	41.3±0.5	Veh	35.0±5.9	Veh: Veh	47.8±2.4	Veh	42.7±2.6
0.3	49.6±2.0	46.3±2.1	3	36.7±0.9	0.1	46.3±2.6	20	47.6±4.7
1	44.2±2.5	43.7±2.0	10	38.8±1.6	0.5	53.8±5.9	40	36.9±3.8

Results are mean food intakes (g/kg rat weight) ± s.e.mean; n=6-8; doses of antagonists are given in mg/kg; antagonists were given i.p. with the exception of SB200646 (p.o.).; *P<0.05 vs vehicle (veh), †P<0.05 vs sibutramine (sib); ANOVA and Dunnett's test.

169P d-AMPHETAMINE-CUED DRUG DISCRIMINATION IN RATS: PREDICTIVE VALUE FOR DETECTING STIMULANT DRUGS OF ABUSE

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Abuse potential has always been a major issue for prescribed drugs which treat obesity by reducing food intake; in fact, all drugs of this type are currently Controlled Substances in the UK and USA. The d-amphetamine-cued drug discrimination test using rats is an established model for assessing abuse potential (Barry, 1974). We have now determined its ability to predict the abuse potential of various antiobesity drugs by measuring the responses of rats to a range of weight-modifiers, stimulants and monoamine reuptake inhibitors, including sibutramine which is a noradrenaline/5-hydroxytryptamine reuptake inhibitor in development for treating obesity.

Female PVG rats (150 - 200 g) were trained using a sweetened milk reward to distinguish (≥75% correct lever choice) between d-amphetamine (0.5 mg/kg ip) and saline (1 ml/kg ip) in a 2-choice lever-pressing model. Test drugs were injected ip and 15 min later rats were allowed to lever-press for 2.5 min (test schedule, non-rewarded) followed by 7.5 min (reinforcement schedule, rewarded). Drug doses were increased until rats showed either an unequivocal generalisation to amphetamine or marked suppression of lever-pressing (≤mean total - 1 s.d. mean of a rats normal response assessed in 8 trials).

In order of potency, the following drugs generalised to d-amphetamine:- d-amphetamine > methamphetamine > methylphenidate = fencamfamine = mazindol = nomifensine > amfonelic acid = cocaine > bupropion (Table 1). Desipramine, venlafaxine and sibutramine generalised to saline (Table 1); higher doses suppressed lever-pressing.

This model detected as "amphetamine-like" dopamine releasing agents and combined dopamine releasers/reuptake inhibitors for which abuse potential is marked (d-amphetamine, methamphetamine, cocaine, methylphenidate) and moderate/low (mazindol, fencamfamine, amfonelic acid). The dopamine uptake inhibitors nomifensine and bupropion, which are not substances of abuse, also yielded positive results. This model, is therefore, an extremely sensitive test for detecting stimulant drugs of abuse. Moreover, the data also demonstrate that sibutramine is unlikely to have stimulant abuse potential.

Barry, H. (1974) *Fed Proc* 33, 1814-1824.

Table 1. Profiles of weight-modifiers, stimulants and monoamine reuptake inhibitors in the d-amphetamine-cued drug discrimination model

Drug		AMP	SAL	Drug		AMP	SAL
d-Amphetamine	(0.3)	6/6	0/6	Methylphenidate	(3)	6/6	0/6
Methamphetamine	(0.5)	6/6	0/6	Bupropion	(30)	5/7	0/7
Mazindol	(3)	4/5	0/5	Nomifensine	(3)	5/6	0/6
Amfonelic acid	(10)	4/4	0/4	Desipramine	(5)	0/8	5/8
Cocaine	(10)	6/9	1/9	Venlafaxine	(10)	0/5	5/5
Fencamfamine	(3)	5/5	0/5	Sibutramine	(3)	0/16	9/16

Drugs: doses mg/kg in brackets. Results are number of rats generalising/number tested (some animals failed to generalise to either AMP (amphetamine) or SAL (saline) or displayed suppressed lever-pressing).

170P ROLE OF β-ADRENOCEPTORS IN MEDIATING THE THERMOGENIC EFFECTS OF SIBUTRAMINE

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Sibutramine (BTS 54 524) is a potent in vivo inhibitor of noradrenaline and 5-hydroxytryptamine reuptake (Buckett *et al.*, 1988). Sibutramine has been previously shown to alter the energy balance of rats by reducing food intake (Halford *et al.*, 1995) and by stimulating thermogenesis (Connoley *et al.*, 1995). In rodents, thermoregulatory thermogenesis mainly results from sympathetic activation of β-adrenoceptors in brown adipose tissue (BAT), and the present study was performed to determine whether thermogenesis induced by sibutramine administration also results from β-adrenoceptor activation.

Oxygen consumption (VO_2) of 16 adult (200-250g) female Wistar rats was measured in closed circuit calorimeters at thermoneutral ambient temperature (29°C). Recordings were made for 1.5h (Basal) before dosing with sibutramine (10mg/kg po), and measurements continued for a further 4h (Test). Each rat received, in addition to sibutramine, vehicle, atenolol (1mg/kg sc), ICI 118551 (erytho-DL-1-(7-methylindan-4-yloxy)-3-(isopropylamino)butan-2-ol; 1mg/kg sc) or atenolol (20mg/kg sc) + ICI 118551 (20mg/kg sc). Every rat received each treatment in a balanced design with 1 week between each test.

As shown in Table 1, sibutramine alone enhanced VO_2 by 21% and this increase was abolished by combined treatment with high, non-selective doses of atenolol and ICI 118551. However, the lower selective doses of the antagonists, atenolol (β_1) and ICI 118551 (β_2) failed to decrease VO_2 in sibutramine-treated rats.

As sibutramine potently inhibits noradrenaline reuptake in vivo (Buckett *et al.*, 1988), but has no affinity (Ki>1000nM) for β_1-, β_2- or β_3-adrenoceptors (unpublished), the data argue that sibutramine stimulates thermogenesis by indirectly increasing the sympathetic activation of β-adrenoceptors. Furthermore, this activation is resistant to inhibition by low doses of atenolol or ICI 118551 which have previously been reported to selectively block β_1- and β_2-adrenoceptors (Carlisle & Stock, 1992), but susceptible to inhibition by a combination of high doses of these antagonists. Together, these findings suggest sibutramine-induced thermogenesis is mediated via an atypical β-adrenoceptor, probably the β_3-adrenoceptor on BAT.

Buckett, W.R., Thomas, P.C. & Luscombe, G.P. (1988) *Neuropsychopharmacol. Biol. Psychiat.* 12, 575-584.
Carlisle, H.J. & Stock M.J. (1992) *Am. J. Physiol.* 263, R915-R923.
Connoley, I.P., Heal, D.J. & Stock, M.J. (1995) *Br. J. Pharmacol.* 114, 388P.
Halford, J.C.G., Heal, D.J. & Blundell, J.E. (1995) *Br. J. Pharmacol.* 114, 387P.

Table 1. Effects of β-adrenoceptor antagonists on sibutramine-induced thermogenesis

	Basal VO_2	Test VO_2		Basal VO_2	Test VO_2
Sibutramine (10) + Vehicle	12.9±0.4[a]	15.4±0.4***[a]	Sibutramine (10) + Atenolol (20) + ICI 118551 (20)	12.6±0.4[a]	13.1±0.5[b]
Sibutramine (10) + Atenolol (1)	13.6±0.6[a]	14.8±0.6***[a]			
Sibutramine (10) + ICI 118551(1)	13.1±0.4[a]	14.7±0.5***[a]			

VO_2 (ml/kg$^{0.75}$/min). Values ± s.e.mean; n=16. **P<0.01, ***P<0.001 vs respective basal value (paired t-test). Values in columns sharing same superscript letter are not significantly different (P>0.05) than Sibutramine VO_2 (ANOVA for repeat measures + Dunnett's test).

171P BEHAVIOURAL EVALUATION OF SX-3228, A POTENT ANTICONVULSANT

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Previous studies suggest that the non-benzodiazepine 3-(1,3,4-oxadiazol-2-yl)-1-6-naphthyridin-2(1H)-one derivative, SX-3228, has high affinity for benzodiazepine (BZ) receptors (IC_{50}: 9.79 nM) and in addition it binds preferentially to BZ1 receptors (BZ1: IC_{50} 17.0 nM, BZ2: IC_{50} 127 nM; Ohno *et al.*, 1994). In the present studies SX-3228 was compared to the BZ1 selective receptor agonist, zolpidem (BZ1: IC_{50} 26.7 nM, BZ2: IC_{50}156.0 nM) and the non-selective BZ receptor agonist, diazepam (BZ1: IC_{50} 14.0 nM, BZ2: IC_{50} 19.6 nM) using several behavioral tests. In a model of epilepsy, male Swiss Webster mice (20-25g) were pre-treated with the test compounds (i.p.) thirty min prior to injection of pentylenetetrazole (PTZ, 120 mg/kg, s.c.) and observed for tonic seizures for 30 min. SX-3228 was 10 fold more potent in blocking PTZ-induced tonic seizures (ED_{50}=0.086 mg/kg) than diazepam (ED_{50}=0.48 mg/kg) and was 100 fold more potent than zolpidem (ED_{50}=4.86 mg/kg). In an operant procedure, male PVG rats (240-340 g) were trained to pull a chain for food reward which was delivered on a random interval 30s schedule. On test days, rats were given either drug or vehicle (i.p.) 30 min (SX-3228) or immediately prior to testing and a reduction in chain-pulling rates used as an index of motor disruption. In this test zolpidem reduced chain-pulling rates with a minimum effective dose (MED) of 3.0 mg/kg (mean % baseline chain-pulls± s.e. mean=33.6 ±9.9) which was significantly lower ($p<0.05$) than vehicle treated rats (mean % baseline chain-pulls ±s.e. mean=74.6 ±8.9). Intermediate doses of 0.3 mg/kg and 1.0 mg/kg of zolpidem did not significantly reduce chain-pulling rates (mean % baseline chain-pulls; 77.4 ±7.4 and 47.6 ±12.4 respectively). In the same test, SX-3228 and diazepam also reduced chain-pulling rates and, like zolpidem, were found to have an MED of 3.0 mg/kg (see table 1). In the rat conditioned suppression of drinking (CSD) test of anxiety, thirsty male PVG rats (240-340 g) that were trained to lick a spout for water rewards, were given conditioning sessions in which the presentation of a light signalled the delivery of a mild electric foot shock (0.4 mA, 0.5s). On the test days, SX-3228 and diazepam were given 30 min (i.p.) before testing. Mean pre- and during-light (dur) lick rates were used to calculate a suppression ratio, (SR=dur/pre+dur), which was used as an index of conditioned fear. SX-3228 induced an anxiolytic-like effect and had a minimum effective dose of 3.0 mg/kg whereas the MED for diazepam was 10.0 mg/kg (see table 1). These data suggest that in animal models of motor disruption SX-3228, zolpidem and diazepam have equipotent effects and that in models of anxiety, SX-3228 and diazepam have similar potencies. However, SX-3228 is 10-fold more potent than diazepam and 100-fold more potent than zolpidem against PTZ seizures. SX-3228 was fully anticonvulsant when given to male Swiss Webster mice (20-25 g) at a dose of 0.3 mg/kg (i.p.), 30 min before PTZ (8/8 mice protected). This effect was attenuated by administration of the BZ receptor antagonist, flumazenil, at a dose of 40.0 mg/kg (i.p.) given 15 min after SX-3228 (2/8 mice protected). Therefore, it is unlikely that the greater potency of SX-3228 in the anticonvulsant assay is due its effects at a receptor other than the BZ receptor.

Compound	Dose (mg/kg)	% baseline chain-pulls	pre	dur	SR
SX-3228	Vehicle	57.2±8.6	92.9±15.9	23.1±7.4	0.13
	0.3	77.1±8.7	nt	nt	nt
	1.0	63.9±9.7	88.4±17.9	21.3±6.7	0.17
	3.0	22.5±9.5*	76.3±10.5	45.8±7.5	0.38*
	10.0	12.3±4.6*	119.4±42.5	87.3±42.9	0.36*
	30.0	nt	149.4±46.6	135.1±49.2	0.49*
Diazepam	Vehicle	77.2±4.6	77.0±9.4	7.6±3.7	0.07
	0.1	78.5±4.7	nt	nt	nt
	0.3	70.3±6.9	nt	nt	nt
	1.0	62.4±7.1	72.9±13.6	16.1±8.3	0.15
	3.0	9.1±2.4*	104.8±8.8	25.2±7.3	0.19
	10.0	nt	62.2±13.8	30.0±7.9	0.30*

Table 1. Effects of BZ receptor agonists on the chain-pulling test and CSD test. *P<0.05 ANOVA followed by Newman-Keuls test compared with vehicle control. nt= not tested.

Ohno, K., Odai, O., Tominaga, Y. *et al.* (1994) *The XIII International Symposium on Medicinal Chemistry*, 19-23rd September, Paris

172P THE GLYCINE/NMDA RECEPTOR ANTAGONIST, L-701,324, REVERSES ISOLATION REARING-INDUCED HYPERLOCOMOTION IN THE RAT

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We have previously reported that the glycine/NMDA receptor antagonist, L-701,324 (7-chloro-4-hydroxy-3(3-phenox)phenyl-2(*H*)quinolone), reverses the deficits in prepulse inhibition (PPI) induced following isolation rearing in the rat (Bristow et al., 1995). In addition to impaired PPI, however, numerous studies have also reported increased locomotor activity in isolation reared animals, a response normalised by tricyclic antidepressants (Garzon et al., 1979). Thus the present study has determined whether L-701,324 will also attenuate isolation-induced hyperactivity in the rat.

Male hooded Lister rats (21-22 days old at delivery) were housed either singly or in groups of five and maintained on a 12 h light:dark cycle (lights on 0700 h) in the same temperature and humidity controlled holding room for 40 weeks prior to testing. On test days, rats were intraperitoneally injected with either L-701,324 (0.1 - 3 mg/kg), vehicle (1 ml/kg; 25% polyethylene glycol (molecular weight 300) in water, pH 10) or desipramine (DMI; 5 mg/kg) and placed in individual photocell activity cages 30 min (L-701,324) or 60 min (DMI) later. Each box was equipped with 5 infrared beams, 2 of which were positioned at each end of the base of the cage to record cage crossings i.e consecutive beam breaks which were monitored in 10 min intervals for a total of 30 min.

Vehicle-pretreated, socially isolated rats showed a significant increase in the number of cage crosses recorded in the first 10 min period after being placed in the novel environment of a photocell activity cage (Table 1). Thereafter, locomotor activity was comparable in both group housed and isolation reared animals (mean no. cage crosses ± s.e. mean; 10-20 mins:- group housed = 6.0 ± 1.1, isolates = 7.7 ± 1.9; 20-30 min:- group housed = 3.6 ± 0.8, isolates = 3.5 ± 0.9). Consistent with previous studies, pretreatment with DMI (5 mg/kg, i.p.) significantly attenuated the increased locomotor response observed in isolation-reared rats (Table 1). In addition, pretreatment with L-701,324 also dose-dependently and significantly attenuated isolation-induced hyperactivity at doses not affecting spontaneous locomotor activity in group-housed animals (Table 1).

Table 1 Effect of L-701,324 on isolation-induced hyperactivity

Treatment		Cage Crossings (0 - 10 min) Group housed	Isolates
L-701,324 (mg/kg)			
	0	11.5 ± 1.9	19.2 ± 1.8†
	0.1	11.0 ± 1.5	16.3 ± 1.9
	0.3	13.4 ± 1.8	11.8 ± 1.9*
	1	11.3 ± 1.5	7.5 ± 0.9*
	3	9.3 ± 1.2	7.7 ± 1.3*
DMI	5	7.1 ± 1.3	10.5 ± 1.4*

Results are expressed as the mean number of cage crossings ± s.e. mean (n=12 rats/group) and data were analysed by analysis of variance followed by Students t test; †$P < 0.05$ compared to group housed rats, *$P < 0.05$ compared to isolated rats.

In conclusion, the present results are consistent with our previous studies showing that L-701,324 can reverse the behavioural abnormalites induced in rats reared in social isolation following weaning.

Bristow, L.J. et al. (1995). *Psychopharmacol.*, **118**, 230-232.
Garzon, J. et al. (1979). *Eur. J. Pharmacol.*, **59**, 293-296

173P THE GLYCINE/NMDA RECEPTOR ANTAGONIST, L-701,324, ATTENUATES THE DEFICIT IN PREPULSE INHIBITION OF ACOUSTIC STARTLE RESPONDING INDUCED BY AMPHETAMINE IN THE RAT

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Prepulse inhibition (PPI) of the acoustic startle response is attenuated in rats treated with amphetamine (Mansbach et al., 1988), an effect thought to result from increased dopaminergic transmission in the nucleus accumbens (Swerdlow et al., 1992). Given that the glycine/NMDA receptor antagonist, L-701,324 (7-chloro-4-hydroxy-3(3-phenoxy)phenyl-2(*H*)quinolone) blocks amphetamine-induced hyperlocomotion (Bristow et al., 1994), we have examined whether L-701,324 will also attenuate amphetamine-induced disruption of PPI in the rat.

