57P METABOTROPIC GLUTAMATE RECEPTORS (mGLuR1): 2-SUBSTITUTED PHENYLGLYCINES AS SELECTIVE ANTAGONISTS

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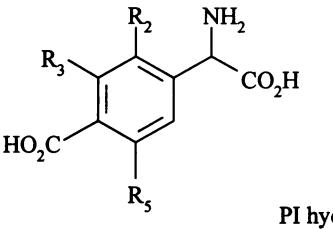
There are three subclasses of G-protein linked metabotropic glutamate receptors. Group 1 subclass (mGluR1, mGluR5) activates phospholipase C and leads to inositol triphosphate and diacylglycerol formation by phosphoinositide (PI) hydrolysis. Groups 2 and 3 (mGluR2, 3 and mGluR4, 6, 7, 8) are negatively coupled to adenylyl cyclase and their activation leads to decreased intracellular cyclic-AMP levels. We have found that 2-substituents (figure 1) can increase the antagonist potency of phenylglycine derivatives compared to (S)-4-carboxyphenylglycine ((S)-4CPG) against PI hydrolysis at human mGluR1α receptors expressed in AV-12 cells stimulated with quisqualate (0.3μM on mGluR1α and 0.1μM on mGluR5a) (Kingston *et al.*).

The compounds were selective for mGluR1α compared to mGluR5a and none showed agonist activity up to 100μM. The 2,3-substitution pattern of **2** was ten times more potent than the 2,5-substituents of **3**. The (+)-isomer of **1** was obtained by resolution of the racemate via the crystalline D-lysine salt. The synthesis and SAR of other 2-substituents will be shown. In conclusion, compounds **1** and **2** are more potent mGluR1 antagonists than the phenylglycines previously reported (Sekiyama *et al.*).

Figure 1



	R_2	R_3	R_5	PI hydrolysis IC_{50} (μM) mGluR1*	mGluR5
(S)-4CPG	H	H	H	58±14	>100
1 (+)-isomer	Me	H	H	12±1.8	>100
2	Me	OH	H	8.1±1.7	>100
3	Me	H	OH	77	>100

*mean value ± SD from 2-3 experiments

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58P LIGANDS OF GROUP 1 METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) EXHIBIT LOW AFFINITY BINDING CHARACTERISTICS TO mGluR1α

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The metabotropic glutamate receptors (mGluRs) are a family of receptors coupled to membrane GTP binding proteins. They are divided into three major groups of which, mGluR1α belongs to the group 1 receptors which are positively coupled to phosphoinositide hydrolysis.

Binding profiles of the mGluRs have been difficult to achieve due to the unavailability of specific, potent radioligands. In this study we have characterised the binding of [^{3}H] glutamate to a clonal human mGluR1α expressed in Syrian hamster tumour cell line (AV12-664). This cell line also expresses a rat glutamate-aspartate transporter, GLAST.

The methods used were adapted from Eriksen & Thomsen (1995) and Thomsen *et al* (1993). Briefly, cell membrane preparations (300ug protein) were incubated for 60 min with [^{3}H] glutamic acid in the presence or absence of displacing ligands in a final volume of 250μl. Non-specific binding was determined in the presence of 10μM quisqualate. The reaction was terminated by rapid filtration and radioactivity measured by liquid scintillaton counting. Data was analysed using EBDA/LIGAND software.

It was found that [^{3}H] glutamate bound to the membranes in a saturable manner with a dissociation constant (Kd) of 130± 37nM (s.e.m.) and a maximum binding capacity (Bmax) of 1.34± 0.32 pmoles/mg protein (n=10).

In the presence of the non hydrolysable GTP analogue, GTPγS (100uM), the Kd and Bmax showed a trend towards a reduction with mean values of 70±12nM and 0.69±0.18 pmol/mg protein respectively (n=5). This was not statistically significant (p>0.05, unpaired t-test)

The inhibition constant (Ki) values obtained for both the agonists and antagonists reflect relatively low affinities for mGluR1α compared to other neurotransmitter receptors. However, the order of potencies obtained from binding studies correlated with functional data measured by phosphinositide (PI) hydrolysis assay (table1).

Table 1. Ki values (±s.e.m.n>3) compared to functional activities (EC_{50} or IC_{50} ±s.e.m n>3) at the mGluR1α receptor.

Agonists	Ki (μM)	Hill	EC_{50} (μM)
Quisqualate	0.01±0.06	0.85±0.18	3±0.5
(RS)-DHPG	0.17±0.08	0.75±0.23	30±7
1S,3R-ACPD	0.40±0.13	0.66±0.12	81±24
L-CCG-1	0.73±0.10	0.67±0.14	93±7
L-HCSA	0.44±0.20	1.42±0.14	39±19
L-CSA	3.51±0.56	1.28±0.08	48±10
L-SOS	5.61±3.27	1.64±0.70	110±2
Antagonists			IC_{50} (μM)
(S) 4C3HPG	2.91±0.93	0.73±0.11	26±15
(S) 4CPG	15.4±2.99	1.05±0.23	58±14
M4CPG	>15	<0.3	57±20

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59P LY339434, A GluR5-SELECTIVE KAINATE RECEPTOR AGONIST

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Kainate receptors, GluR5 and GluR6, appear to form functional ion channels although kainate, does not discriminate between these channel subtypes. We have performed (a) ligand binding studies in human AMPA and kainate receptor transfected HEK293 cells and (b) whole-cell voltage clamp electrophysiological recordings in human kainate receptor transfected cells and acutely isolated dorsal root ganglion neurons to evaluate the pharmacological profile of (2S, 4R)-4-[3-(2-napthyl)-2(E)-propenyl]glutamic acid (LY339434). In addition, we have compared the activity of LY339434 to (2S,4R)-4-methylglutamic acid, a compound reported to act as a potent and selective kainate receptor agonist (Gu *et al.*, 1995).

From ligand binding studies at human AMPA and kainate receptor transfected HEK293 cells (Fletcher *et al.*, 1995; Hoo *et al.*, 1994; Korczack *et al.*, 1995), LY339434 produced K_i values > 10μM at GluR1, 2, 4 and 6 and an approximate K_i value of 15 nM at GluR5. Kainate produced K_i values of 177 ± 22 nM (n=3) and 32 ± 14 nM (n=4) at GluR5 and GluR6, respectively.

Functional studies were performed using whole-cell voltage clamp electrophysiology in human GluR5 (Korczack *et al.*, 1995), human GluR6 transfected HEK293 cells (Hoo *et al.*, 1994) and acutely-isolated dorsal root ganglion (DRG) neurons. DRG neurons were isolated from 5-8 day old Harlan rats (Bleakman *et al.*, 1996). Prior to use, cells were exposed to concanavalin A (250μg/ml, ≥10min) in order to remove agonist-induced desensitization. All experiments were performed at room temperature (22°C) and results are given as the mean ± s.e.mean.

LY339434 evoked inward currents and concentration-response curves were generated in GluR5 and GluR6 transfected cells with EC_{50} values of 2.5 ± 0.9 μM and >300 μM respectively (n=3 cells). EC_{50} values for kainate at human GluR5 and GluR6 were 16.2 ± 1.0 μM (n=4 cells) and 1.5 ± 0.2 μM (n=3 cells), respectively. In DRG neurons, LY339434 yielded an estimated EC_{50} value of 0.8 ± 0.2 μM (n=3-6 cells) in comparison to an estimated EC_{50} value of 11.6 ± 0.2 μM (n=4 cells) for kainate. Inward currents evoked by LY339434 (3μM) were inhibited by NBQX (3-10 μM).

We have also examined the pharmacological profile of (2S,4R)-4-methylglutamic acid at kainate receptors in DRG neurons and at human GluR6 expressed in HEK293 cells. EC_{50} values of 0.17 ± 0.04 μM (n=4 cells) and of 0.7 ± 0.1μM (n=3 cells) were obtained for these cells, respectively.

The present studies suggest that (2S,4R)-4-methylglutamic acid is a potent agonist at kainate receptors in DRG neurons and at human GluR6 receptors expressed in HEK293 cells. However, LY339434 shows selectivity as an agonist for human GluR5 expressed in HEK293 cells and kainate receptors in rat DRG neurons and weak activity at AMPA and GluR6 receptors. Such a selectivity profile should allow LY339434 to be of use as a pharmacological tool to examine the functional role of GluR5 receptors in the CNS.

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Fletcher *et al.* (1995) *Recept. Channels* **3**, 21-31.
Gu *et al.* (1995) *J. Med. Chem.* **38**, 2518-2520.
Hoo *et al.* (1994) *Recept. Channels* **2**, 327-337.
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60P ACTIVATION OF GROUP III mGluRs IS UNLIKELY TO ACCOUNT FOR QUISQUALATE-INDUCED SENSITISATION TO L-2-AMINO-4-PHOSPHOBUTANOATE IN NEONATAL RAT SPINAL CORD

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L-2-Amino-4-phosphonobutanoate (L-AP4) can evoke depolarisation of rat hippocampal or neocortical neurones and spinal motoneurones but only after prior exposure of the tissue to certain glutamate analogues, most notably quisqualate (QUIS; Robinson *et al.*, 1986; Sheardown, 1988; Ishida *et al.*, 1995). The mechanism of this so-called 'QUIS effect', has not yet been identified; L-AP4 is itself a glutamate analogue which does not act on ionotropic glutamate receptors but which is an agonist at group III metabotropic glutamate (mGlu) receptors. The present study has examined the contribution of group III mGlu receptors to the QUIS effect.

We used a grease-gap technique (Bufton *et al.*, 1995) to record from the L3, L4 or L5 ventral root of superfused, *in vitro*, hemisected spinal cords taken from Wistar rats (3-5 days old). Tissue viability was assessed by inducing mono- and poly-synaptic reflexes from the ventral root with single stimuli (0.5 ms, 10 x threshold) delivered to the ipsilateral dorsal root prior to any drug administration and at various times thereafter. Drugs were superfused at 1.0 ml min^{-1} in a Krebs-bicarbonate buffer (in mM): NaCl 124, KCl 3, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 26 and (+)-glucose 11, maintained at 25°C and gassed with 95% O_2/5% CO_2. Superfused drug additions were made at 22-min intervals with drug exposure periods of 2 min.

Superfusion of L-AP4 alone (0.05-300 μM) did not cause any measurable depolarisation but it did inhibit both the evoked, monosynaptic potential as well as spontaneous, irregular spiking activity in the preparation. This latter activity is considered to be motoneurone action potentials, that may derive from phasic input from excitatory interneurones. A single, 2 min application of L-AP4 suppressed this activity from 5 min (0.1 μM) for up to 40 min (1000 μM), effects which were respectively prevented or shortened by 300 μM *(RS)*-α-methyl-4-phosphonophenylglycine (MPPG, a group III mGlu antagonist), added prior to and during application of L-AP4. A single bolus of 15 μM QUIS, which typically evoked a 4 mV depolarisation, allowed a 100 μM superfusion of L-AP4 to produce an initial depolarisation of 0.74 ± 0.39 mV (n=13; the QUIS-effect), an action which persisted in a declining fashion for 2-5 hr. Co-superfusion with MPPG (300 μM; n=8), either during the QUIS application itself and/or during the subsequent L-AP4 superfusion, did not in any way alter these post-QUIS, L-AP4-induced depolarisations.

One explanation of the QUIS effect invokes an action at mGlu receptors. In the case of QUIS, these would be of the group I subclass, blockade of which has already been shown not to influence the QUIS effect in spinal cord (Ishida *et al.*, 1995). Our present data, using a demonstrable functional blockade of group III and perhaps even group II mGlu receptors by MPPG, indicate that an action at these receptors by L-AP4 or any other substance is also unable to account for the phenomenon.

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61P STUDIES OF THE INTERACTIONS OF (2S,4R)-4-METHYLGLUTAMATE AND ATPO WITH AMPA AND KAINATE RECEPTORS

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We are currently studying the non-NMDA receptors which are naturally expressed in cultured cortical (Cx) and spinal cord (SC) neurones. Two substances that can distinguish between AMPA and kainate (KA) receptor subtypes recently came to our attention. (2S,4R)-4-Methylglutamic acid (MeGLU) is purported to be a highly selective KA receptor agonist (Gu et al., 1995) while (RS)-2-amino-3-[5-tert-butyl-3-(carboxymethoxy)-4-isoxazolyl]phosphonic acid (ATPO) is a potent selective antagonist of AMPA responses (Madsen et al., 1996). Neither substance has, however, been investigated at the single neurone level using quantitative pharmacological techniques. Here we use whole-cell patch-clamp recordings to characterize the actions of MeGLU and ATPO on non-NMDA ionotropic glutamate receptors of cultured rat Cx and SC neurones.

Semi-rapid application of MeGLU induced a concentration-dependent, non-desensitizing inward current on both types of neurone. Responses to MeGLU were competitively antagonized by NBQX with a K_i(Cx) of 280 nM. This is 3.5 times less potent than for the action of NBQX on responses to AMPA (K_i(Cx) = 83 nM) and KA (K_i(Cx) = 79 nM). Cyclothiazide (CTZ, 100 μM), an established blocker of desensitization at AMPA receptors, enhanced responses to AMPA (100 μM) of both Cx (to 483±36%, n=7, P<0.001) and SC (to 680±64%, n=8, P<0.001) neurones. Responses of Cx neurones to KA (30 μM) were also potentiated by CTZ (to 351±17%, n=7, P<0.001), while responses of SC neurones were unaffected (to 108±7%, n=8, P>0.05). Responses of both neurone types to MeGLU (100 μM) were unaffected by CTZ (Cx to 112±5.7%, n=9, P>0.05: SC to 101±5.9%, n=15, P>0.05). Concanavalin A (Con A, 300μg ml^{-1}), which blocks desensitization at KA receptors, enhanced responses to both KA (Cx to 128±6.3%, n=13, P<0.001: SC to 150±5.5%, n=14, P<0.001) and, to a significantly greater extent, to MeGLU (Cx to 306±25.1%, n=11, P<0.001: SC to 315±21.8%, n=12, P<0.001).

ATPO reduced peak responses to AMPA in a competitive manner (K_i(Cx) = 18 μM), but had variable actions on the plateau phases of responses, which were not significantly altered (P>0.05). ATPO also antagonised responses to KA and MeGLU competitively, with K_i(Cx) values of 30 and 24 μM respectively. The Hill slopes for the action of ATPO were all close to 1.

In conclusion, results from manipulations of desensitization of naturally expressed non-NMDA receptors indicate that MeGLU is a more selective agonist at KA receptors than KA itself. ATPO behaves as a competitive inhibitor of non-NMDA receptors, with a slight preference for AMPA responses. Its weak effect on plateau responses to AMPA may, nevertheless, provide a basis for discriminating between responses at KA and AMPA receptors.

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62P DIFFERENTIATION OF KCl-, NMDA- AND GLUTAMATE-EVOKED cGMP ACCUMULATIONS IN THE GUINEA-PIG CEREBELLUM

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Sodium nitroprusside (SNP) evokes a biphasic cGMP accumulation in guinea-pig cerebellum (Hernández *et al.*, 1994), which is enhanced by cAMP stimuli such as NECA. Here, we have examined the nature of cGMP responses to KCl, glutamate and NMDA in this tissue.

The accumulation of [^{3}H]-cGMP was measured at 37°C with guinea-pig (Dunkin-Hartley, male, 200 - 800g) cerebellar slices pre-incubated with [^{3}H]-guanosine as previously described (Neil *et al.*, 1996). Results were expressed as a percentage conversion from the total [^{3}H]-guanine nucleotides with basal levels subtracted, from experiments carried out on at least 3 separate occasions. Statistical significance was evaluated using a Student's paired t test.

Exposure of guinea-pig cerebellar slices to KCl (30 mM), glutamate (10 mM) and NMDA (1 mM) evoked time-dependent accumulations of [^{3}H]-cGMP with distinct patterns (Figure 1).

In a second series of experiments, accumulations of [^{3}H]-cGMP evoked by KCl, glutamate and NMDA at 200 s were 0.91 ± 0.18, 0.21 ± 0.02 and 0.12 ± 0.05 % conversion over basal, respectively. Basal levels were 0.24 ± 0.03 % conversion. Dizocilpine (100 μM) completely inhibited glutamate and NMDA-stimulated cGMP accumulation (2 ± 5 %; P=0.97 %, and 4 ± 5 % control response, P=0.84 %) and partially reversed the KCl response (73 ± 1 %, P=2.62 %). In the presence of 10 μM NECA, KCl- and NMDA-evoked [^{3}H]-cGMP production were significantly potentiated to 157 ± 9 % (P=0.84%) and 167 ± 11 % (P=4.67 %) control, respectively. However, the effect of NECA on glutamate-stimulated cGMP failed to reach significance (141 ± 28 % control, P=15.88%).

Thus, differences in time courses and dizocilpine-sensitivity suggest distinct mechanisms for the generation of cGMP by KCl, glutamate and NMDA in the guinea-pig cerebellum. In addition, the differential sensitivity of cyclic GMP responses in the presence of NECA suggests a complex interaction between glutamate and NECA, possibly involving Group III metabotropic glutamate receptors (Neil *et al.*, 1996).

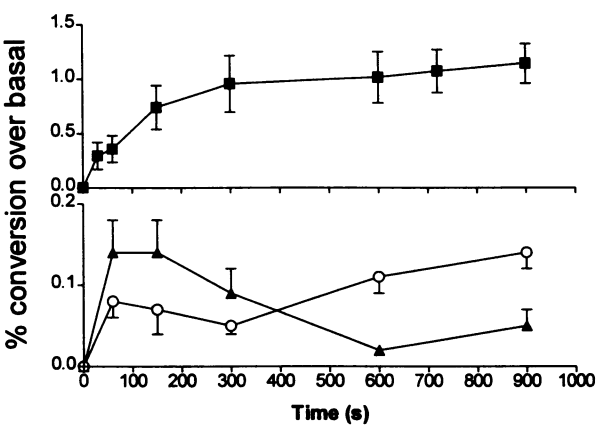

Figure 1. Time course for cGMP production in the presence of KCl (■), glutamate (○) and NMDA (▲).

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KEN is a BBSRC Glaxo Wellcome CASE Student.

63P RECEPTOR BINDING CHARACTERISTICS OF THE NOVEL NMDA RECEPTOR GLYCINE SITE ANTAGONIST [^{3}H]-GV 150526A IN RAT CEREBRAL CORTICAL MEMBRANES

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GV 150526A (3-[2-(Phenylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic acid sodium salt) is a new, high affinity, selective antagonist at the glycine site of the NMDA receptor (Di Fabio *et al.*, 1997). This compound has been shown to reduce the infarct area and to protect somatosensory evoked potentials (SEP) in the middle cerebral artery occlusion (MCAo) model of focal ischemia in the rat (Bordi *et al.*, in press). The aim of this work was to evaluate the binding characteristics of [^{3}H]-GV 150526A as a radioligand for the glycine site of the NMDA receptor, in comparison with [^{3}H]-glycine, the first radioligand to be used to identify this class of binding sites.

Crude synaptic membranes were obtained from the cerebral cortex of adult (250g) male Sprague-Dawley rats as described by Kishimoto *et al.* (1981). The final binding assay was performed by incubation (90 min, 4 °C) of the membrane suspension in buffer solution (50 mM Tris citrate, pH 7.1) containing 1 nM [^{3}H]-GV 150526A. The reaction was terminated by filtration. In the case of [^{3}H]-glycine, displacement binding curves were obtained with 20 nM radioligand and the incubation (20 min, 4 °C) was terminated by centrifugation.

Initial kinetics experiments revealed that the association and the dissociation constants of [^{3}H]-GV 150526A were 0.071 ± 0.01 $(nM)^{-1}$ min^{-1} ($t_{1/2}$ = 5.1 ± 0.5 min) and 0.066 ± 0.001 min^{-1} ($t_{1/2}$ = 10.5 ± 0.1 min), respectively (mean ± s.e.mean, n=3). An equilibrium dissociation constant of 0.9 nM (pK_D = 9.03) was calculated from these values. Saturation experiments, performed at radioligand concentrations ranging from 0.3 to 30 nM, showed that [^{3}H]-GV 150526A interacted with rat cortical membranes in a saturable way and to a single binding site, with a K_D = 0.8 nM (pK_D = 9.08 ± 0.08, n = 4) and a receptor density of 3.4 ± 0.2 pmol/mg protein. By means of homologous displacement experiments, a K_i = 1.8 nM (pK_i = 8.76 ± 0.02, n = 5) was determined. These values were in line with that found by displacement of [^{3}H]-glycine by GV 150526A (K_i = 3.2 nM, pK_i = 8.49 ± 0.02, n = 5). The pharmacological profile of [^{3}H]-GV 150526A binding was then investigated by studying the effect of a series of agonists, partial agonists and antagonists of the glycine site of the NMDA receptor. A strong, linear correlation (r = 0.99, P < 0.01), with a slope not significantly different from unity (P > 0.05), was found when comparing the pK_i values obtained using [^{3}H]-GV 150526A and [^{3}H]-glycine (see Table 1). In contrast, ligands specific for other recognition sites (namely strychnine, kainic acid, AMPA, NMDA and dizocilpine) did not inhibit the binding of both radioligands.

In conclusion, this study supports the identity of the high affinity site labelled by [^{3}H]-GV 150526A with that of the strychnine-insensitive glycine site of the NMDA receptor. Moreover, the high affinity and slow dissociation kinetics of [^{3}H]-GV 150526A make this radioligand a valuable tool to further investigate the properties of the glycine site.

Table 1.: Effect of some glycine site ligands on [^{3}H]-GV 150526A and [^{3}H]-glycine binding to rat cerebral cortical membranes (mean ± s.e. mean, n = 3-5 experiments).

Drug	[^{3}H]-GV 150526A pK_i	[^{3}H]-glycine pK_i
Glycine	6.78 ± 0.07	6.74 ± 0.06
D-serine	6.29 ± 0.06	6.49 ± 0.04
D-alanine	5.98 ± 0.08	6.30 ± 0.12
1-aminocyclopropyl-1-carboxilate	6.67 ± 0.04	6.78 ± 0.11
(±)-3-amino-1-hydroxypirrolid-2-one	5.00 ± 0.05	5.19 ± 0.06
D-cycloserine	4.02 ± 0.07	3.95 ± 0.08
1-amino-1-carboxycyclobutane	4.66 ± 0.09	5.05 ± 0.15
GV 150526A	8.76 ± 0.02	8.49 ± 0.02
7-chlorokynurenic acid	6.51 ± 0.01	6.69 ± 0.05
5,7-dichlorokynurenic acid	7.16 ± 0.02	7.19 ± 0.03
5,7-dinitroquinoxaline-2,3-dione	6.90 ± 0.14	7.02 ± 0.08
6,7-dinitroquinoxaline-2,3-dione	6.18 ± 0.10	6.13 ± 0.18
5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione (ACEA 1021)	8.44 ± 0.12	8.21 ± 0.11

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64P COMPARISON OF NMDA RECEPTORS EXPRESSED IN *XENOPUS LAEVIS* OOCYTES FOLLOWING INJECTION OF RAT BRAIN RNA AND mRNA ENCODING THE NR1A SUBUNIT

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The long-term expression of native and cloned, rat *N*-methyl-D-aspartate (NMDA) receptors has been studied in *Xenopus* oocytes to test for changes in the pharmacological properties of these ionotropic glutamate receptors.

Xenopus oocytes were injected with rat brain RNA and mRNA encoding the rat NR1A subunit. Single oocytes were studied using two-electrode voltage-clamp between days 1 to 60 post-injection. The amplitude of the response to NMDA, its dependence on glycine, its potentiation by spermine and its antagonism by philanthotoxin-343 (PhTX-343) were investigated. All experiments were performed at a holding potential of -80 mV in Mg^{2+}-free frog saline at 20-23 °C. Unless otherwise stated, glycine was co-applied with NMDA at a concentration that maximised the response to the latter.

(i) Oocytes injected with rat brain RNA: Responses to 100 µM NMDA were first observed 2 days after RNA injection. The amplitude of these responses increased up to day 30 and then declined. The EC_{50} for NMDA was: 16.5 ± 3.9 µM (± s.e.mean, n = 9 oocytes) at day 6; 54.7 ± 7.8 µM* at day 10; 39.7 ± 4.0 µM* at day 20; 26.8 ± 3.9 µM* at day 30; and 25.7 ± 6.3 µM at day 40. The effect of glycine on the response to NMDA also varied. For 100 µM NMDA, the concentration of glycine required to induce the smallest measurable (20 nA) response was: 522 ± 112 µM (n = 9) at day 3; 0.54 ± 0.17 µM* at day 6; 37.0 ± 12.8 µM* at day 10; 203 ± 70 µM* at day 20; 533 ± 130 µM* at day 30; 700 ± 150 µM* at day 40; and 7000 ± 1200 µM* at day 50. Potentiation of responses to 100 µM NMDA by 1 mM spermine was: 25 ± 2 % (n = 7) at day 6; 41 ± 3 %* at day 10; 87 ± 4 %* at day 20; 221 ± 7 %* at day 30; and 263 ± 6 %* at day 40. Antagonism of responses to 100 µM NMDA by PhTX-343 decreased with time post-injection. The IC_{50} values for PhTX-343 were: 5.6 ± 1.6 µM (n = 7) at day 6; 27.5 ± 7.4 µM* at day 10; 41.3 ± 10.9 µM* at day 20; 52.6 ± 11.7 µM* at day 30; and 64.4 ± 17.0 µM* at day 40.

(ii) Oocytes injected with NR1A mRNA: Responses to 100 µM NMDA were observed from day 2 onwards and increased in amplitude until 14 to 21 days post-injection. The glycine concentration required to elicit a maximal response to 100 µM NMDA remained constant at 100 µM throughout the 60-day period. 1 mM spermine potentiated the responses to 100 µM NMDA by 319 ± 14 % (n = 7), an effect that was unchanged as the oocytes aged. The IC_{50} for antagonism of responses to 100 µM NMDA by PhTX-343 was 12.0 ± 4.8 µM (n = 7); this was also unchanged as the oocytes aged.

The properties of native NMDA receptors expressed in *Xenopus* oocytes from rat brain RNA change with time, whereas those of cloned NR1 receptors do not. This is probably due to different rates of expression of subunits comprising the native NMDA receptors. Similar results to *(i)* above were obtained after injection of rat brain RNA into oocytes kept for 30 days *in vitro*. Thus, the changes in the pharmacological properties were not due to oocyte ageing. Care must be taken when comparing pharmacological data for native NMDA receptors expressed for different lengths of time in *Xenopus* oocytes.

** Significantly different from the previous value, (P < 0.05; Student's t test)*

65P AUTORADIOGRAPHICAL LOCALISATION OF IMIDAZOLINE I_2 SITES LABELLED WITH [^{3}H]2-(2-BENZOFURANYL)-2-IMIDAZOLINE IN RABBIT BRAIN

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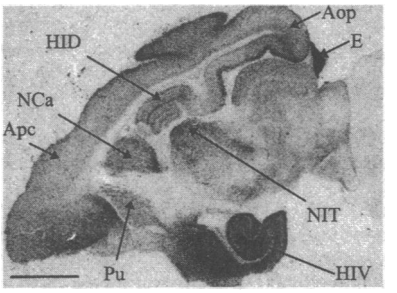
Imidazoline I_2 sites are present in a range of mammalian tissues in multiple forms, I_{2A} and I_{2B}, the latter site being amiloride insensitive. Recently [^{3}H]2-(2-benzofuranyl)-2-imidazoline (2-BFI) has been characterised as a radioligand that selectively labels I_2 sites with high affinity in rabbit brain and kidney (Lione *et al.*, 1996; Hosseini *et al.*, 1997). Rabbit brain represents an interesting model for studying I_2 sites, given the high density of sites compared with other species and, that it expresses I_{2A} and I_{2B} sites (Lione *et al.*, 1996; Hosseini *et al.*, 1997). To date [^{3}H]idazoxan, bound in the presence of rauwolscine to preclude binding to α_2-adrenoceptors, has been used to identify a profuse distribution of I_2 sites throughout rabbit brain (Renouard *et al.*, 1993). In the present investigation receptor autoradiography has been used to further study the distribution of I_2 sites, labelled with [^{3}H]2-BFI, in rabbit brain.

New Zealand white rabbits of either sex (2.0 - 2.5 kg) were sacrificed with an overdose of sodium pentobarbitone (60 mg kg^{-1} i.v.) followed by rapid exsanguination. Whole brains were rapidly removed and then frozen by immersion in cold isopentane (-40 °C). Cryostat cut 12 µm thick sections of rabbit brain were used for autoradiography based on the methods of Mallard *et al* (1992). Sections were incubated with 0.3 nM [^{3}H]2-BFI alone or containing 10 µM 2-(4,5-dihydroimidaz-2-yl)-quinoline (BU224), to define non-specific binding. Autoradiograms were analysed by computer assisted densitometry (Quantimet 970) with reference to the Monnier & Gangloff, (1961) rabbit brain atlas.

Results are given as mean ± s.e.mean of fmol mg^{-1} tissue (n = 4). The highest density of [^{3}H]2-BFI binding was observed in the epiphysis (E; 112 ± 16), and ventral hippocampus (HIV; 80 ± 11), with moderate binding in the caudate (Nca; 44 ± 5), cuneate (62 ± 6), and interpeduncular nuclei (59 ± 7), cortex (Aop; 35 ± 4), putamen (Pu; 24 ± 3), thalamus (NIT; 36 ± 5), hypothalamus (40 ± 4), solitary tract (45 ± 7), and dorsal hippocampus (HID; 42 ± 3). [^{3}H]2-BFI binding was lowest in the corpus callosum (3 ± 0.2) and internal capsule (4 ± 2). Total [^{3}H]2-BFI binding was reduced to background levels by the addition of 10 µM BU224 (> 95 % specific binding).

Fig. 1. Autoradiographic distribution of total 0.3 nM [^{3}H]2-BFI binding to a parasagittal section of rabbit brain. Scale bar = 5 mm.



Autoradiography of [^{3}H]2-BFI has revealed I_2 sites to be present in many areas of rabbit brain, its widespread distribution being comparable to that observed using [^{3}H]idazoxan labelling of I_2 sites in rabbit brain (Renouard *et al.*, 1993). The selectivity and low non-specific binding further supports the notion that [^{3}H]2-BFI represents a superior radioligand for the study of I_2 sites.

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66P AFFINITIES OF EFAROXAN DERIVATIVES FOR α_2-ADRENOCEPTORS AND I_2-BINDING SITES IN RAT BRAIN MEMBRANES

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Efaroxan is an α_2-adrenoceptor antagonist and also a potent insulin secretagogue in the pancreas. This effect on insulin is thought to be mediated *via* an imidazoline (I) receptor (Chan *et al.*, 1995), distinct from the I_2 imidazoline binding site in rat brain, which may indicate efaroxan has potential in the study of diabetes. For this reason a series of efaroxan derivatives were synthesised as potential tools in studying this mechanism. These compounds KU14R (imidazole-efaroxan), nitro-efaroxan, amino-efaroxan, JCF7 (2,3-dihydro-2-benzofuranyl-2-imidazole), EF10750 (tetrazole-efaroxan and JCF9 (phenylimidazoline-efaroxan) have been examined for their affinities at α_2-adrenoceptor and I_2-binding sites in the rat brain.

Rat (male, Wistar 275g) brains were homogenised (10vol 50mM Tris-HCl buffer, pH 7.4 containing 320mM sucrose) and centrifuged (1000g, 10min). The precipitate was discarded and supernatant centrifuged (32,000g, 20min). The resulting P_2 membrane preparations were washed twice by centrifugation (32,000g 20min, 50mM Tris-HCl, pH 7.4) and frozen (-70°C) until use. Aliquots of thawed membrane (300µg protein) were incubated (45min, 22°C) with either 1nM [^{3}H]RX821002 or 1nM [^{3}H]2-BFI (2-(2-benzofuranyl)-2-imidazoline) to label α_2-adrenoceptors and I_2-binding sites respectively (Lione *et al.*, 1996). α_2-Adrenoceptors specific binding was defined by rauwolscine (10µM) and I_2 specific binding was defined by BU224 (Hudson *et al.*, 1994) (10µM). Efaroxan and its analogues were examined for their ability to compete with the labelled ligands over the range of 0.1nM - 100µM. Bound ligands were separated by filtration and determined by liquid scintillation counting. Results were analysed by Prism (GraphPAD Software, 1994).

Displacement curves for all seven compounds were best fit to a single site in each case. KU14R, nitro-efaroxan, amino-efaroxan and JCF7 showed low affinity for the α_2-adrenoceptor with amino-efaroxan showing the highest affinity (Table 1.). However, of all the compounds investigated only JCF7 had any affinity for I_2-binding sites in the rat brain and this was only 2.4 fold greater than its affinity at α_2-adrenoceptors.

Table 1 Affinities of Efaroxan and its analogues for α_2-adrenoceptors and I_2-binding sites in rat brain membranes. Data are mean K_i values ± s.e.mean from three to four experiments performed in triplicate.

	K_i (nM)	
	α_2-adrenoceptors	**I_2-binding sites**
Efaroxan	3.77 ± 0.112	>10,000
KU14R	349.8 ± 68.7	>10,000
Nitro-efaroxan	714.5 ± 38.8	>10,000
Amino-efaroxan	189.8 ± 20.9	>10,000
JCF7	643.0 ± 11.9	1546.7 ± 120.5
EF10750	>10,000	>10,000
JCF9	>10,000	>10,000

Clearly these efaroxan derivatives have very little affinity for either α_2-adrenoceptors or I_2-binding sites in the rat brain. Whether they show affinity or functional activity for the pancreatic imidazoline binding site is currently under investigation.

This work was supported by the Wellcome Trust.

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67P CHRONIC CLORGYLINE AND DEPRENYL TREATMENT DECREASE IMIDAZOLINE$_2$ SITE DENSITY IN RAT INTERPEDUNCULAR NUCLEUS

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Although imidazoline-2 (I_2) binding sites are widespread in rat brain, they are particularly dense in discrete brain nuclei such as the interpeduncular (IPN), arcuate and area postrema. (Mallard *et al.*, 1992). There is evidence (Parini *et al.*, 1996) that the I_2 site is a domain on the isoforms of the enzyme monoamine oxidase (MAO). Brain homogenate binding studies have shown chronic treatment of rats with the irreversible MAO_A inhibitor clorgyline downregulates I_2 site binding (Olmos *et al.*, 1993). We have now used receptor autoradiography to examine the effects of several chronic drug treatments on I_2 sites in the rat IPN.

The drugs or saline (controls) were administered i.p., twice daily for 14 days, to male Wistar rats (250g). The drug doses used were 3mg kg^{-1} clorgyline, deprenyl (irreversible MAO_B inhibitor) and ethoxy-idazoxan (α_2-adrenoceptor antagonist), and 10mg kg^{-1} moclobemide (reversible MAO_A inhibitor), 2BFI (2-(2-benzofuranyl)2-imidazoline, I_2-site ligand) and BU224 (2-(4,5-dihydroimidaz-2-yl)-quinoline, I_2-site ligand). 24 hours after their last injection the rats were anaesthetised (pentobarbitone 60 mg kg^{-1}) and the brains removed and frozen. Following cryostat sectioning (12µm slices), I_2 sites were labelled with 1nM [^{3}H]2BFI (for methods see Mallard *et al.*, 1992), with the specific component defined with 10µM BU224. Autoradiograms were allowed to develop with tritium standards for 28 days and then quantitated on a densitometer.

I_2 site densities in the IPN are shown in Figure 1. Relative to the saline controls (96.2±5.5 fmol mg $^{-1}$ tissue) chronic deprenyl and clorgyline treatment markedly decreased I_2 site binding to 23.0±4.4 and 25.0±8.4 fmol mg^{-1} tissue respectively. In contrast, moclobemide treatment failed to affect binding in this nuclei (84.0±12.5). Ethoxy-idazoxan treatment resulted in a small but insignificant decrease in I_2 site density (61.0±31.0). The I_2 site selective ligands 2BFI and BU224 also failed to affect I_2 site binding in the IPN.

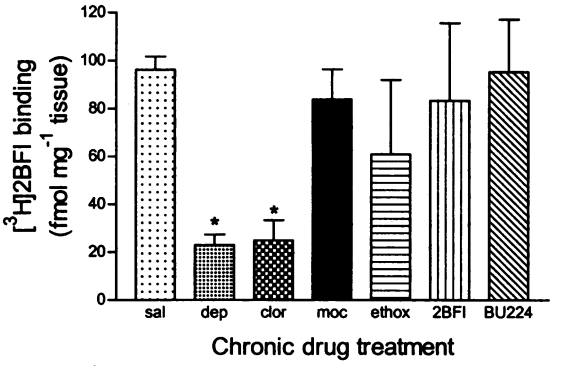

Figure 1. [^{3}H]2BFI binding to IPN following various chronic drug treatments.(*P<0.001, unpaired t-test, n=3-4 rats).

These data support the proposal by Parini *et al* (1996) that there is a link between MAO and I_2 sites. Alternatively, since moclobemide was without effect, the action of the irreversible MAO inhibitors may be an indirect effect on an I_2 site binding protein, as previously discussed by Olmos and coworkers (1993).

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68P AUTORADIOGRAPHY OF [^{3}H]MIVAZEROL BINDING IN THE RAT HEART

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The new anti-ischaemic drug mivazerol is a known α_2-adrenoceptor agonist. Peripherally, this α_2-agonist works by decreasing noradrenaline release (Zhang *et al.*, 1995), possibly from the right atrium which has a known distribution of α_2-adrenoceptors (Rump *et al.*, 1995). Consequently, 4 male Wistar rats (250g) were perfused and their hearts removed and analysed by the technique of radioligand receptor autoradiography. Hearts were sectioned transversely at 12 micron thickness from the aortic arch through to the ventricles. Autoradiography using either of the α_2-agonists [^{3}H]mivazerol or [^{3}H]clonidine (3 nM) was performed on the sections either in the presence or absence of rauwolscine (10 µM). Sections were apposed to tritium-sensitive hyperfilm (6 weeks) or tritium-sensitive emulsion coated coated coverslips (4 weeks). Results failed to detect binding of [^{3}H]clonidine to the rat heart. Conversely, [^{3}H]mivazerol displayed high binding throughout the rat heart, in particular the aorta and tricuspid annulus fibrosis (Table 1), which was not displaceable by the α_2-adrenoceptor antagonist rauwolscine. These results suggest binding to sites other than α_2-adrenoceptors. [^{3}H]Mivazerol binding was higher in the atria than in the ventricles, this may be explained by the lack of vagal innervation reaching the ventricles in comparison to that found in the atrial musculature and sinoatrial and atrioventricular nodes (Schlant and Sonnenblick., 1990).

Atrial chamber				
Rat heart	Total		Non-specific binding	
area analysed	Mean	s.e.mean	Mean	s.e.mean
Left atria	60.73	11.55	45.1	5.7
Right atria	59.48	8.07	62.48	19.73
Tricuspid annulus fibrosis	113.2	5.85	128.7	5.01
Aorta	145.28	31.8	155.23	46.21
Intra-atrial myocytes	49.83	4.3	55.63	6.08
Ventricular chamber				
Left ventricle	34.01	6.8	36.49	6.42
Right ventricle	36.47	9.28	29.32	5.46
Tricuspid annulus fibrosis	42	5.64	44.11	7.8
Aorta	~	~	~	~
Intraventricular septum	29.9	5.88	31.85	6.92

Table 1 Quantitated data of [^{3}H]mivazerol binding to rat hearts at the level of the atrial and ventricular chambers in fmol mg^{-1} protein (n=4).

[^{3}H]Mivazerol has been shown in this study not to bind to α_2-adrenoceptors, but the site to which this ligand acts is not known at present. The identification of this site may help to explain the anti-ischaemic actions of this drug.

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This study was funded by UCB Pharma.

69P THE ALLOSTERIC INTERACTIONS OF ANTAGONISTS WITH AMILORIDE ANALOGUES AT THE HUMAN α_{2_A}-ADRENOCEPTOR

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Apart from the primary binding site, G protein-coupled receptors may also possess a second, or allosteric, binding site. The binding of an allosteric agent to the allosteric site can influence the association and/or dissociation kinetics of a ligand binding at the primary binding site. Allosterism has been characterized in detail with the muscarinic receptors (Lazareno & Birdsall, 1995, and references therein), but has only been studied to a more limited extent at other G protein-coupled receptors. With adrenoceptors, it has been reported that the amilorides increase the dissociation rates of antagonists from the α_2 receptor, and it has been postulated that the amilorides were acting via an allosteric site (Howard *et al.*, 1987, Nunnari *et al.*, 1987). However, the same workers also reported that amiloride caused a decrease in the B_{max}, which is not compatible with the ternary complex allosteric model.

To examine whether the amilorides can allosterically modulate antagonist binding, the effect of amiloride on the equilibrium binding of [^{3}H]yohimbine and [^{3}H]rauwolscine was first examined. Under the assay conditions used (20mM Hepes, 100mM NaCl, 10mM $MgCl_2$, pH7.4, 30°), 0.1mM or 0.3mM amiloride did not significantly affect ($p>0.5$) the B_{max}. The effect of amiloride and its 5-N-alkyl derivatives on [^{3}H]yohimbine dissociation from the membrane-bound human α_{2a} adrenoceptor, expressed in CHO cells, was then studied in detail. The kinetic data fitted well to an equation derived from the ternary complex allosteric model (Lazareno & Birdsall, 1995), with amiloride concentration and time as two independent variables. The estimated maximal increase in the dissociation rate caused by the amiloride analogues varied from (2.02 ± 0.01)-fold (n=2) for the parent amiloride, to (138 ± 15) -fold (n=4) for 5-(N,N-hexamethylene)-amiloride (HMA). The log affinity constants of the amilorides at the yohimbine-occupied receptor ranged from 2.47 ± 0.02 (n=4) for HMA to 1.75 ± 0.07 (n=3) for 5-(N-ethyl-N-isopropyl)-amiloride. Using affinity estimates for the amilorides at the unliganded receptor determined from competition experiments, the observed cooperativity factor was calculated to vary from (3.6 ± 2) x 10^{-3} (n=2) for amiloride to (3.8 ± 0.2) x 10^{-5} (n=4) for HMA. The pronounced effect of HMA on [^{3}H]yohimbine dissociation could be reversed by increasing concentrations of amiloride in a manner which was quantitatively compatible with amiloride and HMA binding to the same site.

Thus the amiloride compounds do appear to interact with a separate allosteric site on the α_{2a} receptor, and to exert a high negative cooperativity on [^{3}H]yohimbine binding at the primary binding site.

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70P EFFECT OF INTRAVENOUSLY-ADMINISTERED CLONIDINE, RILMENIDINE AND MOXONIDINE ON BLOOD PRESSURE AND HEART RATE IN CONSCIOUS WILD-TYPE AND MUTANT D79Nα_{2A}-ADRENOCEPTOR (α_{2A}-AR) MICE

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The selective imidazoline$_1$ (I_1) receptor agonists rilmenidine (RIL) and moxonidine (MOX) are considered representatives of a new class of centrally-acting antihypertensive agents (Yu & Frishman, 1996). Selectivity over α_2-adrenoceptors (α_2-ARs) is claimed to reduce side effects associated with central activation of α_2-ARs (i.e. sedation and depression). In this study, we investigate the cardiovascular effects of the I_1 receptor agonists clonidine (CLO), RIL and MOX in conscious wild-type (129Sv) and mutant D79N α_{2A}-AR (functional 'knock-out'; MacMillan *et al.*, 1996) mice.

Male mice (22-34 g) were anesthetized with pentobarbital (50-60 mg kg^{-1}, ip). The left carotid artery and jugular vein were cannulated with PE-10 tubing. The cannulae were tunneled under the skin and placed into a fabric pocket secured to the back of the mouse. Mice were allowed to fully recover from anesthesia for at least 18 hrs. On 4 consecutive study days, mice were placed in restraint boxes and the arterial cannula was connected to a pressure transducer for the measurement of mean blood pressure (MBP) and heart rate (HR). Vehicle or 1 of 3 doses of test agonist was administered to each mouse via the venous cannula (0.5 ml kg^{-1}; 4 X 4 Latin square). CLO and RIL were dissolved in 0.9% saline. MOX was dissolved in distilled H_2O (pH 7.0). MBP and HR were measured every 5 min for 2 hrs post-dose.

All 3 agonists produced dose-dependent decreases in MBP in conscious 129Sv mice which lasted the duration of the 2 hr study. In contrast, agonist-induced decreases in HR returned to baseline within 2 hrs. In D79N mice, the decrease in MBP and HR produced by RIL and MOX were abolished. For CLO, the decrease in MBP was not observed and a dose-dependent pressor effect was exposed. The bradycardia observed with CLO in D79N mice mirrored the pressor effect suggesting a reflex mechanism of action (Table 1).

Table 1. Baseline values and the maximum decrease in MBP and HR following intravenous administration of agonists in conscious mice. Values are means ± s.e. mean.

Agonist	Dose	Wild Type 129Sv		Mutant D79N α_{2A}-AR	
		MBP	HR	MBP	HR
	(μg kg^{-1})	(mmHg)	(beats min^{-1})	(mmHg)	(beats min^{-1})
CLO	baseline	136±5	473±20	146±5	516±21
	Veh	8±4	64±23	10±4	24±7
	30	18±2	112±31	16±2	35±18
	100	24±3[1]	195±33[1]	14±3	109±36[1]
	300	30±4[2]	291±28[2]	13±4	180±55[2]
RIL	baseline	137±3	559±14	142±4	472±24
	Veh	11±2	62±14	14±5	121±58
	100	12±3	109±39	15±1	112±16
	300	17±5	103±23	11±4	87±27
	1000	28±1[2]	161±16[1]	9±4	109±57
MOX	baseline	141±2	481±22	150±3	493±28
	Veh	9±1	27±11	10±3	90±44
	100	16±1[1]	63±19	13±4	112±62
	300	23±3[2]	98±38	16±5	123±39
	1000	27±3[2]	142±28[2]	19±7	68±10

[1]$p<0.05$ or [2]$p<0.01$ versus vehicle; baseline = values at time 0.

This study fails to demonstrate hypotension which can be attributed to I_1 receptors. The hypotension and bradycardia observed in 129Sv mice are consistent with actions at α_{2A}-ARs as described previously (MacMillan *et al.*, 1996). These data support the contention that α_2-ARs mediate the antihypertensive effects of clonidine and other selective I_1 receptor agonists (Stornetta *et al.*, 1995; MacDonald *et al.*, 1997).

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71P COMPARISON OF THE SPINAL *VERSUS* SUPRASPINAL ANTINOCICEPTIVE ACTIONS OF FOUR α_2-ADRENOCEPTOR AGONISTS IN CHLORALOSE ANAESTHETISED RATS

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The clinical profiles of claimed α_2-adrenoceptor agonists vary in their antinociceptive, sedative, muscle relaxant and cardiovascular actions. We have compared the effects of four clinically available agonists, medetomidine, xylazine, romifidine and tizanidine, mediated at spinal and supraspinal sites, on spinal nociceptive reflexes.

Methods were as used by Herrero & Headley (1991). Male Wistar rats were anaesthetised with halothane and the trachea, carotid artery and both jugular veins were cannulated. After a laminectomy at T10-11 the cord was either sectioned or left intact. Subsequently anaesthesia was maintained with α-chloralose (15-20 mg kg^{-1} h^{-1}). Single motor unit activity was evoked in hindlimb flexor muscles by noxious pinch to the receptive field (mean 1.8 N over 16 mm^2 in spinalised; 1.5 N in intact). Drugs were given i.v. in cumulative doses and effects calculated as mean percentage of the last three pre-drug responses ± s.e. mean, from which estimates were made of ID_{50} values (taken as the dose producing 50% inhibition).

In spinalised animals, medetomidine (1.25-10 μg kg^{-1}) and xylazine (20-320 μg kg^{-1}) dose dependently reduced responses to pinch (Table 1). Responses to pinch stimuli recovered from xylazine within 20 min to 93 ± 5 % control. The longer-lasting inhibition caused by medetomidine (10 μg kg^{-1}) was reversed from 33 ± 13 % to 81 ± 5 % by 160-320 μg kg^{-1} of the α_2-adrenoceptor antagonist atipamezole. In contrast romifidine (40-320 μg kg^{-1}) and tizanidine (80-640 μg kg^{-1}) tended to enhance pinch responses (at top dose of romifidine to 123 ± 11 %, n = 8; tizanidine to 135 ± 14 % control, n = 8). The α_1-adrenoceptor antagonist prazosin (0.5-1 mg kg^{-1}) converted this enhancement to inhibition (romifidine to 64 ± 13 %; tizanidine to 32 ± 13 %). After prazosin the inhibition could be reversed towards control by atipamezole. Pretreatment with prazosin unmasked the inhibitory actions of tizanidine and romifidine, allowing ID_{50} values to be estimated for spinalised animals (Table 1).

In sham spinalised animals medetomidine and xylazine had similar effects on nociceptive reflexes. Under these conditions tizanidine (5-40 μg kg^{-1}; n = 7) and romifidine (0.31-5 μg kg^{-1}; n = 4) reduced rather than enhanced responses (tizanidine to 48 ± 19 %; romifidine to 54 ± 3 % at top doses). These inhibitions were little affected by prazosin (1 mg kg^{-1}) but were reversed by atipamezole (160 μg kg^{-1}), (tizanidine to 98 ± 7 %; romifidine to 90 ± 6% control).

Table 1. ID_{50} values on nociceptive reflexes of four α_2-adrenoceptor agonists in spinalised and spinally intact rats.

DRUG	SPINALISED		SPINALLY INTACT		POTENCY RATIO
	μg kg^{-1}	n	μg kg^{-1}	n	
MEDETOMIDINE	3.4 ± 0.4	9	2.0 ± 0.2	7	1.7
XYLAZINE	310 ± 40	14	61 ± 5	17	5.0
TIZANIDINE	670 ± 102 *	6	41 ± 15	4	16.3
ROMIFIDINE	122 ± 42 *	6	5 ± 1	4	24.4

* ID_{50} from data obtained in the presence of 1 mg kg^{-1} of prazosin

Comparing ID_{50} data (Table 1) revealed that medetomidine and xylazine were similarly potent in spinalised and spinally intact animals whereas tizanidine and romifidine were appreciably more potent under spinally intact conditions.

The data indicated that some claimed α_2-adrenoceptor agonists activate α_1- as well as α_2-adrenoceptors. The α_1- component results in excitation at spinal level but supraspinally enhances the antinociception mediated by α_2-adrenoceptor activation.

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Herrero J.F. & Headley P.M. (1991) Br. J. Pharmacol. 104, 166-170.

72P Mrz 2/576, A GLYCINE SITE ANTAGONIST, BLOCKS SPINAL NEURONAL RESPONSES AND SHOWS ANALGESIC PROPERTIES IN BEHAVIOURAL TESTS

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Mrz 2/576 is one of a new series of tricyclic pyrido-phthalazine diones that are selective and systemically active antagonists at the strychnine insensitive glycine modulatory site of the NMDA receptor complex (Chizh *et al.*, 1996; Parsons *et al.*, 1977). We have compared Mrz 2/576 with uncompetitive NMDA channel blockers on electrophysiological tests of nociception. In behavioural tests the activity of this agent was compared with morphine using mechanical and thermal nociceptive thresholds with concurrent motor function assessments.

Extracellular action potentials were recorded from hindlimb flexor muscle single motor units in α-chloralose anaesthetised spinalised male Wistar rats (280-350 g). Cumulative i.v. doses of antagonists were tested on responses evoked by 'high' and 'low' intensities of noxious pinch stimuli applied to the hindpaw receptive field. Effects are expressed as % of the last three pre-drug responses (mean ± s.e.m.). Slope values were obtained when the log of the doses reducing responses to 50% control (ID_{50}) were plotted against control response amplitude using linear regression analysis. Conscious male Sprague-Dawley rats (250-350 g) underwent paw pressure, thermal (Hargreaves) and rotarod (accelerating) tests. Baseline values were obtained prior to i.p. administration of test compounds and data were analysed as differences from these values (n=8 per point).

Mrz 2/576 reduced spinal nociceptive reflex responses in a dose dependent manner (ID_{50} 2.8 ± 0.6 mg kg^{-1}, n=6). The slope values (log ID_{50} (mg kg^{-1}) per spike s^{-1}) obtained with the uncompetitive NMDA antagonists were: memantine ($8.5x10^{-2}$, n=28)>ketamine ($5.4x10^{-2}$, n=49)>MK801 ($4.7x10^{-2}$ n=21) with Mrz 2/576 being the least dependent on control firing rate ($2x10^{-2}$, n=12). The rank order of slope values *in vivo* matches the rank order of voltage dependence reported *in vitro* (Parsons *et al.*, 1993, 1997.

Time course analysis following i.p. administration showed that the effects of Mrz 2/576 peaked at 5 minutes while morphine peaked at 30 minutes. Mrz 2/576 and morphine both increased mechanical and thermal thresholds. The lowest dose of Mrz 2/576 that produced an enhancement of paw pressure threshold (2.5 mg kg^{-1}) did not impair rotarod performance. A similar analgesic effect was seen with morphine at this dose.

We have previously shown *in vivo* correlates of the voltage dependence of channel blocking NMDA antagonists (Jones *et al.*, 1996). As predicted the $glycine_B$ site antagonist Mrz 2/576 is least affected by spike discharge rate. In normal animals Mrz 2/576 is a selective and systemically active NMDA antagonist that is effective at reducing nociceptive reflex responses, is analgesic at doses below those causing motor impairment and has a similar potency to morphine in these tests.

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73P RESPONSES OF NEURONES IN THE OUTER DORSAL HORN OF RAT LUMBAR SPINAL CORD SLICES TO A SELECTIVE NEUROKININ 1 RECEPTOR AGONIST

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Cutaneous primary afferents containing Substance P (SP) terminate predominantly in lamina I (LI) and the outer part of LII (LIIo) of the dorsal horn of the spinal cord. In LI a population of neurones with non-spiny dendrites running mainly in the rostral caudal and medio-lateral orientations express the neurokinin 1 (NK1) receptor which on the basis of affinities is the probable target for SP. The actions of a selective NK_1 agonist on neurones in LI and LIIo which do, or do not, express NK_1 receptors has been investigated.

Neonatal (15-25 day old) rats were anaesthetised with ether and decapitated. Parasagital slices of the 3-6 lumbar segments were prepared with intact dorsal roots, dorsal root ganglia and peripheral nerves. Intracellular recordings were made from neurones in LI-LIIo with electrodes containing 2% Neurobiotin in 3M potassium methyl sulphate. Following the characterisation of the neurones responses to intracellular current injection, bath application of the selective NK_1 receptor agonist, [Sar^9, $Met(O_2)^{11}$]SP (1μM) and peripheral nerve stimulation, the neurones were labelled by microiontophoretic injection of the neurobiotin. Subsequent histological analysis was used to investigate the co-localisation of NK_1 receptors, immunocytochemically, on labelled neurones using a double fluorescence protocol. The neurones were then further stained to permit their detailed anatomical reconstruction.

Thirteen neurones were located and labelled adequately to determine their receptor expression and morphology. Of these five expressed the NK_1 receptor. Four of these were strongly depolarised by 1μM [Sar^9, $Met(O_2)^{11}$]SP (3-15mV). Neurones not expressing the NK_1 receptor were not directly depolarised by this agonist. The NK_1 receptor expressing neurones produced EPSPs in response to peripheral nerve stimulation at intensities which activate both Aδ- and C-fibre primary afferents. Depolarising current injection produced multiple firing in these neurones with limited spike frequency adaption. Their action potentials were followed by marked after-hyperpolarisations. Hyperpolarising current injection revealed a small inward rectification but no delayed rectification. These neurones had dendrites and soma which were confined principally to LI with a few entering LIIo but not deeper. Their local axon branches were more extensive with some extending into LIIi and LIII. In contrast the neurones not expressing NK_1 receptors had much more diverse structure with several neurones having dendrites descending into LIIi and LIII which bore spines. The responses of these neurones to afferent stimulation and intracellular current injection were also more heterogeneous.

From these observations SP released from primary afferents would be predicted to act on a sub-population of LI neurones with a characteristic structure. These neurones have biophysical and pharmacological charcteristics which are homogeneous and will underlie their specific function role. They are a prime target for analgesic action and their detailed charcterisation may suggest new targets for drug development.

74P SINGLE AMINO ACID SUBSTITUTION STABILIZES HIGH AFFINITY STATE OF THE NK-1 TACHYKININ RECEPTOR FOR NEUROKININ A AND B

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The present study examines the pharmacological properties of genetically engineered human NK1 tachykinin receptors in which residues at the extracellular surface of the fourth transmembranal domain were substituted with the corresponding aminoacids from NK2 receptor.
The G166C, Y167F and the G166C substitutions equally induced high affinity binding of a group of tachykinin ligands, including neurokinin A, neurokinin B, septide ([pGlu6, Pro9]-substance P(6-11)) and substance P-methylester, otherwise characterized by the apparent lack of binding competence of the wildtype NK1 receptor.
Direct assessment to the binding constant employing radiolabelled neurokinin A ([^{125}I]-NKA) confirmed its high affinity interaction with the G166C mutant receptor. Surprisingly, [^{125}I]-NKA also bound strongly to the wildtype receptor, in sharp contrast to its low apparent affinity to displace [^{125}I]-Substance P. Interestingly, we found that the binding constant of neurokinin A did not change in response to the mutation.
The use of multiple radioligands in parallel and simultaneous data analysis (Munson *et al.*, 1980) demonstrated that, the experimental data can only be adequately accounted for when tachykinins are depicted as binding to two independent classes of binding sites. These two classes of the NK-1 receptor display equal affinity for SP, while NKA strongly discriminate them as shown in table 1. The high and low affinity component of NKA binding do not change in response to the mutation. We do observe, however, that the single residue substitution greatly affects the relative proportion of the two forms of the NK-1 receptor (table 1). A similar trend is seen for other mutation sensitive ligands. Hence, the single aminoacid mutation seems to define two pharmacologically distinct forms of the NK-1 receptor.
Finally, analysis of the effect of N-ethylmaleimide (Li H. *et al.* 1996) and dithiothreitol, which alkylates sulfhydryl groups and reduces cysteine bound, on radiolabeled Substance P and neurokinin A binding confirm the different mode in which these ligands interact with the receptor and support the existence of a mutation induced change in the conformational status of the NK-1 receptor.

Table1.
Dissociation constants of Substance P and neurokinin A for binding to wildtype and G166C NK1 receptors.

Ligands	Dissociation Constant (Kd)(nM) Wildtype		G166C	
	Kd^H	Kd^L	Kd^H	Kd^L
SP	0.54	0.54	0.25	0.25
NKA	3.7	860	1.4	1200
Bmax(%)	31	69	96	4
S.E.means of Bmax	4	4	1	1

All reported data, n=7, have a standard error of the mean lower than 30% (Kd^H, high affinity and Kd^L, low affinity component).

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75P THE NEUROKININ 1 (NK) RECEPTOR ANTAGONIST GR205171 REDUCES CARRAGEENAN-EVOKED FOS-LIKE IMMUNOREACTIVITY IN THE RAT LUMBAR SPINAL CORD

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Intraplantar carrageenan elicits mechanical hyperalgesia, paw oedema and Fos-like immunoreactivity (Fos-LI; peak response time 3h) in discrete areas of the rat lumbar spinal cord associated with nociceptive input (Honoré *et al.*, 1995). We have investigated the effect of the NK_1 antagonist GR205171 (rat pKi 9.5, human pKi 10.5, Gardner *et al.*, 1996), its lower affinity enantiomer GR226206 (human pKi 6.8 [2-2-methoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-(2S-phenyl-piperidin-3R-yl)-amine dihyrochloride), and morphine, on carrageenan-evoked Fos-LI. Male Random Hooded rats (160-220g) received 2% carrageenan (0.1ml; i.pl.) and were sacrificed under anaesthesia (60mgkg^{-1}; pentobarbitone; i.p.) by cardiac perfusion 3 hours later. Drugs or vehicle (saline) were administered 30mins. prior to carrageenan. Following fixation, the spinal cord (L3-L5) was removed, and Fos-LI investigated using standard immunocytochemistry. For counting Fos-LI nuclei, sections were divided into 5 areas: area 1 (laminae I&II), area 2 (III&IV), area 3 (lateral V&VI), area 4 (medial V&VI) and area 5 (VII-X). Data is presented as mean ± s.e.mean (total in 6 sections; L4). Statistical significance assessed using Student's unpaired t-test with Bonferroni correction for multiple comparisons where appropriate ($P<0.05$ significant).

Following carrageenan and vehicle (s.c. saline) total numbers of ipsilateral Fos-LI nuclei were significantly increased to 1533±31 (n=5; $P<0.01$) vs 94±35 (n=6; i.pl. saline), with 684±33 in area 1, 98±10 in area 2, 297±13 in area 3, 180±9 in area 4, and 275±14 area 5. GR205171 (3mgkg^{-1}s.c.) significantly reduced total Fos-LI nuclei by 20% (1228±47; $P<0.01$; n=4), with reductions of 38% and 49% in areas 1 and 3 respectively ($P<0.01$). There was no significant change in areas 2 and 5; however, there was a significant increase (45%; $P<0.01$) in area 4. GR226206 (3mgkg^{-1} s.c.) had no significant effect on total numbers of Fos-LI nuclei (1482±24; n=4), however a significant reduction of 23% ($P<0.01$) in area 3 was observed, with a significant increase of 46% ($P<0.01$) in area 4. There was no significant inhibition in areas 1, 2 and 5. Morphine (2mgkg^{-1}s.c.) significantly reduced total Fos-LI nuclei by 58% (514±70; n=4; $P<0.01$), with reductions of 70%, 27%, 64%, 28%, and 50% in areas 1-5 respectively ($P<0.05$ throughout). Drug treaments did not visibly reduce paw oedema. In conclusion, GR205171 inhibited carrageenan-induced Fos-LI in laminae I&II in an enantiomer specific manner. Thus, NK_1 receptors may mediate nociceptive transmission. Conversely, increases in Fos-LI nuclei in medial V&VI were not enantiomer specific. As yet, we have no explanation for the responses seen in lateral and medial V&VI. Morphine reduces Fos-LI in all areas.

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76P CHARACTERISATION OF THE TACHYKININ RECEPTORS MEDIATING THE JEJUNAL DISTENSION-INDUCED VISCERAL PAIN REFLEX IN RATS

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Distension of the jejunum in anaesthetised rats is considered to be a noxious stimulus and elicits an intraluminal pressure-dependent, capsaicin-sensitive fall in diastolic blood pressure. This reflex depressor response is considered to be predictive of visceral nociception as it is potentiated by naloxone, blocked by morphine (Ness & Gebhart, 1990) and abolished by capsaicin (Lembeck & Skofitsch, 1982). Since tachykinins are involved in capsaicin-sensitive nociceptive neurotransmission, we tested the effect of neurokinin receptor antagonists on the jejunal distension-induced reflex.

Male Wistar rats (300-400 g) were anaesthetised with pentobarbitone sodium (60 mg kg^{-1}, s.c.). The proximal jejunum was distended with a latex balloon (12.5-100 mm Hg, 25 s every 5 min) via a polyethylene catheter and blood pressure recorded via the left common carotid artery. Animals were maintained at approximately 35°C by placing them on a heat pad. Drugs were given intraperitoneally (i.p.) and intestinal distension recommenced 10 min after drug administration and repeated at 5 min intervals up to 25 min following administration.

The jejunal distension-induced depressor response was inhibited in a dose-dependent fashion by the tachykinin NK_2 receptor antagonist SR48968 (0.1-10 mg kg^{-1}, i.p., ED_{50} = 0.7 mg kg^{-1} (95% C.L., 0.4-1.5); n=19) and the NK_1 receptor antagonist CP99994 (0.3-3 mg kg^{-1}, i.p., ED_{50} = 0.8 mg kg^{-1} (95% C.L., 0.1-6.1); n = 11). The tachykinin NK_3 receptor antagonist, SR142801 (0.3-10 mg kg^{-1}, i.p., n = 21) did not significantly affect the responses to jejunal distension. In separate experiments, SR48968 (3 and 10 mg kg^{-1}, i.p.) and CP99994 (3 mg kg^{-1}) also reduced the sensitivity to jejunal distension producing a 2.5-fold (SR48968, 3 mg kg^{-1}), 4.7-fold (SR48968, 10 mg kg^{-1}) and 2.7-fold (CP99994, 3 mg kg^{-1}) increase in threshold pressure. The administration of each of the antagonists did not significantly affect resting blood pressure or the relationship between intestinal pressure and volume i.e. compliance.

These results indicate that tachykinin NK_1 and NK_2 receptors mediate the jejunal distension-induced visceral pain reflex in the anaesthetised rat and suggest either that NK_3 receptors are not involved or are not inhibited by the concentration of SR142801 used in the study. The apparent antinociceptive effects cannot be attributed to changes in intestinal compliance and are likely to be as a result of an effect on the reflex pathway.

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77P *PHONEUTRIA NIGRIVENTER* SPIDER VENOM INDUCES OEDEMA FORMATION IN RAT SKIN BY ACTIVATION OF CAPSAICIN-SENSITIVE PRIMARY AFFERENT NEURONS

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Stimulation of sensory neurons can lead to neurogenic oedema formation. Previous studies have revealed that the neurogenic oedema is inhibited by using rats pretreated with capsaicin as neonates (Jancso *et al.*, 1967). The venom from the spider *Phoneutria nigriventer* (PNV) induces rat skin oedema which is partially mediated via a mechanism involving the tachykinin NK_1 receptor (Palframan *et al.*, 1996). The aim of the present study was to determine whether PNV acts directly or indirectly via activation of sensory neurons to cause NK_1 mediated oedema formation.

Capsaicin (50 mg kg^{-1}) was s.c. administered to neonatal (2 days old) Wistar rats under anaesthesia, in order to destroy capsaicin sensitive afferent nerve fibers. Control neonatal animals were s.c. injected with the capsaicin vehicle (10% ethanol and 10% tween 80, in saline). Sixty to seventy days later, the rats (200-280 g) were anaesthetised with sodium pentobarbitone (50 mg kg^{-1}, i.p.) and intradermally (i.d.) injected with PNV (1-10 µg $site^{-1}$) either alone or with the NK_1 receptor antagonist SR140333 (1nmol $site^{-1}$). The skin oedema was measured as the extravascular accumulation of previously injected i.v. ^{125}I-human serum albumin (Palframan *et al.*, 1996). Statistics were by one way ANOVA and Bonferroni's modified t-test.

Intradermal injection of PNV (1-10 µg $site^{-1}$) caused a dose-dependent and immediate inflammatory oedema in vehicle-pretreated animals (µl $site^{-1}$, n=4), which was significantly reduced ($P<0.05$, n=5) in rats pretreated with capsaicin as neonates, see Table 1.

Table 1. Effect of capsaicin pretreatment on oedema formation induced by PNV. Results are expressed as µl plasma extravasated per site. Mean ± s.e.mean, n=4-5. *$P<0.05$ and #$P<0.05$ compared to PNV sites in vehicle pretreated rats.

GROUP	Vehicle Pretreated		Capsaicin Pretreated	
	Alone	+ SR140333	Alone	+ SR140333
PNV 1 µg	28 ± 4.2	16 ± 0.3*	21 ± 2.8#	23 ± 0.3
PNV 3 µg	37 ± 1.5	22 ± 0.3*	26 ± 3.4#	26 ± 3.4
PNV 10 µg	58 ± 4.8	29 ± 2.0*	26 ± 4.7#	26 ± 4.4

Our results suggest that PNV directly activates the capsaicin-sensitive primary afferent neurons to release substance P or a related peptide which acts via NK_1 receptors to induce oedema formation. These findings indicate that PNV may constitute an useful tool to investigate the pathophysiological role of sensory neurons. Further analysis of the mechanism by which PNV activates sensory nerves is under study.

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78P INHIBITION OF NERVE GROWTH FACTOR-INDUCED PLASMA EXTRAVASATION IN RAT SKIN BY IMMUNOGLOBULIN-LIKE DOMAINS OF NERVE GROWTH FACTOR trkA

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Nerve Growth Factor (NGF) is known to induce plasma extravasation when injected into rat skin (Otten *et al*, 1985) and is considered to be involved in the generation of inflammatory pain. NGF binds to the immunoglobulin (Ig)-like domains of the trkA receptor, and recombinant Ig-like domains (trk-$Ig_{1/2}$) have been shown to inhibit NGF-induced neurite outgrowth of PC12 cells (Asopa *et al*, 1996). The aim of this study was to investigate the ability of trk-$Ig_{1/2}$ to inhibit NGF oedema-inducing responses *in vivo*.

Male Wistar rats were anaesthetized with sodium pentobarbitone (50mgkg^{-1}, i.p.) the dorsal skin shaved and test agents (0.1ml$site^{-1}$ in Tyrode), injected intradermally (i.d.) in a balanced, randomized pattern. Oedema formation was measured by the extravascular accumulation of ^{125}I-albumin (100kBq, i.v.) over 0-30 min (Brain & Williams, 1985). Statistical comparisons between treatments were made using ANOVA followed by Bonferroni's modified t-test.

Injection of 7S NGF (8 pmol $site^{-1}$ produced a significant plasma extravasation that was inhibited by co-injection of trk-$Ig_{1/2}$ (24 pmol $site^{-1}$), see table 1. In a further experiment to assess selectivity, pretreatment of skin sites with trk-$Ig_{1/2}$ (24 pmol $site^{-1}$). significantly inhibited plasma extravasation induced by NGF (8 pmol $site^{-1}$ 38.6 ± 9.8 µl, $p<0.01$ compared with 74.2 ± 8.3 µl for NGF alone) but not that induced by the NK_1 agonist GR73632 (30 pmol $site^{-1}$; 43.1 ± 9.5 µl, compared to 47.2 ± 9.5 µl $site^{-1}$ for GR73632).

All results are mean ± s.e.mean µl plasma $site^{-1}$, n=4.

These results indicate that trk-$Ig_{1/2}$ can bind to NGF and prevent its' action *in vivo* and presents a useful model for the evaluation of NGF receptor agonists and antagonists.

Gavin Bennett is the recipient of a BHF PhD studentship.

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Table 1 The effect of trk-$Ig_{1/2}$ co-injection on plasma extravasation induced by NGF and Tyrode. Results are mean ± s.e.mean µl plasma $site^{-1}$ n=6 **$p<0.01$ compared with sites receiving NGF alone.

	NGF (8pmol $site^{-1}$)	Tyrode (vehicle)
Agent alone	57.7 ± 9.2	16.7 ± 3.8
8 pmol trk-$Ig_{1/2}$	57.0 ± 4.6	20.0 ± 3.6
24 pmol trk-$Ig_{1/2}$	29.4 ± 5.0	18.2 ± 2.8

79P METABOLISM OF SUBSTANCE P IN RAT STRIATUM STUDIES USING A COMBINATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AND CAPILLARY ELECTROPHORESIS (CE)

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Substance P (SP) and several SP fragments modulate dopamine release from striatal slices (Khan, *et al.*, 1996). However, it is not clear whether all these fragments are formed *in vivo*. The aim of our study was to investigate the metabolism of SP in rat striatum using HPLC and CE.

Male Wistar rats (250-300 g)(3 per time point) were anaesthetized with Hypnorm (1 ml kg^{-1}, i.p.) and 1 μl SP in saline was injected into the right striatum. The animals were killed at 2, 10 or 20 min. The left striata were used as controls. Tissues were homogenised in ice-cold 1M HCOOH and centrifuged (7,000 g, 4°C, 30 min). The supernatant was chromatographed on an Ultrasphere 5 μm ODS column with a gradient of CH_3CN (5% to 30% over 20 min and held for 10 min) in 0.1% CF_3COOH. The peaks were detected at 214 nm. Fractions (0.5 ml) were collected for CE analysis. CE was performed with 57 cm x 75 μm i.d. silica capillaries with a run buffer of 50 mM phosphate, pH 2.44, at 20 kV. Detection was at 200 nm.

Pilot studies showed that when 1 pmol [^{3}H-Leu^{10}]SP was injected the disappearance was so rapid that only [^{3}H]leucine was detected. Injection of 10 nmol SP allowed the decay to be monitored. A plot of SP concentration *vs.* time gave a straight line of slope -4 x 10^{-7} M min^{-1}, R^2 = 0.986, suggesting the reaction was zero order. The rate of metabolism was 0.2 nmol min^{-1}. The background UV signal was such that it was difficult to discern the presence of metabolites, however some peaks appeared to be larger after treatment with SP (Figure 1). When the appropriate fraction was subjected to CE, SP(1-7) was undetectable in the control but present in the treated striatum (Figure 1).

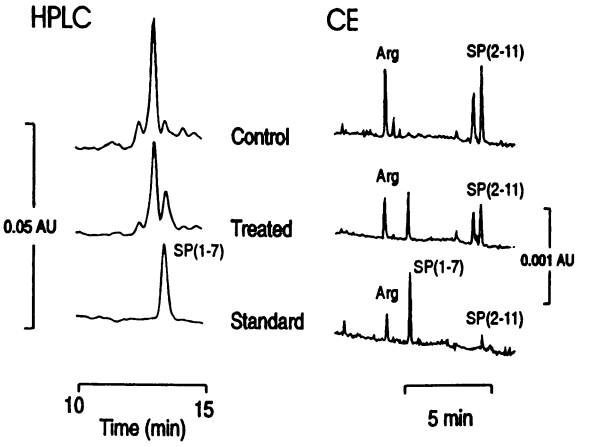

Figure 1. Chromatograms of treated, control and standard samples (left) and electropherograms (right) of the fraction corresponding to the retention time of SP(1-7). Arg and SP(2-11) were added as migration time markers.

HPLC combined with CE represents an important advance over either technique alone. SP(1-7) appeared to be a major *in vivo* metabolite and no other species appeared to be formed in such significant amounts. This is in contrast with the data reported when SP was infused intrastriatally over 2 h (Andrén & Caprioli, 1995).

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80P ENDOTOXIN-INDUCED CYCLOOXYGENASE EXPRESSION IN THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS

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Cyclooxygenase (prostaglandin H synthase, COX) exists in at least two isoforms. The COX-1 isoenzyme is constituently expressed in most tissues while the COX-2 isoform is inducible and is expressed in migratory cells e.g. macrophages in response to infection or inflammatory stimuli (Vane & Botting, 1995). Antigenic stimuli can also stimulate the hypothalamo-pituitary-adrenal (HPA) axis by mechanisms which involve eicosanoid generation but the respective roles of COX-1 and COX-2 in this process are unclear. This study utilizes semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) to examine the effects of endotoxin, which stimulates the release of pro-inflammatory mediators, on the expression of COX-1 and COX-2 in the hippocampus, hypothalamus, pituitary gland and adrenal gland of the rat.

Male Sprague Dawley rats (approximately 200 g) were injected with endotoxin (E. Coli K-235, 250 µg/rat i.p.), or with saline vehicle (0.2ml) alone. Tissue was collected at 3 h and 6 h after injection and from untreated control animals and frozen immediately on dry ice. Total RNA was extracted from the tissues and reverse transcription was performed using random hexanucleotide primers. The resultant cDNA was then used as a template for subsequent PCR amplification using specific PCR primers for COX-1, COX-2 or ß-actin. The RT-PCR products were analysed by gel electrophoresis and subsequent hybridisation with relevant probes. Dot blots were excised and radioactivity measured by Cerenkov counting.

In all tissues studied 3 h and 6 h vehicle treatment had no significant effect on COX-2 expression however, exposure to endotoxin for 3 h produced significant increases in COX-2 expression (fold increases vs vehicle controls) in the hippocampus (3.7 ± 0.7, $P<0.05$), hypothalamus (3.0 ± 0.9, $P<0.05$), adrenal gland (58.7 ± 16.3, $P<0.001$) and pituitary gland (49.1 ± 4.5, $P<0.001$) as analysed by ANOVA plus Fischer's test (n=4). After 6 h treatment there were no significant differences in COX-2 expression between the endotoxin and control groups. Northern blot analysis of hypothalamic tissue hybridised with the COX-2 probe confirmed the presence of the 4.4kb COX-2 transcript. Endotoxin treatment did not induce COX-1 gene expression in any tissue studied, to the contrary COX-1 expression was reduced significantly in the hypothalamus (47.1 ± 2.6%, $P<0.01$ vs vehicle), pituitary gland (16.0 ± 8.8%, $P<0.001$ vs vehicle) and adrenal gland (31.0 ± 3.6%, $P<0.001$ vs vehicle) although not in the hippocampus. After 6 h treatment there were no significant differences in COX-1 expression between the endotoxin and control groups.

Our finding that endotoxin increases the expression of COX-2 but decreases COX-1 expression in neural and endocrine tissue suggests that COX-2 plays a significant role in effecting HPA responses to endotoxin and that it may also contribute to other aspects of the brain-neuroendocrine response.

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81P THE CELLULAR EXPRESSION OF LIPOCORTIN 1 IS UNAFFECTED BY DRUGS WHICH BLOCK THE CLASSICAL PATHWAY OF PROTEIN SECRETION

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Lipocortin 1 (LC1) is strongly implicated as a protein second messenger of glucocorticoid action in the brain-neuroendocrine system as well as in the periphery. We have previously demonstrated that dexamethasone stimulates the translocation of LC1 from an intracellular pool to a pericellular pool in a number of tissues, a process which may be necessary for LC1 to gain access to cell surface binding sites and thereby exert its biological effects (Buckingham et al., 1996). The mechanism of transportation is unknown but, as LC1 does not possess a signal sequence which would direct it to the endoplasmic reticulum, it is unlikely to involve the classical pathway of protein exocytosis. To verify this we have compared the effects of three drugs (monensin, brefeldin A and nocodazole) which inhibit protein secretion at different steps along the classical secretory pathway on the exportation of LC1 by various rat CNS and peripheral tissues and on the secretion of ACTH by the pituitary gland.

In experiments measuring LC1, samples of cortex, hippocampus, hypothalamus, anterior pituitary gland and peritoneal macrophages were collected *post mortem* from adult male CFY rats (200 ± 10g) and incubated for 2-3h at 37°C in an atmosphere of 95%O_2/5%CO_2 in the presence and absence of dexamethasone (0.1μM) and/or monensin (10μM), brefeldin A (1.4μM) or nocodazole (3.3μM). LC1 in the pericellular and intracellular pools was then analysed by SDS-PAGE and western blotting. The blots were scanned and the data (in parenthesis) expressed as a percentage of the drug free control. These values are semi-quantitative and are only intended to give a relative numerical guide to the ratios of band intensities; statistical comparisons have therefore not been made. Anterior pituitary segments were incubated *in vitro* for 3h ± monensin (10μM), brefeldin A (1.4μM) or nocodazole (3.3μM); where appropriate CRH (200nM) was included in the medium for the final hour. ACTH in the final incubate was measured by radioimmunoassay.

In all tissues studied the 'native' biologically active 37 kDa species of ir-LC1 was detected within the intracellular compartment. Incubation of the tissue with dexamethasone (dex.) had no obvious effects on intracellular ir-LC1 levels; however, in all tissues studied, the steroid produced a pronounced increase in the amount of ir-LC1 contained within the pericellular pool (e.g. in anterior pituitary tissue 1294% vs. control 100%). The steroid induced externalisation of LC1 was unaffected by monensin (1747% vs. dex. alone 1680%), brefeldin A (1269% vs. dex. alone 1294%) and nocodazole (1066% vs. dex. alone 1033%). By contrast, the significant ($P<0.01$; n=6) rises in ir-ACTH release by CRH (e.g. 512±37 vs. basal control 202±21 pg/ml) were blocked by monensin (196±31 vs. CRH alone 512±37 pg/ml, $P<0.01$), brefeldin A (213±19 vs. CRH alone 351±29 pg/ml, $P<0.01$) and nocodazole (258±50 vs. CRH alone 533±62 pg/ml, $P<0.01$), none of which influenced basal ACTH release. In conclusion, these results suggest that the steroid-induced exportation of LC1 from cells is effected via a route other than the classical endoplasmic reticulum-Golgi complex pathway.