Startle responses were recorded in male Wistar rats (320 - 450 g) using 6 SR-LAB (San Diego Instruments) stabilimeter chambers. Animals were pretreated with either L-701,324 (1 or 3 mg/kg, i.p.) or vehicle (25% Peg 300; 1 ml/kg) 20 min prior to injection of amphetamine (4 mg/kg, s.c.) or saline (1 ml/kg) and placed in individual startle chambers 20 min later. Following a 5 min acclimation period to background noise (65 dB), startle responses were recorded to 12 repetitions of 7 different stimuli:- (i) a 40 ms 120 dB pulse-alone, (ii) - (iv) a 40 ms 120 dB pulse preceeded 100 ms before by either a 5 ms 70 dB, 75 dB or 80 dB prepulse, (v) - (vi) a 5 ms 75 dB or 80 dB prepulse-alone and (vii) a 100 ms period when no stimulus was presented. Stimuli were presented in a random order for each subject (n = 12 rats/group) and PPI calculated as the percentage reduction in startle responding i.e. 100 x [(pulse - (prepulse+pulse))/pulse].

Amphetamine injection significantly attenuated PPI in the rat (Table 1), an effect resulting from an increase in startle amplitude to the prepulse + pulse stimuli (mean startle amplitude ± s.e. mean; veh/veh = 546 ± 57, 349 ± 43 and 265 ± 38; veh/amph = 672 ± 98, 633 ± 114* and 538 ± 96* at 70, 75 and 80 dB prepulses respectively) in the absence of any effect on startle responses to the 120 dB pulse-alone. Pretreatment with 1 mg/kg L-701,324 significantly attenuated the deficit in PPI induced by amphetamine (Table 1). This resulted from a marked reduction in the increased amplitude to the prepulse + pulse stimuli seen in amphetamine-treated rats, to values not significantly different from vehicle-treated controls (mean startle amplitude ± s.e. mean for L-701,324/amph treated rats = 547 ± 68, 413 ± 53 and 310 ± 44 at 70, 75 and 80 dB prepulses). In contrast, a higher dose of L-701,324 significantly impaired startle responding to the pulse-alone and failed to significantly reverse the deficit induced by amphetamine.

Table 1 Effect of L-701,324 on amphetamine disruption of PPI

Dose	% PPI			Pulse
(mg/kg, s.c.)	70 dB	75 dB	80 dB	120 dB
Veh/veh	10 ± 5*	42 ± 5*	54 ± 6*	615 ± 67
Veh/amph	-11 ± 5	-1 ± 8	13 ± 9	615 ± 81
1 mg/kg 324/amph	-3 ± 4	21 ± 6*	39 ± 6*	528 ± 60
3 mg/kg 324/amph	-3 ± 3	14 ± 5	25 ± 6	336 ± 45*

Results are expressed as the mean ± s.e. mean % PPI or startle amplitude and analysed by analysis of variance followed by Dunnetts t test, *P<0.05 compared to Veh/amph

Whilst the effects of higher doses are compromised by impaired startle responding to the pulse-alone, the present study shows that 1 mg/kg L-701,324 reduced the disruption in PPI induced by amphetamine in the rat. This observation is consistent with previous studies suggesting that L-701,324 can attenuate activation of mesocorticolimbic dopamine systems in the rat.

Bristow, L.J. et al. (1994). *Br. J. Pharmacol.*, **114**, 321P.
Mansbach, R.S. et al. (1988). *Psychopharmacol.*, **94**, 507-514.
Swerdlow, N.R. et al. (1992). *Psychopharmacol.*, **108**, 189-195.

174P THE TRH ANALOGUE, RX77368, IMPROVES A WORKING MEMORY DEFICIT IN AMPA-INDUCED SEPTAL-HIPPOCAMPAL LESIONED RATS

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We have previously shown that the thyrotrophin-releasing hormone (TRH) analogue, RX77368 (RX), improved performance in an operant delayed non-matching to position test in AMPA-induced septal-hippocampal lesioned rats, on test days when a significant impairment was present (Ballard *et al.* 1995). The aim of the present study was to determine the effect of this analogue in lesioned rats using different protocols in the Morris water maze.

Prior to surgery, male Lister Hooded rats (234±1.0g; Charles River, UK) were divided into 4 treatment groups (n=12 each) based on latency to find a visible platform (cue trial) and average swim speed (120s swim trial): sham-saline; AMPA-saline; sham-RX; AMPA-RX. AMPA ((RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; 1.8µl of 9mM) or vehicle was infused into the medial septum/diagonal band area, under halothane (1.5%) anaesthesia and was followed by a recovery period of 10-14 days. RX (1.0mgkg^{-1}, i.p.) or saline was administered 30min prior to trials, each day of testing in the water maze. The experimental protocol was as follows: (i) 2 swim trials; (ii) cue trial; (iii) acquisition of fixed platform position (2 trials/day, 11 days) and probe trial; (iv) reversal learning (2 trials/day, 8 days) and probe trial; (v) working memory test (2 trials/day, inter-trial interval=30s, different platform position on each of 4 days). Maximum time for all trials was 120s, except for probe trial: 60s. A computer tracking system was used to trace each rat's swim path and to measure latency, distance travelled and speed to find the platform. Following behavioural testing, lesion placement was assessed using histological (cresyl violet staining) and biochemical (hippocampal choline acetyltransferase activity) techniques.

Statistical analysis, using an ANOVA with repeated measures followed by post hoc Newman-Keuls test showed no difference between the 4 groups across all trials during acquisition (acq) and reversal (rev) learning. However, during the acquisition and reversal probe trials, lesioned rats (acq: 8.6±1.2s; rev: 8.6±1.2s) spent significantly (acq: $p<0.01$; rev: $p<0.05$) less time in the platform area than sham rats (acq: 16.0±2.0s; rev: 13.6±1.9s). These results show that the rate of learning a platform position is unaffected in lesioned rats, yet the probe trial reveals impaired localisation in this group, which is not improved by RX treatment.

In the working memory test, sham rats showed significantly reduced ($p<0.001$, 1-factor ANOVA) latency to find the platform in trial 2 (t2) compared to trial 1 (t1) (saline-treated, t1: 60.5±5.6s, t2: 27.7±3.4s; RX-treated, t1: 60.7±5.4s, t2: 18.7±1.7s). In contrast, lesioned rats showed no difference in latency between the trials (t1: 48.1±5.8s, t2: 37.0±4.9s), but the RX-treated lesioned rats did show a significant ($p<0.001$) reduction in latency in trial 2 (t1: 48.0±5.8s, t2: 24.1±3.8s). This suggests that lesioned rats have impaired working memory, which was significantly improved by RX.

In conclusion, this study shows that the TRH analogue, RX, did not improve long-term memory of a platform position, but significantly improved working (short-term) memory in AMPA-induced septal-hippocampal lesioned rats.

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175P EFFECTS OF 5-HT$_3$ ANTAGONISTS BRL 46470A AND ONDANSETRON ON LEARNING AND MEMORY IN MICE

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Ondansetron (OND) and BRL 46470A (BRL) (endo-N-(8-methyl-8-azabicyclo[3,2,1]oct-3-yl)-2,3-dihydro-3,3-dimethyl-indole-1-carboxamide hydrochloride) are highly selective 5-HT$_3$ receptor antagonists.which have previously been shown to exert anxiolytic-like activity in animal models (Costall et al., 1988; Blackburn et al., 1993). 5-HT$_3$ receptor antagonists also appear to enhance cognitive performance in rats (Bentley & Barnes, 1995) although the apparent inactivity of BRL on learning in marmosets (Samson et al., 1994) might suggest that this compound has less efficacy. To examine this further we have compared the effects of OND and BRL in an active avoidance memory paradigm in mice.

Seven groups of BALB/C male albino mice (20-24g) (8 per group) were used. The mice were injected i.p. with saline (0.1ml/10g body weight), BRL 0.1, 1 or 10 mg/kg or OND 0.1, 1 or 10 mg/kg 30 min before placing in a shuttle box. The effects on learning and memory retention were studied using the two-way punishment reinforced active avoidance method (shuttle box). We used the method of Gozzani & Izquierdo (1976) according to which the mice were trained in a conventional shuttle box (Ugo Basile) with 50 trials single learning session and 7 days later a single retention session with 30 trials. In every learning trial the mice received 5s buzzer with 670 Hz and 70dB followed within 2s by random 0.4mA foot shocks. In the retention trial the mice received the same stimulation only with foot shocks of 0.2 mA. A one-way ANOVA was used for statistics.

Mice treated with BRL at doses of 0.1 and 10 mg/kg showed an increased number of avoidances ($p<0.01$ and $p<0.05$) compared with control (Table). Mice injected with OND at a dose of 0.1 mg/kg also showed an increase in the number of avoidances ($p<0.05$). In the retention test 7 days later mice pretreated with BRL or OND, at all doses tested, exhibited an increase in the number of avoidances compared with control. The dose-effect relationships for the two compounds were complex. BRL exhibited an a inverted bell-shaped dose-response relationship, whereas OND had a linear inverse relationship over the dose range used. Nevertheless it can be concluded that both compounds are able to improve learning and memory retention in the avoidance paradigm.

TABLE 1

Drugs (mg/kg i.p.)	*Number of avoidances per session*	
	Learning	Retention
control (saline)	2.00 ± 0.53	3.62 ± 1.39
BRL 0.1	12.5 ± 1.62**	23.62 ± 3.15**
BRL 1.0	4.00 ± 0.53	17.62 ± 1.39*
BRL 10.0	7.00 ± 1.18*	21.25 ± 3.20**
OND 0.1	5.50 ± 0.90*	25.75 ± 3.95***
OND 1.0	5.00 ± 0.87	15.75 ± 1.12**
OND 10.0	4.75 ± 0.70	9.50 ± 1.71*

* $p<0.05$, ** $p<0.01$ and *** $p<0.001$ compared to control

DG is Wellcome Travel Fellow.

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176P REDUCED NOVEL OBJECT EXPLORATION IN RATS PERINATALLY EXPOSED TO COCAINE

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Prenatal exposure to cocaine is associated with impaired development in human infants. Recent studies indicate that rodent offspring exposed to cocaine prenatally also show behavioural abnormalities (Woods et al, 1988). Perinatal cocaine exposure reduces dopaminergic function in the nucleus accumbens of young-adult rats (Giustino & Marsden, 1995) which is involved in locomotor activity and novelty-induced exploration (Hooks & Kalivas, 1995). The present study assesses the effects of perinatal cocaine on young adult rats on locomotor activity and short-term memory.

Gravid dams (Lister hooded) were given either saline or cocaine (20 mg/kg/sc) on gestation day 10 and then every other day until the weaning age of the pups (25 days old) when only males were selected (Giustino & Marsden, 1995). Behavioural tests were made 4 weeks post-weaning using 8 rats per experimental group.

The first experiment monitored locomotor activity in an open field arena in rats perinatally exposed to either cocaine or saline and given either acute cocaine (15 mg/kg i.p.) or saline. Activity was monitored for 5 min from 10 min post injection using a computer based video tracking system (Beckett & Marsden, 1995). In the second experiment novel object exploration was monitored using a modified version of a previous method (Ennaceur & Delacour, 1988). Rats were given two familiarisation sessions separated by 24 hrs where they were allowed 5 min per session to explore the open field with no objects present. The following day rats were individually placed in the open field and given two 3 min trials separated by 1 min. In the first trial (T1) rats were exposed to two identical objects. During the second trial (T2) rats were exposed again to two objects, placed in the same position, with one familiar object (ie. present in T1) and one novel object. Data were analysed by non parametric ANOVA, Kruscall-Wallis, followed by Mann-Whitney U test.

Perinatal cocaine (n=8) had no effect on spontaneous activity compared to perinatal saline (n=8) exposed rats with respect to active time and average speed but reduced rearing ($p<0.05$). Acute cocaine administration significantly increased locomotor activity (active time, $p<0.01$, average speed, $p<0.01$, rearing activity, $p<0.01$) in the rats exposed perinatally to saline but not in those exposed to cocaine. In the latter group activity tended to increase but this did not reach significance. In the novel object exploration test there was a significantly ($p<0.01$) higher exploration during T1 in the rats perinatally exposed to saline (33 ± 4 secs n=8) compared to those exposed to cocaine (7 ± 3 secs n=8). During the discrimination trial (T2) rats exposed perinatally to saline showed significantly ($p<0.01$) lower total exploration levels (11 ± 3 secs) compared to the first trial (T1), an effect not observed in cocaine exposed rats (5.5 ± 3 secs). Furthermore maternal treatment had a significant effect on exploratory activity at the familiar object (df=32; H=11.34; $p<0.01$) when the saline exposed group explored the familiar object significantly less ($p<0.01$) than the novel object, an effect not observed in the cocaine group .

The results demonstrate that low maternal cocaine consumption during pregnancy and lactation causes enduring behavioural changes ranging from reduced ability to perform a short-term memory task to altered locomotor responsiveness to a psychostimulant drug. The diminished behavioural sensitivity to acute cocaine supports previous work showing perinatal cocaine reduces dopaminergic function (Giustino & Marsden, 1995). The reduction in performance of the short-term memory test, expressed as reduced exploratory activity, reduced habituation and lack of discriminative ability, may be due to impaired attentional mechanisms as well as reduced capability to acquire and keep information over the testing session.

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177P EFFECT OF THE I_2-LIGAND 2-BFI ON PASSIVE AVOIDANCE PERFORMANCE IN THE RAT

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The α_2-adrenoceptor antagonist/imidazoline-2 (I_2)-site ligand idazoxan improves performance in animal models of learning and memory (Sara, 1991). 2-BFI (2-(2-benzofuranyl)-2-imidazoline) is an analogue of idazoxan with high affinity (Ki=1.2 nM) and 1550-fold selectivity for I_2-sites over α_2-receptors in rat brain (Hudson *et al.*, 1995). Since I_2-sites are located in brain regions (e.g. cortex and hippocampus; Mallard *et al.*, 1992) that are involved in cognitive function, we have explored the effects of 2-BFI on passive avoidance in the rat.

Male Wistar rats (200-450g, University of Bristol, n=8-10) were injected i.p. and 30 min later placed in the light side of a two compartment step-through avoidance chamber. Latencies to cross to the dark compartment were recorded (300 s cut-off). A footshock training stimulus (0.5 mA; 0.5 s) was applied 3 s after the rat had entered the dark box. After 24 h or 7 d, retention of the adversive experience was assessed i.e., latencies to cross to the dark box were measured again (600 s cut-off). In one study, 2-BFI was given 30 min after training on day 1. Effects of 2-BFI on locomotor activity were also evaluated. Rats (n=8) were placed in novel activity boxes after injection and the number of infrared beam breaks (counts) recorded over 60 min.

2-BFI significantly increased latencies to cross into the dark box during training (Table 1). This was most marked in rats treated with 10 mg/kg which also significantly decreased locomotor activity in the 30 min after injection. 2-BFI (3 mg/kg), a dose which did not decrease activity and induced only a small increase in latency on day 1, significantly increased latencies to enter the dark box on day 2. By contrast, 2-BFI (1-10 mg/kg) did not improve retention after 7 d or when given after training.

Thus, 2-BFI improved passive avoidance in rats but only when given before training suggesting that it enhanced perception and /or attention rather than memory/retrival mechanisms. Similar effects are produced by idazoxan (Dickinson *et al.*, 1989).

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Table 1. Effect of i.p. administration of 2-BFI on passive avoidance behaviour and locomotor activity in the rat

Treatment	Training Day 1	Retention Day 2	Retention Day 7	Retention Day 2[a]	Locomotor Activity 0-30 min	30-60 min
Saline	18(5-43)	113(26-391)	17(2-250)	196(33-600)	8248±1156	2482±579
1 mg/kg	37(9-300)*	122(48-600)	24(11-495)	170(36-600)	7920±1236	2403±596
3 mg/kg	51(18-136)*	600(145-600)*	93(18-600)	179(12-600)	8106±726	2531±679
10 mg/kg	281(58-300)*	196(35-600)	87(17-600)	159(50-600)	4401±849*	2220±468

Median(range) latency (s), Mann Whitney U test; mean±s.e.mean counts, ANOVA+Dunnett's test; *P<0.05; [a]=2-BFI post-training

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178P IS BW 723C86-INDUCED HYPERPHAGIA AN *IN VIVO* MODEL OF RAT CENTRAL 5-HT_{2B} RECEPTOR FUNCTION?

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The 5-HT_{2B} receptor is found in both the rat stomach fundus (Baxter et al., 1995) and hypothalamus (Duxon et al., 1995). We have therefore investigated the effect of BW 723 C 86 [(±) 1-[5-(2-thenyloxy)-1H-indol-3-yl] propan-2-amine HCl] (BW), an agonist with approximately 10 fold selectivity for the rat 5-HT_{2B} receptor (Baxter et al., 1995), on rat food intake.