We are grateful to the Wellcome Trust for generous financial support (Grant No. 141943/Z94/Z/MP/JF).

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82P INFLAMMATORY RESPONSES INDUCED BY TWO SECRETORY PHOSPHOLIPASES A_2 (sPLA$_2$) HOMOLOGUES ISOLATED FROM SNAKE VENOM

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The ability of sPLA$_2$ to induce inflammation is thought to parallel their enzymatic activity. Bothropstoxin I (BthTX-I) and BthTX-II are sPLA$_2$ homologues isolated from *Bothrops jararacussu* snake venom which are devoid (BthTX-I) or have very low (BthTX-II) PLA$_2$ activity (Homsi-Brandeburgo *et al.*, 1988). The aim of the present study was to investigate the ability of BthTX-I and BthTX-II to induce local oedema formation in both rat paw and skin *in vivo*. We have also investigated the effects of both BthTXs in rat peritoneal mast cells *in vitro*.

Male Wistar rats (150-200 g) were used. Paw oedema was induced by a subplantar injection of sPLA$_2$ into the left hind-paw of animals and paw volume was measured using a hydroplethysmometer. Skin oedema formation was measured as the local accumulation of i.v. injected [^{125}I]-human serum albumin into the skin sites of rats anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.p). *In vitro* peritoneal mast cell degranulation was carried out by measuring the released [^{14}C]5-HT using a β counter.

BthTX-I and BthTX-II (12.5-100 μg^{-1} paw each) caused dose-dependent rat paw oedema. Similarly, BthTX-I and BthTX-II (0.125-5 μg site^{-1} each) caused dose-dependent rat skin oedema formation (n=8). Pretreatment of the animals with the histamine and 5-HT antagonist cyproheptadine (Cypro, 2 mg kg^{-1}, i.p.), markedly reduced the oedema formation induced by both BthTXs either in the paw or skin (Table 1). Previous incubation of BthTX-I and BthTX-II with *p*-bromophenacyl bromide (*p*-Bpb; a compound known to inhibit phospholipasic activity, Warner *et al.*, 1988) also significantly inhibited the oedema formation induced by these enzymes (Table 1). The polyanion heparin significantly reduced the oedema formation induced by both BthTXs (5 μg site^{-1} each; n=7) in the rat skin as well as the paw oedema induced by BthTX-I (50 μg paw^{-1}, n=10; Table 1). BthTX-I and BthTX-II (10-100 μg ml^{-1} each; n=5) caused dose-dependent *in vitro* mast cell degranulation. Heparin (50 IU ml^{-1}) inhibited the [^{14}C]5-HT release induced by both sPLA$_2$ homologues (n=4; $p<0.05$); however, *p*-Bpb significantly inhibited the [^{14}C]5-HT release induced by BthTX-II but not by BthTX-I.

Our results indicate that oedema formation induced by BthTX-I and BthTX-II is mostly dependent on *in vivo* mast cell degranulation. Since heparin reduced the oedematogenic activity induced by both (BthTX-I) and (BthTX-II) it is likely that the cationic charge of these substances plays a major role in the mast cell activation. The findings that oedema formation by these sPLA$_2$ is reduced by *p*-Bpb indicate that *p*-Bpb may not be a suitable pharmacological tool to investigate the correlation between enzymatic activity and inflammatory effects of PLA$_2$s.

We thank the FAPESP support.

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Table 1. Oedema formation (mean ± s.e.mean) induced by BthTX-I and BthTX-II in the rat. *P<0.05 as compared to control values.

	PAW VOLUME (ml at 15 min)				SKIN OEDEMA (μl site^{-1}, 0-30 min)			
	Control	Cypro	*p*-Bpb	Heparin	Control	Cypro	*p*-Bpb	Heparin
BthTX-I	0.52 ± 0.06	0.05 ± 0.02*	0.18 ± 0.02*	0.06 ± 0.03*	57 ± 8.8	10.2 ± 3*	21 ± 3.2*	21 ± 1*
BthTX-II	0.60 ± 0.12	0.14 ± 0.02*	0.19 ± 0.02*	0.48 ± 0.05	97 ± 11	20.8 ± 3.7*	39.7 ± 4*	71 ± 3.4*

83P EXTRACELLULAR GABA LEVELS ARE INCREASED IN BRAIN REGIONS ASSOCIATED WITH THE GENERATION OF ABSENCE SEIZURES

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Absence epilepsy is characterised by the demonstration of spike and wave discharges (SWD) associated with behavioural arrest. The precise mechanisms responsible for producing these seizures are unknown, although they appear to originate within the thalamocortical circuitry. In animal models, antagonists at the $GABA_B$ receptor can block these seizures whilst $GABA_B$ agonists exacerbate them. We have previously shown that in a well characterised animal model (Genetic Absence Epilepsy Rat from Strasbourg, GAERS) extracellular GABA levels in the ventrolateral thalamus are significantly higher than in non-epileptic control rats (Richards et al., 1995). In the present study, we have extended this study to examine the frontal cortex and hippocampus, the former region being closely involved with the generation of SWD, and the latter in which SWD cannot be detected during absence seizures.

Female Wistar rats (200-250g, GAERS and non-epileptic controls) were anaesthetised with ketamine/medetomidine ($75mg.kg^{-1}/0.5mg.kg^{-1}$ i.p.) and implanted with concentric microdialysis probes (2mm length) in either the ventrolateral thalamus (2.1P, 2.4L, 6.6V from bregma), the frontal cortex (2.8P, 1.5L, 1.5V) or the ventral hippocampus (CA1)(5.2P, 4.8L, 8.0V). On the following day, probes were perfused with artificial CSF at 0.5 µl/min. After a 1h stabilisation period, 6 x 20 min basal samples were collected, the mean of the last 5 giving the basal GABA level. Rats from both strains then received (-)baclofen ($2 mg.kg^{-1}$ i.p.), and a further 6 x 20 min samples were collected. GABA levels were determined by HPLC with fluorimetric detection following derivatisation with o-phthalaldehyde/mercaptoproponoic acid.

In the hippocampus (CA1) there was no difference in basal GABA levels between GAERS and non-epileptic controls (0.23±0.06, 0.32±0.12, µM, mean±s.e.mean, GAERS (n=6) and controls (n=5) respectively). In agreement with our previous findings, basal GABA levels in the ventrolateral thalamus were higher in GAERS than controls (0.14±0.01, 0.09±0.01, µM, GAERS and controls (both n=5) respectively, $p<0.05$, Student's t-test). Furthermore, basal GABA levels were also higher in the frontal cortex of GAERS (0.32±0.10, 0.18±0.03, µM, GAERS (n=5) and controls (n=6) respectively, $p<0.05$, Student's t-test). Administration of the $GABA_B$ agonist, (-) baclofen, did not significantly alter GABA levels in any of the brain regions investigated.

The demonstration of elevated basal GABA levels in the two brain regions associated with the generation of SWD, together with normal GABA levels in a region where these seizures are not recordable, is further evidence of the importance of GABAergic neurotransmission in the aetiology of absence epilepsy. Whether these elevated levels are a cause or a consequence of absence seizures remains to be determined.

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84P EPILEPTIFORM POTENTIALS INDUCED BY MUSCARINIC BUT NOT METABOTROPIC-GLUTAMATE RECEPTOR ACTIVATION IN IMMATURE RAT OLFACTORY CORTEX, *IN VITRO*

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Activation of muscarinic cholinergic (mAChRs) or metabotropic-glutamate receptors (mGluRs) in adult mammalian olfactory cortex results in a sustained increase in cell excitability, due to a number of postsynaptic mechanisms (Constanti et al., 1993; Libri et al., 1997), as well as a decrease in evoked synaptic transmission mediated by presynaptic inhibitory receptors (Libri et al., 1997). However, age-associated changes in the level of mAChRs and muscarinic agonist efficacy in the CNS have been described (Araki et al., 1996). In addition, an enhanced susceptibility of immature CNS to epileptogenesis is well recognized (Johnston 1996). In the present study, the development of pre- and postsynaptic mAChR and mGluR responsiveness was studied in olfactory cortex from both immature (P14-P22) and adult (≥ P40) rats, using a conventional single-electrode current clamp recording technique *in vitro*.

Transverse slices of olfactory cortex were prepared as previously described (Constanti et al., 1993). Stable intracellular recordings were made from neurones in the deep cell layer III, using microelectrodes (60-80 MΩ) filled with 2M potassium acetate. Subthreshold excitatory post-synaptic potentials (EPSPs) were evoked in response to orthodromic stimuli delivered to local association fibres (layer II), through a bipolar nichrome wire electrode (50 µm diameter) insulated except at the tip. Measurements were performed before and during bath-application of drugs so each neurone served as its own control.

In adult neurones, a 2 min bath-application of the mAChR agonist oxotremorine-M (OXO-M; 10 µM; *n*=6) or the selective mGluR agonist 1-aminocyclopentane-1S-3R-dicarboxylic acid (1S-3R-ACPD; 10 µM; *n*=5) evoked persistent postsynaptic excitatory effects (*e.g.* membrane depolarization, increase in input resistance and intense repetitive firing) accompanied by a depression of the evoked EPSPs (70.5 ± 5.6 %, mean ± S.E.M, in OXO-M; 42.3 ± 11.5 % in 1S-3R-ACPD, measured at -80 mV resting potential). In contrast, in immature neurones (*n*=17) the 10 µM OXO-M-induced membrane depolarization was followed by the appearance of spontaneous synchronous epileptiform activity consisting of large amplitude (10.8 ± 1.4 mV) and voltage-*in*dependent (between -60 to -120 mV) bursting potentials, lasting for 17.5 ± 3.6 s and occurring at a frequency of 2.6 ± 0.5 discharges/min. This activity resembled the epileptiform discharges seen in adult (*n*=5) or immature (*n*=4) rat olfactory cortex treated with 4-aminopyridine (4-AP; 200 µM) and was reversible within 30 minutes of drug washout. Bursts were also abolished by co-application of tetrodotoxin (1 µM; *n*=3), atropine (1µM; *n*=3), the NMDA receptor antagonist DL-APV (100 µM; *n*=3), the non-NMDA receptor antagonist CNQX (20 µM; *n*=3), the $GABA_A$ receptor modulator pentobarbitone Na (100 µM, *n*=2) or by raising extracellular Mg^{2+} to 5.2 mM (4 times normal; *n*=3). In addition, during superfusion with 10 µM OXO-M, evoked EPSPs rather than being reduced appeared as large amplitude (20-40 mV), long-lasting (5-8 s) depolarizing potentials with superimposed population spikes. By contrast no obvious age-dependent differences were observed in the nature or time-course of metabotropic-glutamate agonist-evoked pre- and post-synaptic responses. Thus, 1S-3R-ACPD (10-50 µM; *n*=4) consistently failed to elicit spontaneous epileptiform potentials even in immature cells where muscarinic epileptiform activity had previously been demonstrated.

We suggest that in immature olfactory cortical neurones the enhanced susceptibility to muscarinic agonist-induced 4-AP-like epileptiform discharges may arise from the absence, a delayed maturation or a decreased efficacy of presynaptic inhibitory mAChRs

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85P COMPARISON OF THE EFFECTS OF THE ANTI-CONVULSANT DRUG LAMOTRIGINE WITH SODIUM VALPROATE AND GABAPENTIN ON HUMAN BRAIN TYPE IIa Na^+ CHANNELS EXPRESSED IN A MAMMALIAN CELL LINE

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Epilepsy is a disorder characterised by hyper-excitability of brain neurones leading to recurrent seizures. Current therapies for epilepsy include lamotrigine (LTG), sodium valproate (NaVPA) and gabapentin. A suggested mechanism of the anti-convulsant action of LTG is state-dependent modulation of Na^+ channels (Xie *et al*, 1995), whereas the mechanisms of action for NaVPA and gabapentin are less clear. The aim of the present study was to compare the effects of LTG with NaVPA and gabapentin on the α-subunit of human brain type IIa Na^+ channels stably expressed in a mammalian cell line (Peakman *et al*, 1995).

Concentration-, voltage- and use-dependent effects of these drugs were studied using whole-cell patch clamp techniques. The methods and solutions were as previously described by Xie *et al* (1995). Voltage-dependence was examined by comparing the potency of drugs at different holding potentials (V_h). LTG inhibited Na^+ currents with a half-maximal inhibitory concentration (IC_{50}) of 641μM at a V_h of -90mV and 56μM at a V_h of -60mV. NaVPA and gabapentin, at 3mM, produced no inhibitions at a V_h of -90mV, and only small inhibitions of 23±5% (mean±s.e.mean, n=5) and 19±4% (n=4), respectively, at a V_h of -60mV. Use-dependent inhibition of the drugs was investigated with trains (10 Hz) of 20 pulses of varying durations. A ratio between the twentieth and first pulse was calculated and the inhibition was expressed as a percentage of control. Use-dependent inhibition by LTG (100μM) was 6.7±3.3% ($P>0.1$, paired Student's t-test, n=4), 12.2±3.0% ($P<0.01$, n=7) and 20.7±4.8% ($P<0.01$, n=7) with 0.7, 3.5 and 20ms pulses, respectively. NaVPA or gabapentin, at 1mM, did not cause any use-dependent inhibitions ($P>0.1$).

The voltage- and use-dependent actions of LTG may be due to preferential interactions with an inactivated state. Therefore, the drug effect was examined on fast and slow inactivated states generated using 10ms or 1s conditioning pulses. LTG induced a hyperpolarising shift in the slow, but not fast inactivation curves. An estimated potency on the slow inactivated state (K_i) for LTG was calculated to be 21μM (n=6). NaVPA or gabapentin, at 1mM, caused small (<2.8mV) hyperpolarising shifts in the slow inactivation curves (K_i values could not be calculated).

The results demonstrate that LTG, but not NaVPA or gabapentin, inhibits human brain type IIa Na^+ currents in a voltage- and use-dependent manner. A possible explanation is that LTG stabilises channels in the slow-inactivated state, thus reducing the number of channels available for opening during subsequent depolarisations (Xie *et al*, 1995). The low activity at type IIa Na^+ channels suggests that NaVPA and gabapentin exert their anti-convulsant effects via other mechanisms.

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86P OPIOID RECEPTOR ACTIVATION DOES NOT MODULATE POTASSIUM CHANNEL CURRENT IN NG108-15 AND SH-SY5Y CELLS

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In NG108-15 cells which possess δ–opioid receptors and SH-SY5Y cells which possess μ- and δ-opioid receptors, opioid receptor activation inhibits voltage-dependent calcium channel currents (Seward et al., 1991; McFadzean & Docherty, 1989) and evokes calcium mobilisation (Connor et al., 1994). In other neurones activation of δ- and μ-opioid receptors evokes an inwardly rectifying potassium current (North, 1993). In the present study we have investigated whether opioid receptor activation results in an alteration of potassium conductances in NG108-15 and SH-SY5Y cells.

Cells were cultured as described previously (Connor & Henderson, 1996). NG108-15 cells were differentiated by 3-day exposure to IBMX (50 μM) and PGE_1 (10 μM), SH-SY5Y cells by 6-day exposure to the retinoic acid analogue AM580 (1 μM). Whole-cell patch clamp experiments were performed at 19-21 °C. For calcium channel current recordings the bath solution consisted of (mM): TEA-Cl 121, $BaCl_2$ 10, CsCl 5.4, $MgCl_2$ 1, HEPES 10, glucose 10, sucrose 50, pH 7.4 and the pipette solution (mM): CsCl 120, $MgCl_2$ 5, BAPTA 8, MgATP 5, NaGTP 0.5, HEPES 25, pH 7.2. For potassium channel current recordings the bath solution consisted of (mM): NaCl 140, KCl 2, $CaCl_2$ 2.5, $MgCl_2$ 1, HEPES 10, glucose 10, sucrose 40, pH 7.4 and the pipette solution (mM): KCl 140, $MgCl_2$ 1, $CaCl_2$ 1, EGTA 10, NaATP 5, NaGTP 0.2, HEPES 10, pH 7.2. The cells were superfused at 2.5 ml/min. Pipette resistances were 2-5 MΩ.

In NG108-15 cells, the δ-opioid agonist DPDPE (300 nM) reversibly inhibited N-type voltage-dependent calcium channel currents, reducing peak calcium current by 44 ± 8% (n = 8). When the voltage-dependent calcium currents were blocked by cadmium (100 μM) the steady state current/voltage relations over the voltage range of -150 to +40 mV showed outward current at depolarising potentials but no inward rectification. The potassium channel blocker, tetraethyl ammonium (20 mM) reduced the depolarisation-evoked outward current by 56 ± 7% (n = 7) at +40 mV. DPDPE (1 μM) had no effect on the steady-state current/voltage relation (Table 1). In SH-SY5Y cells, the μ-opioid agonist DAMGO (300 nM) inhibited peak N-type voltage-dependent calcium channel currents by 62 ± 4% (n = 5). In these cells depolarisation also evoked outward currents. DAMGO (1 μM) had no effect on the steady state current/voltage relation (Table 1).

The results show that activation of δ-opioid receptors in NG108-15 and μ-opioid receptors in SH-SY5Y cells does not modulate outward voltage-activated potassium current, nor does it activate an inwardly rectifying potassium current. It is therefore unlikely that membrane hyperpolarisation as a result of an increase in potassium current is involved in opioid-induced elevation of intracellular calcium. The absence of opioid-activated inwardly rectifying potassium currents in these cell lines could be due to a lack of channel proteins, or appropriate G protein subunits for potassium channel activation, or both.

Table 1. Steady state current (pA) in neuroblastoma cells. Data are presented as mean ± s.e.mean (n = 8 for all data).

	NG108-15 cells		SH-SY5Y cells	
Em(mV)	control	DPDPE	control	DAMGO
-150	-41 ± 8	-46 ± 14	-15 ± 3	-17 ± 2
+40	532 ± 44	555 ± 35	229 ± 43	213 ± 44

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87P DUAL EFFECTS OF BENZOCAINE ON A HUMAN CARDIAC CLONED POTASSIUM CHANNEL (hKv1.5)

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hKv1.5 is a *Shaker*-related K^+ channel cloned from human ventricle. The current generated by these channels seems to be the counterpart of the ultrarapid delayed rectifier described in native human atrial cells. Recently, an apparently hydrophobic binding site for antiarrhythmic and tertiary local anesthetics has been identified in the internal mouth of this human K^+ channel (Yeola *et al.*, 1996). In addition, it has been demonstrated that interaction of cationic drugs with an extracellular receptor site on Kv1.2 channels increased the current amplitude (Tseng *et al.*, 1996). The present study was undertaken to determine whether the uncharged local anesthetic, benzocaine (BZ): 1) blocks hKv1.5 channels, 2) interacts with the proposed internal receptor, and 3) can exert an agonist effect.

The experiments were performed at room temperature on *Ltk*$^-$ cells transfected with the gene encoding the sequence of the hKv1.5 channel. Currents were recorded using the whole-cell configuration of the patch clamp technique (Delpón *et al.*, 1996).

BZ (100-700 μM) is a weaker blocker of hKv1.5 channels, the apparent affinity constant (K_D) calculated assuming a Hill coefficient of 1 being 910 $\pm$ 96.9 μM. The blockade was voltage-dependent in such a way that it increased steeply in the voltage range of channel opening (between -30 mV and 0 mV). This result suggests that BZ could act as on open channel blocker. At more depolarized potentials, when the channel opening reached saturation, the blockade induced by 700 μM BZ decreased progressively, from 50.7$\pm$ 2.5% at 0 mV to 40.1 $\pm$ 2.4% at +60 mV (BZ 700 μM, n= 9, $P<0.01$). BZ induced a fast phase of decline of the tail current elicited on return to -40 mV after depolarization. In the absence of drug, the time constant of decline of the tail current averaged 57.1 $\pm$ 9.7 ms, whereas in the presence of BZ 700 μM two time constants were obtained: τ_f 13.7 $\pm$ 2.3 ms and τ_s= 92.8 $\pm$ 15.4 ms (n=7).

In the presence of high external K^+ (140 mM) the blockade induced by BZ (500 μM) was independent of the duration of the pulse, reaching 30.9 $\pm$ 3.6% and 37.5 $\pm$ 8.1% when the depolarizing pulses were of 500 ms or 5000 ms of duration, respectively (n=5, $P>0.05$). This result indicated that BZ does not interact with the slow inactivating state of the channel. Finally, when BZ was added in the presence of bupivacaine, a potent blocker of this channel (K_D= 9.4 $\pm$ 1.4 μM), a partial relief of block can be observed, thus suggesting a competition between both drugs.

Moreover, low concentrations of BZ (10 nM) exerted an agonist effect shifting the activation curve 8.4 $\pm$ 2.7 mV in the negative direction (n=5) and slowing the deactivation of the tail currents from 48.1 $\pm$ 5.9 ms to 85.9 $\pm$ 11.1 ms (n=6, $P<0.01$).

It is concluded that BZ produced a voltage-dependent block of hKv1.5 channels binding to the same or overlapping receptor site as the tertiary amine local anesthetic bupivacaine. Additionally, at low concentrations, BZ can also exert an agonist effect.

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88P BLOCK OF hKv 1.5 CHANNELS BY R(+)-ROPIVACAINE AND R(+)-MEPIVACAINE: STRUCTURE-ACTIVITY RELATIONSHIP

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Bupivacaine is an amide type local anesthetic widely used in the clinical practice. It is marketed as the racemic mixture of S(-)- and R(+)-bupivacaine, although the cardiotoxicity of bupivacaine has been related to the higher potency of R(+)-bupivacaine *versus* the S(-) enantiomer to block cardiac Na^+ (1.6-fold more potent) and hKv1.5 channels (7-fold more potent). Ropivacaine is the pure S(-)- enantiomer of AL381, and has been developed as a less toxic alternative to bupivacaine (Åkerman et al., 1988). The differences between bupivacaine, ropivacaine and mepivacaine are only the lengths of the substituent at position 1 which is a butyl, propyl or methyl, respectively. In order to assess the structural determinants of these drugs to block hKv1.5 cardiac channels, we have studied the effects of the R(+)-enantiomers of ropivacaine and mepivacaine on hKv1.5 channels. The results have been compared with those previously reported for R(+)-bupivacaine (Valenzuela et al., 1995).

Cloned human cardiac K^+ channels (hKv1.5) were stably transfected in *Ltk*$^-$ cells and the effects of R(+)-ropivacaine and R(+)-mepivacaine on the expressed hKv1.5 currents were studied using the whole-cell configuration of the patch-clamp technique.

Neither R(+)-ropivacaine nor R(+)-mepivacaine modified the activation time course of the hKv1.5-like current. However, 100 μM R(+)-ropivacaine induced a fast initial decline of the current during the application of a depolarizing pulse from a holding potential of -80 mV to +60 mV with a time constant of 6.12$\pm$0.64 ms (mean$\pm$s.e.m.). Block reached steady state at the end of 250 ms depolarizing steps to +60 mV averaging 79$\pm$2% (n=8) of inhibition. The apparent dissociation constant (K_D) was 32.2$\pm$1.5 μM (n=22) (i.e. 2.5 times lower than that exhibited by S(-)-ropivacaine: 81 μM), and the Hill coefficient (n_H) 1.003$\pm$0.044.Block of hKv1.5 channels by R(+)-ropivacaine was voltage dependent and described by a fractional electrical distance (δ) of 0.156$\pm$0.003, similar to that described for bupivacaine enantiomers and S(-)-ropivacaine (Valenzuela et al., 1995; 1997). R(+)-ropivacaine (100 μM) slowed the deactivation course of the tail currents recorded at -40 mV [43.5$\pm$6.6 ms (n=8) *versus* 216.2$\pm$83.0 ms (n=5); $P<0.01$], thus resulting in a "crossover" phenomenon. The association (k) and dissociation rate (l) constants of R(+)-ropivacaine binding to the hKv1.5 channel were (1.37$\pm$0.14) $\times 10^6$ $M^{-1}s^{-1}$ and 44.2$\pm$4.4 s^{-1} (n=11), respectively. The effects of R(+)-mepivacaine on hKv1.5 channels were qualitatively similar to those obtained in the presence of R(+)-ropivacaine. In fact, R(+)-mepivacaine block of hKv1.5 channels was also voltage-dependent, consistent with a δ of 0.158$\pm$0.009. However, the K_D was 17-fold higher for R(+)-mepivacaine (537.3$\pm$79.0 μM; n_H=0.902$\pm$0.111; n=15) than for R(+)-ropivacaine. This decrease in potency was attributed to its higher dissociation rate constant (540 s^{-1}) and, therefore, to a more unstable drug-channel complex.

All these results, together with those previously reported for bupivacaine enantiomers and S(-)-ropivacaine, strongly suggest that the length of the substituent at position 1 in the molecule is a structural determinant for: 1) the potency of the drug to block hKv1.5 channels and 2) the higher or weaker stereoselectivity. Thus, compounds with longer substituents will be more potent and their block of hKv1.5 channels will display a marked stereoselectivity.

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89P PHARMACOLOGICAL STUDIES USING HUMAN VOLTAGE-DEPENDENT CALCIUM CHANNELS

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Extracellular calcium (Ca^{++}) primarily enters excitable cells via voltage-dependent calcium channels (VDCCs). Multiple VDCC subtypes have been identified and designated L, N, P, Q, R and T based on their electrophysiological and pharmacological properties (Olivera *et al.*, 1994). Each VDCC is a multimeric protein complex comprised of at least three tightly coupled subunits α1, α2δ and β. Although β subunits contribute to the biophysical properties the major pharmacological differences depend on the α1 subtype. Five genes encoding for α1 subunits are present in neuronal tissue. Alpha 1_C and 1_D encode for L-type channels and $\alpha 1_B$ for N-type VDCC. It remains unlear however, which channel (P, Q, or R) are encoded by the $\alpha 1_A$ and $\alpha 1_E$ genes.

We have investigated the pharmacology of calcium flux into HEK293 cells stably transfected with different human VDCCs viz., $\alpha 1_A\alpha 2\delta\beta 4a$, $\alpha 1_B\alpha 2\delta\beta 1b$ and $\alpha 1_E\alpha 2\delta\beta 1b$ (Williams *et al.*, 1992 & 1994) using a fluo-3 based fluorescence assay and stimulation with potassium $[K^+]_o$.

The cells were maintained in Dulbecco's modified Eagles medium and plated out into polylysine coated (10μg/ml) 96 well plates ($2x10^5$ cell/well) 18 h prior to the experiment. The cells were then washed with Tyrodes solution and loaded with calcium sensitive dye Fluo-3AM (20μM) for 1h. Excess dye was removed by washing and compounds added to the wells prior to depolarisation with $[K^+]_o$.

All three cell lines exhibited an increase in fluorescence corresponding to a rise in intracellular calcium $[Ca^{++}]_i$ to an increase in $[K^+]_o$. The $\alpha 1_B$ and $\alpha 1_E$ transfected HEK293 cells showed maximal responses at 40mM $[K^+]_o$ equivalent to 500-800nM $[Ca^{++}]_i$ which peaked within 10s and returned to normal basal levels within 3-4min. The $\alpha 1_A$ cell line was less sensitive to $[K^+]_o$ requiring 70mM for a maximal response. The rise in $[Ca^{++}]_i$ was lower 200-500nM and was slower to peak.

Omega-conotoxin GVIA (CgTx GVIA), ω-agatoxin IVA (Aga IVA), and ω-conotoxin MVIIC (CmTx MVIIC) were used to identify N, P and Q-type VDCCs respectively.

Aga IVA and CmTx MVIIC reduced the rise in $[Ca^{++}]_i$ in the $\alpha 1_A$ cell line with IC_{50}s of 254 ± 21nM and 297 ± 31nM respectively whereas CgTx GVIA (1μM) was inactive (<20%). The rise in $[Ca^{++}]_i$ in the $\alpha 1_B$ cell line was completely blocked by CgTx GVIA (IC_{50}=1.5 ± 0.3nM) and CmTx MVIIC (IC_{50}= 3.8 ± 1.8 nM) but was unaffected by Aga IVA (1μM). The $\alpha 1_E$ cell line was unaffected by all three toxins at concentrations upto 1μM. These results suggest that the $\alpha 1_A$, $\alpha 1_B$, and $\alpha 1_E$ cell lines have properties in common with Q, N and R VDCCs respectively.

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90P EVIDENCE FOR THE INVOLVEMENT OF DIFFERENT CALCIUM CHANNEL SUBTYPES IN MEDIATING TRANSMISSION FROM PRIMARY AFFERENT SUBSETS IN THE NEONATAL RAT SPINAL CORD

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The neonatal rat spinal cord contains both excitatory and inhibitory neurones, many of the latter being GABAergic or glycinergic interneurones (Evans, 1989). The peptides, ω-conotoxin GVIA (ω-CgTx GVIA) and ω-conotoxin MVIIC (ω-CmTx MVIIC), both block the N-type voltage-sensitive calcium channel (VSCC) whilst ω-CmTx MVIIC also blocks the P-/Q-type VSCCs (Birnbaumer *et al.*, 1994). We have studied the influence of these peptides on synaptic reflexes in both control and combined bicuculline- + strychnine-treated cords.

Dorsal root stimulation elicits a multiphasic reflex in the corresponding ventral root whose initial phase largely derives from monosynaptic inputs to motoneurones from Aβ primary afferents (Evans, 1989). Polysynaptic linkages yield potentials of both short and long, stimulus-to-peak latencies that may arise from recruitment of Aβ-/Aδ-fibres and C-fibres, respectively (Thompson *et al.*, 1992). Here, we used a grease-gap technique (Bufton *et al.*, 1995) to record from the L3, L4 or L5 ventral root of *in vitro*, hemisected spinal cords taken from Wistar rats (3-5 days old). Amplitudes of the evoked monosynaptic, early polysynaptic and late polysynaptic potentials were measured from the ventral root at respective latencies of approximately 7, 70 and 2000 ms following a single, supramaximal stimulus (0.5 ms) delivered at 5-min intervals to the ipsilateral dorsal root. Drugs were superfused at 0.5 ml min^{-1} in a Krebs-bicarbonate buffer maintained at 25°C and gassed with 95% O_2/5% CO_2. Peptides were delivered in added bovine serum albumin (0.5 mg ml^{-1}). Data show the mean ± s.e.mean and were analysed using Student's *t*-test.

In control cords, ω-CgTx GVIA (1 μM) abolished both the monosynaptic and late polysynaptic potentials but only partially reduced the early polysynaptic potential (-58 ± 3% of control; n=10; see Bufton *et al.*, 1995). All synaptic components were abolished by superfusion with ω-CmTx MVIIC (n=4). Combined treatment with the glycine antagonist, strychnine (1 μM) and the $GABA_A$ antagonist, bicuculline (10 μM), increased both the early (+128 ± 23%; $p<0.001$, n=7) and late polysynaptic components (+153 ± 25%; $p<0.002$, n=4), leaving the monosynaptic element largely unaltered. In the combined presence of both these antagonists, ω-CgTx GVIA (1 μM) abolished the monosynaptic and late polysynaptic reflexes. However, its action on the enhanced, early polysynaptic component was limited to a 16 ± 6% reduction from control amplitudes (n=3; $p<0.05$). Application of ω-CmTx MVIIC (3 μM) again abolished all components of this enhanced synaptic reflex (n=3).

The observation that application of inhibitory amino acid antagonists increased the early polysynaptic component without affecting the monosynaptic portion of the reflex suggests that the increase in this early polysynaptic component is due to an unmasking of Aδ-fibre influences. In the combined presence of bicuculline and strychnine, the lack of sensitivity of this early component to ω-CgTx GVIA, together with its continued sensitivity to ω-CmTx MVIIC, may indicate that transmission from Aδ-fibres is largely mediated via P-/Q-type VSCCs. In contrast, transmission from C-fibres would appear to be mediated via N-type VSCCs. In order to substantiate these claims, further studies using NMDA antagonists are underway.

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91P EVIDENCE FOR A Na/K/2Cl COTRANSPORTER IN TYPE I HAIR CELLS ISOLATED FROM THE MAMMALIAN VESTIBULAR SYSTEM

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Type I vestibular hair cells, found only in amniotes, are innervated by a nerve calyx encasing the basolateral membrane of the hair cell. Isolated type I cells shorten in response to depolarizing steps, cell cooling or an increase in $[K^+]_o$. Since these shortenings may underlie mechanotransduction, we have studied these responses in type I hair cells dissociated from the vestibular end organs of gerbil and guinea pig.

Extracellular solution contained (in mM): NaCl (145), KCl (2), $MgCl_2$ (1.8), $CaCl_2$ (1.1), HEPES (10), glucose (3); pH 7.4, 305 mOsm. Dissociated type I cells were distinguished from type II cells by neck to plate and neck to body ratios (Ricci et al. 1996). Images were recorded on a VCR, digitized and measurements made using SigmaScan. High $[K^+]_o$ (125 mM, equimolar substituted for Na^+), caused type I hair cells to shorten significantly to 96.7 ± 3.1 % (n = 48, mean ± SD) after 60 s. This recovered to 98.4 ± 2.8 % of original length 60 s after the K^+ pulse. Depolarizations from -70 mV in whole cell patch-clamp resulted in type I cell shortenings to 72.9 ± 16.0 % (recovery 94.6 ± 7.4 %, n = 7) of original length. Shortening was often accompanied by swelling of the cell body and neck. The time course of voltage and K^+-induced shortenings was similar (10-30 s). Type II cells did not shorten in response to K^+ or voltage depolarizations.

Removal of external calcium did not inhibit K^+ or depolarization-induced shortening. Shortening was not prevented by K^+ current blockade by pipette-applied tetraethylammonium (20 mM) and/or Cs^+ (140 mM), or external 4-aminopyridine (5 mM). K^+-induced shortening was abolished by replacing external Na^+ with N-methyl-D-glucamine (100.1 ± 1.5%, n = 9) or external chloride with gluconate and sulphate (100.8 ± 0.7 %, n = 6). The chloride/sodium dependence suggests the involvement of a Na/K/2Cl cotransporter. Bumetanide (10-100 μM), a cotransporter blocker, significantly reduced K^+ induced shortenings to 99.1 ± 1.5 %, (paired t-test, $P < 0.05$, n = 14). K^+-induced shortening was unaffected by 100 μM amiloride, or 1 mM ouabain, indicating that Na/H exchange or Na/K/ATPase are not involved. Type I cells shortened by ~8 % and swelled in hypotonic solutions (250 mOsm) whilst reversibly increasing in length (n = 8, 102.4 ± 2.2%) in hypertonic solutions (345-350 mOsm, with sucrose). Shortening in hypotonic media resembled that in high K^+, suggesting that, as in vestibular dark cells (Wangemann et al. 1990), high K^+ induces a cell volume increase by activation of Na/K/2Cl cotransport. In the flask-shaped type I cell, this is manifested as a shortening and swelling of the cell.

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92P INTERACTION BETWEEN THE $Na^+/K^+/2Cl^-$ AND K^+/Cl^- CO-TRANSPORTERS IN HUMAN PLATELETS

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$Na^+/K^+/2Cl^-$ and K^+/Cl^- co-transport systems belong to the recently described CCC family and exhibit 25% amino acid sequence identity with one another (Gillen et al., 1996). Although the mechanisms of their regulation are not fully understood, it has been proposed that a key role is played by direct phosphorylation and dephosphorylation of the transporters or by a regulatory protein. Specifically, it has been suggested that the co-transporters may be regulated by one and the same complex of proteins, phosphorylation of which activates the $Na^+/K^+/2Cl^-$ co-transporter, and dephosphorylation of which activates the K^+/Cl^- co-transporter (Cossins, 1991). We have previously shown the presence of these two co-transporters in human platelets loaded with $^{86}Rb^+$ (as an analogue of K^+; Andersson & Vinge, 1991): KCl 30 mM stimulated a $^{86}Rb^+$ efflux that was sensitive to bumetanide, and N-ethylmaleimide (NEM) stimulated a $^{86}Rb^+$ efflux that was insensitive to bumetanide but sensitive to R(+)-[(dihydroindenyl)oxy]alkanoic acid (DIOA); we have attributed these effluxes respectively to $Na^+/K^+/2Cl^-$ co-transport (De Silva et al., 1996) and K^+/Cl^- co-transport (De Silva & Aronson, 1997). We report here an interaction between these two co-transporters in human platelets.

We obtained platelets from healthy volunteers, preloaded them with $^{86}Rb^+$, immobilized them on an inert filter, and measured $^{86}Rb^+$ efflux using a superfusion technique (De Silva et al., 1996) in the following medium (mM): NaCl (119), KCl (4.6), $CaCl_2$ (1.5), NaH_2PO_4 (1.2), $NaHCO_3$ (15), glucose (11), pH 7.4. The platelets were allowed to stabilize for 20 min before measurements of efflux were made for a further 14 min. When the cells were stimulated with NEM (1 mM), it was superfused throughout the experiment.

The results are shown in Table 1. NEM 1 mM stimulated an increase in $^{86}Rb^+$ efflux from the platelets and this efflux was insensitive to bumetanide 10 μM (not shown). KCl 30 mM, which normally stimulates a bumetanide-sensitive $^{86}Rb^+$ efflux (De Silva et al., 1996), had no effect on the NEM-stimulated efflux. Nor did bumetanide 10 μM in the presence of KCl 30 mM have any effect on the NEM-stimulated efflux. However, the phosphatase inhibitor okadaic acid (OA) 300 nM, inhibited the NEM-stimulated $^{86}Rb^+$ efflux. In contrast to its lack of effect on the NEM-stimulated $^{86}Rb^+$ efflux, KCl 30 mM in the presence of both NEM and okadaic acid stimulated an increase in $^{86}Rb^+$ efflux.

These results suggest that when the K^+/Cl^- co-transporter is stimulated with NEM, the $Na^+/K^+/2Cl^-$ co-transporter is inactivated, and that when the K^+/Cl^- co-transporter is inhibited with okadaic acid, the $Na^+/K^+/2Cl^-$ co-transporter can be activated. Thus, when one co-transporter is activated the other is inactivated, consistent with the view that these two co-transporters may be regulated by the same complex of proteins.

Table 1 Cumulative $^{86}Rb^+$ efflux (pmol)

	Control	NEM (1 mM)	NEM (1 mM) + KCl (30 mM)	NEM (1mM) + KCl (30 mM) + Bumet (10 μM)	NEM (1 mM) + OA (300 nM)	NEM (1 mM) + OA (300 nM) + KCl (30 mM)
$^{86}Rb^+$ efflux	68	152	149	156	86*	115*
s.e.mean	3	10	7	7	4	5
n	6	6	5	5	6	6

*Significantly different from NEM 1 mM and from each other ($P<0.0001$; complete efflux curves tested by ANOVA with repeated measures).

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93P A COMPARISON OF THE EFFECTS OF MORPHINE AND NALOXONE ON VENTRAL TEGMENTAL AREA, LOCUS COERULEUS AND SUPRACHIASMATIC NUCLEUS NEURONAL ACTIVITY *IN VITRO*

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Using electrophysiological techniques Aghajanian (1978) demonstrated that naloxone, an opiate receptor antagonist, produced an increased basal neuronal activity of the locus coeruleus in morphine-treated anaesthetised rats. To date however, there have been few studies on the acute effects of morphine and naloxone in isolated sections of the brain known to be important in the central manifestation of opiate-dependence. In the present study we have examined the consequence of acute exposure to morphine, and subsequent addition of naloxone, on the neuronal firing rate recorded in isolated slices of the locus coeruleus (LC) and ventral tegmental area (VTA), and also the hypothalamic suprachiasmatic nucleus (SCN). In addition we have also examined the acute effect of morphine and naloxone in the guinea-pig isolated ileum, a putative peripheral model of opiate dependence.

Male Wistar rats (150-200g) and male Dunkin Hartley guinea-pigs (500-1000g) were used. Sections (500μm) taken from rat LC, VTA and SCN were incubated at 35-37^{0}C as a submerged perfused preparation for extracellular recording. Data has been expressed as mean firing rate (spikes s^{-1}) ± s.d. Segments (5cm) of the terminal portion of the guinea-pig ileum were prepared for isometric recording as previously described (David et al., 1993). They were stimulated electrically (1.0Hz, 0.3ms, 200mA) and responses to drugs have been expressed as the mean percentage ± s.e.mean of the electrically-evoked contractions.

LC neurones, firing rate 4.2±2.2 spikes s^{-1} (n=13) were inhibited by morphine in a concentration-dependent fashion (0.01, 0.1, 1.0μM) with 1.0μM totally abolishing neuronal activity. Naloxone (0.1μM, n=8) antagonised the inhibition back to the control (4.4±1.6 spikes s^{-1}) for all doses of morphine with no evidence of enhanced firing rate. Similarly VTA neurones, firing rate 3.87±1.62 spikes s^{-1} (n=14), were also inhibited by morphine (0.01, 0.1, 1.0μM) in a concentration-related manner, with 1.0μM causing complete inhibition of firing. Naloxone (0.1μM, n=5), as with the LC, restored firing to the control rate (3.2±1.8 spikes s^{-1}) within 3-5mins of application.

In contrast, SCN neuronal firing rate (4.5±1.8 spikes s^{-1}, n=17) was not affected by morphine (0.01-10.0μM). However, in some cases subsequent application of naloxone (0.01-1.0μM, 6 out of 17 neurones tested) elicited a substancial increase in neuronal activity. For the 6 preparations, firing rate increased from a control of 3.9±1.0 to 81.3±13.8 spikes s^{-1}. Naloxone (0.01-10μM) administered to naive sections did not affect the basal rates of firing in LC, VTA or SCN (n=5-8).

In the guinea-pig isolated ileum, morphine (0.3μM) inhibited electrically-evoked contractions by 84.5±4.8% (n=9) but had little effect on basal contractile activity. Subsequent addition of naloxone (1.0μM) following termination of electrical field stimulation (EFS), resulted in a transient contraction (41.5±6%, (n=7) of the response to EFS) in 7 out of 9 preparations. As with the brain sections naloxone (1.0μM) did not appear to affect naive tissue (n=5).

This study has shown that while acute exposure to morphine inhibits firing in the LC and VTA *in vitro*, the subsequent addition of naloxone was not associated with a large increase in firing rate, as observed in morphine-dependent rats *in vivo* (Aghajanian 1978). However, the SCN (identified as the biological clock), a region of brain not generally associated with opiate dependence, responds to acute application of morphine and naloxone in a manner qualitatively similar to that observed in the guinea-pig isolated ileum. Evidence for a common mechanism in these two preparations remains to be determined.

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94P SELECTIVE DEPRESSION OF NMDA RECEPTOR-MEDIATED COMPONENT OF MONOSYNAPTIC CURRENTS OF RAT SPINAL MOTONEURONES BY MORPHINE *IN VITRO*

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Previous experiments have shown that morphine selectively depresses only those components of segmental synaptic reflexes that are sensitive to NMDA receptor antagonist. The dorsal root evoked monosynaptic response of motoneurones recorded extracellularly, which is mediated via non-NMDA receptors, is resistant to morphine (Faber et al, 1997). In the present study this phenomenon has been investigated at single motoneurones of the *in vitro* spinal cord using the patch clamp technique as detailed before (Cao et al, 1997) with the following exceptions:- the rats were 12 to 14 days old, the patch pipette solution additionally contained 5 mM N-(2,6-Dimethylphenyl-carbamoylmethyl) triethylammonium bromide (QX-314) and the recording temperature was 32 to 34 °C.

Motoneurones were identified by their antidromic responses to electrical stimulation of ventral roots. Monosynaptic excitatory postsynaptic currents (EPSCs) were evoked by single shocks at 40 s intervals applied either to a dorsal root (1~2 times threshold intensity) or to a glass microelectrode placed within 0.5 mm of the patch pipette. The EPSCs recorded at holding potential I=0 had a mean (± s.e.mean) latency of 2.5 ± 0.4 ms, duration of 60 to 80 ms, initial down slope of -448 ± 33 pA/ms and peak amplitude of -487 ± 58 pA (n=35). Following bath application of morphine (1 μM), the peak amplitudes and initial down slopes of EPSCs were depressed by 28.4 ± 4.0 % and 26.9 ± 1.9 % respectively (n=15, P<0.01, Mann-Whitney test).

When the Mg^{2+} concentration was raised from 1 to 2 mM and 50 μM R-2-amino-5-phosphonopentanoate (D-AP5) was introduced in order to block NMDA receptors, the slope and amplitude values were reduced by 20.8 ± 2.7 % and 22.0 ± 2.9% respectively (n=14). When morphine (1 μM) was applied to preparations (n=14) in the presence of the NMDA blocking mixture, it had no detectable depressant action on the residual component of the monosynaptic EPSCs.

The results show firstly that in the present preparation monosynaptic currents of motoneurones contain NMDA receptor-mediated components whether elicited from direct focal stimulation of ventral grey matter or from dorsal roots. Secondly, morphine is able to depress these monosynaptic components whether they be from primary afferent or other terminals. Thirdly, opiate receptors modulate monosynaptic transmission onto motoneurones mainly by depressing the NMDA receptor-mediated components. The mechanism causing this selective depressant effect of morphine on NMDA-mediated EPSCs remains to be elucidated.

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95P PHARMACOLOGICAL MODULATION OF DEPOLARIZING $GABA_A$ RECEPTOR-MEDIATED IPSPs IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONES

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Synaptic activation of $GABA_A$ receptors classically produces a fast hyperpolarizing inhibitory postsynaptic potential ($IPSP_H$) in hippocampal pyramidal neurones. However, during periods of repetitive stimulation an additional depolarizing $GABA_A$ receptor - mediated response can be evoked ($IPSP_D$). This response increases neuronal excitability by causing bursts of action potentials and facilitating *N*-methyl-D-aspartate receptor activity (Staley *et al.*, 1995). As such, mechanisms are likely to exist that control the level of activation of $IPSP_D$ such that overexcitability and ensuing neuronal damage do not occur. We have investigated whether $IPSP_D$ is subject to presynaptic regulation similar to that which occurs for hyperpolarizing $GABA_A$ responses by evaluating the effects of a range of agonists known to depress $IPSP_H$.

Experiments were performed on hippocampal slices prepared from 2-4 week old female Wistar rats and maintained in an interface chamber at 30-32°C. Intracellular recordings were made from stratum (s.) pyramidale in area CA1 using electrodes (60-110 MΩ) filled with potassium methylsulphate (2M). In all experiments 6-nitro-7-sulpamoylbenzo-[f]-quinoxaline-2,3-dione (NBQX; 3 μM), D-(E)-2-amino-4-methyl-5-phospho-3-pentanoic acid (CGP40116; 50 μM) and ketamine (50 μM) were present in the perfusing medium to block ionotropic glutamate receptor-mediated synaptic transmission. In those experiments in which the function of $GABA_B$ receptors was not investigated 3-*N*-[1-(*S*)-(3,4-dichlorophenyl) ethyl] amino-2-(*S*)-hydroxypropyl-*p*-benzyl-phosphonic acid (CGP55845A; 1 μM) was also present. Monosynaptic IPSPs were evoked by delivering 10 stimuli @ 100 Hz using bipolar stimulating electrodes placed in s. oriens and s. radiatum close to the recorded neurone. Test compounds were added to the perfusing medium until effects reached equilibrium. $IPSP_D$s were compared at the same membrane potential using DC injection to compensate for any drug induced hyperpolarization or depolarization.

Alternate stimulation in s. oriens and s. radiatum every minute evoked reproducible $IPSP_D$s that were depressed reversibly by 10 μM bicuculline (n = 3). (-)-Baclofen (5 μM), [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAGO; 0.5μM) and carbachol (10 μM) caused substantial reductions in the amplitude of the $IPSP_D$s evoked by stimulation in either dendritic field. Thus, (-)-baclofen depressed $IPSP_D$s evoked by s. oriens and s. radiatum stimulation by (mean ± s.e.mean) 65 ± 4 (n = 3) and 86 ± 9% (n = 3), respectively, DAGO by 53 ± 13 (n = 4) and 61 ± 15 % (n = 4) and carbachol by 45 ± 3 (n = 6) and 40 ± 4% (n = 4). In contrast, 2-chloroadenosine (10 μM) caused only a small reduction in both $IPSP_D$s, reducing s. oriens and s. radiatum responses by 28 ± 11 (n = 3) and 19 ± 13 % (n = 4), respectively. The effects of (-)-baclofen and DAGO were reversed by CGP55845A (1 μM, n = 3) and naloxone (10 μM, n = 4), respectively.

These data indicate that agonists known to presynaptically regulate $IPSP_H$ can also attenuate the $GABA_A$ receptor-mediated $IPSP_D$ evoked by repetitive stimulation in s. oriens and s. radiatum.

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96P INFLUENCE OF NICOTINE ON CAFFEINE-STIMULATED DOPAMINE RELEASE FROM RAT STRIATUM AND NUCLEUS ACCUMBENS

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Epidemiological reports suggest a link between tobacco smoking and caffeine intake. The priming effects of caffeine on nicotine's behavioral effects have been reported (Shoaib, M. *et al.*, 1996). However, the effects of nicotine pretreatment on the actions of caffeine have not been reported. Similarly, while the ability of systemic and locally applied caffeine to augment mesolimbic dopamine (DA) release in conscious and anaesthetised rats is known (Museo, E. & Pert, A., 1995) it is still unclear if caffeine, like nicotine and other psychostimulants, can preferentially increase DA release from this dopaminergic pathway. Using *in vivo* microdialysis of anaesthetized male Sprague Dawley rats, we compared the ability of caffeine to increase DA release in the mesolimbic and nigrostriatal pathways, and the influence of nicotine pretreatment on this action.

On days 1-7 rats received either 0.4 mg/kg nicotine base or saline by s.c. injection. On day 8 rats (250-350g) were anaesthetized with equithesin (3ml/kg i.p.) and a dialysis probe implanted in the striatum or accumbens. Animals received either caffeine (50 or 100 mg/kg) or saline i.p. injection. In a separate experiment, some animals received 1000 μM caffeine via the dialysis probe for 15 min. Dialysate samples were collected every 15 min and analysed for DA using HPLC with electrochemical detection. Data were analyzed using two way ANOVA for repeated measures.

We report that although 50 mg/kg caffeine failed to increase dopamine overflow in either striatum or accumbens (n=4), 100 mg/kg caffeine increased release to a peak of 177 ± 38% and 248 ± 83 % over basal in the accumbens (n=9, $p<0.05$) and striatum (n=8, $0.05<p<0.1$), respectively. There was no significant difference in the size of the response in the two brain regions. Pretreatment with nicotine failed to prime either pathway to the action of 50 or 100 mg/kg caffeine (n=4). Indeed, pretreatment with nicotine significantly reduced the peak effect of 100 mg/kg caffeine in the accumbens ($p<0.05$) by 85%. Local application of 1000 μM caffeine significantly increased accumbal dopamine release to 183 ± 39 % of basal in saline treated rats (n=4) and to 154 ± 27 % in nicotine pretreated rats (n=4). The two treatments were not significantly different.

These data suggest that systemic caffeine does not preferentially stimulate DA release from the mesolimbic system, and that nicotine pretreatment does not prime either dopaminergic pathway to the neurochemical effects of caffeine.

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97P EFFECTS OF L-SERINE-O-PHOSPHATE ON DOPAMINE OVERFLOW IN RAT STRIATUM *IN VIVO* AND *IN VITRO*

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L-Serine-O-phosphate (LSOP) is an excitatory amino acid receptor agonist which is found at relatively high concentrations in the mammalian brain (Klunk *et al.*, 1991). At metabotropic glutamate receptors (mGlus), LSOP appears to show some selectivity for members of the Group III family of mGlus (Knöpfel *et al.*, 1995). We have investigated the possibility that this agent may have effects upon dopamine release in the rat striatum, a response which has previously been shown to be regulated by Group I mGlus (Knöpfel *et al.*, 1995).

Experiments were conducted on male Wistar rats (250-350 g). Dopamine release was evaluated at 20 minute intervals in conscious, freely-moving rats using microdialysis probes implanted into the striatum (Cadogan & Marsden, 1997). The perfusate was analysed for dopamine content using HPLC coupled to electrochemical detection, and expressed as a percentage of the mean of six pre-treatment estimations. Data are means ± s.e.mean of 6-8 experiments. Dopamine release *in vitro* was assessed using striatal slices pre-labelled with [^{3}H]-dopamine (Cadogan *et al.*, 1997). Slices were subjected to two periods of electrical stimulation (S1, S2) preceded by two periods in the absence of stimulation (C1, C2). Data were expressed as ratios of the second and first periods of stimulation (S2/S1), or absence of stimulation (C2/C1). Where indicated, LSOP (1 mM) was present during the second control and stimulation periods. Data are means ± s.e.mean of 4 experiments. Statistical significance was evaluated by use of ANOVA with post-hoc Dunnetts' t-test.

Perfusion of the rat striatum *in vivo* with artificial CSF gave basal levels of dopamine in the microdialysate of 45.2 ± 7.2 fmol (n=14). Switching the perfusion medium to a second reservoir containing artificial CSF led to a modest rise in dopamine overflow (e.g. 145 ± 20 % at 20 minutes), which returned to near-basal levels at 60 minutes (112 ± 11 %). Perfusion with 30 mM LSOP for 60 minutes led to a maintained elevation of dopamine levels (e.g. at 100 minutes: control 101 ± 9 %; LSOP 182 ± 21 %; P<0.01).

In the absence of LSOP, release of dopamine *in vitro* was maintained during the two control periods (C2/C1 ratio = 106 ± 30 %), and slightly reduced during the second stimulation period (88 ± 5 %, P<0.05). The presence of LSOP did not significantly alter the release of dopamine in either the absence (81 ± 3 %) or in the presence of electrical stimulation (88 ± 4 %).

We conclude that LSOP facilitates the release of dopamine *in vivo* but not *in vitro*, indicating that LSOP activity may require the presence of some intact neural circuitry which may be lost in the preparation of striatal slices.

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98P EFFECTS OF 3,4-METHYLENEDIOXY-METHEMPHETAMINE ON DOPAMINE AND SEROTONIN RELEASE AND UPTAKE

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The abilities of 3,4-methylenedioxy-methamphetamine (MDMA) to release or block the uptake of endogenous dopamine (DA) or serotonin (5-HT) were examined in rat brain slices using fast cyclic voltammetry (FCV). 5-HT release was measured in the dorsal raphé nucleus (DRN) and substantia nigra pars reticulata (SNr). DA release was measured in the caudate putamen (CPu) and the nucleus accumbens (NAc). The rate of uptake was expressed as time (s) for a 5-HT or DA signal to decay to half its maximum ($T_{1/2}$).

350 µm coronal slices of DRN, SNr, CPu or NAc were prepared from male Wistar rats (250-300 g), set up in a brain bath and superfused with 95% O_2 / 5% CO_2 gassed artificial cerebrospinal fluid at a rate of 1ml / min at 32° C. FCV was carried out using a 1.5 cycle triangular waveforms scanning from -1.0 to + 1.4 V or -1.0 or +1.0 V at 2 Hz. 5-HT and DA release was measured at a 7 µm carbon fibre electrode (CFE). MDMA (1 mM) was pressure ejected at 10 psi for 1s, 20 µm away from the CFE, from a micropipette (5 µm tip) using a Neurophore device as described previously (Iravani and Kruk, 1995; 1997).

Using the -1.0 V to +1.4 V waveform, MDMA was electroactive, with two oxidation peaks at +500 and +1100 mV. The first oxidation peak (+1100 mV), appeared on the first scan. With subsequent scans a second oxidation peak (at +500 mV) was seen. When the input waveform was set at -1.0 V to +1.0 V, MDMA was not electroactive, and further experiments were carried out using this waveform. This waveform did not affect DA or 5-HT sensitivity.

5-HT or DA release was evoked with electrical stimulation (50 pulses / 50 Hz, 0.2 ms, 20 V, every 5 min.) using a bipolar tungsten electrode placed 80 µm inside the slice. Once stable DA or 5-HT release to electrical pulses were obtained, MDMA was pressure ejected. Pressure ejection cycle preceded electrical stimulation by one minute. Application of MDMA in the vicinity of the CFE did not release any detectable quantity of 5-HT or DA in any of the nuclei studied. Following pressure ejection of MDMA electrically-evoked 5-HT release was significantly potentiated in DRN and SNr, while DA release was potentiated in CPu only (table 1). $T_{1/2}$ values were significantly potentiated in all cases (see table 1).

The results indicate that in contrast to (+)amphetamine, MDMA when applied directly to brain slices, does not release DA or 5-HT, but like (+)amphetamine, it inhibits DA and 5-HT uptake (Iravani and Kruk, 1995; 1997). It is likely that in *in vivo* studies in which MDMA-induced release of 5-HT or DA were reported, increases in extracellular concentration are attributable to inhibition of monoamine uptake. In those studies, biotransformation of MDMA to a form capable of carrier-mediated 5-HT or DA release, cannot be ruled out.

*Table 1 The effects of MDMA on electrically evoked DA and 5-HT release and uptake. *P<0.05, paired Student's t-test.*

Nuclei	Potent'n by MDMA (% of Control)	$T_{1/2}$ (s) Control	$T_{1/2}$ (s) +MDMA
DRN (n=4)	121 ± 8*	11.5 ± 0.9	24 ± 2.3*
SNr (n=6)	232 ± 27*	12.3 ± 1.5	32 ± 8.5*
CPu (n=4)	186 ± 33*	5.4 ± 0.3	11 ± 2*
NAc (n=4)	105 ± 6	6.5 ± 0.9	10 ± 2*

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99P A SEROTONERGIC LESION DOES NOT AFFECT INHIBITION OF UPTAKE OF [^{3}H]NORADRENALINE INTO RAT CORTICAL SYNAPTOSOMES BY FLUOXETINE, CITALOPRAM AND DESIPRAMINE

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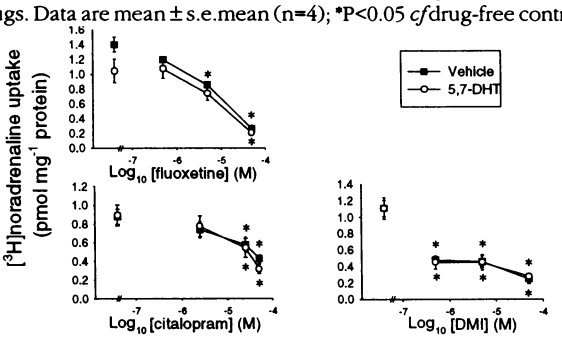
Experiments using microdialysis *in vivo* indicate that local infusion of the selective serotonin reuptake inhibitor (SSRI) fluoxetine into the frontal cortex of rats causes a substantial increase in the concentration of extracellular noradrenaline (NA) (Hughes & Stanford, 1996). Inhibition of neuronal uptake of NA by fluoxetine is likely to contribute to this increase in efflux (Hughes & Stanford, 1996). The more selective SSRI, citalopram, also inhibited uptake of [^{3}H]NA *in vitro*, albeit with lower potency. Because this action of fluoxetine and citalopram was unaffected by a selective lesion of noradrenergic neurones, it is likely to involve a non-noradrenergic site. The question arises as to whether the fluoxetine-induced increase in NA efflux involves inhibition of NA uptake into serotonergic neurones? For this reason, we have investigated the effects of a lesion of serotonergic neurones, induced by the neurotoxin, 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT), on inhibition of synaptosomal uptake of [^{3}H]NA by fluoxetine, citalopram and the selective NA uptake inhibitor, desipramine (DMI).

Male SD rats (260-350 g) were used. Rats were injected with DMI 25 mg kg^{-1} i.p and 45 min later, under halothane anaesthesia, they received an i.c.v. injection of 150 µg 5,7-DHT, dissolved in 10 µl saline containing 0.02 % ascorbate: AP -0.8; ML ±1.4; DV 3.9 mm from bregma. Controls were injected with DMI (i.p.) and vehicle (i.c.v.). The rats were killed 7 days later, synaptosomes prepared from the cerebral cortex and uptake of [^{3}H]NA measured as described in Dalley & Stanford (1995). To confirm the extent of the lesion, the monoamine content of each cortex was determined using HPLC-ECD. Statistical analyses used the Mann-Whitney U-test or ANOVA with post-hoc tests, as appropriate.

The cortical content of 5-HT was reduced by 54% after the lesion (ng g^{-1}: control 380 ± 50 (n=14); 5,7-DHT 176 ± 14 (n=19); P<0.001) but neither NA (P=0.675) nor dopamine content (P=0.33) was affected. The lesion did not alter specific [^{3}H]NA uptake (P=0.526). The inhibition of [^{3}H]NA uptake by fluoxetine, citalopram or DMI was also unaffected by the lesion (Figure 1).

Figure 1 Graphs show [^{3}H]NA uptake into synaptosomes from 5,7-DHT-lesioned or vehicle-treated rats in the presence of test drugs. Data are mean ± s.e.mean (n=4); *P<0.05 *cf* drug-free control.



These results suggest that NA is not taken up by 5-HT-containing neurones. The non-noradrenergic site which is the target for inhibition of uptake of [^{3}H]NA by fluoxetine remains to be identified. However, the dopamine transporter on dopaminergic neurones is a strong possibility (Buck & Amara,1995).

Z.H is an MRC scholar

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100P COMPARISON OF THE EFFECTS OF SIBUTRAMINE AND *d*-AMPHETAMINE ON THE CONCENTRATION OF EXTRACELLULAR NORADRENALINE IN RAT FRONTAL CORTEX: A MICRODIALYSIS STUDY

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Intracerebral microdialysis *in vivo* was used to compare changes in extracellular noradrenaline concentration ("NA efflux") in rat brain induced by sibutramine (BTS 54 524) or *d*-amphetamine. Both compounds inhibit neuronal uptake of NA (Buckett *et al.*, 1988; Thornburg & Moore, 1973), but *d*-amphetamine, a CNS stimulant, also releases NA (Aspley *et al.*, 1996).

Microdialysis probes were implanted in the frontal cortices (mm: AP+3.5; L+1.5; V-5.0 relative to bregma) of male SD rats (260-350g) under halothane anaesthesia which was maintained throughout the experiment. After perfusion (1 µl min^{-1}) with modified Ringer's solution (Dalley & Stanford, 1995) for 2 h, samples were collected at 20 min intervals. After collection of 4 basal samples, 0.25, 0.5, 1, 3 or 10 mg kg^{-1} sibutramine or 1, 3 or 10 mg kg^{-1} *d*-amphetamine or 2 ml kg^{-1} saline were injected i.p. and samples were collected for a further 4h. NA was measured by HPLC-ECD. Rats were used to test only one dose of drug or vehicle. Raw data were analyzed using split-plot ANOVA.

Both drugs increased extracellular NA concentration (Table 1). For sibutramine, but not *d*-amphetamine, the relationship between drug dose and the magnitude of the increase in efflux was described by a bell-shaped curve. At 1 mg kg^{-1}, these two drugs caused a similar increase in NA efflux. However, at 10 mg kg^{-1} *d*-amphetamine, the increase was significantly greater than that evoked by an equivalent dose of sibutramine. The results suggest that sibutramine's action as a NA reuptake inhibitor is modulated by autoinhibitory control mechanisms, e.g. by inhibition of firing and activation of α_2-adrenergic autoreceptors. This contrasts with the action of *d*-amphetamine; although it inhibits the firing of central noradrenergic neurons (Grahame & Aghajanian, 1971), this does not prevent a large, dose-dependent release of NA in rat frontal cortex over this dose-range.

KW is a Knoll Scholar; ZH is an MRC Scholar; KM is a BBSRC-CASE Scholar.

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Table 1 Mean ± s.e. mean efflux of NA in each time bin comprising 4 consecutive samples (fmol 20µl^{-1}). Drug or vehicle was injected at 80min. *:P<0.05 (*cf* basal), †: P<0.05 (*cf* corresponding dose of sibutramine), n=9 rats/group (sibutramine), n=4-5 rats/group (amphetamine).

Time (mins)	Saline	Sibutramine (mg kg^{-1})					*d*-amphetamine (mg kg^{-1})		
		0.25	0.5	1.0	3.0	10.0	1.0	3.0	10.0
0-80 (basal)	31.4 ± 0.5	22.0 ± 2.6	19.6 ± 1.3	29.8 ± 1.5	31.5 ± 1.0	32.1 ± 1.0	30.9 ± 2.5	34.9 ± 1.9	25.3 ± 2.9
100-160	32.3 ± 1.0	32.7 ± 8.9	33.8 ± 8.7*	55.1 ± 10.7*	49.1 ± 6.9*	43.9 ± 1.1*	75.5 ± 8.6*	66.0 ± 5.9*	113.2 ± 7.5*†
180-240	31.7 ± 1.1	41.6 ± 2.1*	50.9 ± 2.4*	70.8 ± 0.6*	70.6 ± 3.8*	51.1 ± 3.0*	69.8 ± 6.6*	79.5 ± 4.1*	104.5 ± 6.0*†
260-320	30.6 ± 1.2	37.0 ± 2.2*	53.6 ± 0.8*	70.2 ± 3.1*	75.5 ± 3.7*	53.6 ± 1.4*	63.9 ± 4.1*	73.7 ± 3.4*	97.4 ± 5.6*†

101P STIMULATION OF A PROLONGED, NON-DESENSITIZING PHOSPHOLIPASE D ACTIVITY IN CHINES HAMSTER OVARY (CHO) CELLS HETEROLOGOUSLY EXPRESSING M_1- OR M_3- MUSCARINIC CHOLINOCEPTORS

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Receptor-mediated activation of phospholipase D (PLD) has been shown to involve a range of transduction mechanisms, including heterotrimeric and small G proteins, protein tyrosine kinases, and protein kinase C (Klein *et al.*, 1995). A common observation is that receptor activation causes a rapid, but transient increase in PLD activity. Furthermore, following PLD activation prolonged desensitisation of this response has been observed (Schmidt *et al.*, 1995). Here we have studied receptor-mediated regulation of PLD activity in CHO-m1 and CHO-m3 cells to assess the regulatory mechanisms involved.

Cell monolayers were incubated for 24 h in phosphate-free medium containing $^{33}P_i$ (2 μCi/well). Monolayers were washed with oxygenated Krebs-Henseleit buffer and pre-incubated ± 50 mM butan-1-ol for 10 min at 37°C. Agonists were added for times of up to 30 min, and reactions terminated by addition of ice-cold trichloroacetic acid. Lipids were extracted and [^{33}P]-phosphatidic acid (PA) and [^{33}P]-phosphatidylbutanol (PBut) separated by thin layer chromatography (Boarder & Purkiss, 1993). Data are expressed as mean ± s.e.mean of at least 3 experiments performed in duplicate.

Addition of methacholine (MCh; 1 mM) to CHO-m1 cells caused a time-dependent accumulation of PBut which was prevented by prior addition of atropine (1 μM). Following a brief initial lag of 1-2 min, a linear accumulation of PBut was observed over a 30 min period of stimulation (basal, 301 ± 33; +MCh (30 min), 14976 ± 431 d.p.m. well^{-1}; EC_{50} 0.6 μM). In contrast, PA levels increased immediately upon MCh addition, with a new steady-state level being achieved within about 15 min (basal, 1418 ± 159; +MCh (30 min) 9806 ± 771 d.p.m. well^{-1}; EC_{50} 2 μM). In the presence of butanol the agonist-stimulated increase in PA was reduced by 14 ± 2%. The non-desensitizing nature of the PLD response to muscarinic cholinoceptor activation was further supported by the observation that similar PBut accumulations were seen in CHO-m1 cells pre-challenged with MCh (1 mM) or vehicle. A similar profile of agonist-stimulated PBut and PA accumulations were observed in CHO-m3 cells, except that responses were smaller in magnitude.

In CHO-m1 cells, removal of extracellular Ca^{2+} dramatically attenuated the agonist-stimulated accumulation of PBut (by 90 ± 1%), and more modestly reduced the PA response (by 38 ± 7 %). Phorbol 12-myristate 13-acetate (1 μM) evoked only modest accumulations of PBut and PA (9 ± 3 and 3 ± 2 % of MCh-stimulated increases). In contrast, the Ca^{2+} ionophore ionomycin (5 μM) evoked an increase in PBut accumulation which was 81 ± 15 % of that evoked by MCh (1 mM) at 15 min.

In contrast to the rapid onset and attenuation of agonist-stimulated PLD responses seen in many other cell types, these data demonstrate a markedly different profile of receptor-mediated PLD activation in CHO-m1 and -m3 cells. The brief initial lag phase may indicate that PLD activation in this system is 'downstream' of other second messengers produced in response to M_1 or M_3-muscarinic cholinoceptor activation (e.g. $Ins(1,4,5)P_3$ and diacylglycerol). PKC is likely to play a very minor role in PLD activation in CHO-m1 or -m3 cells, however the present data demonstrate a requirement for elevated intracellular Ca^{2+} concentration, arising either from Ca^{2+}-mobilisation or via Ca^{2+}-influx pathways.

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102P EVIDENCE FOR THE INVOLVEMENT OF A SRC-LIKE KINASE IN SIGNALLING BY A COLLAGEN-RELATED PAPTIDE IN WASHED HUMAN PLATELETS

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Collagen activates platelets via a pathway similar to that used by immune receptors, requiring the Fc receptor γ-chain, the tyrosine kinase syk and phospholipase C-γ2 (PLC-γ2) (Poole *et al.*, 1997). Src-kinases play a crucial role in signalling by immune receptors, so here we have investigated whether they are also involved in collagen signalling, using the collagen-related peptide (CRP) GCP*(GPP*)$_{10}$GCP*G as a stimulus (single letter amino acid code, P*=hydroxyproline).

Human platelets were obtained from platelet rich plasma by centrifugation (640*g*, 20 min). After loading with either [^{3}H]5-HT (60 min, 30°C) or Fura-2AM (60 min, 37°C), platelets were washed and resuspended in Tyrode's buffer at 2-4 x 10^8 ml^{-1}, then stimulated in a stirred suspension at 37°C. For secretion measurements, reactions were stopped with 6% glutaraldehyde. Cytosolic calcium levels ($[Ca^{2+}]_i$) were measured in Fura-2AM loaded platelets by calculating the ratio of the 340/380 nm excitation wavelengths read at 510nm. Immuneprecipitations, whole cell phosphorylation studies and immunoblotting were carried out as described previously (Poole *et al.*, 1997).

Activation of src-like kinases has been associated with small increases in their tyrosine phosphorylation. Stimulation of platelets with CRP (1μg/ml, 90s) caused an increase in the tyrosine phosphorylation levels of the src-kinase members p53/56^{lyn} and p59^{fyn} as assessed by densitometry (1.50±0.03 and 1.22±0.03 fold over basal, respectively, mean±s.e.mean, n=3; $p<0.05$ vs basal, single sample t-test). In contrast, tyrosine phosphorylation levels of other src-kinases in platelets (p54/56^{hck}, p60^{src} and p62^{yes}) were not significantly increased (n=3). After stimulation, p53/56^{lyn} and p59^{fyn} also became associated with other tyrosine phosphorylated proteins with apparent molecular weights of 14, 36, 38 and 72kDa. The role of these kinases was further investigated using the selective src-kinase inhibitor CP118,556 (1-t-butyl-3-p-tolyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-ylamine) (Hanke *et al.*, 1996). CP118,556 caused a concentration-dependent inhibition of CRP-induced (0.1μg/ml) platelet aggregation and [^{3}H]5-HT secretion, with 50% inhibition occurring at 300±50nM and 500±60nM, respectively (n=3). Further investigations showed that whilst [^{3}H]5-HT secretion in response to CRP (0.1μg/ml) was prevented by 10μM CP118,556 (31.3±1.9 and 2.5±0.5%, control and CP118,556 treated, respectively, n=3, $p<0.01$, unpaired t-test) the response to thrombin (0.1 units/ml) was unaffected (38.1±3.4% and 36.6±5.7%, for control and CP118,556 treated, respectively, n=3). Measurement of changes in $[Ca^{2+}]_i$ in Fura-2AM labelled platelets showed a similar profile. CP118,556 (10μM), whilst decreasing basal levels of tyrosine phosphorylation, also prevented CRP-induced increases in whole cell tyrosine phosphorylation, and specifically, phosphorylation of syk and the Fc receptor γ-chain.

These results suggest that p59^{fyn} and p53/56^{lyn} are involved in signalling by CRP and that this involvement is likely to be upstream of the Fc receptor γ-chain, syk and PLC-γ2.

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103P VON WILLEBRAND FACTOR INDUCES TYROSINE PHOSPHORYLATION OF THE TYROSINE KINASE SYK, P95VAV AND PHOSPHOLIPASE C Γ2 IN HUMAN PLATELETS

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Von Willebrand factor (vWF) is an adhesion molecule which plays a central role in the interaction between platelets and the subendothelium. It induces multiple functional responses in platelets, including aggregation and release of granule contents, through sequential binding to the glycoprotein GPIb in the complex GPIb-IX-V and the integrin $\alpha_{IIb}\beta_3$. The underlying signalling mechanisms are not however clearly understood.

In the present study we use native vWF derived from human plasma and a recombinant protein mutated in the A1 region of vWF (ΔA1), the region responsible for binding to GP Ib. This mutant is unable to bind to GP Ib whilst retaining the ability to bind to $\alpha_{IIb}\beta_3$. Activation of platelets by plasma vWF (10 μg/ml) in the presence of the antibiotic ristocetin (1 mg/ml) induces platelet agglutination (63.2 ± 1.74% decrease in optical density) and phosphorylation of multiple proteins on tyrosine. Within 60s on addition of agonist, and extending over a 5 minute period, proteins of 42, 44, 50, 85, 95, 100, 105, 120 and 150 kDa become tyrosine phosphorylated. Immunoprecipitation studies show the non-receptor tyrosine kinase Syk , phospholipase Cγ2 and p95vav to be phosphorylated on tyrosine over the same time period. In contrast, ΔA1 vWF (3 μg/ml) in the presence of ristocetin (1 mg/ml) induces a significantly reduced agglutination response (6.5 ± 0.15% decrease in optical density; $p<0.05$) by comparison with plasma vWF. In addition ΔA1 vWF induces markedly less phosphorylation of proteins on tyrosine by comparison with plasma vWF.

Syk has been previously shown to be essential for adhesion molecule signalling in platelets activated by collagen (Poole et al., 1997) and has recently been shown to be phosphorylated and activated upon binding of specific antibody to GP Ib (Yanabu et al., 1997). This is the first report of the involvement of Vav and PLCγ2 in vWF-induced activation of platelets. It is possible that phosphorylation and activation of all these proteins is required for adhesion signalling downstream of vWF. Studies using additional mutant vWFs with selective binding properties for GPIb and $\alpha_{IIb}\beta_3$ are being carried out to determine which signals occur through which receptor.

S. Falati holds a BBSRC Studentship.

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Yanabu, M., Ozaki, Y., Nomura, S. *et al.* (1997) *Blood* 89, 1590-1598.

104P PROTEIN KINASE A-DEPENDENT MODULATION OF HISTAMINE H1 RECEPTOR COUPLING IN CULTURED HUMAN AIRWAY SMOOTH MUSCLE CELLS

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We have previously demonstrated that histamine H_1 receptor-induced activation of phospholipase C in bovine trachealis muscle is inhibited by elevation of tissue cyclic AMP content. Cultured human airway smooth cells (HASM) express a classical H_1 receptor and the aim of the current study was to determine whether coupling through this receptor is modulated by changes in cell cyclic AMP content.

HASM cells were grown as previously described (Daykin et al., 1993) and subcultured in 24 well plates or on glass cover slips for experiments. Histamine stimulated concentration-dependent [^{3}H]-inositol phosphate formation as previously described (EC50 4.8μM). The responses to all concentration of histamine which produced a significant ($p<0.05$) rise in inositol phosphate accumulation were reduced by agents we have shown previously to elevate cyclic AMP content in these cells: namely isoprenaline (1μM), 3-isobutylmethylxanthine(IBMX, 100μM), PGE_2 (1μM) and VIP(1μM) (all $p<0.05$) (table 1).

In order to determine whether cyclic AMP-induced inhibition of phospholipase C activation alters calcium release evoked by histamine, we next studied the effect of microinjection of the catalyic subunit of protein kinase A (100UI/ml) on the intracellular release of calcium in single cultured HASM cells stimulated by histamine (1μM). Cells were microinjected with fura 2 (pentasodique salt) and either native or boiled PKA catalytic subunit and then stimulated with histamine 20min later. The peak calcium response to histamine was reduced from 253±36 nM over basal to 65±25nM over basal(n=33, $p<0.001$) in cells microinjected with active (cf boiled, inactive) PKA catalytic subunit.

These results demonstrate that agents known to elevate cyclic AMP content in cultured HASM cells inhibit histamine induced phospholipase C activation , and that active catalytic subunit of PKA reduces the subsequent calcium response to histamine H_1 receptor stimulation.

Table 1. Inhibition of the inositol phosphate response to histamine stimulation by agents which elevate intracellular cyclic AMP.

Agent	**% inhibition of response to 100μM histamine**	**n**
Isoprenaline	45 ± 3	8
IBMX	43 ± 9	3
Isoprenaline + IBMX	69 ± 1	3
PGE_2	33 ± 8	5
VIP	49 ± 7	3

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105P SELECTIVE INHIBITION OF THE CARBACHOL-INDUCED CALCIUM RESPONSE BY CRF IN THE CATH.a CELL LINE

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Corticotrophin releasing factor (CRF) is the primary hypothalamic releasing hormone responsible for the activation of the pituitary-adrenal axis in response to stress. A region of the brain believed to be important in the CNS response to stress is the locus coeruleus, the predominant site of noradrenergic cell bodies in the brain. In this study we demonstrate, using a locus coeruleus-like cell line, CATH.a (Suri *et al.*, 1993), that CRF modulates other receptor signalling pathways, in particular, CRF selectively inhibits the muscarinic receptor-mediated calcium response.

CATH.a cells were grown in DMEM with 10% FCS. Single cell calcium imaging was performed using Fura2 (5μM, 30mins) loaded cells grown on gelatin coated coverslips. Fluorescence was analysed with ImproVision software. Data are expressed as changes in 340:380nm ratio units (ΔR), which are indicative of changes in intracellular calcium concentrations. Phosphoinositide (PI) hydrolysis was estimated using a *myo*-[^{3}H]inositol prelabelling technique. Cells loaded with *myo*-[^{3}H]inositol for 18hr were washed twice with Hank's HEPES buffer and were then incubated at 37°C with buffer containing 20mM LiCl and various agonists. The reaction was terminated after 40 minutes by aspiration of the media and addition of methanol. Total [^{3}H]-inositol phosphates ([^{3}H]-IP) accumulating were measured and expressed as fold basal.

The pituitary adenylate cyclase-activating peptide (1-27) (10nM) and vasoactive intestinal peptide (1μM) stimulated rises in intracellular calcium concentration (0.85±0.16 and 0.75±0.15 ΔR, respectively, n=4) which were unaffected by CRF (1μM) (n=2). However, the calcium response to carbachol (1mM) (0.40±0.08ΔR, n=5) was completely abolished by CRF (1μM) (n=4). Forskolin (10μM) also eliminated the carbachol-induced calcium response (n=4). Incubation of cells with dibutyryl cyclic AMP (dbcAMP) (1mM), an activator of protein kinase A (PKA), for 12 minutes prior to agonist exposure similarly abolished the intracellular calcium response to carbachol. Rp-adenosine 3',5'-cyclic monophosphothioate triethylamine (100μM), an inhibitor of PKA, reversed the attenuation of the carbachol response by dbcAMP. Carbachol increased [^{3}H]-IP accumulation to a maximum of 2.4±0.11 fold basal (EC_{50}=6.75±0.26μM, n=3). In the presence of forskolin (10μM), the carbachol-induced PI response was not significantly different from control (Students t test, $p>0.05$, n=3) reaching a maximum of 2.57±0.17 fold basal (EC_{50}=7.76±0.37μM).

The present results indicate that CRF selectively inhibits the muscarinic receptor-mediated intracellular calcium response in a locus coeruleus-like cell line. The finding that forskolin, and more particularly, dbcAMP can mimic the effects of CRF on the carbachol response suggests an involvement of PKA. The target for PKA is unclear but the lack of effect of forskolin on carbachol-stimulated [^{3}H]-IP accumulation indicates that the muscarinic receptor may not be involved. Clarification of the mechanism of the CRF response might provide an insight into its role in the locus coeruleus under stressful conditions.