Male SD rats (200-250 g) were held under a 12 h light cycle (lights on 0700 h) with free access to food and water. Some were implanted with guide cannulae in the right lateral ventricle [dorsal ventral + 0.64 mm from interaural line, rostral caudal -0.8 mm from the bregma, medial lateral +1.5 mm (Paxinos and Watson, 1986)]. After 5 days, rats were placed alone in clear perspex cages (26 x 26 x 22 cm) with free access to water. After 15 min, a cannula was inserted and 1 ul saline or BW (1-30 ug) infused over 2 min. Two min later, it was removed, 3 pellets of normal food added and behaviour scored for 15 min. Intact rats given BW s.c. 30 min pretest were scored similarly after 15 min habituation to the cages. Alternatively, intact rats were singly housed on day 1, food deprived for 1 h on day 2 at 13.00 h and given antagonists p.o. 1 h and BW s.c. 30 min prior to restoring normal food and weighing the amount eaten 2 h later. BW was made up in 0.9% NaCl. Antagonists were given p.o. in 1% methyl cellulose (2 ml/kg). Results are cited as means ± s.e.m. and analysed by 1-way ANOVA and Dunnett's test or 2-way ANOVA and Newman-Keuls test (antagonist studies).

Feeding duration was increased by BW given i.c.v. (saline, 2.9 ± 1.5, BW 1 ug 26.1 ± 10.2 $p<0.05$, 3 ug 73.2 ± 15.6 $p<0.01$, 10 ug 39.2 ± 15.6, $p<0.01$ secs) or s.c. (saline 11.8 ± 7.2 secs, BW 10 mg/kg 183.3 ± 58.5 secs $p<0.05$, 20 mg/kg 163.2 ± 77.6 secs $p<0.05$) as was feeding frequency. BW also increased 2 h food intake in free feeding studies (saline, 0.55 ± 0.11 g, BW 20 mg/kg 1.28 ± 0.23 g, $p<0.05$, 50 mg/kg 1.45 ± 0.34 g, $p<0.05$). No increase in feeding was seen in BW dosed rats pretreated with the 5-$HT_{2C/2B}$ receptor antagonists, SB 200646A (Kennett et al., 1994) 20 or 40 mg/kg p.o.(data not shown) or SB 206553 (Kennett et al., this meeting) (vehicle + saline 1.4 ± 0.3 g, vehicle + BW 20 mg/kg 3.0 ± 0.4 g, $p<0.05$, SB 206553 20 mg/kg + saline, 2.1 ± 0.3 g, n.s. vs vehicle + saline, SB 206553 20 mg/kg + BW 2.1 ± 0.3 g, n.s. vs SB 206553 alone) although when given alone, both antagonists tended to increase basal food intake.

In conclusion, BW 723C86 causes hyperphagia probably via stimulation of central 5-HT_{2B} receptors, although further proof may require more selective tools. The hypophagic effect of mCPP, a 5-$HT_{2C/2B}$ receptor agonist, (Kennett et al., 1994) is thus likely to be 5-HT_{2C} receptor mediated.

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179P FURTHER EVIDENCE FOR BEHAVIOURAL DIFFERENCES BETWEEN SELECTIVE BZ-1 (ω1) AND NON-SELECTIVE BZ (ω) RECEPTOR LIGANDS IN RATS

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Although the molecular characterization of benzodiazepine (BZ) receptor subtypes is now well documented (Sieghart, 1995), the role of each subtype in the behavioural effects of BZ (ω) receptor ligands remains unclear (Sanger et al., 1994). The present experiments were carried out to investigate further behavioural differences between selective BZ-1 (ω1) (the only available compounds selective for a subtype, e.g. zolpidem) and non-selective full (diazepam) and partial (bretazenil) BZ (ω) receptor agonists in the rat elevated plus-maze test, a procedure designed for evaluating anxiety-modulating agents. Behaviours recorded comprised the traditional indices of anxiety as well as ethologically derived responses.

The apparatus consisted of a maze elevated to a height of 50 cm with two open (50 x 10 cm) and two enclosed arms (50 x 10 x 50 cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10 x 10 cm). Male Sprague-Dawley rats (160-220g) were injected intraperitoneally 30 min before the beginning of the experiment and observed during a 4 min period

When administered alone, the non selective BZ (ω) receptor ligands diazepam and bretazenil not only increased the proportion of time spent in the open arms (considered as a traditional index of anxiety) and the total number of arm entries (which may be considered to reflect a disinhibitory action) (see figure 1) but also affected head-dippings which can be considered as an index of risk assessment. In contrast, while displaying a similar behavioural profile to diazepam and bretazenil with respect to open arm entries and head-dips, the selective BZ-1 (ω1) agent zolpidem failed to affect the total number of arm entries. This difference may be related to the preferential sedative properties of BZ-1 (ω1) ligands which might mask the disinhibitory action of non-selective compounds.

In order to investigate further this hypothesis, zolpidem (0.3-1 mg/kg) was simultaneously injected with either diazepam (1.5 mg/kg) or bretazenil (1 mg/kg). When diazepam was co-administered with zolpidem, the behavioural effects closely resembled those observed with zolpidem when administered alone as the total number of entries measure remained unaffected. In contrast, the co-administration of bretazenil and zolpidem produced a profile very similar to that obtained with bretazenil alone. These

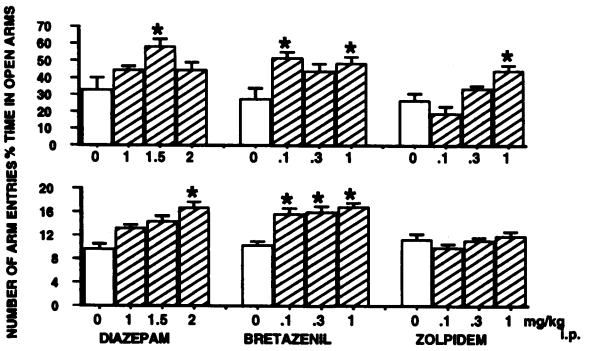

Fig. 1: Effects of three BZ (ω) receptor ligands on the behaviour of rats exposed to the elevated plus-maze test. P<0.05 (Dunnett).

results can be interpreted as showing that zolpidem, behaving as a full agonist at the $GABA_A$ receptor containing the α1 subunit (Pritchett and Seeburg, 1990), was able to mask the disinhibitory action of diazepam, probably by potentiating its sedative properties. In contrast, the lack of a similar effect of zolpidem with bretazenil may be related to the partial agonist properties of the latter drug (Puia et al., 1992) which antagonised the sedative action of zolpidem.

These results underline differences between the preferential depressant activity of zolpidem (involving BZ-1 (ω1) receptor subtype activation) as compared to the disinhibitory activity observed with non-selective BZ (ω) receptor ligands. We can tentatively suggest that this latter effect involves the activation of a BZ (ω) receptor subtype on which zolpidem is inactive.

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180P EFFECT OF THE SELECTIVE 5-$HT_{2B/2C}$ RECEPTOR ANTAGONIST, SB 206553, ON mCPP- AND ENVIRONMENTAL STRESS-INDUCED INCREASE IN PLASMA ADRENOCORTICOTROPIC HORMONE IN RATS

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5-Hydroxytryptamine (5-HT) may be involved in the control of stress-induced adrenocorticotropic hormone (ACTH) release (Van de Kar, 1991) and there is evidence that 5-HT_{2C} receptor stimulation may have a part to play in this response. For example, m-chlorophenylpiperazine (mCPP), a 5-HT receptor agonist with some selectivity for 5-$HT_{2B/2C}$ receptors, causes a dose-dependent, centrally-mediated increase in plasma ACTH (Calogero et al., 1990) which is reportedly partially reversed by non-selective antagonists that have a high affinity for 5-$HT_{2B/2C}$ receptors (Bagdy et al., 1989). We have investigated the effects of the selective 5-$HT_{2B/2C}$ receptor antagonist, SB 206553 (5-methyl-1-(-3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo-[2,3-fr]indole - Kennett et al., this meeting), on an mCPP-induced increase in plasma ACTH in rats. Also, in order to investigate whether an mCPP-induced increase in plasma ACTH correlates pharmacologically with a physiological release of the hormone, we have conducted an experiment to examine the action of SB 206553 on an environmental stress-induced increase in plasma ACTH.

Male Sprague-Dawley rats (approx. 320g; n=9-12/group) were group housed in a 12 hour light/dark cycle (lights on at 7.00am). They were handled and dosed p.o with vehicle (1% methyl cellulose soln., 2 ml/kg) and/or i.p. with 0.9% saline solution (1 ml/kg) each day for one week prior to sample collection (performed 11.00am-1.00pm). On the test date of the first experiment, rats were administered vehicle or SB 206553 (20 mg/kg p.o., 1 hour pre-sample collection) and 0.9% saline or mCPP (2.5 mg/kg i.p., 30 min pre-sample collection). In the second experiment, rats were dosed with vehicle or SB 206553 (20 mg/kg p.o., 1 hour pre-sample collection), 45min before being placed alone in an unfamiliar, white social interaction box under white light for 15 min (see Kennett et al., this meeting). Trunk blood was collected in pre-chilled EDTA vials and centrifuged for 5 min at 2000 r.p.m.. Plasma was removed, stored at -70°C and analysed for hormone content by radioimmunoassay (Biogenesis Ltd.). Results (mean ± s.e.m) were analysed by 2-way ANOVA and Newman Keul's test.

mCPP and the novel, aversive environment each significantly ($p<0.01$) increased basal plasma ACTH (from control levels of 117.6 ± 6.6 and 145.7 ± 8.9 to 273.9 ± 24.6 and 273.3 ± 13.8 pg/ml, respectively). SB 206553 had no effect alone, but produced a signficant ($p<0.01$) partial reversal of the mCPP- and stress-induced increases (to 205.2 ± 20.1 and 218.7 ± 13.0 pg/ml, respectively).

These results suggest that 5-$HT_{2B/2C}$ receptor stimulation is, at least in part, important in the release of ACTH when produced either by mCPP or a physiological challenge. It is noteworthy that a higher degree of receptor occupation, by SB 206553, may be required to reverse this hormonal response than a 5-HT_{2C} receptor mediated, mCPP-induced behavioural response (Kennett et al., this meeting). However, it should be borne in mind that since SB 206553 produces an anxiolytic-like response in rats (Kennett et al, this meeting), the reduction in ACTH seen in this study may not be due to pharmacological antagonism, but may represent an end product of response competition between anxiogenesis and anxiolysis.

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181P DSP-4 NEUROTOXICITY IN RAT BRAIN: EFFECT ON HEAT-SHOCK PROTEIN EXPRESSION AND *IN VIVO* NORADRENALINE EFFLUX

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Heat-shock protein synthesis is enhanced by various stressors (Kelley & Schlesinger, 1982). Stress also enhances the firing rate of noradrenaline (NA)-containing neurones originating in the locus coeruleus (LC; Aston-Jones & Bloom, 1981). DSP-4 is a neurotoxin which preferentially inactivates noradrenergic neurones from the LC (Jonsson *et al*, 1981). We have used DSP-4 to determine the involvement of NA in controlling heat-shock protein (hsp70 and αβ-crystallin) expression in rat brain induced by physical/psychological stress (saline injection).

Various groups of male Sprague-Dawley rats (250-300g) were injected with 40mg/kg DSP-4 or saline (i.p.). Histology with haematoxylin and eosin staining and immunohistochemistry with peroxidase detection were performed on coronal sections of paraffin-embedded brains at hind- and midbrain levels 2h after injection (n=3). Anti-hsp70 and αβ-crystallin antibodies were raised to 10 amino acid synthetic peptide sequences. Whole brain levels of NA were determined 2h after DSP-4 by HPLC with electrochemical detection (ECD; n=5). Intracerebral microdialysis was performed in frontal cortex (mm; AP +3.5, L -1.5, V -5.0, jawbar -3.3) of halothane-anaesthetised rats (n=6). Microdialysis probes (Hospal membrane) were perfused with Ringer's buffer (pH6.6; mM; 145 NaCl, 4 KCl, 1.3 $CaCl_2$) at 1μl/min. Samples were collected (20min intervals) into 5μl 0.01M $HClO_4$. Four basal samples were collected before DSP-4 injection and were analysed by HPLC with ECD. All data are mean ± s.e. mean.

DSP-4 did not cause microscopically visible (40x magnification) damage to neurones in brain sections. Compared with saline controls, DSP-4 produced a 9% depletion ($P<0.05$; Student's unpaired 't'-test) in whole brain levels of NA (ng/g; saline, 393±7; DSP-4, 358±12), with no change in 5-hydroxtryptamine (ng/g; saline, 644±27; DSP-4, 693±22) and dopamine (ng/g; saline, 1044±12; DSP-4, 1058±24). Microdialysis studies showed increased (maximal 231% at 60min) extracellular NA over basal levels (0-80 min, $F_{1,9}=6.82$, $P<0.05$; 80-160 min, $F_{1,10}=6.96$, $P<0.05$; split-plot ANOVA). When compared with untreated controls, saline injection increased immunoreactivity of hsp70 in neurones and reduced glial levels of hsp70 and αβ-crystallin in spinal cord and cerebellum. In midbrain, saline injection enhanced hsp70 expression in neurones and reduced glial levels of both proteins in cortex, hippocampus, corpus callosum, thalamic nuclei and basal ganglia. DSP-4 treatment markedly diminished or abolished these changes.

DSP-4 does not cause morphological degeneration of NA neurones confirming the earlier report of Booze *et al* (1988). We now provide the first *in vivo* evidence that DSP-4 produces marked efflux of NA from neurones originating in the LC. Saline injection, previously shown to increase brain levels of the proto-oncogene, c fos, mRNA and protein (Asunama *et al*, 1992), has now been shown to alter both hsp70 and αβ-crystallin expression in rat brain. DSP-4 inhibited the changes in heat-shock proteins at 2h when NA efflux was markedly increased. Therefore, these results provide evidence that NA neurotransmission is involved in modulating the altered brain expression of hsp70 and αβ-crystallin after saline injection.

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182P EXOGENOUS CORTICOSTERONE ADMINISTRATION INCREASES ANXIETY IN RATS

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Previous research (Bhatnagar *et al.*,1994) has demonstrated that intra-hippocampal administration of the cholinergic antagonist scopolamine (SCOP) prior to restraint stress in rats results in hyper-secretion of corticosterone (CORT). We have shown that SCOP increases indices of anxiety in rats (Smythe *et al*, in press), and we sought to investigate if this effect might be due to changes in CORT. In the present study we have examined anxiety, as measured by the Black-White box task, following injections of CORT at various pre-test times. Adult male Lister rats were injected with CORT (15 mg/kg ip) or its vehicle (VEH; 30% propylene glycol in 0.9% saline), at 0, 5 and 10 min prior to being placed into the white chamber of the Black-White box (n=7/group). Rats were videotaped and the tapes were scored for latency to exit the white chamber, latency to re-enter the black chamber, and time spent in the white chamber. Data were analysed by analysis of variance (ANOVA) and post hoc testing was performed using a Bonferroni corrected t-test.

ANOVA revealed no differences amongst groups in latency to enter the black chamber. However, there was a main effect of drug on latency to re-enter the white chamber $F(1, 36)=4.80$, $p<.04$. Overall, CORT treated rats avoided entering the white chamber after leaving the black compartment ($p<.03$), as revealed by the increased latency to re-enter the white compartment. These data are depicted in Fig. 1. ANOVA revealed a significant interaction between injection pretreatment time and drug on time spent in the white compartment, with $F(2, 36)=3.41$, $p<.04$. Group comparisons showed that CORT injected at 5 and 10 min before testing decreased time spent in the white chamber (p's <.01), while VEH injected at 10 min prior to testing also reduced time in the white area ($p<.01$). These data are shown in Fig. 2.

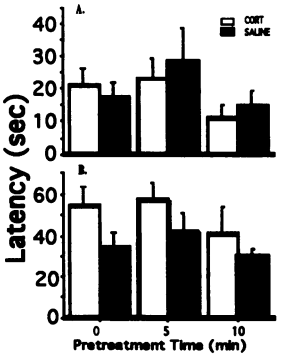

Fig. 1. Panel A (top) shows latency to enter black chamber after initial placement in the box. Panel B (bottom) shows time until re-entry of white chamber. Values shown are means ±SEM.

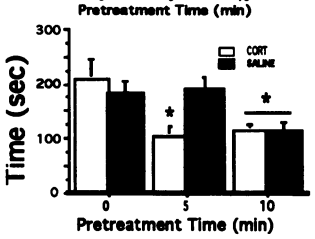

Fig. 2. CORT treated rats spent less time in the white area compared to the VEH treated rats, although injection stress alone affected the VEH rats at the 10 min pretreatment time point. *significantly different from respective control and 0 min time points (p's<.01).