Suri C., Fung B.P., Tischler A.S. and Chikaraishi D.M. (1993). *J.Neurosci.* **13**, 1280-1291.

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106P THE MODE OF ACTION OF BRADYKININ AND OTHER BASIC SECRETAGOGUES ON HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS

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Bradykinin (BK), a proinflammatory peptide, induces histamine release (HR) from rat peritoneal mast cells (RPMC) via a mechanism, independent of B_1 and B_2 receptors, which is sensitive to pertussis toxin (Bueb *et al*, 1990). However, the detailed signal transduction mechanisms for BK stimulation in RPMC is not clear. In the present work, we studied the effect of calphostin C and hydrogen peroxide (H_2O_2), both known to inhibit the activity of protein kinase C (PKC) (Taher *et al*, 1993), on BK-induced HR. We also investigated whether HR induced by basic secretagogues (BK, substance P and 48/80) could be antagonised by heparin and dextran sulphate (DS) due to their negative charge density (Niitsuma *et al*, 1996).

RPMC were obtained by peritoneal lavage. The washed cells were incubated at 37 °C in HEPES-buffered solution with the test drugs for a specified time period; then challenged with the stimulant (at submaximal concentration) for another 15 min. The reaction was stopped by cold buffer. Histamine was assayed fluorometrically. The results were expressed as a percentage of total histamine content and corrected for spontaneous release (< 10%). Student's *t*-test was used for statistical analysis, and $p<0.05$ was considered as significant.

Table 1. Effect of DS or heparin (Hep) on HR induced by BK, 48/80 or substance P (Sub P)

	DS+	Hep+		DS+	Hep+		DS+	Hep+
BK	BK	BK	48/80	48/80	48/80	Sub P	Sub P	Sub P
48.9	52.1	53.7	52.1	55.6	54.6	44.6	52.8	45.6
±2.6	±1.8	±1.8	±1.7	±1.3	±2.1	±2.8	±5.1	±3.4

Results are expressed as HR (% of total). Cells were incubated with DS (560 μg/ml) or heparin (100 U/ml) for 30 min prior to the challenge with BK (60 μM), 48/80 (0.5 μg/ml) or substance P (20μM) for a further 15 min. Each point is the mean±SEM of results from 2-4 experiments performed in triplicate.

The results (Table 1) show that DS (5.6, 56 and 560 μg/ml) or heparin (1-100 U/ml) given at 0, 30, and 45 min prior to the challenge by BK, 48/80 or Sub P (15 min) had no effect on stimulated HR. H_2O_2 and calphostin C had pronounced potent and dose-related inhibitory effect on BK-stimulated HR (Fig.1). The effect of H_2O_2 was non-cytotoxic, and was time-dependent at 0, 15 and 30 min incubation. H_2O_2 was able to inhibit BK-induced HR even when it was removed from incubating medium after pre-incubation before BK stimulation.

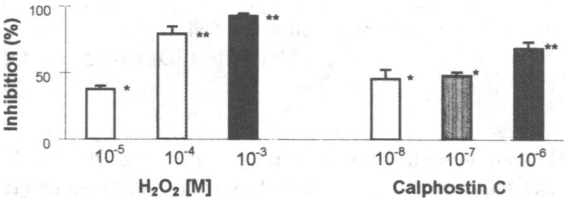

Fig.1. Inhibition of BK-induced HR by H_2O_2 or calphostin C. Cells were incubated with H_2O_2 or calphostin c for 30 min prior to BK stimulation (15 min). Results are mean±SEM of 2-4 experiments in triplicate. $^*p<0.05$, $^{**}p<0.01$, compared to BK-induced HR of 48.1±2.1 for H_2O_2 and 52.4±3.4 for calphostin C.

The results indicate that high negatively charged molecules such as heparin or DS are unable to inhibit HR induced by BK, 48/80 or substance P. The potent inhibition of BK stimulation by H_2O_2 or calphostin C suggests that a PKC sensitive pathway is involved in BK stimulation.

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107P VISUALISATION OF TISSUE KALLIKREIN AND KININ RECEPTORS IN HUMAN ASTROCYTOMAS

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The serine protease, tissue kallikrein, is known to be present in gastric carcinoma, ductal breast cancer cells, pituitary prolactin secreting adenomas and Lewis lung tumours. In addition to increasing cell proliferation, the vasodilatory effect of the bioactive kinin peptides (generated by tissue kallikrein), might be expected to increase vascular permeability and thereby enhance metastasis of tumours. Since kinins act by signal transduction mechanisms that are linked to B1 and B2 kinin receptors, the aim of this study was to elucidate the localisation of tissue kallikrein and B1 and B2 receptors in normal human brain and in astrocytic tumours, in order to determine whether the kallikreinkinin system plays a role in the pathogenesis of this disease state.

Ethical permission for the work was applied for and received from the University of Natal Faculty of Medicine Ethical Committee and from the Forensic Surgeon. Tumour tissue obtained at surgery, and control tissue from forensic autopsies performed between six and twentyfour hours after death, were fixed in 5% formol saline and embedded in paraffin wax. Sections were probed for the presence of tissue kallikrein, B1 and B2 receptor-like immunoreactivity using standard immunolabelling techniques, detected with either diaminobenzidine or fluoroscein isothiocyanate viewed respectively by trans-illumination and confocal light microscopy (Raidoo & Bhoola, 1997).

Thirty two samples were taken from different brain areas of each of six normal brains. In these samples immunoreactive tissue kallikrein and B2 receptors were detected in cerebrocortical neurones and not in glial cells. Immunolabelling for B1 receptors was absent in cortical areas.

Individual tumour samples were obtained from six brains. In all these six samples, immunolabelling for tissue kallikrein and B1 receptors was observed in the cytoplasm of the astrocytic cells. The morphology of these cells varied from round to oval to star shaped. Immunolabelling for B2 receptors was observed also in some astrocytic cells but with reduced intensity compared to tissue kallikrein and B1 receptor-like immunoreactivity. Immunoreactive tissue kallikrein and kinin receptors was also present in endothelial cells of the stromal blood vessels.

These observations suggest that tissue kallikrein and B1 receptors are abundant in proliferating tissue, whether of inflammatory or neoplastic origin. Therefore specific inhibitors of tissue kallikrein, B1 and B2 receptors, or of their gene expression may be of therapeutic value in the treatment of tumours of the CNS.

Raidoo DM & Bhoola KD (1997) *J Neuroimmunol* **77(1)**, 39-44

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108P MUSCARINIC RECEPTORS IN THE LOCUST FOREGUT ARE LINKED TO INOSITOL PHOSPHATES AND DIACYLGLYCEROL

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Acetylcholine causes concentration - dependent, atropine -sensitive contraction of the isolated foregut of the locust *Schistocerca gregaria* (Wood *et al.*, 1992). Here we describe the effects of Li^+ and atropine on ACh-stimulated tissue inositol phosphate production as well as the effect of H7 on ACh- and phorbol 12,13-dibutyrate (PDBu)-induced foregut contraction.

Locust foregut homogenates, prepared and incubated with [^{3}H]-*myo*-inositol as described by Hinton & Osborne (1995), were challenged for 2 min with ACh (1μM and 0.5mM) in the absence and presence of either Li^+ (50 mM) or atropine (10μM). [^{3}H]-inositol phosphates (IPs) were eluted from anion exchange columns (DOWEX-1, X8, formate form, mesh 100-200, Bio-Rad) as described by Berridge *et al.* (1983). Anion exchange chromatography yielded peaks corresponding to the known elution profiles of IP_1, IP_2, IP_3 and IP_4. Radioactivity was quantified by liquid scintillation counting and the results, which were normalised by subtracting the Tris buffer treated controls, expressed as d.p.m. mg^{-1} protein. Isolated foreguts (oesophagus to proventriculus) were incubated in Clarke Insect Ringer containing neostigmine (50μM) at 18±2°C for twenty min. prior to testing the contractile effects of ACh (1μM - 0.5mM) and PDBU (1 - 10μM) in the absence and presence of Li^+ (50 mM), H7 (10 nM - 10 μM).

ACh caused concentration-dependent production of inositol phosphates which was reduced significantly in the presence of Li^+ and atropine. The contractile effect of ACh (0.5 mM) was reduced by 45 ± 5.1 % (n = 8) in the presence of 50mM Li^+ and by 33 ± 2.9 % (n = 8) in the presence the protein kinase C (PKC) inhibitor H7 (1 μM; Hidaka *et al.*, 1984) respectively. In comparison, H7 (1μM) caused 95% inhibition of the contractile efffect of PDBu (10 μM).

These data suggest that ACh-induced contraction of the locust foregut involves inositol phosphates and diacylglycerol. Thus, Li^+ reduces ACh-induced production of inositol phosphates as well as antagonising tissue contraction. Furthermore, the effects of H7 on contraction caused by both ACh and PDBu suggest that activation of muscarinic receptors leads to activation of PKC. However, the inability of H7 to abolish the effects of ACh suggests that both parts of the bifurcating phosphatidylinositol-derived second messenger system are involved in contraction.

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109P TIME DEPENDENCY AND AGONIST SELECTIVITY FOR REDUCTION OF A_{2B} ADENOSINE RECEPTOR-EVOKED CYCLIC AMP RESPONSES IN THE GUINEA-PIG CEREBRAL CORTEX

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Our understanding of the mechanisms of receptor desensitisation derives mainly from studies of the β-adrenoceptor (e.g. Lohse, 1993). In this report, we have investigated the potential for desensitization of A_{2B} adenosine receptor-evoked cyclic AMP (cAMP) responses in slices from the guinea-pig cerebral cortex.

Generation of [^{3}H]-cAMP in [^{3}H]-adenine labelled slices of cerebral cortex from male, Dunkin-Hartley guinea-pigs (300-800 g) was assessed essentially as previously described (Alexander *et al.*, 1996). Agonist-evoked responses were initially expressed as a percentage conversion from the total [^{3}H]-adenine nucleotides with basal levels subtracted, from experiments carried out on at least 4 separate occasions. Thereafter, receptor-mediated cAMP responses evoked in the presence of near-maximally active concentrations of adenosine (100 μM) or NECA (10 μM), were compared to the NECA-evoked response at 15 minutes. Statistical significance was evaluated by use of ANOVA with post-hoc Newman-Keuls tests.

Table: time-dependency of cAMP responses (means ± s.e.mean) to NECA or adenosine in guinea-pig cerebral cortex slices.

Time (min)	cAMP accumulation (percentage of NECA response at 15')		
	NECA	Adenosine	Adenosine + dipyridamole (1 μM)
15	100*	84 ± 8*	77 ± 10*
30	156 ± 28*$^+$	86 ± 8*	ND
60	177 ± 33*$^+$	54 ± 8	ND
90	176 ± 28*$^+$	34 ± 7	ND
120	114 ± 24*	10 ±8$^+$	11 ± 5$^+$

*P<0.05 compared to cAMP response in the absence of agonist, $^+$P<0.05 compared to respective agonist-evoked cAMP response at 15 minutes, ND not determined.

Basal accumulations of [^{3}H]-cAMP in guinea-pig cerebral cortex slices were 0.74 ± 0.19 % and 0.51 ± 0.11 % conversion at 15 and 120 minutes, respectively. A 15 minute incubation in the presence of 10 μM NECA led to a significant cAMP generation of 2.53 ± 0.97 % over basal (P<0.001). The cAMP responses to NECA and adenosine at different time points are shown in the Table.

Thus, continuous exposure of guinea-pig cerebral cortex slices to adenosine leads to a transiently maintained elevation of cAMP levels which declines over time to levels not different from basal. The reduced cAMP response to adenosine with increasing time is unlikely to be due to uptake since incubation in the presence of the uptake inhibitor, dipyridamole, fails to enhance adenosine-evoked cAMP responses. The distinct time profile for the partial agonist, adenosine, and the "full" agonist, NECA, suggests a role for receptor occupancy in the mechanism of this phenomenon. Extracellular metabolism of adenosine, but not NECA, may also account for this distinction.

JKS is a BBSRC Glaxo Wellcome CASE Student.

Alexander SPH, Cooper JA, Shine J & Hill SJ (1996) *Br.J.Pharmacol.* **119**, 1286-1290

Lohse MJ (1993) *Biochem.Biophys.Acta.* **1179,** 171-188

110P MODULATION OF OF A_{2B} ADENOSINE RECEPTOR-EVOKED CYCLIC AMP GENERATION IN THE GUINEA-PIG CEREBRAL CORTEX

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We have previously observed that adenosine evokes a reduced maximal cyclic AMP (cAMP) response compared to the non-selective adenosine receptor agonist 5'-*N*-ethylcarboxamidoadenosine (NECA) in slices from the guinea-pig cerebral cortex (Alexander *et al.*, 1996). In this report, we have investigated whether responses to adenosine and NECA are differentially affected in the presence of agents which potentiate the A_{2B} adenosine receptor-evoked cAMP response (Hill & Kendall, 1989).

Generation of [^{3}H]-cAMP in [^{3}H]-adenine labelled slices of cerebral cortex from male, Dunkin-Hartley guinea-pigs (300-800 g) was assessed essentially as previously described (Alexander *et al.*, 1996). Agonist-evoked responses were initially expressed as a percentage conversion from the total [^{3}H]-adenine nucleotides with basal levels subtracted, from experiments carried out on at least 4 separate occasions. Concentration-response curves were constructed to NECA (10^{-7} M to 10^{-4} M) and adenosine (10^{-7} M to $10^{-3.5}$ M). Statistical significance was evaluated by use of ANOVA with post-hoc Newman-Keuls tests.

Basal accumulations of [^{3}H]-cAMP in guinea-pig cerebral cortex slices at 15 minutes (0.60 ± 0.08 % conversion, mean ± s.e.mean) were not significantly changed in the presence of 1 mM histamine (HA; 0.77 ± 0.12) or 100 μM 5-hydroxytryptamine (5HT; 0.58 ± 0.08). cAMP generation evoked by 100 μM NECA (1.94 ± 0.25 % conversion) was significantly enhanced in the presence of HA (12.39 ± 0.86; P<0.001) or 5HT (8.27 ± 1.25; P<0.001). NECA evoked concentration-dependent responses under all three conditions with maximal responses of 98 ± 3 %, 91 ± 4 % and 91 ± 3 % of the respective 100 μM NECA responses. pEC_{50} values for NECA (5.24 ± 0.11) were significantly increased in the presence of HA (5.93 ± 0.14; P<0.001) and 5HT (5.56 ± 0.17; P<0.05).

Adenosine also evoked a concentration-dependent stimulation of cAMP generation which was significantly enhanced in the presence of HA (P<0.001) or 5HT (P<0.01). Maximal responses to adenosine were 69 ± 9, 69 ± 12 and 64 ± 10 % of the NECA response in the absence of a second agonist, or in the presence of HA or 5HT, respectively. pEC_{50} values for adenosine were 4.35 ± 0.09, 4.61 ± 0.12 and 4.37 ± 0.07 in the absence of a second agonist, or in the presence of HA or 5HT, respectively.

Thus, the partial agonist nature of adenosine is conserved in the presence of agents which potentiate A_{2B} adenosine receptor-evoked cAMP responses. Furthermore, these data suggest that the modulation of the A_{2B} adenosine receptor-evoked cAMP response is likely to take place distal to the receptor.

JKS is a BBSRC Glaxo Wellcome CASE Student.

Alexander SPH, Cooper JA, Shine J & Hill SJ (1996) *Br.J.Pharmacol.* **119**, 1286-1290

Hill SJ & Kendall DA (1989) *Cell.Signal.* **1,** 135-141

111P INHIBITORS OF RECEPTOR INTERNALIZATION BLOCK ADENOSINE A_{2a} RECEPTOR RESENSITIZATION

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Adenosine A_{2a} receptors expressed in NG108-15 neuroblastoma x glioma hybrid cells are G_s-coupled receptors and are subject to agonist-mediated desensitization (Keen *et al.*,1992). In this study we have investigated the effect of inhibitors of receptor internalization on the desensitization and resensitization of A_{2a} adenosine receptor responsiveness in NG108-15 cells. Cells were cultured in DMEM containing 6% fetal bovine serum. Following drug treatment (if any), cells were harvested, washed and frozen at -70°C until required. Adenylyl cyclase activity was then assessed in cell homogenates using a binding protein assay (Mundell *et al.*, 1997) and expressed as pmol cAMP / min / mg protein.

Pretreatment of NG108-15 cells for 40 min with 0.4 M sucrose or 0.25 mg/ml concanavalin A (con A) did not affect acute adenosine A_{2a} receptor-stimulated (with 3 μM CGS21680) adenylyl cyclase activity, or the desensitization of this response produced by 30 min pretreatment with 10 μM NECA (the inhibitors of internalization were added 10 min before NECA). For sucrose experiments, CGS21680-stimulated activity in the absence of any pretreatment was 17.3 ± 0.7, following sucrose was 18.4 ± 1.1, following NECA pretreatment was 5.6 ± 0.6 and following sucrose and NECA was 7.0 ± 0.8 pmol cAMP/min/mg protein, n=5. For con A experiments, CGS21680-stimulated activity in the absence of any pretreatment was 22.4 ± 1.9, following con A was 21.8 ± 1.1, following NECA was 11.4 ± 1.3, and following con A and NECA was 10.3 ± 1.5 pmol cAMP/min/mg protein, n=4.

The effect of sucrose on resensitization of the A_{2a} response was then investigated. Cells were pretreated with NECA for 30 min in the presence or absence of sucrose, following which cells were washed and incubated in the presence or absence of sucrose for a further 30 or 90 min. Values obtained for CGS21680-stimulated adenylyl cyclase activity were as follows: following 30 min NECA alone 5.7 ± 0.6, following 30 min NECA in the presence of sucrose 7.1 ± 0.8, following 30 min NECA alone and 30 min resensitization 12.7 ± 0.9, following 30 min NECA and 30 min resensitization in the presence of sucrose 7.2 ± 0.3, following 30 min NECA alone and 90 min resensitization 16.1 ± 0.8 and following 30 min NECA and 90 min resensitization in the presence of sucrose 7.2 ± 0.8 pmol cAMP/min/mg protein, n=5. Similar results were obtained with con A. Treatment of cells with sucrose alone for 120 min did not affect CGS21680-stimulated adenylyl cyclase activity as compared to non-sucrose treated controls (control activity 17.3 ± 0.7 and activity following sucrose 16.9 ± 0.9 pmol cAMP/min/mg protein).

These results indicate that blockers of receptor internalization inhibit resensitization but not desensitization of endogenously expressed adenosine A_{2a} receptors.

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112P DISRUPTION OF ENERGY METABOLISM BY PYRIDYLISATOGEN IS MEDIATED BY THE MITOCHONDRIAL PERMEABILITY TRANSITION IN RAT HEPATIC MITOCHONDRIA

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2, 2'-Pyridylisatogen tosylate (PIT) has been shown to be a non-competitive antagonist of ATP responses (Spedding *et al.*, 1975). In addition to antagonism of ATP responses, PIT produces a direct relaxation of the smooth muscle and tone must be restored with a spasmogen Hooper *et al.*, 1974). Direct relaxation of the smooth muscle has been attributed to an inhibition of ADP-stimulated respiration indicating that disturbance of mitochondrial function causes loss of smooth muscle tone (Foster et al., 1978). Interference with normal Ca^{2+} homeostasis, in particular opening of the mitochondrial permeability transition pore leads to an inhibition of oxidative phosphorylation (Menton and Markham, 1995). The aim of this study was to determine whether the deleterious effects of PIT upon mitochondrial oxidative phosphorylation could be prevented by the inhibitor of the mitochondrial permeability transition pore cyclosporin A (CsA).

Rat liver mitochondria were prepared from female Wistar rats. Mitochondria, incubated in the presence of 5mM glutamate + 5mM malate, displayed a respiratory control index (RCI) of 7.2 ± 0.4 (n=4). Oxygen consumption was measured using a Clark type O_2 electrode (Rank Bros, Bottisham, Cambridge). Ca^{2+} influx was measured indirectly as a stimulation of mitochondrial respiration.

Ca^{2+} (50 μM) influx was significantly ($P<0.01$) increased by PIT (10-50 μM) from 61.3 ± 8.3 to a maximum of 103.9 ± 13.1 ng atoms O min^{-1} mg $protein^{-1}$ at PIT (33.3 μM). Energy production, in the presence of Ca^{2+} (100 μM), was inhibited by PIT (10 and 50 μM) measured as a decrease in state 3 and increased state 4 respiration value. Incubation of mitochondria with CsA (10 μM) protected energy production in the presence of PIT (10 μM) by decreasing state 4 respiration but had no effect at higher concentrations of PIT (50 μM; Table 1).

	State 3	State 3 + CsA	State 4	State 4 + CsA
Control	68.7 ± 3.7	64.5 ± 3.6	29.1 ± 3.3	28.1 ± 2.3
PIT (10 μM)	53.8 ± 4.8	51.1 ± 3.7	52.3 ± 5.4	30.9 ± 1.8**
PIT (50 μM)	39.2 ± 1.5	46.9 ± 4.6	36.7 ± 1.7	37.2 ± 1.5

Table 1. Effect of CsA (10 μM) upon state 4 and state 3 respiration, in the presence of Ca^{2+} (100 μM), in rat hepatic mitochondria measured in ng atoms O min^{-1} mg $protein^{-1}$; mean ± s.e. mean; n=4; **$P<0.05$.

PIT stimulates Ca^{2+} influx into rat hepatic mitochondria and induces opening of the mitochondrial pore as CsA protects mitochondrial energy production. These conditions would facilitate pore opening, severly compromising cellular ATP levels, and if reflected in smooth muscle, maybe responsible for the direct relaxation produced by PIT.

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113P IDENTIFICATION AND CHARACTERISATION OF ENDOGENOUS P2Z ($P2X_7$) RECEPTORS IN CHO-K1 CELLS

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CHO-K1 cells do not possess endogenous receptors for most neurotransmitters and are therefore often used for studies on recombinant receptors. Although it is known that these cells express endogenous P2Y receptors (Iredale & Hill, 1993) they are not known to express P2X receptors. In this study we have examined these cells using electrophysiological and biochemical methods to determine whether they possess endogenous P2X receptors.

^{45}Calcium ($^{45}Ca^{2+}$) influx was measured as described (Michel et al., 1996). Influx of the DNA-binding dye, YO-PRO-1 (Hickman et al., 1994), was measured fluorometrically while lactate dehydrogenase (LDH) release was measured using a Promega kit. These studies were performed in buffer comprising (mM) sucrose 280, glucose 10, KCl 5, $CaCl_2$ 0.5 and Hepes 10 (pH 7.4, 22°C). Electrophysiological studies were performed as described previously (Chessell et al., 1997). Values are the mean±s.e.mean of 3-5 experiments.

ATP and dibenzoyl-ATP (DbATP) activated inward currents in CHO-K1 cells that reversed at ~ 0 mV suggesting activation of non-selective cation-channels. DbATP (pEC_{50} =4.0±0.1) was a more potent agonist than ATP. DbATP also stimulated $^{45}Ca^{2+}$ influx into CHO-K1 cells. Significant $^{45}Ca^{2+}$ influx was detected within 10s. Thereafter, influx increased linearly with time for 20min and was maximal after 60min. Further studies were undertaken using a 4min incubation. Monovalent cations inhibited DbATP-stimulated $^{45}Ca^{2+}$ influx by >95% at a concentration of 140 mM (pIC_{50} values for NaCl, KCl and choline chloride were 2.08±0.02, 1.74±0.02 and 1.16±0.03). $MgCl_2$ reduced maximal $^{45}Ca^{2+}$ influx (pIC_{50}=3.51±0.08) and decreased the potency of DbATP. DbATP (pEC_{50}=5.5±0.1) was the most potent agonist at stimulating $^{45}Ca^{2+}$ influx (8.1±1.8 fold over basal). ATP and ATPγS also stimulated $^{45}Ca^{2+}$ influx but only at concentrations in excess of 10μM. Adenosine (0.1mM), UTP (1mM) or αβ-methyleneATP (1 mM) did not stimulate $^{45}Ca^{2+}$ influx. DbATP-stimulated $^{45}Ca^{2+}$ influx was blocked by pre-incubating cells with oxidised-ATP (1mM, 45mins), while pyridoxal 5-phosphate and coomassie blue were more potent non competitive antagonists (pIC_{50} of 6.2±0.1 and 5.9±0.1, respectively, against 50μM DbATP).

DbATP (pEC_{50}=5.1±0.1) stimulated YO-PRO-1 influx (3.0±0.8 fold over basal). This response was delayed and only detectable after 8min but thereafter increased with time and was maximal at 90min. DbATP-stimulated YO-PRO-1 influx was inhibited by NaCl (pIC_{50}= 1.12±0.08). Finally, DbATP (pEC_{50}=5.4±0.1) increased LDH release (1.6±0.04 fold over basal). The increased LDH release was not significant until 60 mins but thereafter increased with time.

This study suggests that CHO-K1 cells possess a P2 receptor with pharmacological properties similar to the recombinant $P2X_7$ receptor (Surprenant et al., 1996). Thus, receptor activation leads initially to membrane depolarisation and $^{45}Ca^{2+}$ influx. Prolonged activation leads to a delayed influx of the larger YO-PRO-1 molecule (MW=375) and ultimately to cell lysis. Receptor function was markedly suppressed by monovalent cations and by $MgCl_2$. The presence of an endogenous $P2X_7$ receptor should be considered if CHO-K1 cells are used for studying recombinant P2X receptors.

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114P MIVAZEROL PREVENTS THE TACHYCARDIA CAUSED BY EMERGENCE FROM HALOTHANE ANAESTHESIA PARTLY THROUGH ACTIVATION OF SPINAL α_2 ADRENOCEPTORS

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Mivazerol (MIV) is a new and selective α2-adrenoceptor agonist (Noyer et al.,1994) designed to prevent adverse cardiac outcome in perioperative patients with, or at risk for coronary heart disease (McSPI-Europe Research Group, 1997). The present study was undertaken to determine whether the hyperdynamic state observed in patients at emergence from anaesthesia could be modelled in rats upon withdrawal from halothane (HAL) anaesthesia and to explore the effect and mode of action of MIV under such conditions.

SD male rats were anaesthetised with 1% HAL (N20/02 70/30%) (tidal vol=2.5 ml, 90 cycles/min with body temperature comprised between 37-38°C) so providing (mean ± s.d.) stable heart rate (HR= 334±23 beats/min) and arterial blood pressure (Systolic SAP= 96±12 mmHg) (n=56). Both HR and SAP were continuously recorded during (60 min) and at withdrawal (30 min) of HAL anaesthesia in the presence or absence of MIV administered on i.v. infusion or intrathecally (i.t. T2) respectively 30 min and 2 min prior to withdrawal of the anaesthetic.

Emergence from HAL anaesthesia was associated with sustained increases in heart rate (67±29 beats/min, +20%), and in systolic blood pressure (25±12 mmHg, +26%). The increase in HR observed upon withdrawal from anaesthesia was abolished by bilateral removal of the stellate ganglia. Continuous intravenous infusion for 60 min of 2.2 - 15.3 μg kg^{-1} h^{-1} of MIV prevented, in a dose-dependent manner, the increase in HR upon withdrawal of the anaesthetic without affecting basal HR or SAP during anaesthesia. At an infusion rate of 15.3 μg kg^{-1} h^{-1} i.v., MIV (plasma drug level of 1.7 ng/ml) reduced the increase in HR at emergence by 98% and this effect was completely abolished by 0.34 mg/kg, i.v. rauwolscine. Prevention of tachycardia by MIV was also observed when the drug was administered i.t. (T2), 2 min prior to withdrawal of HAL (65% reduction of HR increase at 2.5 μg/kg). Pre-treatment with 0.5 μg pertussis toxin (PTX) i.t. (T2) 7 days prior to the experiment abolished the antitachycardic effect of 2.5 μg/kg MIV i.t. and partially (-50%) the effect caused by intravenous infusion of 15.3 μg kg^{-1} h^{-1} MIV without affecting HR in its own.

These results demonstrate that withdrawal of HAL anaesthesia in the rat produces immediate and sustained increases in HR which are due to activation of the sympathetic nervous system. Furthermore, pre-treatment with MIV completely abolished this tachycardia via activation of α2-adrenoceptors located at least in part in the spinal cord.

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115P REGIONAL HAEMODYNAMIC EFFECTS OF HUMAN ADRENOMEDULLIN-(22-52) IN THE CONSCIOUS RAT

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Adrenomedullin (ADM) shows some structural homology with calcitonin gene-related peptide (CGRP) (Kitamura *et al.*, 1993), but the widespread vasodilator effects of ADM in conscious rats are not inhibited by the $CGRP_1$-receptor antagonist, CGRP-(8-37) (Gardiner *et al.*, 1995). Since *in vitro* studies indicate ADM-(22-52) may act as an antagonist of ADM-induced cyclic AMP accumulation (Eguchi *et al.*, 1994), we have assessed the regional haemodynamic effects of ADM-(22-52) and its influence on responses to ADM or CGRP, in conscious rats.

Male, Long Evans rats (350-450g) were chronically instrumented with pulsed Doppler probes and intravascular catheters to allow monitoring of changes in renal, mesenteric and hindquarters haemodynamics in the conscious, unrestrained state; all surgery was carried out under sodium methohexitone anaesthesia (40-60mg kg^{-1} i.p.) (see Gardiner *et al.*, 1995 for details).

Rats (n=11) were given i.v. bolus doses of ADM (1 nmol kg^{-1}) before and 10 min after the onset of i.v. infusion of ADM-(22-52) (500 nmol kg^{-1} h^{-1}). The latter had no effect on heart rate (HR: 330 ± 7 to 330 ± 7 beats min^{-1}, mean ± s.e. mean), mean blood pressure (MBP: 106 ± 2 to 102 ± 3 mm Hg), renal vascular conductance (RVC: 53 ± 3 to 58 ± 3 [kHz mm Hg^{-1}]10^{-3}, or hindquarters vascular conductance (HVC: 32 ± 2 to 36 ± 3 [kHz mm Hg^{-1}]10^{-3}), but caused an increase in mesenteric vascular conductance (MVC: 60 ± 7 to 71 ± 8 [kHz mm Hg^{-1}]10^{-3}; P < 0.05, Wilcoxon's test). In the presence of ADM-(22-52), the maximum (at 1 min) fall in MBP and rise in HVC in response to ADM were significantly (P<0.05 Wilcoxon's test) smaller than in the absence of ADM-(22-52)(-12 ± 2 vs -18 ± 2 mmHg; 15 ± 3 vs 25 ± 4 [kHz $mmHg^{-1}$]10^{-3}, respectively) although the maximum increases in RVC and in MVC in response to ADM were not different in the two conditions. However, when assessed from areas under or over curves, the hypotensive and renal, mesenteric and hindquarters vasodilator effects of ADM were reduced by ADM-(22-52) (Table 1).

In other experiments, ADM-(22-52) had no significant effects on the hypotensive or vasodilator responses to CGRP (0.1 or 1 nmol kg^{-1}).

Although these data are consistent with ADM-(22-52) acting as an antagonist of the cardiovascular actions of ADM, they also indicate ADM-(22-52) may exert agonistic effects, particularly in the mesenteric vascular bed. Our results are the opposite of those of Champion *et al.* (1997) who showed ADM-(22-52) antagonised responses to CGRP, but not to ADM, in anaesthetised cats.

Table 1. Integrated (area under curve (AUC) or over curve (AOC)) cardiovascular responses to ADM alone or after (+) ADM-(22-52) in conscious rats. Values are mean ± s.e. mean: *P < 0.05 (Wilcoxon's test). Measurements were made over 15 min.

		+ ADM-(22-52)
HR (AUC, beats)	690 ± 124	508 ± 113
MBP (AOC, mm Hg min)	161 ± 11	77 ± 14*
RVC (AUC, [kHz mm Hg^{-1}]10^{-3} min)	229 ± 20	143 ± 22*
MVC (AUC, [kHz mm Hg^{-1}]10^{-3} min)	250 ± 27	99 ± 15*
HVC (AUC, [kHz mm Hg^{-1}]10^{-3} min)	172 ± 22	94 ± 17*

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116P CARDIOVASCULAR RESPONSES TO N-NITRO-L-ARGININE METHYL ESTER (L-NAME) IN CONSCIOUS, HYPERTENSIVE TRANSGENIC [(mRen-2)27] RATS

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It is feasible the hypertension and widespread vasoconstriction reported in conscious, transgenic [(mRen-2)27] rats (Gardiner *et al.*, 1995) is due to loss of nitric oxide (NO)-mediated vasodilator tone. If so, then the pressor and regional constrictor effects of an NO synthase inhibitor, such as L-NAME, might be less in hypertensive, transgenic (TG) rats than in control, normotensive, Hannover Sprague-Dawley (SD) rats. In the present work we assessed cardiovascular responses to L-NAME (3 $mgkg^{-1}h^{-1}$) in male, SD (n = 8) and TG (n = 10) rats, chronically instrumented with intravascular catheters and pulsed Doppler probes to monitor changes in renal, mesenteric and hindquarters blood flows. All surgery was carried out under anaesthesia (sodium methohexitone, 40-60 mg kg-1 i.p.) (for details see Gardiner *et al.*, 1995).

Resting values and maximal responses (at 90 min) to L-NAME are shown in Table 1. L-NAME had a greater absolute pressor effect in TG than in SD rats, but this was accompanied by smaller absolute reductions in renal and in mesenteric vascular conductance. One possible explanation is that L-NAME caused a reduction in cardiac output (Gardiner *et al.*, 1990) that was less in TG than in SD rats. While this appears consistent with the differential, L-NAME-induced changes in heart rate in the two groups, we have reported that reduction in cardiac output after L-NAME is independent of heart rate (Widdop *et al.*, 1992). If, following any vasoconstrictor challenge, the reduction in cardiac output was less in TG than in SD rats, the former might always show greater pressor responses. Therefore, in other groups we assessed the pressor and regional vasoconstrictor responses to co-infusion of angiotensin II (2 $\mu gkg^{-1}h^{-1}$) and vasopressin (0.2 $\mu gkg^{-1}h^{-1}$). Here, pressor responses were similar in SD (n = 10) and TG (n = 7) rats (43 ± 2 and 40 ± 5 mmHg, respectively), although, as with L-NAME, the reductions in regional vascular conductance were greater in SD than in TG rats (renal, -38 ± 6 and -14 ± 3; mesenteric, -45 ± 7 and -19 ± 3; hindquarters, -28 ± 3 and -12 ± 3 (kHz $mmHg^{-1}$)10^{-3}, respectively; P<0.05). Thus, a different relation between cardiac output and afterload may exist in SD and TG rats, and the relation may vary, depending on the vasoconstrictor agent(s) used. If this proposal is correct, and it has more general validity, it means pressor effects of constrictor agents should not be used as a measure of their vascular effects. Specifically, we suggest the absolute change in blood pressure caused by L-NAME cannot be taken as a straightforward index of NO-mediated vasodilator tone, since the latter may be reduced when the former is enhanced.

Table 1. Resting cardiovascular values and changes with L-NAME in SD and TG rats. Values are mean ± s.e. mean; *P< 0.05 for change (Wilcoxon test) †P<0.05 versus SD rats (Mann Whitney test). HR = heart rate (beats min^{-1}); MBP = mean blood pressure (mmHg); RVC, MVC, HVC = renal, mesenteric, hindquarters vascular conductance, respectively ([kHz $mmHg^{-1}$]10^{-3})

	Resting		Δ with L-NAME	
	SD rats	TG rats	SD rats	TG rats
HR	364 ± 15	349 ± 11	-62 ± 21*	-7 ± 9†
MBP	98 ± 3	166 ± 4†	+27 ± 5*	+42 ± 3*†
RVC	67 ± 8	36 ± 4†	-28 ± 3*	-19 ± 3*†
MVC	69 ± 7	31 ± 4†	-31 ± 4*	-17 ± 3*†
HVC	36 ± 6	24 ± 3†	-15 ± 4*	-11 ± 2*

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117P NO SYNTHASE AND CYCLIC GMP INVOLVEMENT IN MODULATING NA-INDUCED VASOCONSTRICTION IN THE PERFUSED RABBIT OVARIAN VASCULAR BED

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We have recently characterized the adrenoceptor subtype mediating vasoconstrictor responses of the perfused rabbit ovarian bed as being α_{1A} (Yousif et al.,1996). The present experiments were carried out to determine the role of the vascular endothelium in modulating vasoconstrictor responses to noradrenaline (NA) in this preparation.

Sexually-mature female New Zealand White rabbits (NZW) (3-4 Kg) were anaesthetized with sodium pentobarbitone 50 mg/Kg iv. and exsanguinated. The ovarian artery was cannulated and the ovarian bed *in vitro* was perfused with Krebs' solution at 6 mls/min at 37°C. Basal perfusion pressure was 41.5±3.3 mm Hg (mean±s.e.mean, n=20). Changes in perfusion pressure are expressed as a % of the maximum pressor response elicited by 1 µmole NA, 198±11.2 mm Hg (n=8).

The vasoconstrictor response to NA (0.3µmole) was not potentiated by incubation with indomethacin ($5x10^{-6}$M, 30 min) (71.1±8.2% compared to 90.6±7.1%, n=4, N.S.). The maximal vasoconstrictor response to NA (1µmole) was significantly ($p<0.01$) reduced from 100% to 54.6±10.9% in CHAPS (4.7mg/ml, 30sec)-treated preparations (n=5). However, the potency of NA was increased, pD_2 value rising from 7.67±0.08 to 8.12±0.15 before and after CHAPS respectively ($p<0.05$). The NO synthase inhibitor L-NOARG (10^{-6}-10^{-4}M) alone did not alter the basal perfusion pressure. However, at these concentrations, L-NOARG (30 min) potentiated the vasoconstrictor effect of NA.

Maximum potentiation was observed at L-NOARG (10^{-5} M) and the potency of NA was increased, pD_2 value rising from 7.8±0.07 to 8.14±0.08, n=5, $p<0.05$. No further increase was seen with L-NOARG 10^{-4} M. D-NOARG (10^{-5}-10^{-4} M) did not increase the vasoconstrictor response to NA (n=5). L-arginine ($5x10^{-4}$ M), in the presence of L-NOARG (10^{-5} M), partially reversed the potentiated vasoconstrictor response of NA (0.3µmole) to 105.3±17.1%, compared to a control value of 141.7±7.9% in the presence of L-NOARG (10^{-5} M) only, n=5, N.S. Methylene blue, an inhibitor of guanylate cyclase at a concentration of $3x10^{-5}$ M for 15 min (n=4), enhanced NA-induced vasoconstriction. The potency of NA was increased, pD2 value rising from 8.0±0.1 to 8.6±0.1 ($p<0.01$). Aminoguanidine (10^{-4}M) did not affect NA-induced vasoconstrictor response (n=6).

These results suggest that in the perfused rabbit ovarian bed, there is little or no basal release of NO. However, NA-induced vasoconstriction is accompanied by constitutive NO release which limited the vasoconstriction. This was supported by significant increases in NA vasoconstrictor potency in the presence of L-NOARG or methylene blue. Furthermore, vasoconstrictor responses to NA were not counteracted by the release of relaxant mediators such as prostacyclin.

Yousif, M. H., Williams, K. I. and Oriowo, M. A. (1996) *J Auton Pharmac* 16, 221-227

118P EVIDENCE FOR INCREASED NITRIC OXIDE, BUT NOT ENDOTHELIN-1, PRODUCTION IN THE KIDNEYS OF DOCA-SALT HYPERTENSIVE RATS

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The deoxycorticosterone acetate (DOCA)-salt rat is a volume-dependent model of hypertension which exhibits a progressive loss of renal function. Evidence suggests that endothelin-1 (ET-1) plays an important role in development of hypertension in this model (Schiffrin, 1995). ET-1 is not only a potent renal vasoconstrictor but may also activate NO production in the renal medulla to promote sodium and water excretion. It is possible that NO production may be increased in kidneys of DOCA-salt rats as a result of elevated ET-1. Consequently, we have examined ET-1 mRNA expression and endothelial nitric oxide synthase (eNOS) levels in the kidneys of DOCA-salt rats.