These data suggest that stress may be anxiogenic, and support the possibility that a cholinergic substrate may underlie some aspect of anxiety. In conclusion, we have provided further information as to the interaction between stress and anxiety, and contend that central cholinergic blockade can elicit anxiety by elevating CORT secretion.

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183P L-2-CHLOROPROPIONIC ACID-INDUCED CEREBELLAR NECROSIS IS NOT THE RESULT OF LIPID PEROXIDATION OR DNA DAMAGE

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Oral administration of large doses of L-2-chloropropionic acid (L-CPA; 750 mg/kg) to rats, produces a selective neuronal cell loss of cerebellar granule cells and a small population of Purkinje cells (Widdowson et al., 1995a). Neuronal cell loss occurs between 36 and 48h following L-CPA administration. The development of selective cerebellar neuronal cell death can be prevented following administration of N-methyl-D-aspartate (NMDA) receptor antagonists (Widdowson et al., 1995b) and partially prevented by blockade of nitric oxide synthase (Widdowson et al., 1995c). This suggests that L-CPA neurotoxicity may be a useful model for studying the neurochemistry of excitotoxicity *in vivo* leading to neuronal cell death (Choi, 1988). We examined whether we could find any evidence for lipid peroxidation or damage to DNA prior to, and during the development of the granule cell necrosis, following L-CPA administration, that may account for the damage to granule cells. Male Alderley Park rats (200-220g) were orally dosed with L-CPA (750 mg/kg, pH 7) and killed 36 or 48 h following dosing by CO_2 anaesthesia. Control rats were orally dosed with water. Reductions in cerebellar aspartate concentrations measured 48 h following L-CPA dosing, as measured by HPLC, were used as an indication of the extent of granule cell loss (Widdowson et al., 1995b). Lipid peroxidation was estimated in the cerebellum 48 h following L-CPA administration by measuring 2-thiobarbituric acid reactive substances (TBRS) mainly malondialdehyde formation (Bohme et al., 1977). The amount of TBRS was not significantly increased in the cerebellum 48 h following L-CPA administration, when there is wide scale cerebellar neuronal cell damage (TBRS in ng/mg protein; controls = 4.28 ± 0.17; L-CPA treated = 5.33 ± 0.81; n = 6 animals/ group, mean ± S.E.M.). Furthermore the antioxidants, U74389G(21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione) and U83836E ((-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol) which have been reported to reduce the amount of iron dependent lipid peroxidation (Smith et al., 1995) were not able to prevent the L-CPA-induced neurotoxicity when administered twice daily at 10 mg/kg/i.p. (aspartate concentrations in µmol/g dry weight, mean ± S.E.M for n =5 rats; controls = 14.1 ± 0.2, L-CPA treated = 7.7 ± 1.7* , L-CPA + U73489G = 4.8 ± 0.6 *, L-CPA + U83836E = 4.8 ± 0.9*; *p <0.05 as compared to controls). L-CPA-induced damage to cerebellar DNA was assessed using the DNA single strand break assay (Mullaart et al., 1990) 36 h following L-CPA administration just prior to granule cell necrosis. There was no significant increases in single strand breaks detected in L-CPA treated cerebellum, compared to control cerebellum. We furthermore were unable to detect evidence for single or double strand DNA breaks in necrotic granule cells in sections of rat cerebellum at 48 h following L-CPA administration using the TUNEL method (Gavrieli et al., 1992). In conclusion, L-CPA-induced cerebellar granule cell necrosis does not appear to lead to an increase in lipid peroxidation or DNA damage as a result of possible excessive free radical production.

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184P EFFECT OF ONDANSETRON ON APOMORPHINE-INDUCED CONDITIONED TASTE AVERSIONS IN RATS

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The role of the area postrema in psychotropic drug-induced conditioned taste aversions (CTA) remains unclear (Grant 1987). The presence of 5-HT_3 receptors in this area suggests that these receptors may be involved in this phenomenon. The aim of the present study was to determine whether the 5-HT_3 antagonist ondansetron could prevent CTA induced by apomorphine, which has previously been shown to produce strong CTA in rats (Pratt and Stolerman 1984).

CTA responses to apomorphine (0.25 mg kg^{-1} sc) and ondansetron (0.001 - 0.1 mg kg^{-1} sc) were investigated in male Lister Hooded rats (weight 250-300g, n=6-8/group) using a 2-trial conditioning procedure (Pratt and Stolerman, 1984). Water deprived rats were presented with either a sodium saccharin (0.1%) or a sodium chloride solution (0.9%) and immediately afterwards injected with drug or saline. The drug-paired and the vehicle-paired solutions were then presented simultaneously in a two-stimulus test to assess CTA. The percentage scores for drug-paired flavour intake were subjected to arc-sine transformations to normalise their distribution and analysed by one-way ANOVA followed by the Newman Keuls test for comparisons between treatment groups

During the conditioning trials pretreatment with ondansetron (0.001-0.1 mg kg^{-1}, 30 min prior to flavour presentation) did not affect baseline fluid consumption. Thus fluid intake was 10-13ml in both saline and ondansetron pretreated rats. Results from the 2-stimulus test demonstrated that apomorphine produced clear CTA and that pretreatment with ondansetron (0.1 mg kg^{-1}) blocked apomorphine-induced CTA ($p<0.05$) (Table 1). Conversely, ondansetron alone did not produce CTA (Table 1). There was a significant attenuation ($p<0.05$) of apomorphine-induced CTA following pretreatment with ondansetron (0.001 & 0.01 mg kg^{-1}). Mean ± s.e.mean percentage fluid intakes for drug-paired flavours were 33.1 ± 1.1% and 35.1 ± 1.5% respectively as compared to 27.3 ± 0.5% in the saline pretreated group. In conclusion, ondansetron reduces apomorphine-induced CTA. The neural basis of action of this effect requires further study.

Table 1. Effect of ondansetron on apomorphine-induced CTA in rats

Pretreatment (mg kg^{-1})	Treatment (mg kg^{-1})	Fluid intake (ml) 2-stimulus test		Drug-paired flavour intake (Mean%± SEM)
		drug-paired	vehicle-paired	
saline	apomorphine (0.25)	3.5 ± 0.5*	9.9 ± 0.5	30.7 ± 0.9
ondansetron (0.1)	apomorphine (0.25)	7.3 ± 0.9	6.7 ± 0.9	46.8 ± 4.1†
saline	ondansetron (0.1)	7.4 ± 0.7	6.9 ± 0.7	46.1 ± 2.1†

† $p<0.05$ compared to saline + apomorphine group; ANOVA followed by Newman Keuls test. *$p<0.05$ compared to vehicle paired flavour (paired t-test); indicates CTA.

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185P EFFECTS OF ISRADIPINE ON HORMONE-SENSITIVE LIPASE ACTIVITY OF BROWN ADIPOSE TISSUE IN MICE DURING WITHDRAWAL FROM CHRONIC ETHANOL TREATMENT

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Hormone-sensitive lipase (HSL) is the principal intracellular enzyme regulating lipolysis in adipocytes. We have shown that chronic ethanol treatment (CET) increases the activity of HSL in brown adipose tissue (BAT) of mice, by raising the intracellular cAMP levels (Shih & Taberner, 1995). Withdrawal from CET causes a decrease in adipocyte lipogenesis, a process closely linked to lipolysis. Preliminary studies indicate that calcium entry through dihydropyridine-sensitive (DHP) channels may be implicated in mediating this effect of withdrawal (Hughes *et al.*, 1995). Since calcium is one of the key regulators of intracellular lipid metabolism, including lipolysis, we have used isradipine, a DHP calcium channel antagonist, to investigate the role played by DHP sensitive calcium channels in mediating the effects of CET and withdrawal on HSL activity in mouse BAT.

Groups of 8-9 male TO mice were given pelleted diet and water or 20% (w/v) ethanol solution as sole fluid for 4-6 weeks (ethanol dose: 16-20 g $kg^{-1}day^{-1}$). (±) Isradipine (10mg kg^{-1}) was dissolved in 5% Tween-80 and injected i.p. either at the time of withdrawal (t = 0) or at t = 8h, controls were injected with vehicle. Naive mice received similar treatments. Mice were withdrawn at 24:00h and killed between 9:00 and 10:30h, at t = 9h. Plasma and brain ethanol concentrations were assayed by a standard enzymatic assay. HSL activity was assayed using emulsified [^{3}H] triolein as substrate (Nilsson-Ehle & Schotz, 1976) and expressed as pmol FFA released min^{-1} mg $protein^{-1}$. Differences (means ± s.e. mean) between different treatment groups were analysed by Student's t-test for independent samples.

Mean plasma and brain ethanol levels were 28.9 ± 10.9 and 20.6 ± 6.5 mM at t = 0, and 0.66 ± 0.54 and 0.10 ± 0.06 mM by t = 2h. CET slightly decreased HSL activity in BAT compared to naive animals (0.97 ± 0.1 in naive and 0.87 ± 0.1 in CET). However, 9 hour withdrawal from CET evoked a significant increase in activity to 1.67 ± 0.21 ($p<0.01$). This effect was also seen at 24 h but was less prominent (1.47 ± 0.09, $p<0.01$). Isradipine administration to naive animals 1 or 9 h before the assay had no significant effect on HSL activity in BAT (1.17 ± 0.25 and 2.18 ± 0.58 respectively). Vehicle also had no effect on activity in naive animals (1.13 ± 0.21 and 1.53 ± 0.40 respectively). Neither isradipine nor vehicle had any effect on HSL activity in mice on CET when administered at t = 0 (1.46 ± 0.36 and 1.37 ± 0.09, compared to 0.87 ± 0.10 in ethanol-drinking mice). Administration of isradipine at the onset of withdrawal from CET evoked a non-significant decrease in HSL activity (1.07 ± 0.2), thus tending to oppose the effect of withdrawal. In contrast, its administration at t = 8h significantly raised HSL activity to 5.44 ± 0.61 ($p<0.001$).

Withdrawal from CET precipitates adaptive changes in brown adipocyte lipid metabolism at the level of HSL activation. Calcium entry into adipocytes via DHP sensitive channels may be involved in bringing about these effects as HSL activity in BAT during withdrawal was sensitive to blockade of calcium channels.

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186P CYP2E1 IS EXPRESSED IN DOPAMINERGIC NEURONES IN THE RAT SUBSTANTIA NIGRA

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Endogenous neurotoxins and free radicals have been implicated in Parkinson's disease (PD). The actions of brain cytochrome P450 (CYP) may be important in their formation and/or metabolism. CYP2E1 has previously been shown to be present in the substantia nigra (Hansson *et al.*, 1990; Riedl *et al.*, 1996). CYP2E1 oxidises alcohols to aldehydes which may condense with dopamine to form neurotoxic tetrahydroisoquinolines. In addition, CYP2E1 can produce superoxide anion radicals. The cellular localisation of CYP2E1 is unknown. Hence we have investigated its association with dopaminergic neurones in rat substantia nigra.

Naive male Wistar rats (150-200g; n=4/group) were anaesthetised with sodium pentobarbitone (100mg/kg, i.p.) and transcardially perfused with 0.1M phosphate buffered saline (PBS), followed by PBS containing 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 3 days, cryoprotected in sucrose before being 'snap' frozen in isopentane. Contiguous sections (30µm) were taken through the substantia nigra (SN) and incubated overnight with rabbit anti-rat CYP1A1, CYP2B1/2, CYP2D1, CYP2E1 or CYP3A1 (1:1000). Second layer antibodies consisted of goat anti-rabbit IgG (1:200), and amplified by The ABC method. Sections were examined under a light microscope. In further studies, male Wistar rats (150-200g; n=4/group) were injected with either isoniazid (200mg/kg, i.p.) or saline for 5 days and brains processed as before. Sections from SN were incubated overnight with both rabbit anti-rat CYP2E1 (1:1000) and sheep anti-rat tyrosine hydroxylase (TH) (0.2 µg/ml) antibodies. Second layer antibodies consisted of rhodamine donkey anti-sheep (1:200), and biotinylated goat anti-rabbit IgG (1:200) followed by avidin labelled fluorescein donkey anti-rabbit isothiocyanate (1:50). Sections were mounted onto slides using Vectashield, before being examined under a laser scanning confocal microscope (Leica).

In naive animals, CYP2E1 was strongly expressed in the SN pars compacta. Antibodies against CYP1A1, CYP2B1/2, CYP2D1 and CYP3A1 failed to stain the SN. In the co-localisation study, in saline-treated rats, CYP2E1 was expressed as a faint green colour and TH gave an intense red colour when excited with the laser. The CYP2E1 signals dramatically increased after isoniazid treatment. Using both blue and red light to see co-localisation of CYP2E1 and TH, it was found that CYP2E1 was expressed only in the TH positive neurones of the SN pars compacta.

This is the first study to show co-localisation of CYP2E1 within the dopaminergic neurones of the SN, although NADPH-cytochrome P450 reductase has also been shown to be present in these cells (Haglund *et al.*, 1984). The functional significance of brain CYP2E1 within the SN is unknown. Since brain CYP2E1 produces free radicals, this may be important in nigral cell death in PD.

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187P *IN VITRO* ANTIOXIDANT ACTIONS OF ANTIPARKINSONIAN AGENTS

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Increased formation of reactive oxygen species and oxidative stress in the substantia nigra (SN) have been implicated in the pathogenesis of Parkinson's disease (PD) (Jenner, 1994). The antiparkinsonian agents,deprenyl,apomorphine and bromocriptine have been suggested to possess antioxidant activity and to retard nigral damage (Yoshikawa,*et al.*, 1994, Ubeda *et al.*,1993, Wu *et al.*, 1993). We tested this hypothesis by assessing the ability of a range of dopamine agonists and known antioxidants to inhibit lipid peroxidation, and to react directly with peroxyl radicals (ROO•).

A sample of 2mg/ml ox-brain phospholipid liposomes was incubated with 100μM $FeCl_3$ and 100μM ascorbic acid in 0.15M phosphate buffer (PB), pH 7.4, at 37°C for 1h to induce lipid peroxidation. Colour development occurred on addition of 1ml each of 1% (w/v) thiobarbituric acid (TBA) and 2.8% (w/v) trichloroacetic acid (TCA). TBA-MDA (malondialdehyde) adducts, were measured at 532nm as an index of the amount of lipid peroxidation produced. After addition of a range of concentrations of the named drugs (0-1mM) to the system, inhibition of the control was calculated. Propyl gallate was used as a positive control. For pulse radiolysis experiments, the drugs (0.05%) were dissolved in 10mM PB, pH 7.4 containing 1% CCl_4 and 50% isopropyl alcohol and reacted in a Linear Accelerator Facility. Absolute rate constants for reaction with peroxyl radicals were calculated. The vitamins E and C were used as positive controls.

Apomorphine was a powerful inhibitor of lipid peroxidation (IC_{50}: 4.56±0.86μM c.f. 45.05±4.67μM for propyl gallate).

Bromocriptine shows mild antioxidant ability, but both deprenyl and ropinirole had little effect. In pulse radiolysis experiments, again bromocriptine was moderately effective, deprenyl and ropinirole did not interact to any great extent with peroxyl radicals. Apomorphine had a surprisingly low rate constant suggesting that in the lipid peroxidation assay it acts via a mechanism other than the scavenging of radicals (Table).

Table Inhibition of lipid peroxidation by some anti-PD drugs and rate of reaction with trichloromethyl peroxyl (CCl_3O_2•) radicals (n=3). Values are for 100μM of drug.

Drug	% Inhibition	Rate of Reaction with CCl_3O_2•
Propyl gallate	63.7±3.2	-
Deprenyl	4.9±2.1	1.18×10^7
Bromocriptine	26.9±3.5	7.41×10^7
Apomorphine	100	1.45×10^6
Ropinirole	4.86±2.1	1.61×10^7
Vitamin C	-	1.30×10^8
Vitamin E	-	4.89×10^8

Apomorphine and bromocriptine may be able to inhibit the pathological process that occurs in the SN in Parkinson's disease. The other drugs examined here are unlikely to produce antioxidant activity by the direct scavenging of peroxyl radicals.