Male Sprague-Dawley rats (200-220g) were uninephrectomized under methohexital sodium (50 mg/kg) anaesthesia. A DOCA (200mg) or placebo pellet was implanted subcutaneously. DOCA or placebo rats were then given saline (0.9 % w/v) or tap water, respectively, as their drinking solution. Rats were maintained in metabolism cages so that urine could be collected and assayed for creatinine and nitrate/nitrite (NOx) levels. After 3 weeks, mean arterial pressure (MAP) was recorded in conscious rats via a carotid catheter implanted the previous day. Rats were then anaesthetised and a blood sample removed so that plasma creatinine and ET-1 could be assayed. The kidneys were removed, dissected into cortex and medulla, quick frozen in liquid nitrogen and stored at -80 °C. Total RNA was extracted from the tissue by a guanidine isothiocyanate-phenol-chloroform method, and 20 µg assayed for ET-1 by Northern blot analysis. In addition, total protein was extracted and 60 µg run on a polyacrylamide gel so that eNOS protein levels could be measured by Western blot analysis. Data are expressed as the mean ± s.e.mean and were analysed by ANOVA. $P<0.05$ was taken as significant. For the Northern and Western blots n = 4-5, and for all other data n = 6-11.

DOCA-salt rats had a MAP (197 ± 6 mmHg) significantly elevated over placebo treated rats (133 ± 3 mmHg). Creatinine clearance, a measure of GFR, was reduced in DOCA-salt rats (0.7 ± 0.1 compared to 1.9 ± 0.1 L/day for placebo treated rats). Plasma ET-1 tended to be greater in DOCA-salt animals (1.4 ± 0.5 pg/ml) compared to controls (0.4 ± 0.1 pg/ml, $P<0.08$). ET-1 mRNA expression in medullary tissue was approximately two times greater than in cortical tissue. However, DOCA-salt treatment did not affect this ET-1 mRNA expression compared to controls. Urine NOx excretion was elevated in DOCA-salt treated rats (2.4 ± 0.5 µmol/mg creatinine) compared to controls (1.2 ± 0.1 µmol/mg creatinine). In addition, kidneys from DOCA-salt rats possessed more eNOS protein than placebo treated animals. Medullary tissue also expressed more eNOS than cortical tissue.

An additional group of DOCA-salt rats were given the ETA receptor antagonist A-127722 (Opgenorth *et al.*, 1996) in their drinking solution (8 mg/100ml) for three weeks. Despite a significant decrease in MAP (156 ± 8 mmHg), A-127722 had no effect on creatinine clearance (0.7 ± 0.1 L/day).

Thus, it appears that ET-1 may play a role in the systemic hypertension, but not the reduction in renal function, associated with DOCA-salt treatment. Furthermore, renal eNOS levels and NO production were increased, presumably in an attempt to improve kidney function, but this did not correspond to changes in renal ET-1 mRNA levels.

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119P *IN VITRO* EVIDENCE FOR ENDOTHELIN CONVERTING ENZYME ACTIVITY IN HUMAN KIDNEY

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Endothelin-1 (ET-1) has marked anti-diuretic and anti-natriuretic properties (Simonson, 1993). However, a pathophysiological role of ET-1 has been proposed in renal disorders such as ischaemic acute renal failure (Lopez-Farre *et al.*, 1991). Our aim was to investigate enzymatic processing of big ET-1 in sections of human renal cortex by examining selected binding characteristics of the radiolabelled precursor and cleaved peptide.

Histologically normal human kidneys were obtained from patients undergoing nephrectomy for hypernephroma (50-74 years, n=10). Sections of human renal cortex (10 μm) were incubated with 0.1 nM [^{125}I]-ET-1 or big ET-1 labelled with [^{125}I] at position Tyr^{13} or Tyr^{31} in culture media (M199) for 2h at 37°C to facilitate enzymatic activity. The receptor subtype(s) to which processed [^{125}I]-Tyr^{13} big ET-1 binds was determined by coincubation of sections with ligands that have selectivity for either ET_A (FR139317, 0.1 μM; Aramori *et al.*, 1993) or ET_B receptors (BQ788, 0.1 μM). Specific binding (mean±s.e. mean) was expressed as a percentage of total binding. The Mann-Whitney U-test was used for statistical analysis (significance defined by $P<0.05$).

Specific binding measured from sections incubated with [^{125}I]-Tyr^{13} big ET-1 (which would yield [^{125}I]-ET-1 on enzymatic cleavage) was significantly reduced following coincubation with 10 μM thiorphan, an inhibitor of neutral endopeptidase (NEP) but not the putative endothelin converting enzymes (ECE) (Figure 1). No further reduction in specific binding was obtained with 100 μM thiorphan, indicating that this is a maximal effect. The level of specific binding determined in the presence of phosphoramidon (100 μM), an inhibitor of ECE and NEP, was significantly less than when determined in the presence of thiorphan, indicating that both enzymes cleave big ET-1 in the kidney. No specific binding was detected when sections were labelled with [^{125}I]-Tyr^{31} big ET-1 (which would be expected to yield [^{125}I] labelled C-terminal fragment). Binding of the product of processed [^{125}I]-Tyr^{13} big ET-1 was inhibited mainly by the ET_B selective antagonist (BQ788=75.1±2.1% inhibition, n=5; FR139317=9.7±7.3% inhibition, n=5), consistent with the predominance of this subtype in human kidney.

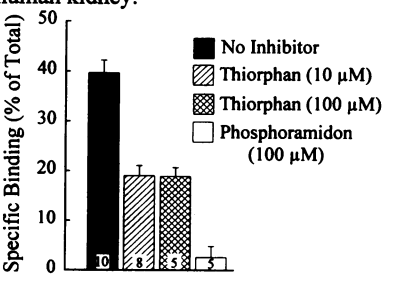

Figure 1. Effect of thiorphan and phosphoramidon on enzymatic processing of [^{125}I]-Tyr^{13} big ET-1 in human kidney.

We conclude that big ET-1 is processed by ECE in human kidney and that the cleaved product binds predominantly to the ET_B receptor subtype. ECE may be a therapeutic target in the attenuation of renal diseases in which ET-1 has been implicated.

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120P EFFECT OF GENDER ON THE RELATIVE CONTRIBUTIONS OF NITRIC OXIDE AND EDHF TO ENDOTHELIUM-DEPENDENT RELAXATIONS IN RAT ISOLATED MESENTERIC ARTERIAL BED

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The present study investigated the relative contributions of nitric oxide (NO) and the endothelium-dependent hyperpolarising factor (EDHF) to endothelium-dependent relaxations in the mesenteric arterial bed in both male and female rats.

Age-matched male (300-360g) and female (180-230g) Wistar rats were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and the superior mesenteric arterial bed was cannulated and perfused at 5ml min^{-1} with gassed (95% O_2 / 5% CO_2) Krebs-Henseleit solution containing 3μM indomethacin. Perfusion pressure was monitored by a pressure transducer placed close to the inflow cannula (McCulloch *et al.*, 1997). Tissues were allowed to equilibrate for 30 min before perfusion pressure was raised by addition of 10-50μM methoxamine. Dose-response curves to the endothelium-dependent relaxant carbachol were constructed in the absence and presence of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100μM) and in the presence of 60mM KCl plus L-NAME. EDHF has recently been suggested to be an endogenous cannabinoid (Randall *et al.*, 1996) and therefore we have used the cannabinoid receptor antagonist, SR141716A (1μM), to investigate the EDHF-mediated component of the endothelium-dependent relaxations.

Basal perfusion pressure was 17.2±0.9mmHg (mean±s.e.mean, n=28) in mesenteries from male rats and 18.5±1.3mmHg (n=22) in mesenteries from female rats. Carbachol induced dose-dependent relaxations of established tone with ED_{50} of 0.43±0.15nmol and a maximum relaxation (R_{max}) of 89.6±1.2% (n=28) in mesenteries from male rats and ED_{50} = 0.72± 0.19nmol and R_{max} = 90.7± 0.9% (n=22) in mesenteries from female rats.

Addition of L-NAME to mesenteries from male rats resulted in a significant (ANOVA with Bonferroni's *post hoc* test) reduction in the potency of carbachol (ED_{50} = 6.45±3.35nmol, $P<0.001$) which was accompanied by a significant reduction in maximal relaxation; R_{max} = 79.2±2.8%, $P<0.01$, n=13). However, in the mesenteries from the females, L-NAME had no effect on relaxation to carbachol (ED_{50} = 0.94±0.20nmol; R_{max} = 86.9±2.3%, n=9). The presence of both L-NAME and 60mM KCl abolished vasorelaxation to carbachol in mesenteries from both males and females. SR141716A did not affect the potency or maximum relaxation to carbachol in mesenteries from male or females. However, in the presence of L-NAME, SR141716A significantly ($P<0.01$) decreased the potency of carbachol (ED_{50} = 53.8±36.8nmol), to a greater extent than that seen in the presence of L-NAME alone in mesenteries from male rats, without affecting maximum relaxation R_{max} = 72.4±4.5%, n=10). In mesenteries from female rats, SR141716A in the presence of L-NAME, significantly ($P<0.01$) shifted the dose-response curve to carbachol to the right 7-fold, (ED_{50} = 6.66±2.46 nmol) compared with L-NAME alone, and significantly ($P<0.001$) reduced R_{max} (70.1±5.5%, n=8).

The results of the present study clearly indicate that the contributions made by NO and EDHF to endothelium-dependent relaxations differ between male and female rats. Specifically, EDHF appears to be functionally more important in females than males. The mechanisms underlying this difference are currently being investigated.

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121P RELATIVE CONTRIBUTIONS OF NITRIC OXIDE AND EDHF TO ENDOTHELIUM-DEPENDENT RELAXATIONS IN THE ISOLATED PERFUSED MESENTERIC BED FROM NORMOTENSIVE AND HYPERTENSIVE BRATTLEBORO RATS

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Early studies using animal models of hypertension demonstrated alterations in endothelium-dependent relaxation in hypertension. However, Randall *et al.* (1991) demonstrated, in the blood perfused rat mesentery, that there was, in fact, no difference in the endothelium-dependent relaxations to carbachol in spontaneously hypertensive rats versus their normotensive controls. A similar observation has also been made *in vivo* in normotensive (DI/N) and hypertensive (DI/H) Brattleboro rats (Gardiner *et al.*, 1994). The present study was carried out to investigate the relative contribution of nitric oxide (NO) and the endothelium-derived hyperpolarising factor (EDHF) to endothelium-dependent relaxations to carbachol in the isolated mesenteric arterial bed of DI/N versus DI/H rats.

Male DI/N and DI/H rats (430-560g) were anaesthetised with sodium pentobarbitone (60mg kg^{-1}, i.p.) and the mesenteric arterial bed was isolated and perfused (95% O_2 / 5%CO_2-gassed Krebs-Henseleit solution containing 3μM indomethacin) via the superior mesenteric artery. Flow rate was constant at 5ml min^{-1} and perfusion pressure was monitored via a pressure transducer placed close to the inflow cannula. (McCulloch *et al.*, 1997). Following 30 min equilibration, perfusion pressure was raised (ca 80-90mmHg) with 10-30μM methoxamine. Dose-response curves were constructed to carbachol in the absence and presence of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100μM), to define the NO-mediated component, or the presence of L-NAME plus 60mM KCl. In the presence of L-NAME, the concentration of methoxamine was reduced to 1-3μM.

Basal perfusion pressure in mesenteric arterial bed from normotensive (MAP=115±8mmHg, n=3) rats was 11.3±1.0 mmHg (mean±s.e.mean, n=10) and 34.6±11.5mmHg (n=8) in the hypertensives (MAP=148±3mmHg, n=8). Following the addition of methoxamine, perfusion pressure was raised by 74.3±5.3mmHg in mesenteries from normotensive animals and 97.7±7.6 in those from hypertensive rats. In mesenteries from normotensive animals, carbachol induced dose-related relaxations of established tone with ED_{50} = 282±74pmol and maximal relaxation (R_{max}) = 81.2±2.0%. This was not significantly (ANOVA with Bonferroni's *post hoc* test) different from the relaxation observed in the mesenteries from the hypertensives (ED_{50} = 778±464pmol, R_{max} = 89.5±1.6%). In the presence of L-NAME (normotensive), the dose-response curve to carbachol was significantly (P<0.001) shifted to the right 50-fold (ED_{50} = 14.2±12.5nmol) and R_{max} was significantly (P<0.05) reduced (61.5±6.4%) compared to control. In mesenteries from hypertensive animals, the ED_{50} was significantly (P<0.001) increased (30.0±20.8nmol) and R_{max} was significantly (P<0.001) reduced (52.1±5.7%) in the presence of L-NAME compared to control. L-NAME caused a greater depression of the vasorelaxation to carbachol in mesenteries from the hypertensives compared to the normotensives. The presence of 60mM KCl resulted in complete inhibition of vasorelaxation to carbachol in mesenteries from both normotensive and hypertensive animals.

The present study demonstrates that, although there are no differences in endothelium-dependent relaxations between mesenteries from normotensive and hypertensive rats, there are differences in the relative contributions of NO and EDHF. Specifically, in mesenteries from hypertensive rats, NO contributes more and EDHF contributes less to endothelium-dependent relaxations compared with normotensive controls.

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122P BIOASSAY OF EDHF IN THE RABBIT ISOLATED FEMORAL ARTERY

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Endothelium-dependent dilatation of the rabbit isolated femoral artery to A23187 is accompanied by hyperpolarisation of the vascular smooth muscle cell membrane potential. (Plane *et al.*, 1995). Both relaxation and hyperpolarisation are largely resistant to the nitric oxide synthase (NOS) inhibitor L-N^Gnitroarginine methylester (L-NAME) leading to the suggestion that relaxation to A23187 is mediated by endothelium-derived hyperpolarising factor (EDHF; Plane *et al.*, 1995). Recently it has been suggested that EDHF may be anandamide, an endogenous cannabinoid , which activates CB_1 receptors (Randall *et al.*, 1996). In the present study, we have further investigated the nature of the mediator of nitric oxide (NO)-independent dilatation of the femoral artery using a bioassay.

New Zealand white rabbits (2-3 kg) of either sex were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.v.) and killed by rapid exsanguination. Femoral and carotid arteries were removed and cleaned of adhering fat and connective tissue. The femoral artery (donor) was cannulated and perfused with warm, oxygenated Krebs buffer (37 °C; 5 ml min^{-1}). An endothelium-denuded ring of carotid artery (detector) was mounted in a myograph for isometric recording of tension and placed below the outflow of the perfused femoral artery (donor). All data are expressed as mean ± s.e. mean and the significance of differences between mean values were calculated using the Students' t-test.

Addition of phenylephrine (PE; 30 μM) to the perfuseate caused constriction of both the donor and detector tissues (15 ± 2 mmHg (n=8) and 19.6 ± 2.3 mN (n=8), respectively). Injection of bolus doses of A23187, in the continued presence of PE, evoked dose-dependent dilatation of the donor tissue which was mirrored by relaxation of the detector ring. The maximum responses to A23187 (2 μmols) were 70.8 ± 5.4 % (n=4) and 78.1 ± 4.2 % (n=4) reversal of PE-induced tone, respectively. Perfusion of the preparations with the NOS inhibitors L-NAME and L-N^Gnitroarginine (Both 100 μM; 30 mins), in the presence or absence of indomethacin (1 μM), did not affect A23187-evoked dilatation in either tissue (n=4; P>0.05). Raising the potassium concentration of the perfusate to 25 mM did not significantly reduce the dilatation of either the donor or detector tissues to A23187 (n=4; P>0.05). However, addition of this concentration of potassium together with the NOS inhibitors significantly inhibited dilatation reducing the maximum responses to 23 ± 6.6 % and 15 ± 7.2 % (n=4; P<0.01), respectively.

Addition of methylene blue (MB; 10 μM) to the perfusate, together with the NOS inhibitors, did not affect dilatation of the donor vessel to A23187 (n=4; P>0.05), but did attenuate dilatation of the detector, tissue reducing the maximum relaxation to 57.9 ± 11.7 % (n=4; P<0.01). Exposure to both the CB_1 antagonist SR141716A (10 μM) and the NOS inhibitors also significantly attenuated dilatation of both the donor and detector tissues to A23187 (n=4; P<0.01), and exposure to the NOS inhibitors, MB and SR141716A together caused a further significant inhibition, reducing the maximum response of the detector tissue to 30.6 ± 7.1 % (n=4; P<0.01).

These data indicate that NO-independent dilatation of the rabbit femoral artery is mediated by a factor, the actions of which are significantly inhibited by 25 mM potassium, which in previous studies, has been shown to inhibit endothelium-dependent hyperpolarisation (Plane *et al.*, 1995). Whereas dilatation of the donor tissue was unaffected by MB, relaxation of the detector was inhibited, indicating that the diffusible factor may be sensitive to extracellular superoxide. In addition, attenuation of dilatation by the CB_1 antagonist SR141716A may suggest a role for a cannabinoid in this NO-independent response.

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123P THE INVOLVEMENT OF AN ENDOCANNABINOID IN EDHF-MEDIATED VASORELAXATION IN THE RAT ISOLATED PERFUSED HEART

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We have recently proposed that anandamide, or related endogenous cannabinoid, is the endothelium-derived hyperpolarizing factor (EDHF), which is responsible for NO-independent, endothelium-dependent relaxations (Randall *et al.* 1996). We have now tested this hypothesis in the rat isolated perfused heart.

Male Wistar rats (300-350g) were heparinized (1,000 U kg^{-1} i.p.) and anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.). In each case, following a thoracotomy, the heart was rapidly excised and perfused in the Langendorff mode at constant flow (20 ml min^{-1}) with oxygenated Krebs-Henseleit buffer containing 2mM pyruvate and indomethacin (10μM). Coronary perfusion pressure (CPP) was raised by L-NAME (100μM) and EDHF-mediated relaxations were induced by bradykinin. The involvement of endogenous cannabinoids in these relaxations was assessed by inclusion of the cannabinoid receptor antagonist SR 141716A (1μM) in the buffer. These responses were compared to those of exogenous anandamide, which was also added in the absence and presence of SR 141716A. The effects of potassium channel blockade (with 300μM tetrabuytlammonium; TBA) and the EDHF inhibitor, clotrimazole (3μM), on these vasorelaxant responses have also been compared.

In the 22 hearts used, L-NAME caused a significant ($P<0.001$; ANOVA) increase in CPP, from 77.5±3.9mmHg (mean±s.e.mean) to 148±8mmHg. Bradykinin caused relaxations (ED_{50}=14.9±5.9pmol) which were antagonised by SR 141716A, which reduced the maximum response from 25.2±2.2% to 8.3±1.2% ($P<0.001$; n=7), with ED_{50}=10.8±6.5pmol. 1μM SR 141716A had no effect on vasorelaxation to the potassium channel activator levcromakalim. The responses to bradykinin were also opposed by TBA (R_{max}=6.7±3.4%; $P<0.01$; n=5) and abolished by clotrimazole (n=3). Anandamide (10nmol-3μmol) also caused coronary vasodilatation, which was antagonised ($P<0.01$) by SR 141716A (R_{max}=32.3±2.3% control vs. 16.4±3.3% in the presence of SR 141716A). The responses to anandamide were also opposed by TBA (R_{max}=18.2±2.8%; n=5) and clotrimazole (R_{max}=5.2±1.3%; n=3). Exogenous arachidonic acid (10μmol) had no effect on coronary perfusion pressure.

We have now shown that anandamide is a potent coronary vasorelaxant, which acts via potassium channel activation and is sensitive to EDHF inhibitors. Furthermore, EDHF-mediated responses to bradykinin in the rat coronary vasculature are opposed by SR 141716A, consistent with our proposal that an endogenous cannabinoid is EDHF.

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Randall, M.D. *et al.* (1996). *Biochem. Biophys. Res. Commun.*, 229, 114-120.

124P COMPARATIVE PHARMACOLOGY OF EDHF-MEDIATED AND ANANDAMIDE-INDUCED VASORELAXATION IN THE RAT ISOLATED SUPERIOR MESENTERIC ARTERIAL BED

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We have recently proposed that an endocannabinoid may be an endothelium-derived hyperpolarizing factor (EDHF) (Randall *et al.*, 1996). We have now compared the pharmacology of EDHF-mediated relaxations with those induced by the prototype endocannabinoid, anandamide.

Male Wistar rats (300-400g) were anaesthetized with sodium pentobarbitone (60mg kg^{-1}, i.p.) and the mesenteric arterial bed was cannulated and perfused with Krebs-Henseleit buffer containing 10μM indomethacin at 5 ml min^{-1} (Randall *et al.*, 1996). Perfusion pressure was raised (ca. 100mmHg) by methoxamine (1-10μM) and dose-response curves (DRCs) were constructed for the vasorelaxants. In order to define EDHF-mediated relaxations the responses to carbachol were determined in the presence of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 100μM). The relaxant effects of carbachol and anandamide have been compared in the presence of L-NAME, high extracellular K^+ (60mM), the potassium channel blocker tetraethylammonium (TEA; 10mM) and the EDHF antagonists clotrimazole (10μM) and proadifen (10μM), which are thought to act through K^+ channel inhibition (Zygmunt *et al.*, 1996).

In the presence of L-NAME, carbachol relaxed established tone (ED_{50}=13.7±4.0nmol and R_{max}=80.7±5.1%; mean±s.e.mean; n=18). Anandamide also caused relaxations described by ED_{50}=39.2±16.1nmol and R_{max} =94.7±9.8% (n=8) which were unaffected by L-NAME (n=4). In the presence of high K^+ relaxant responses to carbachol (R_{max}=3.1±2.1%; n=5) and anandamide (R_{max}=8.9±2.5%; n=5), were almost abolished. TEA caused the DRC to carbachol to be shifted significantly ($P<0.001$; ANOVA) to the right (ED_{50}=579±251nmol) with a significant ($P<0.001$) depression of the maximum (R_{max}=47.7±9.7%). TEA also reduced the maximum relaxation to anandamide (68.9±5.1%; $P<0.05$) but the ED_{50} was unaffected (56.7±13.8nmol). Clotrimazole substantially suppressed the relaxant responses to carbachol, so that at the maximum dose of carbachol used (5.46μmol), the relaxation of tone was 13.1±6.6% (n=3). Clotrimazole abolished vasorelaxation to anandamide (n=3). Proadifen caused a 16-fold rightward shift in the DRC to carbachol (ED_{50}=217±61nmol ($P<0.001$) and R_{max}=44.8±4.1% ($P<0.05$; n=5)). Proadifen caused the DRC to anandamide to be significantly ($P<0.05$) shifted to the right by 10-fold.

In the present study we have shown that anandamide is a potent vasorelaxant, which acts via potassium channel activation. We have also identified parallels between EDHF-mediated and anandamide-induced vasorelaxation, as shown by their sensitivity to a variety of agents, including EDHF antagonists. These results, therefore, add support to our hypothesis that an endocannabinoid may be an EDHF.

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125P EFFECT OF 7-NITROINDAZOLE ON ENDOTHELIUM-DEPENDENT RESPONSES IN THE RAT MESENTERY IN THE PRESENCE AND ABSENCE OF ALLOPURINOL

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Whilst 7-nitroindazole (7-NI) is a potent inhibitor of bovine aortic endothelial nitric oxide synthase (eNOS) in vitro it does not influence endothelium-dependent relaxation of the isolated rabbit aorta or mouse blood pressure (Moore *et. al.*, 1993) suggesting lack of effect on eNOS in isolated blood vessels and in vivo. In an attempt to probe the mechanism(s) underlying this discrepancy we have investigated the ability of 7-NI to influence endothelium-dependent vasodilatation in the perfused rat mesentery in the presence and absence of the xanthine oxidase inhibitor, allopurinol.

Rats (male, Wistar, 250-300g) were used in this study. Mesentery preparations were perfused (5 ml min^{-1}) with Krebs solution containing indomethacin (5 μM) (Moore *et al.*, 1993). After preconstriction with methoxamine (ME, 50 μM) the vasodilator response to carbachol (CCh; 0.01-10 nmol) was determined in the presence/absence of 7-NI (50 μM) and/or allopurinol (50-200 μM). Nitrite concentration in aliquots of the perfusate was determined by chemiluminescence (Harb *et al.*, 1996). In separate experiments, NOS activity in homogenates of rat mesentery was determined as the conversion of [^{3}H] L-arginine (120 nM, 0.5μCi) to [^{3}H] citrulline (Moore *et al.*, 1993) and the catabolism of 7-NI (60 μM) by purified milk xanthine oxidase (XO, 1.4 U ml^{-1}) was assessed (room temperature) in the presence of homoxanthine (0.5 mM) by scanning spectrophotometry.

Rat mesentery homogenates exhibited NOS activity (0.20 ± 0.007 pmol mg $protein^{-1}$ $15\ min^{-1}$, n=6) in vitro. 7-NI, L-N^Gnitro arginine methyl ester (L-NAME) and 1-(2-trifluoromethylphenyl) imidazole (TRIM) inhibited rat mesenteric NOS activity with EC_{50} values of 4.6 ± 0.08 μM, 2.9 ± 0.1 μM and 1014 ± 24.0 μM respectively (all n=6). In perfused mesentery preparations, ME increased perfusion pressure from 13.8 ± 1.4 mm Hg to 83.8 ± 10.2 mm Hg (n=5). Bolus injection of CCh resulted in dose-related vasodilatation (ED_{50}, 0.60 ± 0.07 nmol; maximal response, 89.0 ± 6.0 mm Hg, n=5) accompanied by release of nitrite into the perfusate (e.g. CCh, 5 nmol, 0.10 ± 0.009 pmol μl^{-1}, n=5). Pretreatment (60 min) of ME-preconstricted rat mesentery preparations with 7-NI (50 μM) did not influence either CCh-induced vasodilatation (ED_{50}, 0.80 ± 0.10 nmol, n=5, $P>0.05$) or nitrite release (e.g. 5 nmol, 0.10 ± 0.005 pmol μl^{-1}, n=5, $P>0.05$). Allopurinol (50-200 μM) also failed to affect the vasodilatation or nitrite release triggered by CCh in this preparation. In contrast, 7-NI (50 μM) in combination with allopurinol (200 μM) did reduce vasodilatation due to CCh (ED_{50}, >10 nmol, maximal response, 40.6 ± 10.0 mm Hg, n=5, $P<0.05$) and also abolished the associated release of nitrite into the perfusate. Furthermore, in preliminary experiments, (7-NI, 60 μM; absorbance peak at 360 nm) was broken down by purified XO into an, as yet, unidentified product (absorbance peak, 307 nm). This process was both time-related ($t_{½}$, 6.8 ± 0.1 min, n=3) and inhibited by allopurinol (100 μM).

The present experiments suggest that 7-NI is broken down by XO in the rat mesentery preparation. We propose that the lack of cardiovascular activity of 7-NI in isolated blood vessels and in the intact animal may reflect catabolism by XO in the vasculature.

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126P EFFECT OF 7-NITROINDAZOLE ON ENDOTHELIUM-DEPENDENT RESPONSES IN THE RAT MESENTERY IN THE PRESENCE AND ABSENCE OF ALLOPURINOL

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Vasoconstriction mediated by postjunctional α_2-adrenoceptors has been demonstrated in a number of isolated blood vessels, from a variety of species including pigs (Wright *et al.*, 1995). However, the mechanism for this contraction is still not clear. In porcine blood vessels, stimulation of α_2-adrenoceptors reduces forskolin-stimulated cAMP production. However, basal levels of cAMP are unaffected by α_2-adrenoceptor activation suggesting that the α_2-mediated contractile response involves a novel signal transduction mechanism (Wright *et al.*, 1995). In this present study we have investigated α_2-mediated vasoconstriction in porcine ear artery.

Porcine ear arteries were dissected into 2mm ring segments and suspended in a 5ml isolated organ bath containing Krebs-Henseleit buffer maintained at 37° C. The lower support was fixed and the upper support was connected to a force transducer linked to a MacLab data acquisition system via an amplifier. After a 20 min equilibration period, tension was applied to the tissue which was allowed to relax to a final resting tension of between 1-1.5g wt. Before each experiment, the tissues were contracted 3 times with 60mM KCl.

A submaximally effective concentration of the α_2-adrenoceptor agonist UK14304 (0.3μM) produced a slight contraction (see table 1, UK14304 Alone), while the thromboxane-mimetic U46619 (0.1μM) produced a rapid contraction equal to 115.2% ± 20.3% (n=7) of the KCl response (mean ± S. E. mean). Subsequent addition of 0.3μM UK14304 produced further contraction of the tissue comparable to that seen in the absence of U46619 (table 1). Forskolin (1-2μM) and levcromakalin (1-5μM), a K_{ATP} channel agonist, relaxed the U46619-induced contraction to <10% of the response to 60mM KCl. Subsequent addition of 0.3μM UK14304 produced a 5-fold larger contraction in the presence of forskolin and U46619 compared to that observed in the presence of levcromakalin and U46619 (table 1)

Control	+ U46619	+ U46619 + forskolin	+ U46619 + levcromakalin
7.8% ± 3.3%	10.2% ± 2.6%	46.9% ± 9.6%*	8.1% ± 1.7%

Table 1. Shows responses to UK14304 (0.3μM) in the absence and presence of U46619, U46619 and forskolin, or U46619 and levcromakalin expressed as a percentage of the contraction to 60mM KCl and are shown as the mean ± S. E. mean. * $p<0.01$, Student's unpaired t-test vs UK14304 alone, n=6.

Concentration-response curves were then obtained for UK14304 (1nM to 40μM) in the presence or absence of 0.1μM U46619 and forskolin. In the absence of U46619 and forskolin, UK14304 produced a concentration-dependent contraction of the ear artery reaching a maximum (22.0 ± 10.8% of 60mM KCl response) at approximately 1μM. In the presence of U46619 and forskolin, UK14304 produced an enhanced contraction reaching a maximum (49.6 ± 10.9% of 60mM KCl response; $p<0.05$ vs absence of U46619 and forskolin, Student's paired t-test, n=6) at approximately 1μM. This effect of UK14304 was inhibited by the α_2-adrenoceptor antagonist rauwolscine (1μM) (1μM UK14304 failed to elicit a response), but was unaffected by the α_1-adrenoceptor antagonist prazosin (0.1μM) (7.80 ± 0.24 (pEC_{50} for UK14304 in the presence of prazosin) compared to 7.51 ± 0.24 in the absence of prazosin, n=7), indicating that the effect of UK14304 is indeed mediated via α_2-adrenoceptors.

To conclude, we have demonstrated an α_2-adrenoceptor-mediated, concentration-dependent vasoconstriction in porcine ear artery which is greatly enhanced after the tissue has been preconstricted with the thromboxane-mimetic U46619 and relaxed back to baseline with forskolin. This vasoconstriction appears to be dependent on the agent used to relax the tissue, and the results suggest the involvement of cyclic nucleotides.

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127P EFFECT OF A COMBINATION OF NITRIC OXIDE SYNTHASE INHIBITORS AND POTASSIUM CHANNEL BLOCKERS ON THE ACETYLCHOLINE RELAXATION IN RABBIT MESENTERIC ARTERIES

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Acetylcholine-induced relaxation in small resistance arteries has been shown to be mediated *via* both nitric oxide (NO) and a NO-independent pathway (Waldron & Garland, 1994). The latter pathway may involve endothelium-derived hyperpolarizing factor (EDHF) which is thought to mediate relaxation mainly by the activation of potassium channels (Chen & Suzuki, 1989). In the rat mesenteric arteries it has been shown that nitric oxide synthase (NOS) inhibitors and potassium channels blockers in combination, may produce a greater inhibition than each inhibitor alone (Waldron & Garland, 1994). In the present study the effect of NOS inhibitors and potassium channel blockers on acetylcholine-induced relaxations in the rabbit mesenteric resistance arteries have been investigated.

Female New Zealand White rabbits (2-2.5kg) were anaesthetised with sodium pentobarbitone (60mgkg^{-1}) and killed by rapid exsanguination. The second or third order branches of the mesenteric artery were isolated and mounted in a myograph under normalised tension in oxygenated Krebs solution at 37°C. After precontraction of the tissues with phenylephrine (3-5μM), cumulative concentration response curves to acetylcholine (ACh) were constructed in the absence or presence of the NOS inhibitors N^{G}-nitro-L-arginine methyl ester (L-NAME, 100μM) and N^{G}-nitro-L-arginine (NOARG, 100μM), or potassium channel blockers apamin (50nM) or iberiotoxin (50nM), or the guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10μM) or a combination of these inhibitors. Results are expressed as mean ± s.e. mean and the significant difference between mean values were calculated using the Students' t-test.

ACh induced concentration-dependent relaxation of the rabbit mesenteric artery with a mean pD_2 value of 7.43 ± 0.14 and a maximum relaxation of 96.03% ± 0.92 (n=27). The NOS inhibitors, ODQ or iberiotoxin each significantly inhibited the relaxation to ACh ($P < 0.05$, n=7), whilst apamin did not significantly inhibit the ACh-induced relaxation ($P > 0.1$, n=6). pD_2 values for ACh in the presence of the inhibitors were 6.06 ± 0.32, 6.57 ± 0.27, 5.88 ± 0.20 and 7.89 ± 0.13 respectively. In the presence of a combination of the NOS inhibitors and apamin, or the NOS inhibitors and ODQ, relaxations to ACh were not significantly different from those observed in the presence of the NOS inhibitors alone ($P > 0.1$, n=4-10). However, in the presence of both NOS inhibitors and iberiotoxin, relaxation to ACh was inhibited significantly ($P < 0.05$, n=6). The pD_2 value for ACh in the presence of this combination of inhibitors was 5.05 ± 0.35.

These data show that the ACh-induced relaxation of the rabbit mesenteric resistance arteries is mediated *via* both a NO-dependent and a NO-independent pathway. The latter pathway appears to involve the activation of large conductance calcium activated potassium channel (BK_{Ca}) as iberiotoxin not only caused an inhibition alone but also produced an additional inhibition when used in combination with the NOS inhibitors.

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128P FUNCTIONAL ASSESSMENT OF THE FRESHLY ISOLATED PORCINE SAPHENOUS VEIN

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Porcine saphenous vein-carotid artery interposition graft has been used as a model to study late vein graft failure (Angelini *et al.*, 1990). In the present study, the ability of the freshly isolated porcine sapheous vein to contract and relax to a variety of agonists was examined.

Freshly isolated saphenous vein was obtained from Large White pigs (20-25kg) and collected in warmed Krebs solution. Those veins used for the relaxation studies were collected in 1mM glyceryl trinitrate. Veins were cleaned of adventitia and cut into rings (5mm in length) and mounted in 8ml organ baths. The tissues were bathed in oxygenated Krebs solution at 37°C, under 1g resting tension. Cumulative concentration-response curves were constructed using contractile agonists (noradrenaline, phenylephrine, 5-hydroxytryptamine (5-HT) and U46619) and agonists to produce relaxation (acetylcholine, bradykinin, substance P, A23187 and sodium nitroprusside (SNP) after precontraction with NA (10μM)

Concentration-dependent contractions of the freshly isolated saphenous vein with pEC_{50} values of 7.6 ± 0.07, 5.8 ± 0.06, 5.5 ± 0.06 and 5.1 ± 0.04 were obtained with U46619, 5-HT, noradrenaline and phenylephrine, respectively (n =5). The maximum contractions obtained with 5-HT, noradrenaline and phenylephrine were 12.97g ± 0.85, 15.29g ± 1.04 and 11.18g ± 0.76 respectively at the highest concentration of agonist used (100μM). However, U46619 produced a maximum contraction of 14.40g ± 1.06 at 1μM.

Concentration-dependent relaxations of the freshly isolated saphenous vein were elicted by bradykinin, substance P, acetylcholine and A23187 producing pEC_{20} values of 7.0 ± 0.4, 6.7 ± 0.5, 6.3 ± 0.2 and 5.6 ± 0.3, respectively ($n \geq 4$). At higher concentrations these agonists produced concentration dependent contractions. Maximum relaxation responses were obtained at 10μM with acetylcholine and A23187 (30.3% and 25.4%), 3μM for substance P (33.4%) and 0.3μM for bradykinin (38.5%). A pEC_{50} of 4.7 ± 0.2 was produced by SNP with a maximum relaxation of 63.63% at the highest concentration used (100μM, n =6).

In conclusion, these results show that the porcine freshly isolated saphenous vein responds well to both contractile and relaxant agonists. These data will allow comparison of the functional ability of freshly isolated porcine saphenous vein with explanted porcine saphenous grafts and the human saphenous vein (Lewis *et al.*, 1997).

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129P CHELERYTHRINE INFLUENCES BASAL AND PROSTAGLANDIN F_{2A}-INDUCED INCREASES IN RAT CARDIOMYOCYCTE CONTRACTILITY

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Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induces phosphoinositide breakdown and increases the contractility of rat myocardium (Otani *et al.*, 1988). To test the hypothesis that the positive inotropic effect of $PGF_{2\alpha}$ involves protein kinase C (PKC) activation, we studied the effects of the PKC inhibitor, chelerythrine (Herbert *et al.*, 1990) on the $PGF_{2\alpha}$-induced inotropic responses on rat ventricular myocyte contractility.

Enzymatically dissociated myocytes (Powell *et al.*, 1980) from 280-300g Wistar rats were superfused with 1mM Ca^{2+} Tyrodes buffer and electrically paced at 1Hz at room temperature. Contractile amplitude of single myocytes was measured by a video image and edge detection system. Exposure of myocytes to chelerythrine for 5 minutes augmented contractility in a concentration dependent manner. Contractile amplitude was 102±2%, 108±6%, 122±7%, and 157±14% of basal with 2, 3, 5 and 10μM respectively (mean ± s.e.m, n=4-14). The vehicle for chelerythrine, dimethylsulphoxide (DMSO) did not modify contractility. Therefore, 2μM chelerythrine, which is above the reported IC_{50} of 660nM for PKC inhibition, and which had minimal effects on basal contractility, was used in studies with $PGF_{2\alpha}$.

Myocytes were pretreated with chelerythrine 10 minutes prior to addition of $PGF_{2\alpha}$. The increase in contractile amplitude induced with 3μM $PGF_{2\alpha}$ was attenuated in the presence of chelerythrine, but was unaffected by DMSO (Figure 1).

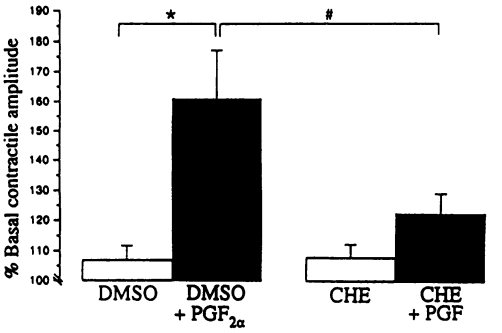

Figure 1. Effect of DMSO and chelerythrine (CHE, 2μM) on $PGF_{2\alpha}$ (3μM) – induced increases in myocyte contractility, *p<0.05 Wilcoxon Signed Rank Test, , #p<0.05 Mann-Whitney Test, n=6/group.

These experiments indicate that PKC plays a role in the positive inotropic effect of $PGF_{2\alpha}$. Also, at concentrations above 2μM, chelerythrine enhances contractile function of cardiac myocytes.

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130P ASSESSMENT OF SUPEROXIDE ANION SCAVENGING ACTIVITY OF SOME COMMON SPIN TRAPS AT PHYSIOLOGICAL TEMPERATURE AND PH BY MICROASSAY *IN VITRO*

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Scavengers of superoxide anion have implicated this reactive oxygen species in the vascular dysfunction associated with a number of conditions including reperfusion injury and nitrate tolerance (Gelvan *et al.*, 1991; Münzel *et al.*, 1995). We have evaluated the utility of the spin trap agents 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), 4-OH-TEMPO (TEMPOL), 4-COOH-TEMPO, 3-carboxy-proxyl (3-CP) and 4,5-dihydroxy-1,3-benzene disulfonic acid (tiron) as potential research tools in the further investigation of oxidant stress by assessing their superoxide anion scavenging activities at physiological temperature and pH *in vitro*.