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188P MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG) INDUCES CHRONIC RELAPSING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (CR-EAE) IN THE DA RAT

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Multiple sclerosis is characterised by CNS inflammation, demyelination and a chronic progressive disease course. Animal models have so far failed to reproduce consistently this triad of features. In particular, the most commonly used model, myelin basic protein induced EAE in the Lewis rat is typically a monophasic, inflammatory disease without demyelination or relapses. More recently it has become apparent that other components of myelin, such as MOG, may also play a role in autoimmune disease of the CNS. Patients suffering from MS show elevated numbers of MOG-reactive T- and B-cells (Sun *et al.* 1991; Kerlero de Rosbo *et al.* 1993) and antibodies to MOG can induce demyelination when given to animals with inflammatory EAE (Linington *et al.* 1993). Also, in the Lewis rat immunisation with MOG causes a demyelinating disease that can lead to relapses (Adelmann *et al.* 1995; Johns *et al* 1995). However, in this rat strain the susceptibility to MOG induced EAE is low, and disease course is highly variable.
In the present study we demonstrate that active immunization against MOG can induce a reproducible and antigenically defined model that combines inflammation, demyelination and a chronic relapsing disease course in the DA rat.
Female DA rats (120-200g, Charles River, Ger.) were injected subcutaneously with 50µg recombinant rat MOG (amino acids (a.a.) 1-125 expressed in *E. coli* using the PQE12 vector, purity >90%, (Adelmann *et al.* 1995)) plus 200µg *M. tuberculosis* H37Ra in 100µl IFA in the base of the tail under light ether anaesthesia. The animals were scored for clinical disease (0: no disease, 1: loss of tail tonicity, 2: hind limb weakness, 3: hind limb paralysis, 4: fore limb weakness) and weighed daily. Results are presented as mean ± stdev. 13 of 14 animals developed EAE (onset on day 10.6±2.0, mean maximal clinical score (m.c.s.) 3.1±1.1) and after recovery within 4.1±1.1 days (m.c.s. 0.5±0.7) suffered an initial relapse on day 20.8±4.0 (m.c.s 2.7±1.2). Four animals which were monitored for a longer period of time developed multiple relapses which occured at intervals of approximately 10 days (range 8 to 12). This pattern of relapse-remission has now been followed for up to a maximum period of 80 days (5 attacks). For histological examination animals were perfused with 4% paraformaldehyde in PBS under terminal ether anaesthesia at different timepoints between days 9 and 80 post immunisation (p.i.). Histological analysis of CNS tissue (for method see Linington *et al.* 1993) revealed the presence of focal demyelinating lesions which consisted of naked axons embedded in a matrix of glial scar tissue, similar to lesions found in patients with MS.
Preliminary studies indicated that the observed pathology is a consequence of synergy between an encephalitogenic MOG -specific T cell response and a demyelinating MOG-specific antibody response. ELISA analysis of sera (days 26 and 34 p.i.) revealed the presence of high titres of MOG specific antibodies (IgG + IgM; 1:400 000 and 1:100 000; OD_{490} > background + 2 stdev). Encephalitogenic T-cell lines (for method see Linington *et al.* 1993) were established, which had a CD4+ phenotype by FACS scan and recognised preferentially epitopes in the regions a.a. 70-91 and a.a. 99-117. The adoptive transfer of 10x10E6 activated MOG -specific T line cells resulted in a lethal, inflammatory disease (onset day 3), whereas injection of 3x10E6 led to acute, monophasic disease (onset on day 4, m.c.s. 2.3±0.3). This relatively mild inflammatory disease was transformed into a lethal, demyelinating disease by cotransfer of anti MOG monoclonal antibody 8-18C5 (4mg/rat, four days after T cell injection), underlining the importance of antibodies in demyelination. This study presents a highly reproducable, antigenically defined animal model which combines hallmarks of MS and is highly suitable for the further immunological and pharmacological study of this crippeling disease.

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189P MECHANISMS OF β-METHYLAMINO-L-ALANINE (BMAA)- AND β-OXALYLAMINO-L-ALANINE (BOAA)-INDUCED DEATH IN RAT CEREBELLAR GRANULE CELLS

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The plant amino acids β-methylamino-L-alanine (BMAA) and β-oxalylamino-L-alanine (BOAA) are structurally related to the endogenous excitotoxin glutamate. They are found in the seeds of the false sago palm (*Lathyrus sativus*) and chickling pea (*Cycas circinalis*), respectively. In cases of excessive dietary exposure, BMAA has been implicated in the aetiology of a syndrome which has features of the motorneurone disease ALS, Parkinsonism and dementia. Likewise, BOAA has been identified as a cause of neurolathyrism. Both of these syndromes are associated with the selective degeneration of motorneurones and both BMAA and BOAA have been used to cause this type of damage *in vivo* (Spencer *et al.*, 1986; 1987). A recent study has shown that immunoglobulins from patients with ALS are able to induce apoptotic death of a motorneurone cell line (Alexianu *et al.*, 1994). However, as yet, there is no direct evidence of apoptosis being involved in BMAA- and BOAA-induced neuronal cell death. This study investigates the excitotoxic properties of BMAA and BOAA in comparison with those of glutamate, and the mechanisms of BMAA- and BOAA-induced cell death.

Cerebellar granule cells (CGC) were prepared from 8 day postnatal rats, using a modified procedure (Messer *et al.*, 1977). The CGC were maintained in culture for 9-14 days after which they were exposed to BMAA, BOAA and glutamate for either 30 minutes, 24 or 48 hours. After a 30 minute exposure the CGC were maintained for a further 24 hours. After 24 or 48 hour exposures the CGC were maintained until the end of these time periods when the amount of cell death was assessed using fluorescein diacetate and propidium iodide. Studies to determine the mechanism of BMAA- and BOAA-induced death were carried out over a 72 hour time period using fluorescein diacetate, propidium iodide and DAPI.

The results of this study show that BMAA kills a maximum of 16.4 ± 5.2% (mean ± SEM, n=3) CGC over a 30 minute time period, 39.4 ± 6% (mean ± SEM, n=3) over 24 hours and 43.4 ± 12.9% (mean ± SEM, n=3) after 48 hours. Likewise, BOAA causes a maximum CGC death of 10.6 ± 2.2% (mean ± SEM, n=3) over 30 minutes, 50.6 ± 2.4% (mean ± SEM, n=3) over 24 hours and 72.5 ± 6.2% (mean ± SEM, n=3) after 48 hours of exposure. Glutamate kills a maximum of 69.8 ± 9.6% (mean ± SEM, n=3) CGC after a 30 minute exposure with an EC_{50} of 170.6 ± 17.6μM (mean ± SEM, n=3). CGC death is significantly greater than that of the controls during a 24 and 48 hour exposure to BMAA or BOAA, and during a 30 minute exposure to glutamate ($P<0.05$, Student's unpaired t-test). Initial studies of the mechanisms of excitotoxin-induced cell death indicate that BMAA and BOAA induce a substantial amount of apoptotic, as well as necrotic, cell death.

These findings show that BMAA, BOAA and glutamate cause CGC death in a concentration-dependent fashion. BMAA and BOAA work slowly, requiring a 24 or 48 hour exposure in which to kill a significant amount of CGC. Glutamate, however, requires only 30 minutes to kill equivalent numbers of neurones. Findings also suggest that BMAA and BOAA induce apoptosis. As apoptosis is an active death process, these findings have implications for the possible therapy of neurodegenerative conditions, such as those caused by dietary exposure to excitotoxins.

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190P COMPARISON OF PROSTANOID DP-RECEPTORS IN THE RABBIT ISOLATED SAPHENOUS VEIN AND HUMAN NEUTROPHIL

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Prostanoid receptors have been designated DP, EP, FP, IP and TP-receptors on the basis of agonist and antagonist data and furthermore, EP-receptors have been subclassified into four distinct subtypes (Coleman *et al.*, 1994). It has also been postulated that there may be subtypes of the DP-receptor (Woodward *et al.*, 1993). In the present study we have examined the effects of selective DP-receptor ligands in the rabbit saphenous vein (RbSV) and human neutrophil (PMN).

Isolated rings of saphenous vein from male NZW rabbits were prepared as described previously (Lydford *et al.*, 1994) with 10μM GR32191B in the buffer. Human PMNs were prepared according to Li *et al.*, 1993, for measurement of fMLP stimulated superoxide production. In both preparations, concentration-effect (E/[A]) curves to DP-receptor agonists were constructed in the absence and presence of appropriate antagonists. Data are presented as mean ± s.e ($n \geq 3$).

In the RbSV, the DP-receptor agonists BW245C, ZK 118.182 and PGD_2 caused concentration-dependent relaxations with mean potencies ($p[A_{50}]$) of 7.58 ± 0.11, 7.36 ± 0.05 and 7.00 ± 0.07 respectively.
The potent DP-receptor antagonist BW A868C (0.01 - 100 μM), caused a rightward displacement of BW245C E/[A] curves in a manner consistent with a two receptor system (Lemoine and Kaumann, 1983). Analysis of the data using their model yielded two pK_B estimates of *8.50 ± 0.07 and 4.89 ± 0.08. The higher estimate corresponds to an activity at DP-receptors, the latter to EP_4-receptors (Lydford *et al.*, 1994). The putative DP-receptor antagonist ZK 138.357 (Thierauch *et al.*, 1995) was without significant effect on BW245C E/[A] curves at a concentration of 1 μM however at 30μM, a three-fold shift of BW245C E/[A] curves was observed. In addition, E/[A] curves to BW245C were antagonised by the EP_1/DP-receptor antagonist AH6809 (3 - 30 μM).

In human PMNs, the $p[A_{50}]$s for inhibition of superoxide production by BW245C, ZK 118.182 and PGD_2 were 8.69 ± 0.10, 7.92 ± 0.12 and 7.73 ± 0.14 respectively.
BW A868C (0.1 μM), ZK 138.357 (1 μM) and AH6809 (3 - 30 μM) inhibited these responses with antagonist potencies similar to previously published estimates for DP-receptor blockade (Table 1).

Table 1. Antagonist potencies against BW245C (pA_2 or $*pK_B$)

Antagonist:	BW A868C	ZK 138.357	AH6809
RbSV	*8.50 ± 0.07	†5.05 ± 0.12	5.93 ± 0.05
PMN	†9.46 ± 0.27	†7.25 ± 0.03	6.59 ± 0.19

† Estimates obtained from a single concentration of antagonist

Whilst the agonist potency order is the same in both preparations; BW245C > ZK 118.182 > PGD_2; data obtained with a range of DP-receptor antagonists appears to indicate differences between the DP-receptors on the RbSV and human PMN. Possible interpretations of the antagonist data is that the differences are due to species variation or interactions with other prostanoid receptors. However, the change in antagonist potency orders between the two preparations may indicate activities at different receptor subtypes.

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191P CHARACTERISATION OF P_{2X} PURINOCEPTORS IN GUINEA-PIG ISOLATED URINARY BLADDER STRIPS USING AGONISTS, ANTAGONISTS AND THE ECTO-ATPase INHIBITOR ARL 67156

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The effects of the three P_2-purinoceptor antagonists suramin pyridoxal 5'-phosphate (P-5-P) and pyridoxal phosphate-6-azo-phenyl-2, 4-disulphonic acid (PPADS) on contractile response to application of adenosine 5'-triphosphate (ATP), P^1, P^5-di-(adenosine-5') tetraphosphate (AP_4A) and P^1, P^5-di-(adenosine 5') pentaphosphate (AP_5A) have been examined in isolated strips of the guinea-pig urinary bladder. The effect of the ecto-ATPase inhibitor 6-N,N,diethyl-D-β,γ-dibromo methyleneATP (ARL 67156) on agonist potency was also assessed.

Isolated strips of guinea-pig urinary bladder were mounted in a modified Krebs solution at 36°C and bubbled with 95% O_2/5% CO_2. Concentration effect curves to ATP, AP_4A and AP_5A showed no clear maximum, so it was not possible to measure EC_{50} concentrations. Therefore, equi-effective concentrations of each agonist were estimated as the concentration which gave a contractile response of about 50% of that obtained to 1000 μM ATP. These concentrations, ATP 300 μM, AP_4A 30 μM and AP_5A 3 μM, were used in subsequent experiments to obtain the IC_{50} values shown in Table 1.

PPADS and P-5-P were effective antagonists against all three agonists, with PPADS being up to thirty-five times more potent than P-5-P. Suramin was an effective antagonist against AP_4A and AP_5A, and at 100 μM almost abolished responses to both agonists. However, responses to ATP were not effectively antagonised by suramin. Even 1000 μM suramin only reduced responses to ATP to 71.6 ± 4.7% of control (n = 6), so that calculation of an IC_{50} was not applicable (denoted as N.A. in table 1). The contrasting effects of suramin on the rapidly hydrolyzable agonist ATP and the more stable diadenosine compounds may be due to inhibition of ecto-ATPases by suramin, preventing the breakdown of ATP, thus enhancing its effect as an agonist. This possibility was examined using the novel ecto-ATPase inhibitor ARL 67156 (Crack *et al*, 1995). ARL 67156 (100 μM) significantly enhanced responses to exogenous ATP (from 2.62 ± 0.54 g to 3.42 ± 0.65 g) and AP_4A (from 2.29 ± 0.43 g to 2.69 ± 0.48 g), whereas responses to AP_5A were not affected (from 2.82 ± 0.73 g to 2.64 ± 0.76 g). All comparisons are by Students' t-test for paired data, and considered significant if $P<0.05$, n = 6-8.

These results imply that the ability of suramin to inhibit responses to AP_4A and AP_5A more effectively than those to ATP cannot be explained on the basis of inhibition of ecto-ATPase activity by suramin.

Table 1. IC_{50} values (μM) for P_2-purinoceptor antagonists in guinea-pig isolated bladder strips. (n = 6).

	ATP	AP_4A	AP_5A
PPADS	4.9 ± 1.8	12.4 ± 2.9	9.1 ± 3.0
P-5-P	178 ± 81	70 ± 15	164 ± 55
Suramin	N.A.	10.7 ± 1.5	24.5 ± 1.0

Crack, B.E., Pollard, C.E., Beukers, M.W. *et al.* (1995) *Br. J. Pharmacol.*, 114, 475-481.

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192P ENDOTHELIN-1-MEDIATED A7r5 SMOOTH MUSCLE CELL ACTIVATION: SEPARATION OF DUAL EFFECT BY VERAPAMIL AND 8Br-cGMP

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Intracellular calcium ions are central in the response of smooth muscle cells to endothelin-1 (ET-1). ET receptor activation results in the turnover of phosphoinositides and subsequent release of calcium ions from intracellular stores (Cioffi & Garay, 1993). In addition, the influx of extracellular Ca^{2+} contributes to the rise in intracellular Ca^{2+} (Yang *et al.*, 1994). The action of ET-1 on the smooth muscle cell line A7r5 was studied using microphysiometry, i.e. extracellular acidification as a functional parameter. For comparison, phosphoinositide turnover in A7r5 cells in response to ET-1 was studied. In addition, we studied the effect of blocking the influx of extracellular Ca^{2+} on the ET-1 mediated response.

A7r5 cells (obtained from ATCC) were grown to confluency in 2 days time after seeding $5x10^4$ cells/cup - in DMEM with 10(v/v)% foetal calf serum - on filters of the 'capsule cups' provided by the manufacturer of the microphysiometer (Molecular Devices). The A7r5 cells - in between two filters - were placed in a 'sensor chamber' of the microphysiometer for the determination of the extracellular acidification rate; to this end cells were cyclically superfused with unbuffered DMEM for a 90 s period followed by a 30 s interval during which the change in pH was recorded. Extracellular acidification rates of each incubation were expressed as percentage of the basal level (that were within the range of -35 to -70 μV/s). ET-1 (0.01 - 1 μM, Sigma) was applied to the cells for 75 s commencing 45 s prior to a 30 s rate data collection period. ET antagonists (BQ-123 from Neosystem and bosentan, kind gift of Roche) were applied to the cells together with ET-1. Verapamil and 8Br-cGMP (Sigma) were given for at least 40 min, commencing at least 20 min prior to ET-1 stimulation. Effects of agents on extracellular acidification were averaged for 2 to 4 channels; EC_{50} and IC_{50} values were calculated from 4 to 5 point dose-response curve fits, and represent means of N determinations with standard error.

ET-1 elicited an increase in extracellular acidification rates of the A7r5 cells for 8 min, the maximal increase in extracellular acidification rate being 50% over basal levels and the EC_{50} for ET-1 was 35.1 ± 5.5 nM (N=3). The ET_A receptor antagonist BQ-123 (0.1-30 μM) blocked the latter response to ET-1 (0.1 μM) with an IC_{50} value of 99.8 ± 81 nM (N=4). Experiments on phosphoinositide turnover in A7r5 cells yielded an EC_{50} of 0.34 ± 0.2 nM (N=3) for ET-1. The response to ET-1 (3 nM) was blocked by BQ-123; IC_{50} value 247 ± 39 nM (N=3). Verapamil (10 nM - 10 μM) applied 30 min before ET-1 (30 nM) did not affect the ET-1 mediated increase in extracellular acidification in A7r5 cells. However, upon withdrawal of verapamil, 20 min after the ET-1 application, a transient increase (max. 10% of basal levels) in extracellular acidification was observed. This delayed response was ET-1 dependent, but not blocked by either BQ-123 (1 μM) or by the less specific ET receptor antagonist bosentan (1 μM). The verapamil dependent delayed response induced by ET-1 were maximal at 1 μM verapamil, EC_{50} was 0.1 ± 0.01 μM (N=5). In comparison to verapamil, 8Br-cGMP (10 μM) also induced a delayed response (upon withdrawal) on the ET-1 elicited cellular response. In contrast to verapamil, however, 8Br-cGMP itself stimulated cellular metabolism, an effect that was abolished by ET-1 (30 nM).

Thus, the dual response to ET-1 of A7r5 smooth muscle cells predicted from intracellular calcium studies can be observed using the microphysiometry response. The main response is likely to be linked to the ET_A receptor and subsequent phospholipase C activation, although the response to ET-1 is observed at a 100 fold higher concentration. A second response to ET-1 in A7r5 cells appears to be delayed by the presence of either verapamil or 8Br-cGMP. In addition, the delayed response was not antagonized by either of the ET receptor antagonists bosentan and BQ-123.

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193P PHARMACOLOGICAL CHARACTERISATION OF THE INWARDLY-RECTIFYING CURRENT IN THE SMOOTH MUSCLE CELLS OF RAT BLADDER

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The aim of the present study was to investigate whether the inwardly-rectifying current in rat detrusor cells (Green *et al.*, 1995) resembled the hyperpolarisation-activated cation current (I_h) described in the rabbit jejunum (Benham *et al.*, 1987) or the arteriolar inwardly-rectifying K-current (Quayle *et al.*, 1993).

Single smooth muscle cells were isolated from segments of rat detrusor by enzymatic treatment and recordings of whole-cell K-currents were made under calcium-free conditions. Cells were subjected to a series of test potentials (-140mV to +50mV) from a holding potential of -10mV. With test potentials negative to -80mV, an inwardly-rectifying current with time-dependent activation was observed. When the external K^+ concentration ($[K^+]_o$) was either increased to 21.2mM or reduced to 2.2mM (from 6mM) the magnitude of inward current at -120mV was either increased by 76.4 ± 12%, n=4 or decreased by 40.1 ± 11%, n=4, respectively. In contrast, the voltage-dependence of the current was little affected by these changes in $[K^+]_o$. Decreasing $[Na^+]_o$ to 13mM (from 128mM) also reduced the magnitude of the inwardly-rectifying current (by 31.3 ± 6%, n=4 at -120mV). The addition of Ca^{2+} (2.5mM) to the external solution had no effect on the magnitude of the inward current. Furthermore, in contrast to the arteriolar inwardly-rectifying K-current (Quayle *et al.*, 1993), the detrusor current was relatively insensitive to 1mM Ba^{2+} but was more sensitive to 1mM Cs^{2+} (at 120mV, the inward current was reduced by 44.1 ± 12%, n=4 and 57.6 ± 6%, n=4, respectively). D7288, a blocker of I_f in the guinea-pig sinoatrial node (BoSmith *et al.*, 1993), reduced the inwardly-rectifying current in a concentration-dependent manner (10-100μM), with almost full inhibition at 100μM, (Figure 1).

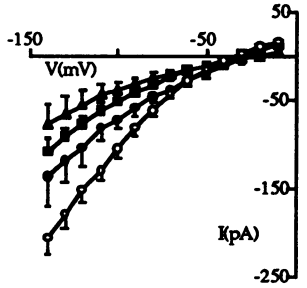

Figure 1: Effects of D7288 on rat detrusor membrane currents. Control, ○; D7288; 10μM, ●; 30μM, ■; 100μM, ▲. Each point represents mean ± s.e.mean, n=4.

Collectively these data demonstrate that the inwardly-rectifying current in rat bladder exhibits the features of the cation current I_h, described in rabbit jejunum.

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194P TRACHEOBRONCHIAL DISTRIBUTION AND DENSITY OF TACHYKININ NK_1 AND NK_2 RECEPTORS IN NORMAL AND MULTIPLE ANTIGEN-CHALLENGED GUINEA-PIGS

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Following multiple antigen challenge, a remarkable hyperresponsiveness to stimulation of NANC vagal nerves associated with an increase in tachykinins (NK_1)-mediated bronchospasm has been recently described (Perretti et al., 1995). Therefore we have studied by '*in vitro*' autoradiography the distribution and density, in trachea and lung, of tachykinin NK_1 and NK_2 binding sites in control and repeatedly antigen-challenged guinea pig. Male Dunkin-Hartley guinea pigs (350-400 g.) were sensitized with 100 mg/Kg of ovalbumin administered before i.p. and immediately after s.c. (vehicle for the controls) (n=3-4 in each group). Two weeks later animals were weekly challenged with an aerosol of water (water-challenged) or ovalbumin (antigen-challenged) for three weeks, then killed seven days later. Tissue sections (10μm thick) were incubated with 0.25nM [^{3}H]CP 96,345 (52.2 Ci/mmol) or 5nM [^{3}H]SR 48968 (23 Ci/mmol) for 30 min at 22°C in 50mM Tris-HCl buffer (pH 7.4) containing 5mM $MnCl_2$ plus 0.02% BSA. Non-specific binding was determined using 1μM unlabeled CP 96,345 or SR 48968, respectively. Specific labelling of [^{3}H]CP 96,345 was found over tracheobronchial smooth muscle, bronchial epithelium and pulmonary blood vessels. Labelling was very dense in bronchial rings both in epithelium and smooth muscle layers. For [^{3}H]SR 48968, autoradiographic localization was restricted to bronchial and tracheal smooth muscles and the density was lower as compared to [^{3}H]CP 96,345 binding sites. No remarkable difference in the distribution of tachykinin receptors was observed between different groups. A significant decrease in the optical density for both tachykinin NK_1 and NK_2 receptors over all structures was observed in antigen-challenged group compared to control. Results are shown in table as mean ± s.e. mean.

Structure	Treatment	NK_1	NK_2
Tracheal	Control	8.1±0.3	3.1±0.2
smooth	Water-challenged	8.4±0.6	3.1±0.3
muscle	Antigen-challenged	3.5±0.3**	2.2±0.3*
Bronchial	Control	14.6±0.8	N.D.
epithelium	Water-challenged	12.4±0.4	N.D.
	Antigen-challenged	9.0±0.4**	N.D.
Bronchial	Control	16.1±0.4	9.6±0.4
smooth	Water-challenged	16.5±0.5	10.5±0.4
muscle	Antigen-challenged	11.6±0.6**	8.5±0.2*
Pulmonary	Control	3.9±0.3	N.D.
smooth	Water-challenged	3.3±0.3	N.D.
muscle	Antigen-challenged	1.9±0.2**	N.D.

*P<0.05 **P<0.01 vs. control (1 way ANOVA and TUKEY tests). N.D.= not detected

Present results are indicative that the increase in functional response to selective tachykinin NK_1 receptor agonists, detected in multiple antigen-challenged guinea pigs, is not linked to an enhanced expression of the tachykinin NK_1 receptors. Other factors such as neuropeptide access to the receptors, reduced metabolic breakdown and/or greater efficacy in transduction mechanism(s) might be involved.

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195P PHARMACOLOGICAL ANALYSIS OF THE CCK_B/GASTRIN RECEPTORS INVOLVED IN PENTAGASTRIN-STIMULATED GASTRIC ACID SECRETION IN THE ISOLATED IMMATURE RAT STOMACH

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Pentagastrin stimulates gastric acid secretion in the rat stomach both directly and indirectly, via histamine release, (Shankley *et al.*, 1992) by interacting with CCK_B/gastrin receptors situated on oxyntic and enterochromaffin-like (ECL) cells, respectively. Established methods and protocols on isolated stomach preparations from immature rats (Welsh *et al*, 1994) were used in this study. The interactions between pentagastrin and three chemically distinct CCK_B/gastrin receptor antagonists (L-365,260, PD134,308 and JB93190 (5-(3S)-2,5-Diaza-5-(3,5-dicarboxyphenyl)-1,4-dioxo-3-(methylphenyl)pentanyl)-6-[3-(1-adamantyl)-2-aza-1-oxopropyl]benzimdazole) (Kalindjian *et al.*, 1996) were investigated in the absence and presence of the histamine H_2-receptor antagonist famotidine (30μM, 1000 × K_B). The CCK_B/gastrin receptors on oxyntic cells alone could then be compared with those mediating the combined response of oxyntic and ECL cells.

Famotidine consistently depressed the acid secretory response of the stomachs to pentagastrin. The degree of depression varied between experiments with direct stimulation of the oxyntic cell by pentagastrin accounting for between 40-75% of the total secretory output. In the presence of famotidine, the three CCK_B/gastrin receptor antagonists shifted, in parallel and to the right, pentagastrin concentration-effect curves without affecting the maximum response. This was also true in the absence of famotidine with the exception of JB93190 which steepened the pentagastrin concentration-effect curves. However, as Schild analysis yielded a slope parameter not differing from unity a pK_B value was calculated. Schild analysis did not reveal any differences between individual antagonist affinities in the absence and presence of famotidine (Table 1). None of the antagonists, at the highest concentrations used, affected the secretory response to exogenous histamine (data not shown). The data could be simulated with a simple model in which the direct and indirect components of the pentagastrin response added to give total secretion. In the model the best fit was obtained when the pK_B values were the same at the two cells. In conclusion, we found no evidence for CCK_B/gastrin receptor heterogeneity between oxyntic and ECL cells or within either cell population in the immature rat stomach.

Table 1.

	control			famotidine treated (30μM)		
	pK_B ± s.e.	slope ± s.e.	df	pK_B ± s.e.	slope ± s.e	df
L-365,260	7.49 ± 0.20*	0.82 ± 0.07*	35	7.10 ± 0.12	0.84 ± 0.10	35
PD134,308	7.61 ± 0.11	0.92 ± 0.10	28	7.36 ± 0.10	1.20 ± 0.11	23
JB93190	9.08 ± 0.10	1.22 ± 0.15	24	8.94 ± 0.15	1.32 ± 0.24	24

*Schild slope significantly different from unity therefore $pK_B = pA_2$

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196P INDUCTION OF B_1-RECEPTOR-MEDIATED FUNCTIONAL RESPONSES IN MOUSE URINARY BLADDER: EFFECTS OF GENETIC DEPLETION OF THE B_2-RECEPTOR GENE

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Bradykinin can stimulate the production of prostanoids and cytokines, an effect which may be mediated via the B_2-receptor. In addition, endogenous IL-1 production can induce responses to [des-Arg^9]-BK (a B_1-receptor agonist, deBlois et al., 1991). To investigate the role of B_2-receptors in the induction of B_1-receptor responses, we investigated the induction of B_1-receptor mediated responses in mouse urinary bladder from animals in which the B_2-receptor gene had been knocked out.

Bladders were obtained from control mice (J129 strain, wild type *Bk2r*$^{+/+}$) or B_2-receptor knockout mice (*Bk2r*$^{-/-}$, Borkowski et al., 1995). The bladders were divided in half longitudinally and mounted for isometric tension recording in organ baths containing Kreb's physiological salt solution (37°C, pH 7.4, aerated with 95%O_2, 5%CO_2). Sequential concentration-effects curves to bradykinin or [des-Arg^9]-BK were obtained in the presence of captopril (1μM) and thiorphan (10μM). For the induction of responses to [des-Arg^9]-BK sections of bladder were incubated (45min or 18h) in vials containing Kreb's physiological salt (10ml, 37°C, pH 7.4, 95%O_2, 5%CO_2). All responses were expressed as a percentage of the reference response 50mM KCl.

In control mice, bradykinin (0.01-3μM) evoked a contractile response (EC_{50} = 128 ± 43nM, E_{max} = 115 ± 10.5%) which was antagonised by Hoe 140 (pA_2 = 10.3 slope = 0.7). [des-Arg^9]-BK (0.001-10μM) was without effect. Following incubation (45min, 37°C) contractile responses to [des-Arg^9]-BK (0.01-3μM, E_{max} = 4.3 ± 1.7%, n = 4) were seen, but the magnitude of these responses were higher following 18h incubation at 37°C (EC_{50} = 83.0 ± 11.6nM, E_{max} 24.9 ± 2.5%; n = 4). Co-incubation (18h, 37°C) with Hoe 140 300nM or bradykinin (3μM) did not effect the induction of the responses to [des-Arg^9]-BK. For B_2-receptor knockout mice, bradykinin (1μM) and [des-Arg^9]-BK (1-30μM) had no effect in non-incubated tissue sections (n = 4). Following incubation (18h, 37°C) a contractile response to [des-Arg^9]-BK was observed (EC_{50} 114.8 ± 71.2nM, E_{max} = 11.2 ± 1.4%, n = 4) and this response was not altered by incubation (18h, 37°C) with IL-1ß (30u/ml, EC_{50} 157.5 ± 60.8nM), E_{max} = 9.9 ± 1.8%, n = 4). For control and B_2-receptor knock-out mice, [des-Arg^9]-BK-evoked contractions were antagonised by [Leu^8]-BK 10μM (a B_1-receptor antagonist) in a non-competitive manner (in the absence and presence of [Leu^8]-BK, the response to [des-Arg^9]-BK 0.3μM in control mice was 16.7% and 6.4% respectively and in B_2-receptor knock-out mice it was 5.3% and 2.0% respectively).

Induction of contractile responses to the B_1-receptor agonist [des-Arg^9]-BK were seen both in control and B_2-receptor knockout mice and this response was blocked by a peptide B_1-receptor antagonist. The induction of [des-Arg^9]BK evoked responses is not dependent on B_2-receptor expression.

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197P ADRENOCEPTOR-MEDIATED MODULATION OF 5-HT RELEASE FROM NEUROEPITHELIAL CELLS OF TRACHEA OF NEWBORN RABBITS

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Neuroepithelial cells (NECs) are distributed throughout the airway mucosa of vertebrates and are rich in 5-HT (see Scheuerman *et al.*, 1989). Recently, we provided evidence that 5-HT release from isolated rabbit tracheae may reflect 5-HT secretion of NECs (Freitag et al., 1995a,b). Moreover, *in vitro* release of 5-HT from tracheae of newborn rabbits was markedly higher than that from tracheae of adults (Freitag et al., 1995a,b), an observation which correlated to the finding that NECs are particularly enriched during the perinatal period. In first pharmacological experiments it was shown that adrenaline, via activation of α-adrenoceptors, enhanced 5-HT release from rabbit tracheae (Freitag et al., 1995b). In the present experiments the adrenergic control of 5-HT secretion from NECs is further characterized.

Isolated tracheae of newborn rabbits were incubated in modified Krebs-HEPES medium. The overflow of 5-HT into the incubation media, which were changed every 10 min, and 5-HT in tissue extracts was determined by HPLC with electrochemical detection. Values given are means (±s.e.m.) of at least 4 experiments.

The spontaneous outflow of 5-HT determined between 50 and 60 min of incubation in the absence of test drugs was 15.2±0.9 pmol/g/10 min (n=30). It declined by about 30 % between 60 and 90 min of incubation. Tissue 5-HT determined at the end of incubation amounted to 612±39 pmol/g. Phenylephrine (1 and 10 μM) enhanced 5-HT outflow by about 70 and 325 %, respectively. The effect of 10 μM phenylephrine was blocked by 1 μM yohimbine and largely inhibited (by 75 %) in the presence of 1 μM prazosin. Isoprenaline inhibited both, the spontaneous outflow of 5-HT and the phenylephrine-evoked release of 5-HT, although with different potencies. At 100 nM isoprenaline inhibited spontaneous outflow almost maximally (by about 35 %), whereas the phenylephrine-evoked release was not significantly affected by 100 nM isoprenaline. However, phenylephrine-evoked release of 5-HT was reduced by about 50 % in the presence of 1 μM isoprenaline and almost abolished in the presence of 10 μM isoprenaline. Forskolin (10 μM) reduced the spontaneous outflow of 5-HT by about 35 % and prevented also the phenylephrine-evoked release of 5-HT.

In conclusion, α-adrenoceptors (possibly of an α_2-subtype) mediate stimulation of 5-HT release from NECs of rabbit trachea. Forskolin causes inhibition of 5-HT release and opposes the α-adrenoceptor-mediated facilitation of 5-HT release. Furthermore, activation of ß-adrenoceptor appears also to mediate inhibition of 5-HT release, and the involvement of cAMP in this response appears to be likely.

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198P ATYPICAL AFFINITIES OF ICI 118,551 AT PORCINE β_2-ADRENOCEPTORS

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Species homologues of adrenoceptor subtypes may have considerably different affinities for certain drugs despite only minor differences at the amino acid sequence level. CGP 20712A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanololmethansulfonate) and ICI 118,551 (erythro-D,L-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol HCl) are among the most selective ligands to differentiate β_1- and β_2-adrenoceptors. Therefore, we have determined affinities for the β_1-adrenoceptor-selective antagonist, CGP 20712A, and the β_2-adrenoceptor-selective antagonist, ICI 118,551, in five porcine tissues expected to contain mainly β_2-adrenoceptors, aortic vascular smooth muscle, cultured aortic endothelium cells, urinary bladder corpus, urinary bladder infundibulum, and prostate. Radioligand binding was performed using [^{125}I]iodocyanopindolol (ICYP) as previously descrbied (Michel et al. 1993) with definition of non-specific binding by 10 μM (-)-isoprenaline. Data are mean ± S.E. mean of 3-6 experiments; drug affinities are given as -log K_i.