The photometric microssay of scavenging activity was essentially as previously described (Laight *et al.*, 1997). Briefly, the assay mixture consisted of (final concentration): ferricytochrome c (100 μM); xanthine oxidase (XO, 5-20 mU/ml); hypoxanthine (HX, 100 μM); and catalase (200 U/ml) dissolved in isotonic phosphate-buffered saline at pH 7.4 to make a total volume of 100 μl. Changes in absorbance (A) at 550 nm were recorded at 37 °C using a kinetic platereader. Data are mean±s.e. mean.

There was a linear relationship betweeen the initial reaction rate and the concentration of XO (5-20 mU/ml) (r=1.000, P<0.005, n=4). XO at 10 mU/ml, which provided an initial reaction rate of 24.7±1.2 mA/min (n=4), was adopted for subsequent inhibition studies. The initial reaction rate was depressed by superoxide dismutase (200 U/ml) by 86.4±0.9 % (n=12, P<0.01).

The pIC_{50} values for TEMPO, 4-COOH-TEMPO, TEMPOL, 3-CP and tiron were determined to be 3.94±0.10 (n=4), 3.55±0.14* (n=4), 3.30±0.08*(n=7), 2.80±0.11* (n=5) and 2.81±0.04* (n=5), respectively (*P<0.05 with respect to TEMPO, Dunnett's test).

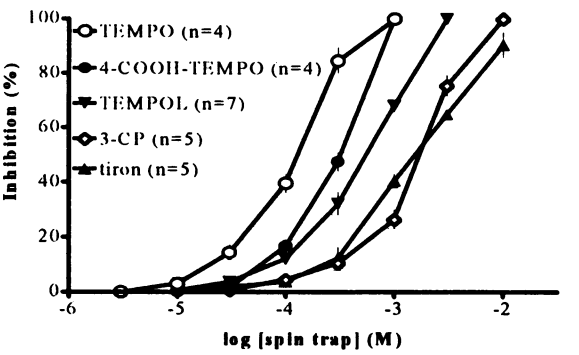

Figure 1. Superoxide anion scavenging activity of spin traps *in vitro*.

In conclusion, this scavenging data will help determine the application of these spin traps to subsequent studies *in vitro* investigating vascular oxidant stress associated with diseases such as diabetes and atherosclerosis (Giugliano *et al.*, 1995).

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131P Cu/Zn SUPEROXIDE DISMUTASE ACTIVITY PRESERVES BASAL ENDOTHELIAL FUNCTION IN THE RAT AORTA *IN VITRO*

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Endothelial dysfunction associated with oxidant stress features in several diseases, including diabetes and atherosclerosis (Giugliano *et al.*, 1995; Harrison & Ohara, 1995). We have evaluated endothelium-dependent and -independent, nitric oxide (NO)-mediated vasodilation in a model of endogenous vascular oxidant stress *in vitro*: the rat aorta treated with the irreversible Cu/Zn superoxide dismutase (SOD) inhibitor, diethyldithiocarbamate (DETCA) (see Omar *et al.*, 1991).

Male Wistar rats (250-300g) were anaesthetised with pentobarbitone sodium (60 mg/kg i.p.), euthanised and thoracic aortic rings prepared and mounted under isometric conditions in Krebs-Henseleit solution (composition in mM: NaCl 133; KCl 4.7; NaH_2PO_4 1.35; $NaHCO_3$ 16.3; $MgSO_4$ 0.61; $CaCl_2$ 2.52; glucose 7.8) containing indomethacin (10μM), gassed with carbogen and warmed to 37° C. Rings were treated with DETCA (10mM) for 30min followed by 30min washout while untreated paired rings served as temporal controls. Contraction to noradrenaline (NA, 1nM-1μM) was then assessed with and without a 10min prior incubation with N^G-nitro-L-arginine methyl ester (L-NAME, 0.3mM). Subsequently, DETCA-treated and control rings were equivalently precontracted with NA to assess vasorelaxation to either the novel phosphodiesterase type V inhibitor ONO-1505 (4-[2-(2-hydroxyethoxy)ethylamino]-2-(1*H*-imidazol-1-yl)-6-methoxyquinazoline methanesulfonate) (Laight *et al.*, 1996) (0.1-10 μM), acetylcholine (ACh, 1nM-3μM) or S-nitroso-N-acetyl penicillamine (SNAP, 0.03-30μM). Data are mean±s.e. mean and were compared by Student's paired t-test.

Contraction to NA (pD_2=7.68±0.08; area under curve (AUC)=2.3±0.2; E_{max}=1.4±0.1g) was enhanced by DETCA (pD_2=7.97±0.10*; AUC=3.1±0.3*; E_{max}=1.7±0.1g) (*P<0.05, n=4). However, in the presence of L-NAME, the DETCA effect was reversed (control group: pD_2=8.41±0.08; AUC=4.9±0.5; E_{max}=2.1±0.2g and DETCA group: pD_2=8.34±0.09* AUC=4.1±0.4*; E_{max}=1.8±0.2g) (*P<0.05, n=5). NA elicited precontraction of 1.6±0.1g (at 30 nM) and 1.5±0.1g (at 100 nM) with and without DETCA, respectively, in the absence of L-NAME (P>0.05, n=14). Vasorelaxation to ACh (pD_2=7.44±0.05; AUC=129.7±7.7; E_{max}=90.6±3.1%, n=5) or SNAP (pD_2=6.06±0.10; AUC=140.0±6.9; E_{max}=99.4±0.6%, n=5) was not significantly affected by DETCA (ACh: pD_2=7.56±0.10; AUC=122.8±7.1; E_{max}=89.4±3.5% (P>0.05, n=5) and SNAP: pD_2=5.93±0.08; AUC=150.1±9.4; E_{max}=100±0% (P>0.05, n=5). In contrast, vasorelaxation to ONO-1505 (pD_2=5.77±0.04; AUC=46.8±7.3; E_{max}=54.3±7.3%) was inhibited by DETCA (pD_2=5.83±0.06; AUC=20.6±4.0*; E_{max}=25.5±4.4 %* (*P<0.05, n=5)

The L-NAME-sensitive ability of DETCA to augment vasoconstriction to NA, coupled with the abrogation in vasorelaxation to a PDE V inhibitor, but not to ACh or SNAP, indicates a selective lesion in basal endothelial function. This emphasizes the role of Cu/Zn SOD in preserving basal, endothelium-derived NO vasodilator activity, probably by preventing the inactivation of NO by endogenous superoxide anion.

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132P UPTAKE AND INTRACELLULAR FATE OF L-3,4-DIHYDROXYPHENYLALANINE, THE DOPAMINE PRECURSOR, IN CACO-2 CELLS

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Endogenous dopamine in the digestive tract has a role in regulating sodium absorption (Finkel et al., 1994). Epithelial cells of intestinal mucosa rich in aromatic L-amino acid decarboxylase (AADC) activity use circulating or luminal L-3,4-dihydroxyphenylalanine (L-DOPA) as a source for dopamine (Vieira-Coelho et al., 1997). The present study was aimed at study the kinetic characteristics of L-DOPA transporter and the fate of newly-formed dopamine in Caco-2 cells (ATCC 37-HTB). Cells (passages 23-30) were grown at 37° C in a humidified atmosphere (5% CO_2) on 2 cm^2 plastic culture clusters or polycarbonate filters in Minimum Essential Medium supplemented with 20% fetal bovine serum and 100 U ml^{-1} penicillin G, 0.25 μg ml^{-1} amphotericin B and 100 μg ml^{-1} streptomycin. After 6 days, the cells formed a monolayer and each 2 cm^2 culture well contained about 100 μg of cell protein; 24 h before the experiments the cell culture medium was changed to a serum free medium. In uptake studies, Caco-2 cells were preincubated (30 min) with Hanks medium with added pargyline (100 μM) and tolcapone (1 μM). L-DOPA and dopamine were assayed by h.p.l.c. with electrochemical detection. Results are arithmetic means with s.e.mean, n=4-5. Statistical differences between experimental groups were determined by ANOVA followed by the Student's t test. In the presence of benserazide (50 μM), L-DOPA was rapidly accumulated in Caco-2 cells. At equilibrium (30 min incubation), the intracellular L-DOPA concentration was 32.9±2.3 μM at medium concentration of 0.5 μM. In saturation experiments, the accumulation of L-DOPA was saturable with a K_m of 60±10 μM and a V_{max} of 6.6±0.3 nmol/mg protein/6 min; at 4°C, the amount of L-DOPA accumulated in the cells was non-saturable. When cells were incubated with increasing concentrations of L-DOPA (10 to 100 μM) in the absence of benserazide a substantial amount of taken up L-DOPA was decarboxylated to dopamine with an apparent K_m value of 27.2±3.8 μM. In experiments performed in cells cultured in polycarbonate filters, the accumulation of L-DOPA in the presence of benserazide was greater when the substrate was applied from the basolateral cell border than from the apical cell border. In the absence of benserazide, L-DOPA applied from the basolateral cell border resulted in a non-linear formation of dopamine (K_m = 43±7 μM; V_{max} = 23.7±1.2 nmol/mg protein/6 min). The amount of dopamine leaving the cell through the apical cell border was lower than that which escaped through the basolateral cell border and was a saturable process (K_m of 623±238 μM; V_{max} of 0.19±0.02 nmol/mg protein/6 min). In conclusion, the data presented here show that Caco-2 cells are endowed with an efficient L-DOPA uptake system and intracellular L-DOPA was found to be rapidly converted to dopamine, some of which diffuses out of the cell. The utilization of Caco-2 cells cultured on polycarbonate filters probably provides a better way to look at processes such as the outward transfer of intracellular molecules, namely the outward transfer of newly-formed dopamine.

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133P COMPETITIVE AND NON-COMPETITIVE INHIBITION OF OF L-3,4-DIHYDROXYPHENYLALANINE UPTAKE IN CULTURED *OPOSSUM* KIDNEY CELLS

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Cyanine derivatives have been found to non-competitively inhibit the uptake of L-3,4-dihydroxyphenylalanine (L-DOPA), the precursor of natriuretic hormone dopamine, in rat renal tubules (Soares-da-Silva, 1995; Pinto-do-Ó & Soares-da-Silva, 1996). The present study was aimed to determine the kinetics of L-DOPA uptake in *Opossum* kidney (OK) cells and define the type of inhibition produced by L-5-hydroxytryptophan (L-5-HTP), cyanine 863 and 3,3'-diethyloxacarbocyanine (3,3'-DOC). OK cells (ATCC 1840-CRL) were grown at 37° C in a humidified atmosphere (5% CO_2) on 2 cm^2 plastic culture clusters (Costar, 3524) in Minimum Essential Medium supplemented with 10% fetal bovine serum and 100 U ml^{-1} penicillin G, 0.25 µg ml^{-1} amphotericin B and 100 µg ml^{-1} streptomycin. After 6 days, the cells formed a monolayer and each 2 cm^2 culture well contained about 100 µg of cell protein; 24 h before the experiments the cell culture medium was changed to a serum free medium. In uptake studies, OK cells were preincubated (30 min) with Hanks medium with added pargyline (100 µM), tolcapone (1 µM) and benserazide (50 µM). L-DOPA and L-5-HTP were assayed by h.p.l.c. with electrochemical detection. Results are arithmetic means with s.e.mean or geometric means with 95% confidence limits, n=4-5. Statistical differences between experimental groups were determined by ANOVA followed by the Newman-Keuls test. Non-linear analysis of the saturation curves revealed for L-DOPA uptake revealed a K_m (in µM) of 129 (114, 145) and a V_{max} (in nmol mg $protein^{-1}$ 6 min^{-1}) of 30.0±0.4. IC_{50} values (in µM) for L-5-HTP (1454 [1214, 1742]) obtained in the presence of a nearly saturating (250 µM) concentration of L-DOPA were almost 4-fold those obtained when non-saturating (25 µM) concentrations of L-DOPA were used (330 [293, 371]). IC_{50} values (in µM) for cyanine 863 and 3,3'-DOC (638 [430, 947] and 353 [234, 531]) obtained in the presence of a nearly saturating (250 µM) concentration of L-DOPA were similar to those obtained when non-saturating (25 µM) concentrations of L-DOPA were used (654 [502, 852] and 339 [184, 627]). V_{max} values (in nmol mg $protein^{-1}$ 6 min^{-1}) for L-DOPA uptake are identical in the absence (36.4±0.7) and the presence of L-5-HTP (39.2±1.3), but K_m values (in µM) are significantly greater ($P<0.05$) when L-DOPA uptake was studied in the presence of L-5-HTP (121 [100, 142] vs 318 [237, 399]). In contrast, the effect of cyanine 863 and 3,3'-DOC is a significant reduction in V_{max} values (15.3±0.7 and 12.1±0.7) without significant changes in K_m (160 [98, 222] and 139 [67, 211]) values. It is concluded that L-5-HTP exerts a competitive type of inhibition of L-DOPA uptake in cultured OK cells, whereas both cyanine 863, an organic cation transport inhibitor, and 3,3'-DOC behave as non-competitive inhibitors.

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134P ATTEMPTS TO ESTIMATE LOCATION PARAMETERS FOR COMPLEX CONCENTRATION-EFFECT CURVES IN HUMAN MYOMETRIUM

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Accurate estimation of the location parameters of concentration-effect (c/e) curves for prostanoids on human myometrium is necessary for the determination of antagonist potencies and for the rigorous assessment of theories (López Bernal *et al.*, 1995) on the regulation of signal-transduction pathways. There are many obstacles to such estimations. Many prostanoids produce biphasic c/e curves by simultaneous activation of functionally antagonistic receptors (Fernandes & Crankshaw, 1995; Popat & Crankshaw, 1997), others produce bell-shaped c/e curves by unknown mechanisms (Senchyna & Crankshaw, 1996).

Several models for the analysis of complex c/e curves have been suggested (Szabadi, 1977; Kühl, 1994; Pliška, 1994). In all cases, to obtain a significant fit requires more data points than can be obtained from a single experiment on the human isolated myometrium. In this study we have used data combination and different normalization procedures in an attempt to fit c/e curves for PGE_2 and sulprostone to the two receptor model of Szabadi (1977).

Table 1 Number of c/e curves significantly fitting the two receptor model.

Agonist	Total analysed	Normalization procedure N.cm^{-2}	Hypotonic shock	Observed maximum
PGE_2	5	2	3	3
Sulprostone	12	3	5	8

Concentration-effect curves were generated in paired strips as described by Popat & Crankshaw (1997) and subjectively divided into monophasic and biphasic groups. Data from paired strips in the apparently biphasic group were combined and analyzed according to the Szabadi (1977) model for two functionally antagonistic receptor populations. Maximal responses were normalized in three ways: as force per cross-sectional area (N.cm^{-2}), to a standard contraction induced by hypotonic shock, and to the maximum recorded response in each strip. The *F* test ($p<0.05$) was used to determine the significance of the fit between the one and two receptor models for each normalization procedure.

Results are shown in Table 1. Curves that appeared monophasic did not fit the two receptor model using any of the normalization procedures.

Human myometrial c/e curves can be analyzed according to complex models, but at the loss of the true tissue maximum. The ability to fit a curve does not prove its applicability. A two receptor model is valid for PGE_2 (Popat & Crankshaw, 1997) but its validity for sulprostone is currently unknown.

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135P CHARACTERIZATION OF EXCITATORY PROSTANOID RECEPTORS IN THE HUMAN UMBILICAL ARTERY *IN VITRO*

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Human umbilical arteries (HUA) functionally express prostanoid TP receptors (Templeton *et al.,* 1991), but the status of other excitatory prostanoid receptors in this tissue is unknown. We have characterized the excitatory prostanoid receptors in HUA using natural and synthetic prostanoids and the selective TP receptor antagonist GR32191B (Lumley *et al.*, 1989).

Sections of umbilical cords within 20 cm of the placenta were collected from full-term vaginal or Caesarean section births. HUA were dissected free of Wharton's Jelly, cut into rings, and mechanically denuded of endothelium. Tissues were mounted in organ baths containing oxygenated (95% O_2, 5% CO_2) physiological salt solution with 30μM indomethacin, and maintained at 37°C under a resting tension of 30 mN. Concentration-effect curves to prostanoids and 5-HT were obtained by cumulative addition to matched rings in the absence and in the presence of 0.1 μM GR32191B.

Table 1. Agonist-induced contraction of HUA *in vitro* in the absence (CONTROL) and presence (TREATED) of 0.1μM GR32191B. Values are means ± s.d.mean.

Compound	n	pEC_{50} CONTROL	pEC_{50} TREATED	Apparent pA_2
I-BOP	3	7.3 ± 0.3	6.9 ± 0.2*	7.2 ± 0.2
U46619	5	6.7 ± 0.2	6.0 ± 0.05*	7.6 ± 0.2
5-HT	6	7.3 ± 0.4	7.2 ± 0.4	-

*significantly different from control (Student's t-test, $p<0.05$)

The selective TP receptor agonists, U46619 and I-BOP (Morinelli *et al.*, 1990), but not 5-HT, were antagonised by GR32191B (Table 1).

The potency order for natural prostaglandins was PGD_2 > PGE_2 = $PGF_{2\alpha}$, with estimated $pEC_{50}<5.3$ (n=3) since complete curves could not be obtained. GR32191B caused rightward shifts of the responses in all cases. Fluprostenol, a selective FP receptor agonist, and sulprostone, a selective EP_1/EP_3 receptor agonist, were inactive at concentrations up to 11.4μM and 4.4μM respectively (n=3). In two of three tissues the FP receptor agonist cloprostenol caused a contraction at 1μM which was abolished in the presence of GR32191B.

These data confirm the presence of TP receptors in HUA (Templeton *et al.,* 1991) and suggest that FP, EP_1, and EP_3 receptors are absent. Although the natural prostaglandins and cloprostenol contract this tissue, they appear to act at TP receptors.

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136P EFFECT OF EXCISION SITE AND MENSTRUAL STATUS ON THE RESPONSE OF NON-PREGNANT HUMAN MYOMETRIUM *IN VITRO* TO U46619

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Heterogeneity of tissue sensitivity to agonist stimulation can often be attributed to gradients in tissue responsiveness which coincide with anatomical location (Kenakin, 1984). The human uterus has been suggested to possess such gradients with respect to prostanoid sensitivity (Wikland *et al.*, 1984). We have investigated the effect of anatomical location and menstrual cycle status on the response of the non-pregnant human myometrium (NPM) to the selective prostanoid TP receptor agonist U46619.

Longitudinal strips of NPM were set up, and cumulative concentration-effect curves to U46619 were obtained as previously described (Senchyna and Crankshaw, 1996). Tissues were excised from the sub-serosal layer of the top, lateral wall and isthmus of the uterine fundus, and from the sub-endometrial (sub-endo) layer of the lateral wall. Tissues were allocated to either proliferative or secretory phase groups based on donor recollection of the date of the last menstrual period.

Table 1 Effect of excision site and menstrual status of donor on responses of NPM to U46619. All strips were taken from the sub-serosal layer except where indicated.

Excision Site	Proliferative Phase pEC_{50}	Proliferative Phase Emax $N.cm^{-2}$	Secretory Phase pEC_{50}	Secretory Phase Emax $N.cm^{-2}$
Top	6.9 ± 0.2	2.6 ± 1.0	6.9 ± 0.3	1.8 ± 0.9
Lateral wall	6.9 ± 0.3	1.4 ± 0.9	7.1 ± 0.3	3.1 ± 1.0
Lateral wall (sub-endo)	6.8 ± 0.1	0.9 ± 0.8	7.0 ± 0.4	1.1 ± 0.4
Isthmus	6.8 ± 0.3	2.4 ± 2.4	7.0 ± 0.2	2.2 ± 0.9

(Values are means ± s.d. means, n=5 in all cases)

Graded responses to U46619 were obtained in all strips tested. Concentration-effect data are summarized in Table 1. There were no significant differences in parameters between groups (Anova, $p<0.05$), although maximal responses were highly variable within groups.

The previously reported large inter-donor differences in sensitivity to U46619 (Crankshaw, 1995) were not seen in the present study, perhaps because only tissues from donors with regular menstrual cycles were used.

U46619 causes contraction of NPM by action at TP receptors (Senchyna & Crankshaw, 1996). Our current data therefore suggest that TP receptors are homogeneously distributed throughout the NPM. Furthermore, they suggest that TP receptor-mediated responses are not significantly affected by regulatory factors that vary with the menstrual cycle. However, important information about maximum responses may be lost due to the imprecision of the technique.

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137P VARIABLE RESPONSES TO PROSTANOID EP RECEPTOR AGONISTS IN NON-PREGNANT HUMAN MYOMETRIUM *IN VITRO*

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Responses to prostaglandin (PG)E_2 are highly variable in both non-pregnant (Senior *et al.*, 1991) and pregnant (Crankshaw & Dyal, 1993) human myometrium. We have investigated this phenomenon further by comparing the responses to both excitatory and inhibitory EP receptor agonists in paired samples of non-pregnant human myometrium.

Strips of human myometrium from non-pregnant donors were obtained and set-up for isometric recording of both excitatory and inhibitory responses as described by Fernandes & Crankshaw (1995). All samples were taken from the mid-lateral wall of the uterus. The effects of PGE_2, sulprostone and butaprost were determined by constructing cumulative concentration-effect curves.

Responses to PGE_2 were donor-specific and found to be either biphasic, monophasic excitatory, or monophasic inhibitory. Assuming PGE_2 was acting at both excitatory (EP_3/EP_1) and inhibitory (EP_2) receptors, biphasic curves were fit according to the Szabadi (1977) model for two functionally antagonistic receptor populations, with modifications as described by Crankshaw & Popat (1997). The *F* test ($p<0.05$) was used to determine the significance of the fit. Biphasic PGE_2 curves (n=3) consisted of an initial excitatory phase (pEC_{50}=7.5±0.2) followed by an inhibitory phase (pEC_{50}=7.5±0.3). The pEC_{50} values calculated for monophasic excitatory (n=2) and monophasic inhibitory (n=6) PGE_2 curves were 8.5±0.9 and 7.7±0.4, respectively. Sulprostone was always excitatory, however a downturn in the curve was observed at high concentrations. In lack of an appropriate model, this component of the curve was excluded when determining the pEC_{50} value for the excitatory phase. In cases where PGE_2 elicited a significant excitatory effect, pEC_{50} values for sulprostone were significantly greater compared to cases where PGE_2 produced only inhibition ($p<0.05$, Table 1). Butaprost was always inhibitory and pEC_{50} values were not significantly different between groups.

Table 1. Effect of response to PGE_2 on pEC_{50} values for sulprostone and butaprost in human myometrium *in vitro*. Values are means ± s.d. means

PGE_2 response	sulprostone pEC_{50}	butaprost pEC_{50}
Excitation (n=5)	9.6 ± 0.5*	5.5 ± 0.3
Inhibition (n=6)	8.9 ± 0.5*	5.8 ± 0.6

* significantly different (unpaired t-test, $p<0.05$)

These data suggest excitatory and inhibitory EP receptor-mediated responses are always present. The small but significant change in sulprostone sensitivity suggests that EP receptor-mediated responses are regulated at the site of the EP_3 or EP_1 pathway rather than the EP_2 pathway.

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138P FURTHER INVESTIGATION OF THE 5-HT_{2B} RECEPTOR IN HUMAN SMALL INTESTINAL LONGITUDINAL SMOOTH MUSCLE

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By the use of selective receptor antagonists, 5-HT-induced contraction of the longitudinal muscle layer of human small intestine has been attributed to stimulation of a 5-HT_{2B} receptor (Borman & Burleigh, 1995; Borman *et al.*, 1996). In this study we have investigated the effects of two partial agonists at the 5-HT_{2B} receptor, mCPP (1-(3-chlorophenyl) piperazine) and BW 723C86 (Ellis *et al.*, 1995).

Longitudinal muscle strips from human terminal ileum were prepared as described previously (Borman & Burleigh, 1995). Two cumulative concentration-response curves to 5-HT, mCPP or BW 723C86 were obtained. The second curve, 60 min after the first, was obtained in the presence of antagonist (SB 204741) or control vehicle (incubated for 30 min). Data were analysed using the Mann-Whitney U Test, with $p<0.05$ indicating significance. pEC_{50} values are geometric mean with 95% confidence limits [95%CL], other data are mean±s.e.mean.

Application of either 5-HT, mCPP or BW 723C86 contracted ileal muscle strips but there were differences between their potencies and intrinsic activities. The pEC_{50} [95% CL] value for 5-HT was 7.7 [7.4-8.0] (n=5). The pEC_{50} values [with 95% CL] and intrinsic activity (I.A.) relative to 5-HT for mCPP (n=5) and BW 723C86 (n=4) are given in Table 1. Also shown are the values at the cloned human 5-HT_{2B} receptor (Thomas *et al.*, 1996) and the rat 5-HT_{2B} receptor (see Baxter *et al.*, 1995 for review). Application of SB 204741 (1μM, n=4) produced a significant displacement of the concentration-response curve to 5-HT, with no significant change in the maximum response, allowing an apparent pA_2 of 7.4±0.4 to be estimated. In the presence of this concentration of antagonist, neither mCPP nor BW 723C86 gave a maximum response within the concentration range tested (up to 100μM, n≥3), ruling out the possibility of quantitative analysis of the effects of SB 204741.

	mCPP		BW 723C86	
	pEC_{50}	I.A.	pEC_{50}	I.A.
Ileum	7.4 [6.9-7.9]	0.3±0.1	5.9 [5.2-6.5]	0.8±0.2
Rat	7.4	0.3	7.9	0.8
Cloned	antagonist		7.2	1.0

Table 1. Activities of mCPP and BW 723C86 at the 5-HT_{2B} receptor in human ileum, and at the rat and cloned human 5-HT_{2B} receptors.

In summary, studies to date with a range of agonists and antagonists support the role for a 5-HT_{2B} receptor in human ileal longitudinal smooth muscle. However, these data appear to indicate a difference both between species and between native and cloned human 5-HT_{2B} receptors.

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139P QUANTITATIVE ANTAGONISM (pA_2) BY PROPRANOLOL AND CGP 12177A OF ISOPRENALINE RELAXATION OF THE ISOLATED HUMAN COLON PROVIDES FUNCTIONAL EVIDENCE OF β_3-ADRENOCEPTORS

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Recent reports on mechanical responses of isolated muscle strips of human large intestine (De Ponti et al., 1996; Kelly et al., 1996; Summers et al., 1996) support a role of β_3-adrenoceptors therein. We investigated human colonic longitudinal (taenia coli) and circular smooth muscle segments, to assess the abundance of β_3-adrenoceptors on functional grounds.

Specimens of human transverse or distal colon were obtained from non-pathological intestinal regions of patients undergoing surgery for colonic cancer. Mucosa and submucosa were gently removed and muscle strips (3-4 mm wide, 2 cm long) were mounted in a 20-ml organ bath containing a warm (37°C) areated (95% O_2, 5% CO_2) Krebs' solution. The strips, stretched with 1 g, were allowed to equilibrate in the presence of phentolamine (10 µM), desipramine (0.5 µM), and hydrocortisone (30 µM). Each strip developed a spontaneous tone that was recorded with an isotonic transducer. (-)Isoprenaline (0.1 nM - 10 µM), dissolved in distilled water containing 1% ascorbic acid, was added cumulatively; the contact time ranged between 10 and 20 min for each concentration. Only one concentration of antagonist (60 min preincubation time) was tested for each preparation. The response was expressed as percentage of the maximal effect of papaverine (0.1 mM), that was added for each strip at the end of the experiment. The pA_2 value for antagonists was obtained from linear regression of mean log (dr-1) values vs the negative log of the antagonist concentrations, as previously described (Manara et al., 1996).

Isoprenaline concentration-dependently relaxed circular and taenia strips, with EC_{50} nM (in parentheses 95% confidence limits) of 21 (16-28) and 136 (112-166) respectively; maximal relaxation vs papaverine (mean±sem) was 90±2% and 93±2%. With 0.1 µM propranolol, the selective β_3-agonist CGP 12177A (De Ponti et al., 1996; 1 and 10 µM, 60 min contact time) relaxed taenia strips (28±4% and 41±6% inhibition) and, to a lesser extent, circular strips (10 µM, 18±4). Propranolol and CGP 12177A both antagonized isoprenaline competitively in the circular strips and non competitively in the taenia strips, with slopes significantly different from unity (Table 1).

These antagonists affinities are consistent with a substantial contribution of β_3-adrenoceptor-mediated relaxation by isoprenaline in the taenia, but not in circular smooth muscle. The latter, however, may contain a smaller but still functionally detectable β_3-adrenoceptor component, as suggested by the relaxant effect of their partial agonist CGP 12177A.

Table 1 - Antagonism of (-)isoprenaline relaxant effect on human circular and taenia smooth muscle strips by propranolol and CGP12177A

		pA_2±sem	Slope±sem	pA_2, slope 1
	Propranolol			
Circular	10 nM - 10 µM	8.71±0.27	0.88±0.10	8.44
Taenia	10 nM - 10 µM	8.51±0.12	0.57±0.03**	
	CGP 12177A			
Circular	1 nM - 1 µM	10.3±0.17	0.83±0.05	9.80
Taenia	3 nM - 1 µM	9.84±0.22	0.47±0.04**	

n = 4 - 10 different human colonic preparations. **, $p < 0.01$, slope ≠ 1

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140P EVIDENCE FOR DIFFERENT MECHANISMS OF RELAXATION BY ETHANOL IN ISOLATED PULMONARY AND CORONARY ARTERIES FROM THE PIG

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Recent studies have shown that ethanol, a commonly used diluent, increased intracellular free Ca^{2+} in smooth muscle and endothelial cells maintained in culture (Johnson *et al.*, 1996: Altura *et al.*, 1996). While examining the effects of various endothelium-dependent vasodilators on the porcine isolated pulmonary artery (PPA), we unexpectedly noted that ethanol produced a relaxation at low concentrations. We therefore decided to investigate this relaxation response, and compare this with the effects of ethanol on the isolated coronary artery from the pig (PCA).

Sections of PPA and PCA (3-4mm diameter) were stored overnight at 4°C in Krebs-Henseleit saline containing 2% Ficoll. The following day, 4-5mm segments were prepared for isometric tension recording and placed under 4 and 6 grams resting tension, respectively, as previously described (Lot & Wilson 1994). Tissues were either left endothelium-intact, or the inside of the vessel was rubbed to remove the endothelium. Following three responses to 60mmol/L KCl, preparations were preconstricted with the thromboxane-mimetic, U46619 (~ 80 to 120% of the KCl response) and the effect of ethanol examined. Where necessary, these responses were further examined in the absence and presence of 100µmol/L L-NAME (a NO synthase inhibitor), 1µmol/L flurbiprofen (a cyclooxygenase inhibitor), or the combination of both.

Responses have been calculated as a percentage of the U46619-induced tone and are shown as the mean± s.e.mean. Where appropriate, two-way ANOVA tests have been carried out. A value for $p<0.05$ was considered significant.

Ethanol (0.1 to 1.65%v/v) produced a concentration-related relaxation of U46619-induced contraction in unrubbed segments of both preparations (E_{max}: PPA 66.5±5.6%, n=16; PCA 81.7±4.8%, n=10). In the PCA, this response to ethanol was unaffected by rubbing the endothelium (E_{max} 83.2±5.6%, n=10), a procedure which abolished the relaxant response to 10nM substance P (81.0±5.4%, n=10 unrubbed preparations). In the PPA, however, no relaxation to ethanol was observed after rubbing the endothelium. Indeed, higher concentrations of ethanol (0.84 to 1.65% v/v) caused a contraction (164.0±11.7% of U46619-induced tone at 1.65%v/v, n=16).

The endothelium-dependent relaxation to ethanol in the PPA (E_{max} 71.3±9.1%, n=8) was significantly reduced by 1µmol/L flurbiprofen (31.9±12.0%, n=7), 100µmol/L L-NAME (33.5±10.2%, n=7), and by the combination of both agents (E_{max} 18.3±7.8%, n=7).

Our data shows that ethanol produces a relaxation response in both preparations even at very low concentrations. Interestingly, the mechanisms underlying these responses are different. In the PCA, an endothelium -independent relaxation is seen, whereas in this PPA, the relaxation is dependent upon the endothelium. The latter response appears to involve NO and a prostanoid.

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141P THE EFFECT OF BARAKOL AGAINST ELECTRICALLY-EVOKED CONTRACTIONS OF THE ISOLATED PORCINE TAIL ARTERY AND GUINEA-PIG ILEUM

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Barakol, an active compound extracted from *Cassia siamea*, has been shown to have sedative and anxiolytic effects via central dopaminergic neurotransmission by causing a decrease both *in vitro* (Thongsaard *et al.*, 1997) and *in vivo* (Thongsaard *et al.*, 1996) striatal dopamine (DA) release. The aim of this study was to investigate the effect of barakol on the peripheral nervous system using a porcine isolated tail artery as a model for adrenergic neurotransmission and the guinea-pig ileum as a model of cholinergic neurotransmission. UK-14304, an α_2-adrenoceptor agonist, was used as an internal control.

Portions of the the pig tail artery (stored overnight at 4 °C in 2% Ficoll Krebs-Henseleit saline) were prepared for isometric recording in 20 ml organ baths containing Krebs-Henseleit saline (37 °C; 95% O_2/5% CO_2). All segments (5 mm) were placed under 5 g wt tension, exposed to 60 mM KCl and then stimulated with electrical pulses (6 Hz, 10 Volts, 0.3 ms pulse width) every 5 min. 5 cm segments of the guinea-pig ileum were prepared as previously described (David *et al.*, 1993) and stimulated at 0.05 Hz, 10 Volts, 0.5 ms pulse width. Once stable contractions were established, UK-14304 or barakol were added cumulatively. Responses were calculated as a percentage of control (pre-drugs) electrically-evoked contractions, and are shown as the mean ± SEM (n = 6). Differences between mean values were compared using unpaired Student's t-test ($p < 0.05$).

The electrically-evoked contractions were reduced (>90 %) by prazosin (0.1 μM) indicating these neurogenic responses were mainly mediated by α_1-adrenoceptors. UK-14304 (1-300 nM) inhibited electrically-evoked contractions in a concentration-dependent manner with a maximum inhibition of >80 % of the control responses (Fig. 1). The effect of UK-14304 was antagonised by the selective α_2-adrenoceptor antagonist, RX-811059 (1 μM) (Fig. 2). In contrast, barakol (1-100 μM), concentration range which caused an inhibition of DA release in the striatum (Thongsaard *et al.*, 1997), had no effect on the electrically-evoked contractions in this preparation except at 300 μM when the responses were reduced by 23 ± 7.6% ($p<0.05$). Similarly, barakol (1-300 μM) had no effect on the neurogenic contractions in the guinea-pig isolated ileum preparation.

Fig. 1

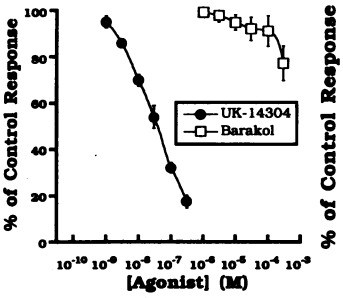

Fig. 2

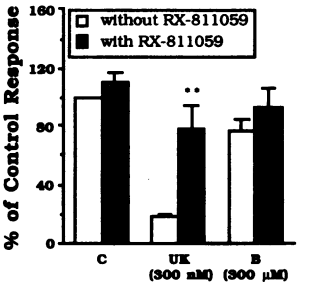

Fig. 1. Effects of UK-14304 (1-300 nM) and barakol (1-100 μM) on the electrically-evoked contractions of the porcine isolated tail artery (n = 6). Fig.2. Effects of RX-811059 (1 μM) on the electrically-evoked contractions in the presences of UK-14304 (UK) or barakol (B). **p<0.01 compared to the response in the absence of RX-811059.

These results show that barakol does not have an inhibitory effect on the peripheral adrenergic or cholinergic neurotransmission. This suggests that barakol may have a specific action on DA function in the central nervous system. Further studies are warranted to assess the effect of barakol on other neurotransmitters in the periphery.

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142P INHIBITION OF NICOTINE-EVOKED RELAXATION OF THE GUINEA-PIG ISOLATED BASILAR ARTERY BY INDOMETHACIN, ASPIRIN AND NITRO-L-ARGININE METHYL ESTER

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O'Shaughnessy & Connor (1994) have described an *in vitro* method for stimulating trigeminal terminals of the guinea-pig basilar artery (GPBA) with nicotine. A nicotine-evoked relaxation was substance P-mediated and blocked by sumatriptan or capsaicin. We have used the cyclooxygenase inhibitors aspirin and indomethacin (indo) and an inhibitor of nitric oxide synthase (nitro-L-arginine methyl ester, L-NAME) to study the mechanisms of the nicotine-evoked relaxation.

Male, Hartley guinea-pigs (250-400g) were killed by stunning and exsanguination. Ring segments (1.5-2.5mm in length) of the GPBA were mounted on tungsten wires (0.1mm diameter) in 10ml tissue baths under a tension (isometric recording) of 0.4g. Tissues were maintained in Krebs' solution at 37°C, gassed with 5% CO_2 in O_2 for 90 min before a concentration-response curve to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10nM-10μM) was obtained. Substance P (3nM) was added to confirm an intact endothelium. Tissues were then washed and equilibrated with guanethidine (3μM) and atropine (3μM). After 1h tissues were re-contracted with $PGF_{2\alpha}$ (1 or 3μM) and the nicotine (0.1mM) relaxation was determined. Tissues were washed for 30min before aspirin (3-30μM), indo (0.3-10μM) or vehicle (1% Na_2CO_3 solution) was incubated with the tissues for a further 40min. Tissues were then re-contracted with $PGF_{2\alpha}$ and the response to nicotine obtained. In other tissues L-NAME (0.1mM) or its inactive D-enantiomer (D-NAME, 0.1mM) was added to the tissue bath 20min before the second response to nicotine. One concentration of inhibitor was tested in a tissue (n=3-6). The ratio of the response to nicotine in the presence of inhibitor (S2) with the initial response to nicotine (S1) was calculated in each tissue. Mean values ± s.e. mean are quoted.

The maximum increase in tension with $PGF_{2\alpha}$ in control tissues was 0.59±0.04g and the relaxation evoked by the first exposure to nicotine was 0.14±0.02g (n=15). Only 50% of tissues responded to nicotine. Concentration-related reductions in the nicotine response were seen with aspirin and indo (Table 1). The nicotine S2:S1 ratios were 0.14±0.05 and 0.83±0.18 in the presence of L-NAME and D-NAME ($p=0.004$) respectively. Responses to $PGF_{2\alpha}$ (1 or 3μM) were not significantly different in aspirin, indo and vehicle treated groups (S2:S1 ratios 1.02±0.05, 1.09±0.03 and 0.99±0.03 for vehicle, indo (3μM) and aspirin (30μM) respectively). The responses to $PGF_{2\alpha}$ were significantly greater ($p=0.01$, t test) in L-NAME (S2:S1, 1.29±0.04) than in D-NAME (S2:S1, 1.08±0.03) treated tissues.

The results suggest that products of cyclooxygenase and nitric oxide synthase contribute to the relaxation evoked by stimulation of sensory terminals in this tissue and block of either enzyme results in more than 70% reduction in response.

This work was supported by the Migraine Trust.

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	Vehicle	0.3 μM	1 μM	3 μM	10 μM	30 μM
Indo	1.07±0.07 (10)	0.53±0.19* (4)	0.42±0.07** (6)	0.37±0.12** (6)	0.27±0.10** (3)	
Aspirin	1.14±0.07 (5)			0.92±0.11 (4)	0.57±0.05** (4)	0.45±0.07** (4)

Table 1. Inhibition by indomethacin and aspirin of nicotine-evoked relaxation of the guinea-pig basilar artery. Mean S2:S1 ratios ± s.e. mean are shown. * $p<0.05$, ** $p<0.01$ Dunnett's t-test. Number of animals in brackets.