ß-Adrenoceptor density as determined from ICYP saturation experiments was 10 ± 1 (vascular smooth muscle), 20 ± 6 (endothelium), 46 ± 7 (bladder corpus), 36 ± 5 (bladder infundibulum), and 58 ± 17 fmol/mg protein (prostate) with K_d values of 12 ± 2, 9 ± 1, 27 ± 10, 32 ± 8, and 55 ± 20 pM, respectively.

In vascular smooth muscle and endothelium CGP 20712A competed for ICYP binding with steep and monophasic curves of low affinity (5.36 ± 0.16 and 5.30 ± 0.12) indicating a homogeneous population of β_2-adrenoceptors. In these two preparations ICI 118,551 also had steep and monophasic curves of rather low affinity (6.90 ± 0.14 and 6.97 ± 0.16). In the urogenital tissues CGP 20712A competed for ICYP binding with shallow and biphasic curves yielding 16 ± 9%, 18 ± 4% and 42 ± 14% high affinity sites in bladder courpus, bladder infundibulum and prostate, respectively. The respective drug affinities were 9.36 ± 0.17, 8.69 ± 0.26 and 9.01 ± 0.20 at the high affinity site and 5.52 ± 0.13, 5.22 ± 0.28 and 5.03 ± 0.11 at the low affinity site indicating the concomitant presence of β_1- and β_2-adrenoceptors. In contrast ICI 118,551 had steeper competition curves in these tissues which could not be resolved into multiple components, and the drug affinities were low (6.83 ± 0.08 in bladder corpus, 6.98 ± 0.03 in bladder infundibulum, 6.85 ± 0.14 in prostate).

Based on our CGP 20712A experiments we conclude that all five porcine tissues contain mainly β_2-adrenoceptors with a minor component of β_1-adrenoceptors in the urogenital tissues. The present and previous data (Nishimura et al. 1987) indicate that ICI 118,551 has only relatively low affinity at porcine β_2-adrenoceptors.

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199P INHIBITORY EFFECTS OF RUBIDIUM ON THE ACTIONS OF POTASSIUM CHANNEL MODULATORS IN MOUSE ILEUM ARE APPARENT AT A PHYSIOLOGICAL POTASSIUM CONCENTRATION

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In previous experiments using intestine, rubidium (Rb) was observed to inhibit relaxant responses to the potassium (K) channel opener, pinacidil (PIN) in Krebs' solution (KS) in which increasing concentrations of Rb progressively replaced K: the action of glibenclamide (GBC), a blocker of ATP-sensitive K channels, was also greatly attenuated (Yeung *et al.*, 1995a, b). To investigate whether this inhibition depends on the absolute concentration of Rb or on reduction in K, the relaxant responses to PIN and antagonism in the presence of GBC were examined in ileum exposed to KS in which a proportion of sodium chloride (NaCl) was progressively replaced by equimolar concentrations of RbCl, the concentration of K being constant (5.95mM).

Mucosa-free preparations from the distal ileum of male BKW mice (28-40g) were placed under 0.5g tension (37°C, 95% O_2/5% CO_2) in normal KS containing 118mM NaCl or in KS where NaCl was substituted by increasing equimolar concentrations of RbCl (0.01-10mM). Isometric contractions were elicited by electrical field stimulation (0.5Hz, 30V, 1msec pulse width). When twitch height was constant, a cumulative concentration-response curve to PIN (0.1-200μM) or vehicle was performed. This procedure was repeated following 20min incubation with a single concentration of GBC (0.1-1μM) or vehicle. Relaxant potency was expressed as geometric mean EC_{50} (the concentration required to reduce twitch height by 50%) with 95% CL. The initial twitch response is expressed as tension (g) ± s.e.mean, and the effect of GBC in terms of PIN dose ratio ± s.e.mean.

Neither PIN-induced relaxation nor the initial twitch height was affected when KS contained 1mM Rb or less (Table 1). However, the relaxant response to PIN and its antagonism by GBC (1μM) was attenuated when Rb reached 10mM. GBC alone and the vehicle for PIN and GBC produced no effect (n≥4).

These results suggest that the inhibitory effect of Rb on the actions of PIN and GBC can still occur in the presence of a fixed concentration of K and therefore the previous results cannot be explained by a reduction in K concentration.

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Table 1 Effect of replacing NaCl in Krebs' solution with increasing concentrations of rubidium on responses to pinacidil alone, and in the presence of glibenclamide in mouse ileum.

			Pinacidil		§Pinacidil + Glibenclamide	
Rb (mM)	Twitch Height (g)	n	EC_{50} (μM) (mean, 95% CL)	n	GBC 0.1μM	GBC 1.0μM
Normal Krebs'	0.68±0.05	14	1.62 (1.33-2.19)	14	2.72±0.37	14.45±1.70
0.01	0.71±0.03	8	1.08 (0.66-1.78)	8	2.85±0.45	11.37±2.56
0.10	0.71±0.04	10	1.04 (0.79-1.40)	8	2.17±0.40	11.44±2.96
1.00	0.61±0.08	8	1.42 (1.00-2.05)	8	2.25±0.35	13.63±3.72
10.00	0.65±0.06	8	5.72 (1.89-12.56)**	8	3.63±1.05	7.37±1.06*

*P<0.05, **P<0.01 (Normal Krebs' solution vs 10mM Rb, Student's unpaired t-test); § (Dose ratio ± s.e.mean; n=4 in all cases).

200P RELAXANT EFFECTS OF LEVCROMAKALIM AND YM 934 IN HUMAN ISOLATED DETRUSOR STRIPS FROM STABLE AND UNSTABLE BLADDERS

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Recent studies have demonstrated the presence of ATP-sensitive potassium channels (K_{ATP} channels) in detrusor muscle of several species, including human where cromakalim reduces spontaneous contractions and contractions elicited by acetylcholine (1). The aims of the present investigation were to compare responses obtained to YM 934 and levcromakalim in preparations of normal, stable bladder with those obtained in 'unstable' bladder muscle.

Stable bladder was obtained from consenting patients undergoing cystectomy for bladder cancer and from organ donors. Unstable bladder was obtained from consenting patients undergoing 'clam' cystoplasty. Following removal of mucosa and serosa the detrusor muscle was cut into strips & were then set-up under 1g tension in 5ml organ baths containing carboxygenated Krebs-bicarbonate solution at 37°C. Isometric tensions were recorded. The concentration of carbachol producing an 80-90% maximal contraction was determined by construction of cumulative concentration-response curves and was used to contract the preparations prior to addition of levcromakalim or YM 934 (2). Geometric mean EC50 values (with 95% confidence limits) or mean maximum values (± sem) were compared using Student's unpaired t-test (two-tailed).
The potency of carbachol in the unstable strips (0.18(0.14-0.24)μM, n = 32) was double its value in stable preparations (0.36(0.27-0.48)μM, n = 25); (p = 0.002). However, the mean maximum contractile response to carbachol in the unstable group was significantly lower (p = 0.02) than in the stable group (6.40±0.62g and 8.83±0.78g, respectively).
The EC50 values for the relaxant effect of YM 934 in stable (0.14(0.09-0.72)μM, n = 13) and unstable bladder (0.19(0.15-0.24)μM, n = 15) were not significantly different. In absolute units a significant difference was observed between the maximum response to YM 934 in the two groups (3.3 ± 0.47g and 2.33 ± 0.23g for stable and unstable detrusor respectively). This, however, reflects differences in the level of pre-contraction by carbachol in stable and unstable bladder. When the concentration-response curves for YM 934 were plotted as a percentage of carbachol-induced pre-contraction, the maximum relaxation was not significantly different for stable and unstable preparations , 70.41 ± 4.33, stable ; 67.71 ± 2.89, unstable).
Similarly, there was no significant difference in the potency of levcromakalim in stable (0.27(0.16-0.47)μM, n = 12) and unstable (0.25(0.16-0.39)μM, n = 17) bladder preparations. Expressed as a percentage of carbachol induced pre-contraction, the maximum relaxatory response to levcromakalim in unstable bladder (69.92 ± 2.84) was not signifiacantly different from its value in the stable preparations (62.07 ± 4.17).

The results confirm an increase in potency of carbachol in the unstable bladder and in addition demonstrate that relaxant responses to K_{ATP} channel openers do not differ between stable and unstable bladder.

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201P FACILITATION BY 5-HT OF ATP-MEDIATED ELECTRICALLY-STIMULATED CONTRACTIONS IN THE PIG URINARY BLADDER

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5-hydroxytryptamine (5-HT) has been implicated in the control of neuromuscular transmission in the urinary bladder and may play a role in disturbances of bladder functioning (Waikar *et al*, 1994). Bladder responsiveness to 5-HT shows significant species variation. When electrically stimulated tissues are examined, both pre-junctional excitatory (human, mouse and guinea-pig) and post-junctional inhibitory (monkey, bullfrog) effects of 5-HT have been reported (e.g. see Hindmarsh *et al*, 1977; Tonini *et al*, 1994). In the present study we have examined the effects of 5-HT on cholinergic and purinergic neuromuscular transmission in the pig bladder detrusor.

Pig bladders, obtained from a local abattoir, were used in electrical field-stimulation (EFS) experiments on the day after removal as described by Corsi *et al* (1991). Longitudinal tissue strips (10mm) were prepared from the bladder dome, suspended between platinum electrodes and placed under 1g tension in 15 ml tissue baths containing aerated Krebs solution. After a 1 hour equilibration period, tissues were electrically stimulated and contractile responses monitored. Trains (5s) of electrical pulses were delivered at intervals of 100s, pulse width 0.1msec with supramaximal voltage (50V) and submaximal frequency (4Hz: ~30% of the maximum contractile response to 32Hz).

Incubation with 5-HT (10nM-10μM) resulted in concentration-dependent enhancement of the response to EFS, giving a mean EC_{50} value of 0.9μM (95% CL 0.79-1.11, n=4) and a maximal enhancement of 176±44% (mean±s.e.mean, n=4). Contractile responses in the presence and absence of 5-HT were abolished by tetrodotoxin (1μM, n=3), indicating a neurogenic mechanism.

Blockade of muscarinic receptors by atropine (1μM) resulted in 46±13% (mean±s.e.mean, n=3) inhibition of the contractile response to EFS. However, atropine had no effect on the enhancement of the contractile response by 3μM 5-HT: 71±24% increase in controls and 68±18% increase in the presence of atropine (mean±s.e.mean, n=3). Conversely, desensitisation of P_2 purinoceptors by α,β-methylene-ATP (30μM) had no significant effect on the contractile response to EFS *per se* (112±16% of control, mean±s.e.mean, n=3) but inhibited the 5-HT(3μM)-induced enhancement by 86±8% (mean±s.e.mean, n=3).

Post-junctional effects of 5-HT on bladder contractility were examined by performing cumulative concentration-effect curves to acetylcholine and α,β-methylene-ATP in the presence and absence of 10μM 5-HT. 5-HT did not potentiate the direct contractile response to either acetylcholine [control EC_{50} value = 15.4μM (12.8-18.5); EC_{50} value in presence of 5-HT = 34.1μM (32.4-36.0)] or α,β-methylene-ATP [control EC_{50} value = 4.3μM (3.4-5.5); EC_{50} value in presence of 5-HT = 9.0μM (6.2-11.3)] (95% CL, n=3).

In conclusion, these results indicate that, in the pig urinary bladder, purinergic but not cholinergic neuromuscular transmission is facilitated by prejunctional 5-HT receptors. Further studies are required to characterise the 5-HT receptor sub-type(s) mediating this response.

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202P *IN VIVO* BLADDER SELECTIVITY OF THE NOVEL MUSCARINIC ANTAGONIST, DARIFENACIN, IN THE CONSCIOUS MINIPIG

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Darifenacin ((S)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide) is a new competitive muscarinic antagonist which has marked *in vitro* selectivity for bladder M_3 receptors over neuronal M_1 and cardiac M_2 receptors (Wallis *et al.*,1995). We have conducted experiments in the conscious minipig to ascertain the oral efficacy and selectivity of darifenacin *in vivo* on bladder parameters in comparison with oxybutynin (Ditropan), a treatment currently available for incontinence.

Experiments were conducted in female Göttingen minipigs (n=4) each of which had chronically-implanted bladder catheters and a venous catheter in the inferior vena cava. Filling cystometry was performed at 30 min intervals. The parameters measured were detrusor pressure and heart rate from a lead II ECG. Compounds were administered orally after three control voids and the responses followed for three hours thereafter.

Darifenacin administration (1-10mgkg^{-1}) induced a dose-related inhibition (up to 75%, ranging 70-82%, $p<0.05$) of peak micturition pressure (PMAX), with a calculated ED_{50} value (dose to reduce PMAX by 50%) of 3mgkg^{-1}. Importantly, darifenacin caused no tachycardia at any dose tested. In the same group of animals oxybutynin (10-60mgkg^{-1}) was approximately 13 fold less potent on PMAX with an ED_{50} value of 43mgkg^{-1}. In addition oxybutynin produced significant tachycardia (up to 61%, $p<0.01$) at a dose level of 30mgkg^{-1}.

As oxybutynin is subject to high first-pass metabolism in man, yielding an active metabolite 10 times as potent as the parent compound (Douchamps *et al.*,1988), minipigs were dosed directly with this metabolite (N-desethyl oxybutynin) at 3 and 10mgkg^{-1}. These experiments were performed in a second group of pigs (n=3) where darifenacin (10mgkg^{-1}) was also administered for comparison. Mean control PMAX and heart rates ranged between 11-14mmHg and 90-101bpm respectively, compared with 10-16mmHg and 83-102bpm respectively in the first group of animals. The metabolite induced a dose-related inhibition (up to 57%, ranging 43-73%) of PMAX, while increasing heart rate by 41% at the highest dose. In comparison, darifenacin induced a similar decrease in PMAX (up to 58%, ranging 36-66%) with no effects on heart rate. Thus, darifenacin has a similar potency on the bladder when administered orally to conscious minipigs, but has greater selectivity over the heart than the N-desethyl oxybutynin.

These data show darifenacin is a novel M_3 selective muscarinic receptor antagonist which exhibits potent inhibitory effects on pig bladder *in vivo* at doses which do not affect heart rate.

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203P EVIDENCE FOR NK_3 RECEPTOR SUBTYPES IN RABBIT ISOLATED IRIS SPHINCTER MUSCLE USING THE NK_3 RECEPTOR ANTAGONISTS SR 142801 AND SR 48968

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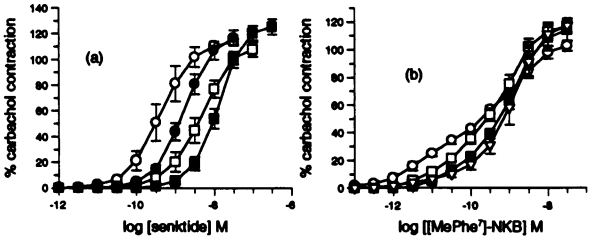
The selective NK-3 receptor agonists senktide and [MePhe^7]-NKB contract the rabbit isolated iris sphincter muscle (Hall et al., 1991; Wang & Hakanson, 1993). We have characterised these responses using the novel NK-3 receptor antagonist, SR 142801 (Emonds-Alt et al., 1995) and the mixed NK-2/NK-3 receptor antagonist SR 48968 (Petitet et al., 1993).

Rabbit iris sphincter muscle strips were prepared for measurement of isometric tension in tissue baths containing Krebs-Henseleit solution (37°C), aerated with 95% O_2 and 5% CO_2. At the start of experiments a reference contraction to carbachol (10 μM) was obtained in each tissue. Experiments were then conducted in the presence of atropine (1 μM) and the NK-1 receptor antagonist CP 99994 (1 μM; McLean et al., 1993). Tissues were incubated with vehicle (DMSO), SR 142801 or SR 48968 for 120 min before concentration-effect curves to senktide or [MePhe^7]-NKB were constructed. Contraction was calculated as % carbachol-induced responses.

Senktide produced monophasic concentration-effect curves (pEC_{50} = 9.49 ± 0.09, n = 10) which were surmountably antagonised by SR 142801 (3-30 nM; pA2 = 8.89, slope = 0.99 ± 0.08, n = 4; Figure 1a). SR 48968 (3-30 μM) produced similar effects but was 630-fold less potent than SR 142801 (pA2 = 6.1, slope = 1.5 ± 0.03, n = 4). Concentration-effect curves to [MePhe^7]-NKB were shallow (n_H = 0.36 ± 0.02, n = 8) and often biphasic. SR 142801 and SR 48968 inhibited responses to low (<1 nM) but not high (>1 nM) concentrations of [MePhe^7]-NKB (Figure 1b). In the presence of SR 142801 (10, 30 and 100 nM) and SR 48968 (3 and 30 μM), concentration-effect curves to [MePhe^7]-NKB were monophasic with n_H values of 0.64 ± 0.03, 0.74 ± 0.04, 0.94 ± 0.1, 0.73 ± 0.08 and 1.32 ± 0.08 respectively (n = 4).

Figure 1. Effects of SR 142801 on responses to senktide and [MePhe^7]-NKB in rabbit iris sphincter muscle (control ○, 3 nM ●, 10 nM □, 30 nM ■, 100 nM ▽)



Collectively these data show that senktide and [MePhe^7]-NKB contract the rabbit iris sphincter muscle via SR 142801-sensitive NK-3 receptors. However, the differing pharmacological profile of the NK-3 receptor agonists and the presence of an SR 142801-insensitive component of [MePhe^7]-NKB-induced contraction, provides the first known evidence for distinct NK-3 receptor subtypes in the same tissue and species.

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204P A COMPARISON OF N-TYPE CALCIUM CHANNELS IN HUMAN AND RAT ISOLATED VAS DEFERENS BY RANK ORDER OF ω-CONOTOXIN ACTIVITY

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Differences in the inhibitory activity of the ω-conotoxins, GVIA and MVIIC, have led to the suggestion that N-type calcium channels in guinea-pig ileum differ from those in rat vas deferens (Boot 1994). In the guinea-pig ileum, both N-channel blockers were equipotent in causing inhibition of electrically-evoked, neuronally-mediated contractions, whereas against neuronally-mediated contractions of rat vas deferens, MVIIC had low potency with respect to GVIA. To elucidate the character of a human N-type channel, we have determined the rank order of inhibitory activity of the conotoxins GVIA, MVIIA and MVIIC in human vas deferens and compared it to that in the rat vas deferens.

Whole rat vas deferens (RVD) were set up using the method of Wardle et al. (1995). Circular muscle preparations of human vas deferens (HVD), obtained from vasectomies with consent, were placed under a resting tension of 10mN in Tyrode's solution, aerated with 95%0_2/5%CO_2 at 37°C, for isometric recording. RVD were continuously stimulated (60V, 0.5ms, 0.2Hz), and HVD were stimulated at 5min intervals for 1s (60V, 0.5ms, 100Hz) between parallel platinum electrodes. A cumulative concentration-effect curve was performed with a single conotoxin in each tissue and the effects compared with time-matched solvent controls.

In RVD, all conotoxins caused concentration-dependent inhibition of the electrically-evoked contractions, with pIC50's (% maximum inhibition) of 9.2 ± 0.1 (93.8 ± 1.3), 9.0 ± 0.1 (92.3 ± 1.8) and 6.3 ± 0.1 (86.5 ± 4.0% at 1μM) for GVIA MVIIA and MVIIC respectively (n=4-7). GVIA was slow to attain a maximal effect (30min), which was apparently irreversible, compared with the faster (5min), reversible actions of MVIIA and MVIIC. In HVD, a similar rank order of conotoxin activity was observed, albeit at a 10 fold lower potency, to that in the rat vas deferens. The pIC50's (% maximum inhibition at 100nM) were 8.1 ± 0.2 (87.5 ± 5.9) and 7.5 ± 0.1 (73.6 ± 7.8) for GVIA and MVIIA respectively (n=5-7). MVIIC was without consistent activity up to 100nM. Contact times of 50-60min were necessary to allow the maximum effects of the conotoxins to be achieved. In addition, the response in the HVD was abolished with either 100nM tetrodotoxin or 30nM prazosin (n=4 each).

We conclude that N-type calcium channels, mediating the effects of electrically stimulating noradrenaline-containing nerves in human and rat vas deferens, have similar (GVIA = MVIIA >> MVIIC) sensitivities to conotoxins. This rank-order differs from the profile (GVIA = MVIIC) reported in guinea-pig ileum (Boot 1995) and atria (Hong & Chang 1995), and at a human brain recombinant α_{1B}-mediated N-type channel (Grantham *et al.*, 1994). We are currently investigating methodological differences between studies that may explain differences in conotoxin activity profiles.

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205P L-NAME HAS NO EFFECT ON THE LENGTH-TENSION RELATIONSHIP OF RAT DETRUSOR MUSCLE *IN VITRO*

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The role of nitric oxide (NO) in the lower urinary tract is unclear. The enzyme nitric oxide synthase may be present in a large proportion of sensory neurones innervating rat bladder (Vizzard *et al.* 1994). There is good evidence to suggest that NO causes relaxation of the trigone and urethra and may therefore have a role in bladder emptying (Andersson and Perrson, 1993). The role of NO in relaxation of the detrusor muscle during bladder filling however is less clear. Our aim was to investigate the possibility that a local release of NO, in response to passive tension, may modulate detrusor tone.

Male rats (200g) were killed and perfused transcardially with modified Krebs–Heinsleit buffer. The bladder was removed and a circular ring of detrusor muscle, 2–3mm wide, was mounted on a micrometer in a 75ml organ bath. The bath contained modified Krebs–Heinsleit buffer and was continuously oxygenated. The tissue was stretched in 0.5 or 1 mm increments and passive length–tension relationships recorded. Three consecutive length–tension relationships were recorded with a 20 minute recovery period between each. L–NAME (10^{-3}M) was then added to the bath and the final length–tension relationship recorded. The tissue was then fixed in 4% paraformaldehyde and proccesed for NADPH–diaphorase histochemistry.

L–NAME was found to have no effect on the length–tension relationship of rat detrusor muscle (Figure 1). Lengthening the muscle by 5mm increased the mean tension in 9 experiments from 0.08g ± 0.01 (s.e.mean) to 1.74g ± 0.15 for control length–tension relationships and from 0.08g ± 0.01 to 1.57g ± 0.11 after treatment with L–NAME. There is no significant difference between these values. NADPH–diaphorase positive fibres were found throughout sections of the tissue.

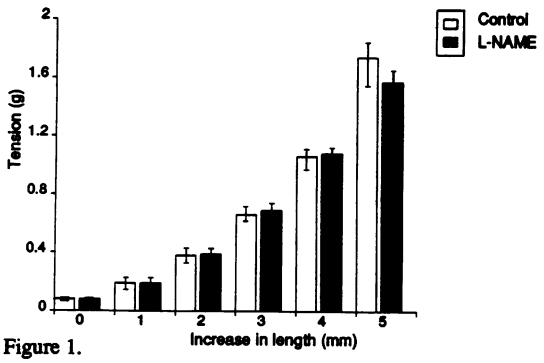

Figure 1.

Thus although fibres containing nitric oxide synthase are present in the tissue we have been unable to demonstrate that release of NO from peripheral nerve terminals, in response to passive tension, causes relaxation of rat detrusor muscle.

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206P CHARACTERISATION OF THE RECEPTORS THAT MEDIATE THE ADRENERGIC COMPONENT OF NON-NICOTINIC VAGALLY-EVOKED RELAXATION OF THE RAT STOMACH *IN VITRO*

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We have previously demonstrated the existence of a non-nicotinic vagal inhibitory pathway to rat gastric smooth muscle which is in part mediated by adrenergic parasympathetic efferent fibres (Carnell & Williams, 1992). The rat stomach is known to express all subtypes of adrenoceptor including the recently characterised $ß_3$-receptor (McLaughlin & MacDonald, 1991). Here we characterise which receptors mediate the adrenergic component of vagally-evoked gastric relaxation.

Male Wistar rats (approximately 200 g) were starved overnight and killed by inhalation of halothane. Following transcardial perfusion (2 min) with modified Krebs-Heinsleit buffer (pH 7.4), the stomach was rapidly excised with both thoracic vagus nerves intact, and cut open along the greater curve. The stomach contents were washed out and surrounding fat tissue was removed. The entire stomach was then mounted in standard 70 ml organ baths containing warmed (37°C) and oxygenated (95% O_2 : 5% CO_2) modified Krebs-Heinsleit buffer (pH 7.4). Tissues were placed under an initial tension of 5 g and tension was measured isometrically between the apex of the fundus and the antrum-pylorus boundary. The thoracic vagi were stimulated by means of an insulated electrode 2 min before and 10 min after the addition of antagonists.

Supramaximal vagal stimulation (20 V, 20 Hz, 1 ms, for 20 s) resulted in a rapid-onset strong contraction followed by a longer duration relaxatory response. In the presence of the nicotinic antagonist mecamylamine (0.1 mM), the contractile response was abolished and a shorter duration relaxation remained.

In the presence of the classical ß-adrenoceptor antagonist (-)-propranolol (0.01 - 10 μM) the magnitude of the mecamylamine-resistant vagally-evoked gastric relaxation was reduced by a maximum of 48 ± 4 % (mean ± s.e.mean; n = 6) in a concentration-dependant manner. The $ß_1$ adrenoceptor antagonist atenolol (10 μM) caused a reduction in the magnitude of the mecamylamine-resistant vagally-evoked gastric relaxation of 40 ± 6 % (n = 4). Neither the $ß_2$-adrenoceptor antagonist ICI 118,551 ((±)-1-[2,3-dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)-amino]-2-butanol) (10 μM; n = 6) nor the novel $ß_3$-adrenoceptor selective antagonist, SR 59230A (Manara *et al.*, 1995; 10 μM; n = 8) had any influence on mecamylamine-resistant vagally-evoked relaxations. The α-adrenoceptor antagonist phentolamine (10 μM) was also without effect (n = 4). Neither phentolamine (10 μM; n = 4) nor ICI 118,551 (10 μM; n = 2) had any effect on vagally-evoked contractions.

We conclude that the adrenergic fibres mediating vagal inhibition of gastric tone act predominantly if not solely via $ß_1$-adrenoceptors.

We thank Dr. L. Manara for the gift of SR 59230A.

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207P EFFECTS OF Ro 20-1724, A SELECTIVE INHIBITOR OF PDE IV, ON THE DEVELOPMENT OF ACUTE ANTIGEN-INDUCED BRONCHIAL HYPERREACTIVITY AND AIRWAY LEUKOCYTE INFILTRATION IN SENSITISED GUINEA-PIGS

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Non-specific bronchial hyperreactivity and airway eosinophilia are two classic features of bronchial asthma. Evidence is emerging that theophylline, a non-specific inhibitor of phosphodiesterase (PDE), exhibits anti-inflammatory effects (Sullivan et al. 1994) in addition to its known bronchodilator properties (Persson, 1986). Ro 20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) is a selective inhibitor of PDE IV, a member of a class of compounds with anti-inflammatory properties in models of airway inflammation (Howell et al. 1993; Banner & Page, 1995).

Male Dunkin-Hartley guinea-pigs (200-250g) were sensitised to ovalbumin (OvA, 10µg/ml, in an alum suspension). 14-21 days later, animals received either Ro 20-1724 (3mg/kg, in DMSO 50% in saline ip.) or vehicle, either 30 min. before (t=0) or 6 hours after (t=6) exposure to nebulised OvA (0.5%, for 10 min.), or at both times. Mepyramine (30 mg/kg ip) was also given 30 min. before challenge to protect against fatal anaphylaxis. 17-24 hours after the OvA exposure, the animals received nebulised U46619 (30ng/ml for 60 sec.). Conscious, whole animal plethysmography (Griffiths-Johnson et al. 1988), incorporating a computerised acquisition system, was used to monitor specific airway conductance (sGaw) over the ensuing 20 minutes. The lungs were then lavaged after pentobarbitone overdose.

Treatment	2 min. response	n	P
Untreated (Sal)	+7.9 +/-9.2%	5	0.02
Untreated (OvA)	-25.8 +/-2.9%	7	N/A
Ro t=0 (OvA)	-19.1 +/-3.4%	6	0.16
Ro t=6 (OvA)	-11.6 +/-5.1%	6	0.04
Ro t=0 & 6 (OvA)	+6.4 +/-8.2%	6	0.01

Table 1. % changes in sGaw from baseline at 2 min. after U46619 exposure.

17-24 hours after the OvA exposure, all control animals bronchoconstricted to a significantly greater degree to the U46619 than did the saline challenged group and were therefore hyperreactive. There was also airway leukocyte infiltration (*Tables 1 & 2*, highlighted). The bronchoconstriction to U46619 peaked at 2 min. after exposure. A single dose of Ro 20-1724 (Ro) administered 30 min. before the OvA exposure had no effect on the development of this hyperreactivity or on the recruitment of eosinophils into the

Treatment	Macrophages	Eosinophils	Neutrophils	Total cell count
Control (Sal)	12.2 +/-1.9*	1.0 +/-0.4*	0.0 +/-0.0*	13.2 +/-2.3*
Control (OvA)	41.8 +/-6.2	19.2 +/-2.9	7.3 +/-1.1	72.3 +/-7.3
Ro t=0	34.1 +/-5.1	21.1 +/-2.9	3.9 +/-0.9*	59.1 +/-8.1
Ro t=6	23.8 +/-5.2*	8.4 +/-1.9*	1.2 +/-0.3*	33.4 +/-7.2*
Ro t=0 & 6	22.0 +/-3.7*	8.4 +/-1.6*	1.3 +/-0.4*	31.7 +/-5.2*

Table 2. Cells recovered /ml in lavage fluid ($x10^5$)

airways. A dose administered 6 hours after the OvA exposure, alone or in addition to the pre-treatment dose, however, attenuated the acute hyperreactivity and the infiltration of leukocytes (*Table 1 & 2.*).

In conclusion, Ro 20-1724 can prevent the development of an acute hyperreactivity and eosinophilia induced by antigen challenge. Interestingly, a dose given 6 hours after the challenge appears essential whereas pre-treatment appears to be of little consequence.

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208P INHIBITION OF NITRIC OXIDE SYNTHASE ISOFORMS BY 1-(2-TRIFLUOROMETHYLPHENYL) IMIDAZOLE (TRIM) AND RELATED PHENYLIMIDAZOLES

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We have previously reported that 1-(2-trifluoromethylphenyl) imidazole (TRIM) inhibits neuronal and inducible isoforms of nitric oxide synthase (nNOS and iNOS) but is a poor inhibitor of endothelial NOS (eNOS) in vitro (Handy *et al.*, 1995). We have now studied the mechanism of inhibition of mouse cerebellar nNOS by TRIM and have evaluated additional substituted imidazole compounds for inhibition of NOS isoforms in vitro.

Homogenates (10,000 x g) of mouse cerebella and lungs removed from urethane-anaesthetised (10 g kg^{-1}, i.p.) rats 6 h after i.p. administration of 5 mg kg^{-1} E. Coli endotoxin (serotype: 0127-B8) and bovine aortic endothelial cells were used as the source of nNOS, iNOS and eNOS respectively (Babbedge *et al.*, 1993). NOS activity was determined as the conversion of [^{3}H] L-arginine to [^{3}H] citrulline.

Kinetic (Lineweaver-Burke) analysis of the effect of TRIM on nNOS (L-arginine concentration, 0.12-10.12 µM) revealed competitive inhibition by TRIM at the substrate binding site (K_m for L-arginine, 2.5 µM, K_i for TRIM, 21.7 µM). Varying the concentration of either $CaCl_2$ (e.g. 0.75 mM, 59.3±2.4%; 7.5 mM, 65.4±2.9%, both n=6, P>0.05) or NADPH (e.g. 0.05 mM, 56.8±1.4%; 5mM, 58.7±1.3%, both n=6, P>0.05) did not affect the nNOS inhibitory effect of TRIM (50 µM). Preincubation of mouse cerebellar supernatant (0°C, 0-60 min) with TRIM (50 µM) did not affect the degree of NOS inhibition observed (e.g. 0 min: 61.4±2.4%, c.f. 60 min: 67.4±1.6%, n=4-6, P>0.05) implying the absence of time-dependent irreversible NOS inhibition.

Imidazole is a poor inhibitor of NOS with IC_{50}s of 290.6 µM, 616 µM and 101.3 µM (n=6) for nNOS, iNOS and eNOS respectively. Addition of a phenyl group at position 1 of the imidazole increased NOS inhibitory effect in an isoform non-selective manner (i.e.1-phenylimidazole, IC_{50}s: nNOS, 72.1 µM, iNOS, 53.9 µM, eNOS, 86.9 µM) whilst substitution of 1-phenylimidazole e.g. 1-(2-chlorophenyl) imidazole (IC_{50}s: nNOS, 43.4 µM, iNOS, 786.5 µM, eNOS, 392.3 µM) and 1-(2,3,5,6-tetrafluorophenyl) imidazole (IC_{50}s: nNOS, 56.3 µM, iNOS, 202.4 µM, eNOS, 559.6 µM) resulted in a disproportionate increase in nNOS (c.f. eNOS) inhibition.

TRIM inhibits mouse cerebellar nNOS by competitive interaction at the L-arginine site. Whether TRIM interferes with L-arginine binding secondary to an interaction at the haem site of NOS remains to be determined. Although less potent, other substituted 1-phenylimidazole derivatives share with TRIM an increased selectivity for nNOS (c.f. eNOS). Such structure-activity data may provide a rational basis for the identification of additional nNOS selective inhibitors.

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