143P PRE-JUNCTIONAL ALPHA$_2$-ADRENOCEPTORS MODULATE THE NON-CHOLINERGIC MOTOR RESPONSE OF THE PORCINE ISOLATED BLADDER

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Sibley (1984) reported that neurogenic contractions to high frequency of stimulation involve acetylcholine, while responses to lower frequencies were mediated by another transmitter. In a separate study, a role for ATP in the non-cholinergic response was suggested by the ability of α, β-methylene ATP to inhibit the associated excitatory junction potential, but complementary contraction-based experiments were not conducted (Fujii, 1988). In the present study we have examined the nature of the non-cholinergic component of neurogenic contractions of the porcine isolated bladder and assessed the potential for pre-junctional, modulation of transmitter release by α2-adrenoceptors.

Pig bladders were collected from a local abattoir and stored overnight at 4°C in modified Krebs-Henseleit (K-H) solution. Transverse segments (4cm long, 7mm wide) from the body of the bladder were placed in K-H solution under 10 g wt. resting tension, maintained at 37°C and gassed with 95%O_2/5%CO_2. Following exposure to 60mM KCl preparations were stimulated electrically as described below. All responses have been expressed as a percentage of either the response to 60mM KCl or the neurogenic response prior to the addition of drugs, and are shown as the mean ± s.e.m.. Differences between mean values were considered significant if $p < 0.05$ (unpaired Student's t-test).

Trains of electrical pulses (0.03-20Hz, 0.3ms, for 10s every 90s) caused transient, frequency-dependent contractions. The response to 20Hz/10s was 95.6±10.2% of the sustained response elicited by 60mM KCl (20.3± 3.0 g wt., n=6).

The response to 1Hz/10s (25.5±3.4%, n=4) was abolished by 0.3μM tetrodotoxin, which also reduced the response to 20Hz/10s to 7.0±4.9% (n=4) of the contraction to 60mM KCl. Atropine (0.3μM) failed to alter the response to 1Hz/10s (control: 51.8±8.7% (n=6) and atropine: 41.9±9.7% (n=6)) but significantly reduced the response to 20Hz/10s (52.4±9.8%, n=6). The subsequent addition of 300μM suramin, a P2x purinoceptor antagonist, failed to significantly affect the response to either 1Hz/10s (40.1±9.0%, n=6) or 20Hz/10s (50.6±10.8%, n=6). Phentolamine (10μM), an α-adrenoceptor antagonist, failed to reduced the atropine-resistant response to 1Hz/10s (n=4).

UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate) (1μM), a selective α2-adrenoceptor agonist, significantly reduced the response to 1Hz/10s from 28.4±6.7% to 7.3±3.4% (n=6) but failed to affect the response to 20Hz/10s (95±10.2% compared to 95±8.2%). UK-14304 (3nM-1μM) produced a concentration-dependent inhibition of the response to 1Hz/10s, with a pD2 value of 7.23±0.26 (n=9). The maximum inhibition produced by UK-14304 was 52.7±4.2% (n=9) of the control response to 1Hz/10s. RX-811059 (2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline) (3μM), a selective α2-adrenoceptor antagonist, caused a slight enhancement of the response to 1Hz/10s (119.7±3.9%, n=4) but reversed the effect of 1μM UK-14304 from 47.3±4.2% to 142.8±10.6% of the control response to 1Hz/10s.

Our results show that the non-cholinergic component of neurogenic contractions of the porcine isolated bladder is neither adrenergic nor purinergic in nature, but can be regulated by pre-junctional α2-adrenoceptors.

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144P IS THERE EVIDENCE FOR GABA$_B$ RECEPTOR SUBTYPES IN THE RAT ANOCOCCYGEUS MUSCLE?

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It has been suggested that subtypes of presynaptic GABA$_B$ receptor exist in both the rat and human CNS (Bonanno & Raiteri 1993). This proposal was based on data obtained in synaptosomal release studies. At least three types of presynaptic/heteroreceptors have been suggested, based on the antagonism or inactivity of phaclofen, CGP 35348, CGP 55845A and the agonist/antagonist activity of CGP 47656 (see Froestl & Mickel 1997 for structures). The purpose of this study was to investigate the action these key compounds on a peripheral presynaptic GABA$_B$ receptor and to compare the profile with the classification in the CNS.

The anococcygeus muscles were dissected from male Wistar rats (250 - 400g) as described by Gillespie (1972) and mounted in an organ bath containing Krebs-Henseleit solution at 37°C and gassed with 95% O_2 / 5% CO_2. A resting tension of about 0.5g was applied and measured by an isometric transducer. The twitch response of the muscle was evoked by a train of 10 pulses at 10 Hz every 10 s at a supramaximal voltage. Twitch responses of constant magnitude were usually obtained after a 30 min equilibration period.

Addition of the agonists GABA and (-) baclofen (0.1 - 100 μM) to the bathing solution caused a concentration dependent depression of the muscle contractions (see table 1 for EC_{50}). At a concentration of 100μM, (+) baclofen inhibited the twitch response by only 24% of the maximum effect of GABA. The GABA analogue, γ-hydroxybutyrate (GHB, 0.1 - 100mM) and CGP 47656 (0.1 μM - 10mM) both inhibited the muscle twitch. GHB was a weak agonist and CGP 47656 was a weak partial agonist

Table 1

	EC_{50} (μM)	n	Relative Potency
GABA	1.3±0.5	6	1
(-) baclofen	3.1±1.0	6	0.42
(+) baclofen	> 100	6	-
CGP 47656	490±50	6	0.0027
GHB	12900±340	6	0.0001

Values are the mean ± s.e.mean.

The inhibitory action of GABA (10μM) was unaffected by bicuculline methochloride (50μM) but was inhibited 49.9±8.1% by 1mM phaclofen, while CGP 47656 (10mM) inhibited the maximal effect of GABA by 35.4±10.0%. The inhibitory effect of 10μM (-) baclofen was antagonised 22.3±6.3% by CGP 35348 (100μM) and 40.6±6.6% by CGP 55845A (2.5 nM)

The pharmacological profile of the compounds acting on the anococcygeus muscle was similar to previous studies in the CNS; thus, phaclofen, CGP 35348 and CGP 55845A were all antagonists at the presynaptic GABA$_B$ receptor and CGP 47656 was a partial agonist as has been previously reported (see Froestl & Mickel 1997). The results of this study suggest that the GABA$_B$ receptor in the anococcygeus muscle is a standard GABA$_B$ receptor which does not correspond to any of the subtypes suggested by Bonanno & Raiteri (1993).

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145P THE EOSINOPHIL RECRUITMENT IN BRONCHEOLAR LAVAGE FLUID AND PLEURAL CAVITY FROM RATS TREATED WITH CAPSAICIN AS NEONATES

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Asthma is a multifactorial disease characterized by reversible obstruction of airways and intense recruitment of eosinophils in the airways. There is some evidence for neurokinin-mediated non-adrenergic non-cholinergic (NANC) neuronal control of smooth muscle tone and leucocyte infiltration in airways of different animal species (Barnes, 1992). The aim of the present study was to examine the antigen-(ovalbumin)-induced eosinophil infiltration in the bronchoalveoalar lavage (BAL) fluid in rats treated with capsaicin as neonates. The carrageenin-induced pleurisy was also investigated in the capsaicin-treated animals.

Capsaicin (50 mg kg^{-1}) was injected subcutaneously in neonates, 2 days old, Wistar rats (Lembeck & Holzer, 1979). Sixty to seventy days later, the animals were used. Control neonatal animals were s.c. injected with the capsaicin vehicle (10% ethanol and 10% tween 80, in saline). For the BAL experiments, the animals were sensitized with 200 µg ovalbumin (OVA) + 8 mg aluminium hydroxide by oral route (0.15 ml). Fourteen days later, antigen challenge was performed by injecting of 0.4 ml OVA (1mg rat^{-1}) into the airways. The BAL was performed at 48h after challenge and the total and differential leukocyte counts were assessed in the BAL fluid. Pleurisy was induced by injecting carrageenan (125 µg $cavity^{-1}$; 0.2ml) into the pleural cavity of anaesthetised rats. 24 h later, the chest was opened and the pleural cavity washed with 5 ml of heparinised PBS.

The number of leucocytes in the pleural exudate was counted by conventional techniques. Statistical analysis was evaluated by ANOVA and non-paired Student's *t* test.

In the BAL fluid of OVA-sensitized control rats (48 h), the number of eosinophils (0.7 ± 0.4 x 10^6 eosinophils/BAL; n=10) was significantly higher as compared to non-sensitized animals (0.03 ± 0.01 x 10^6 eosinophils/BAL, n= 13; $p<0.05$), as expected. In capsaicin-treated animals the eosinophil content in BAL fluid from the non-sensitized group (0.02 ± 0.001 x 10^6 eosinophils/BAL, n=10) did not differ from the control animals. In contrast, in capsaicin-treated and OVA-sensitized animals, the eosinophil numbers (1.4 ± 0.3 x 10^6 eosinophils/BAL, n=14) were markedly increased compared to control, sensitized animals ($p<0.05$). The carrageenan-induced pleurisy (24 h) revealed that the number of eosinophils in the pleural cavity of control animals (10 ± 2.6 x 10^6 eosinophils/cavity; n=11) was not different when compared to capsaicin-treated animals (13 ± 2.4 x 10^6 eosinophils/cavity; n=12).

Our results indicate that eosinophil recruitment induced by antigen, but not by carrageenan, is greatly enhanced in capsaicin-treated rats suggesting that NANC nerves innervating the airways smooth muscle play a role in the late eosinophil influx.

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146P ECTONUCLEOTIDASE ACTIVITY IN THE PROSTATIC AND EPIDIDYMAL PORTIONS OF THE GUINEA-PIG VAS DEFERENS

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The presence of ectonucleotidases responsible for the extracellular degradation of ATP and other nucleotides has been demonstrated on many tissues, including the vas deferens. These enzymes sequentially break down ATP via adenosine 5'-diphosphate (ADP) and adenosine 5'-monophosphate (AMP) to adenosine, which may then act upon its own receptors, be taken up into cells or undergo deamination to the inactive inosine. Responses to ATP are known to be greater at the prostatic end than at the epididymal end of the vas deferens (Sneddon & Machaly, 1992) and the population of adenosine receptors is also different at the two ends (Brownhill *et al.*, 1996). Because its breakdown may influence the responses to ATP, we investigated the ectonucleotidase activity in the prostatic and epididymal portions of the bisected guinea-pig vas deferens.

Isolated vasa deferentia from male Dunkin Hartley guinea-pigs (350-400g) were removed and bisected to give prostatic and epididymal portions. The degradation of ATP (100 µM) was studied as described in detail elsewhere (Bailey & Hourani, 1994). Briefly, the tissues were incubated with ATP (100 µM) in aerated Krebs buffer (1.5ml) at 37°C and 50µl aliquots were taken at various times and frozen at -20°C for later analysis by high performance liquid chromatography. Values (mean ± s.e.mean, n=3) obtained for each end were compared using Student's t test and *P* values are given in brackets.

ATP (100 µM) was dephosphorylated by both the prostatic and epididymal portions of the guinea-pig vas deferens, with ADP, AMP, adenosine and inosine being detected (Figure 1). The half-life of ATP in the presence of the prostatic portion was 16.4 ± 1.9 minutes, whereas that for the epididymal portion was 24.0 ± 3.8 minutes ($P > 0.05$). The rate constants for degradation of ATP at the prostatic and epididymal ends were 0.042 ± 0.005 min^{-1} and 0.029 ± 0.004 min^{-1} respectively ($P < 0.05$). Taking into account the weights of the tissues (35 ± 1mg and 18 ± 1mg respectively), the rate constants per g were 1.2 ± 0.15 $min^{-1}.g^{-1}$ and 1.7 ± 0.24 $min^{-1}.g^{-1}$ respectively. These values are not significantly different ($P > 0.05$).

These results show that the rate of breakdown of ATP does not differ between the prostatic and epididymal portions of the guinea-pig vas deferens, and that differences in responses to ATP are therefore not due to different ectonucleotidase activities.

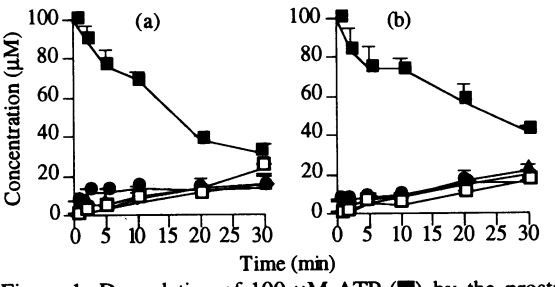

Figure 1. Degradation of 100 µM ATP (■) by the prostatic (a) and epididymal (b) portions of the guinea-pig vas deferens to ADP (●), AMP (▲), adenosine (◆) and inosine (□). Each point is the mean of 3 determinations and vertical bars show s.e. mean.

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147P BRADYKININ RELEASE IN THE ISOLATED PERFUSED BOVINE MAMMARY GLAND

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Kinins are synthesized not only in the circulation but also locally in tissues (Nolly et al., 1994). We have recently reported that healthy bovine milk contains kinins and that during mastitis the levels are significantly increased (Eshraghi, et al, 1995). In this study a unique in vitro model, the isolated perfused bovine mammary gland, was used to study the release of bradykinin (BK).

Udders were dissected immediately after the slaughter of healthy cows which were both CMT (California Mastitis Test) negative and showed no clinical signs of mastitis. The udders (12-18 Kg) were rapidly infused with oxygenated heparinized (1000 IU/L) Tyrode's solution. The udder was then transferred to the laboratory within 40 min, and one half of the udder was perfused with Tyrode's solution (95:5, O_2CO_2) via the external pudendalis artery. Perfusate draining via the superficial epi-gastric vein was collected in tubes containing EDTA (1mg.ml^{-1}) and frozen (-15°C) until assay. The two teat canals of the perfused udder quarters were canulated and the 'pseudo-milk' was collected and also frozen until assay.
The perfused organs physiologically remained viable for at least 3 hours. During an initial equilibration period (30 min) the perfusion rate was set at 110-150 ml.min^{-1} and adjusted to give a basal perfusion pressure of 85 mm Hg (the mean arterial blood pressure of adult cattle). Bradykinin was extracted using Sep-Pak Vac cartridges (C18). The eluates were dried under nitrogen and stored at -15° C until radioimmunoassay against synthetic BK (Moshi et al, 1992). The mean recovery of radiolabelled BK was 92.3 $\pm$1.3 %. A one-way ANOVA was used for statistical analysis of the data (mean, sem). $P \leq 0.05$ was considered statistically significant.

Infusion of BK (0.1-50.0 μg.ml^{-1}) produced a dose-related reduction in perfusion pressure 20.0 ($\pm$ 3.8) - 42.8 ($\pm$ 4.3) % ($P<0.05$, n=5) compared with the basal level. Immunoreactive BK (IR-BK) was detected in the venous effluents from the perfused tissue. The concentration of the IR-BK fell from an initial mean value of 578 $\pm$ 155 pg.ml^{-1} to a minimum mean concentration of 205.7 $\pm$ 92.2 pg.ml^{-1} after 60 min ($P<0.05$). The subsequent IR-BK outflow changed little between 60 min and 180 min ($P>0.05$). The pseudo-milk also contained IR-BK. The mean level of IR-BK in the milk fell from an initial mean value of 1486.4 $\pm$ 67.0 pg.ml^{-1} to plateau after 150 min at 656.0$\pm$89.0 ng.ml^{-1} ($P=0.001$).

In this initial study, the healthy bovine mammary vasculature appeared to produce BK at a concentration 2-3 orders below that necessary to produce vasomotor changes.

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148P THE EFFECT OF PLATELET ACTIVATING FACTOR (PAF) ON LEUKOTRIENE RELEASE FROM RAT CHOPPED LUNG AND TRACHEA

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Voelkel *et al.*(1982) reported that the increased perfusion pressure in rat isolated lungs induced by platelet activating factor (PAF) was prevented in the presence of a lipoxygenase inhibitor. We have previously shown that PAF contracts rat isolated trachea (Warhurst & Court, 1996). The aim of this study was to determine the profile and quantities of leukotrienes (LTs) released by PAF from rat isolated lungs and trachea.

Isolated trachea and chopped lungs were prepared as indicated below:
Male Sprague-Dawley rats (250-300g) were killed by stunning and bleeding. The trachea was dissected from the lungs at the point of bifurcation and cut into two transverse segments. The segments were extensively washed with Greenberg-Bohr buffer (GBB) and placed in 200μl of GBB containing 0.25% (w/v) bovine serum albumin (GBB-BSA). The lung lobes were finely chopped and extensively washed in GBB then 0.5g lung samples were placed in 450μl GBB-BSA.

Following pre-incubation for 15 min at 37°C with shaking, samples were exposed to PAF (0.1 or 1.0μM), lyso PAF (0.1 or 1.0μM) or GBB-BSA (solvent control for PAF and lyso PAF), for 0.5, 1, 3 or 10 min. Alternatively, the samples were preincubated for 15 min with the PAF antagonist 1-0-hexadecyl-2-acetyl-*sn*-glycero-3-phospho(N,N,N)trimethyl -hexanolamine (0.1μM) prior to PAF incubation for 0.5 and 1.0 min. The samples were then centrifuged at 2000×g for 10 s. The supernatant was removed and LTs extracted using Sep-Pak C_{18} cartridges (modified from Antoine *et al.*, 1991). The extract was redissolved in methanol and analysed using reversed phase HPLC (60:40 methanol:water v/v, 0.1% v/v acetic acid, 0.05% w/v EDTA, at 0.8ml/min), measured at 280 nm. This method produced a LT extraction efficiency of 95-100% and a minimum detection level of 1pmol for individual LTs.

Incubation of GBB-BSA or lyso PAF with lungs or trachea resulted in no LTs being detected. Nor were LTs detected in 3 or 10 min incubations with PAF. Incubations with PAF (0.1 or 1.0μM) did, however, result in LT release. For either the trachea or the lung the total amount of LT released at 0.5 and 1 min was not significantly different ($P>0.05$, Student's unpaired t-test), but the profile of the LTs released was altered (see Table 1). PAF incubation for 0.5 min resulted in LTC_4 being the major LT released, whilst at 1 min LTE_4 was predominant. LTB_4 was never detected. The PAF antagonist prevented all PAF induced LT release.

These results show that PAF receptor activation induced a concentration dependent release of LT C_4, D_4 and E_4. The profile of LTs detected was dependent on the time interval from PAF addition to the final sample extraction. This was consistent with previous findings (Warhurst & Court, 1996) that the PAF induced contractile response of rat isolated trachea was maximal at 0.5 min and had returned back to baseline at 3 min.

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Table 1. The amount of LTs detected from lungs and trachea exposed to PAF (0.1 or 1.0μM) for 0.5 or 1.0 min.

PAF conc.	Time	Mean ± s.e.mean LT detected (pmoles/mg wet weight tissue) for n = 4-9					
(μM).	(min)	TRACHEA			LUNGS		
		LTC_4	LTD_4	LTE_4	LTC_4	LTD_4	LTE_4
0.1	0.5	204±48	184±31	144±29	32±8	27±4	22±7
1.0	0.5	836±75	721±69	689±38	114±24	104±20	90±17
0.1	1.0	106±21	159±32	192±39	18±3	25±8	35±9
1.0	1.0	521±81	634±102	801±124	77+9	92±18	123±26

149P IMPROVED INSULIN SENSITIVITY IN ZUCKER AND ZDF RATS FOLLOWING CHRONIC TREATMENT WITH THE NOVEL THIAZOLIDINEDIONE MCC-555

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Thiazolidinediones enhance the intracellular insulin signal transduction mechanism through activation of the nuclear receptor, peroxisome proliferator-activated receptor gamma (PPAR-γ) (Lehmann *et al.*, 1995). MCC-555 [(±)-5-[{6-(2-Fluorbenzyl)-oxy-2-naphyl}methyl]-2,4-thiazolidinedione] is a potent novel thiazolidinedione lacking in haematological and cardiac side effects (Ishii *et al*, 1996). Here we report on the effects of MCC-555 on insulin insensitivity in obese (*fa/fa*) Zucker and fatty Zucker diabetic (ZDF) rats using hyperinsulinaemic euglycaemic clamps. Zucker rats exhibit extreme hyperinsulinaemia, mild glycaemia and impaired glucose tolerance (Bowen *et al*, 1991). ZDF rats display severe insulin resistance and a β cell defect (Peterson, 1994). They provide widely accepted models of non-insulin dependent diabetes mellitus (NIDDM). 15-week old male ZDF rats (300g) were divided into 3 groups of 8, a diabetic group orally dosed with MCC-555 suspended in 0.5% carboxymethylcellulose vehicle at 10 mg/kg/day and a lean and diabetic group receiving vehicle (5 ml/kg) for 21 days before hyperinsulinaemic euglycaemic clamping. This experimental design was also applied to 15-week old male fatty Zucker rats (400 g) and lean Zucker rats (250 g). Rats were anaesthetised with pentobarbitone (30 mg kg^{-1}) and clamps were established as described by Terrattaz and Jeanrenaud (1983). Obese Zucker and fatty ZDF rats treated with MCC-555 required respectively a 280 % ($p<0.005$) and 300 % ($p<0.005$) increase in glucose infusion rate to maintain euglycaemia compared with the untreated obese Zucker and fatty ZDF rats, respectively. MCC-555 treatment produced a 98 % ($p<0.005$) insulin-induced suppression of HGP in fatty ZDF rats compared with an impaired 17 % suppression of HGP in untreated fatty ZDF rats. Whole body glucose uptake (WBU) during clamping was significantly increased in obese, MCC-555 treated Zucker rats 58 % above their basal rates. There was a significant 103 % ($p < 0.05$) increase in WBU in the treated ZDF rats compared with the fatty, untreated rats.

We have therefore confirmed that obese Zucker and fatty ZDF rats display reduced responsiveness to insulin, demonstrated by the lack of suppression of HGP and impaired disposal of glucose during insulin infusion. MCC-555 treatment substantially improved hepatic and peripheral insulin sensitivity in obese Zucker and fatty ZDF rats during hyperinsulinaemic euglycaemic clamping. In view of this improved responsiveness to insulin, MCC-555 may prove a promising candidate for the treatment of NIDDM and impaired glucose tolerance.

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150P EVIDENCE OF CENTRAL NPY RECEPTOR UP-REGULATION IN DIETARY-INDUCED OBESITY IN THE RAT

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Neuropeptide Y (NPY) is a powerful inducer of feeding behaviour when injected into the hypothalamus of rats and NPY hyperactivity has been suggested to be involved in the development of obesity and diabetes by virtue of its hyperphagic, anti-thermogenic and insulinotropic actions (Dryden et al., 1994). In the present study we used autoradiography to quantitate [^{125}I]PYY binding (40 pM) to coronal brain sections (Widdowson, 1996) in order to determine changes in NPY receptor density in male Wistar rats (initial weight = 200g) made obese over 6 weeks by the unlimited availability of highly palatable, energy-rich food, and in rats restricted to 60% of their normal intake of chow throughout 10 days. Control Wistar rats in each experiment received unlimited normal chow. Binding to Y1-type NPY receptors was masked using the selective Y1 antagonist, BIBP3226 (1 μM) leaving binding to Y2 and Y5 receptors.

Rats fed the high diet gained more weight than controls (obese = 588 ± 5g vs controls = 470 ± 5g; mean ± s.e.mean, $p<0.01$, Student's t-test), were hyperinsulineamic (33.1 ± 3.3 vs 20.5 ± 1.9 μU/ml plasma $p<0.05$) but were not hyperglycemic 3.9 ± 0.1 vs 3.9 ± 0.1 mmol/l plasma). Measurements of regional specific [^{125}I]PYY binding in the brains of obese rats showed increased binding ($p<0.001$ vs control; 2-way ANOVA) in the hypothalamus, hippocampus, thalamus and amygdala, but not in the cerebral cortex, as compared to controls. Binding to the hypothalamic perifornical/lateral area, for example, was 15.1 ± 1.2 fmol/mg tissue in obese rats and 9.5 ± 0.6 fmol/mg tissue in controls ($p<0.01$ Student's t-test followed by Bonferonni correction for multiple comparisons). By contrast, food-restricted rats exhibited reduced specific [^{125}I]PYY binding ($p<0.001$ vs control; 2-way ANOVA) in the hypothalamus, hippocampus, amygdala and thalamus with no change to binding in the cerebral cortex. Here, binding to the hypothalamic perifornical/lateral area was 10.1 ± 1.4 fmol/mg tissue in food-restricted rats and 19.6 ± 2.1 fmol/mg tissue in controls; $p<0.01$ Student's t-test followed by Bonferonni correction for multiple comparisons). The changes in specific [^{125}I]PYY binding in dietary-obese and food-restricted rats versus controls persisted in the presence of BIBP3226. These data suggest that diet-induced obesity results in a reduction in NPYergic neuronal activity leading to an up-regulation of BIBP3226-insensitive receptors, presumed to be Y2 and/or Y5 receptors. By contrast, diet-restriction, which produced a reduction in body weight gain, increases NPYergic activity leading to a down-regulation in Y2 and/or Y5 receptors in the brain.

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151P SUBCHRONIC EFFECTS OF SR 58611A ON GLUCOSE TOLERANCE, INSULIN SENSITIVITYAND BODY WEIGHT IN NORMAL MICE

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The selective β3 adrenoceptor agonists have been shown to be effective in some rodent models of obesity and diabetes, but subsequently proved disappointing in clinical use (Himms-Hagen & Danforth, 1996). The potent agonist SR 58611A, given acutely, improves glucose tolerance and the insulin response in normal mice (see abstract p100, this meeting), but its longer term effects on glucose metabolism, energy intake and body weight have not been investigated. In this study normal lean mice were given SR 58611A (0.25 mg kg-1 i.p.) daily for 15 days to determine whether the improvement in glucose tolerance was maintained after sub-chronic treatment and if appetite suppression occurred.

Groups of 4-8 adult male CBA mice were given SR 58611A, saline, or gliclazide (2 mg kg^{-1}) or metformin (60 mg kg^{-1}) as positive controls. Body weight and food intake were monitored every 48h over this period. On day 15, the acute glucose tolerance (GTT) to 1 g kg^{-1} glucose i.p. was measured after an overnight fast. Exogenous insulin sensitivity (IST) was assessed in fed mice given insulin, 2.5 IU kg^{-1} i.p. Differences between groups (mean ± sem) were analysed by unpaired Students t-test.

Although acute SR 58611A did not significantly affect basal (non-fasted) glucose level (7.16 ± 0.25 mM, n = 8), subchronic treatment produced a significant 60 min post-dose reduction in glucose level to 4.94 ± 0.58 mM ($p < 0.05$). Gliclazide also produced a greater decrease in glucose level after the chronic treatment (3.41 ± 0.2 mM compared to 5.05 ± 0.47 mM at the same time after an acute dose, n = 8). Neither acute nor sub-chronic treatment with metformin produced any change in basal glucose level.

The peak rise in blood glucose occurred 30 min after an acute glucose challenge (10.2 ± 1.0 mM, n =8). Gliclazide was less effective at blunting this hyperglycaemia after sub-chronic dosing (peak: 6.46 ± 0.66 compared to 5.16 ± 0.84 mM acutely), as was metformin (peak: 7.08 ± 0.74 mM compared to 5.9 ± 0.92 mM acutely). In contrast, the effect of SR 58611A was enhanced after the sub-chronic treatment (peak: 3.68 ± 0.41 mM compared to 6.1 ± 1.2 mM). The IST response after sub-chronic SR 58611A was similar to that observed following a single dose; blood glucose level at 120 min post-insulin: 4.1 ± 0.7 mM (acute), 5.48 ± 0.71 mM (chronic).

The mean body weight of the mice given SR 58611A did not alter significantly over the dosing period (day 1: 31.25 ± 0.75 g, day 15: 32.0 ± 0.76 g, n = 4) and the average daily food intake also remained consistent at around 9.0 g per mouse over 48 h. Although the results indicate that the improved glucose tolerance observed after a single dose of SR 58611A can be maintained with regular dosing, there is no evidence that this dose regime suppresses appetite or has an anti-obesity effect *per se* in normal mice. Any potential anti-diabetic effect is therefore unlikely to arise from a reduction in body weight or appetite.

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Himms-Hagen, J & Danforth, E (1996) *Curr. Opinion Endocrin. Diab.* **3**: 59-65

152P EFFECTS OF ISRADIPINE AND NITRENDIPINE ON HORMONE-SENSITIVE LIPASE ACTIVITY AND LIPOGENIC RATE OF BROWN AND WHITE ADIPOSE TISSUE IN MICE

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Lipolysis, mediated by intracellular hormone-sensitive lipase (HSL), and lipogenesis are the principal reactions of adipocyte lipid metabolism. Both reactions are under the influence of lipolytic and antilipolytic hormones such as noradrenaline and insulin respectively. Intracellular calcium has an important regulatory role in the resulting hormonal responses in both brown (BAT) and white (WAT) adipose tissues, (Tuchiya & Nagai, 1994). We have previously shown that calcium entry through dihydropyridine (DHP)-sensitive channels may be implicated in mediating the decrease in adipocyte lipogenesis and the increase in lipolysis seen in ethanol withdrawal (Hughes *et al.*, 1995; Jelic & Taberner, 1996). In this study two DHPs, isradipine and nitrendipine, were administered to naive mice in order to examine the role of DHP-sensitive calcium channels in regulating adipocyte lipid metabolism under basal conditions, and to determine whether the effects of the two drugs on HSL activity were direct or secondary to the effects on lipogenesis.

Groups of 8-9 male TO mice weighing 32-45 g were given tap water and pelleted diet *ad libitum*. (±) isradipine (10mg kg^{-1}) and nitrendipine (50mg kg^{-1}), suspended in 5% Tween-80, were injected i.p. between 8:00 and 9:00h. HSL activity was assayed one hour later using emulsified [^{3}H] triolein as substrate (Jelic & Taberner, 1996) and expressed as pmol free fatty acids (FFA) released min^{-1} mg $protein^{-1}$. Lipogenic rate was determined by measuring the incorporation of tritium from ^3H_2O injected i.p. one hour after the DHPs into FFAs (Hughes *et al.*, 1995) and is expressed as μg of H incorporated mg^{-1} fat-free tissue h^{-1}. Differences (means ± s.e. mean) between treatment groups were analysed by Student's t-test for independent samples.

HSL activity in vehicle-treated animals was not significantly different from that in untreated animals (1.13 ± 0.2 in BAT and 2.06 ± 2.0 in WAT, vehicle; 0.97 ± 0.1 in BAT and 1.83 ± 0.2 in WAT, naive); a similar lack of effect on lipogenic rate was also observed (vehicle, 34.0 ± 10.5 in BAT and 9.2 ± 2.8 in WAT; naive, 51.3 ± 8.7 in BAT and 6.9 ± 1.5 in WAT). Isradipine had no effect on HSL activity in both BAT and WAT (1.17 ± 0.3 and 3.76 ± 1.0 respectively), whereas nitrendipine significantly increased ($p<0.005$) HSL activity in both tissues (3.10 ± 0.5 and 4.54 ± 0.8 respectively). Lipogenic rate was significantly attenuated (3.4 ± 0.4, $p<0.05$) in WAT of isradipine-treated mice, but remained unchanged in BAT (22.8 ± 4.4). Nitrendipine had no effect on lipogenic rate in either BAT or WAT (46.8 ± 11.9 and 3.3 ± 0.7 respectively).

Thus isradipine appears to have no effect on either HSL activity (and therefore lipolysis) or lipogenesis in BAT, but elevates lipogenic rate in WAT. Nitrendipine, on the other hand, has no effect on lipogenic rate while it causes an increase in HSL activity in both types of adipose tissue. In conclusion DHP calcium channel antagonists do affect intracellular lipid metabolism in naive mice but the effect sems to be dependent on the type of adipose tissue studied as well as on the drug identity. Lipogenesis and lipolysis are affected to different degrees by the DHPs, suggesting differences in the role of calcium entry through DHP-sensitive channels in the regulation of these two processes.

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153P POTENTIATION OF GLUCOSE TOLERANCE AND INSULIN SENSITIVITY BY SR 58611A, A SELECTIVE β_3 ADRENOCEPTOR AGONIST, IN NORMAL MICE

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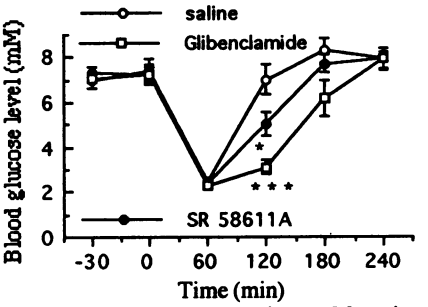
We have shown SR 58611A to be a potent β_3 adrenoceptor (AR) agonist in stimulating adipose tissue hormone-sensitive lipase via an increase of cAMP accumulation (Shih & Taberner, 1995). However, its acute effects on glucose and insulin responses are not known.

Glucose tolerance tests (GTTs): SR 58611A was given (i.p.) to CBA/Ca mice after an overnight fast (group n = 6-7) 30 min prior to glucose (1g kg^{-1}, i.p.) challenge. Blood glucose levels (BGLs) were measured at 30 min intervals for 3 hours. Saline (10 ml kg^{-1}) was given to control mice. Glibenclamide (i.p.) was used as a positive control. Insulin sensitivity test: BGLs were measured immediately before and at 60 min intervals for 4 hours after an acute dose of insulin (2.5 IU kg^{-1}, i.p.) in fed mice. Insulin levels were measured by radioimmunoassay kits (Amersham) in fed mice before and 20 min after administration of drugs. Differences between groups (mean ± sem) were analysed by unpaired Students t-test.

GTT: The peak rise in blood glucose occurred 30 min after the glucose injection in the control mice (from 2.55 ± 0.17 to 10.2 ± 1.0 mM). The rise was dose-dependently reduced by SR 58611A (0.125 to 2 mg kg^{-1}) and glibenclamide (1.25 to 5 mg kg^{-1}). A statistically significant effect was observed at the low dose of 0.25 mg kg^{-1} of SR 58611A (4.1 ± 0.9 mM, $p<0.05$), which was equipotent with 2.5 mg kg^{-1} of glibenclamide (3.32 ± 0.35 mM, $p<0.005$). These doses were used in all subsequent experiments. Glibenclamide decreased basal BGLs 60 min after an acute dose, SR 58611A, however, did not affect the basal BGLs up to 120 min after administration. Insulin (1.19 to 76 IU.kg^{-1}) produced a maximum hypoglyceamia within 60 min which was not dose-dependent (BGLs fell from 6.93 ± 0.56 to 2.75 ± 0.22 mM). However, the return was dose-dependent over 2 to 6 h). The effect of insulin was potentiated by pre-treatment with SR 58611A and glibenclamide (see fig). Plasma insulin levels were significantly higher in SR 58611A (11.91 ± 4.13 ng ml^{-1}) and glibenclamide (5.69 ± 0.7) treated mice than the controls (1.37 ± 0.21 ng ml^{-1} $p < 0.01$).



Saline or drugs were given 30 min prior to insulin administration (at 0 min). *** $p<0.005$; * $p<0.05$ compared to the respective controls.

To summarise, SR 58611A improves GTT through an increase in insulin release. Other β_3 agonists have also been shown to improve glucose tolerance in rodents via increases of insulin release from pancreas (Arch & Kaumann, 1993).

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154P REGULATION OF ENDOTHELIAL FUNCTION BY INSULIN IN THE AORTA OF THE OBESE/LEAN ZUCKER RAT *IN VITRO*

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Insulin may promote glucose delivery to insulin-sensitive sites by enhancing endothelium-dependent vasodilation (Baron, 1996; Petrie *et al.*, 1996; Steinberg *et al.*, 1994). Defects at this vascular level of insulin action may exist in obese/insulin resistant states (Steinberg *et al.*, 1996). We therefore assessed the acute regulation by insulin of both agonist-stimulated and basal endothelial vasodilator function in isolated vascular smooth muscle of the Zucker rat, the obese strain of which is a model of insulin resistance (metabolic Syndrome X) in man.

Male 8-week old obese (298.1±8.8g, n=21) and age-matched lean (213.0±4.6g, n=20) Zucker rats were anaesthetised with pentobarbitone sodium (60 mg/kg i.p.), euthanised and thoracic aortic rings prepared and mounted under isometric conditions in Krebs-Henseleit solution (composition in mM: NaCl 133; KCl 4.7; NaH_2PO_4 1.35; $NaHCO_3$ 16.3; $MgSO_4$ 0.61; $CaCl_2$ 2.52; glucose 7.8), gassed with carbogen and warmed to 37° C. Rings were exposed to human insulin (100 nM) or vehicle (0.1 % (w/v) bovine serum albumin) for 20 min (Laight *et al.*, 1996) and then (i) precontracted with noradrenaline (NA, 100 nM) to assess relaxation to acetylcholine (ACh, 1 nM-1 μM) or S-nitroso-N-penicillamine (SNAP, 0.01-10 μM) or (ii) contracted with phenylephrine (PEP, 0.01-10 μM) with or without a 10 min pretreatment with N^{G}-nitro-L-arginine methyl ester (L-NAME, 300 μM). Data are mean±s.e. mean.

Relaxation to ACh was not significantly different in obese (pD_2=7.06±0.11, area under curve (AUC)=108.1±10.7, n=7) and lean (pD_2=7.24±0.09, AUC=109.0±7.5, n=7) groups. Insulin enhanced relaxation to ACh in the obese group (pD_2=7.29±0.15*; AUC=129.2±14.6* (*$P<0.05$, n=7); but had no significant effect in the lean group (pD_2=7.19±0.07; AUC=108.4±5.8 (n=7). Relaxation to SNAP was similar in obese (pD_2=5.49±0.17, AUC=92.8±13.2, n=5) and lean (pD_2=5.53±0.13, AUC=96.3±10.2, n=5) groups ($P>0.05$). Insulin had no significant effect on relaxation to SNAP in either the obese (pD_2=5.57±0.17; AUC=100.6±13.4 n=5) or lean (pD_2=5.63±0.10; AUC=106.5±8.5, n=5) groups. Contraction to PEP was not significantly different in obese (pD_2=6.98±0.19, n=5) and lean (pD_2=7.23±0.11, n=4) groups. L-NAME elicited a leftward shift in reactivity to PEP in the obese group (ΔpD_2=0.97±0.32, n=5, $P<0.05$) which was not significantly affected by insulin (ΔpD_2=0.88±0.08, n=5, $P<0.05$) ($P>0.05$ for insulin effect). In contrast, L-NAME had no significant effect on reactivity to PEP in the lean group (ΔpD_2=0.17±0.25, n=4, $P>0.05$); but did apparently elicit a leftward shift in the presence of insulin (ΔpD_2=0.42±0.15, n=4, P=0.07).

Insulin enhanced relaxation to ACh in the isolated aorta of the obese, but not lean, Zucker rat, while responses to SNAP were unaffected. In addition, insulin apparently permitted an L-NAME-mediated enhancement of reactivity to PEP in the lean group. This implies an absence of regulation of constrictor reactivity by basal, endothelium-derived NO in the lean Zucker rat and moreover suggests that this endothelial function may be stimulated by insulin. In conclusion, our data indicates both differences in vascular endothelial function *per se* and a differential regulation by insulin in obese and lean Zucker rats.

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155P INHIBITION OF 8-EPI-PROSTAGLANDIN $F_{2\alpha}$ PRODUCTION BY VITAMIN E IS ASSOCIATED WITH THE REVERSAL OF ENDOTHELIAL DYSFUNCTION IN THE CHOLESTEROL-FED RABBIT

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F2-isoprostanes are markers of lipid peroxidation indicating oxidative injury (see Morrow & Roberts, 1996). Oxidant injury may contribute to endothelial dysfunction in various pathological conditions, particularly atherosclerosis. Here, we show that the antioxidant vitamin E (Vit E) prevents the rise in plasma levels of 8-epi-prostaglandin F2α (8-epi-PGF2α) as well as the endothelial dysfunction observed in cholesterol-fed rabbits (this study; Klemsdal *et al.*, 1994).

Three groups of male New Zealand white rabbits (3.5-4.1 kg) were maintained on three separate diets (chow, 1% cholesterol, 1% cholesterol with 0.4% Vit E) for 8 weeks. The animals were sedated with hypnorm (0.1ml/kg, i.m.) and a marginal ear vein was cannulated for drug infusions. Endothelial function was assessed using an infra-red photoplethysmography probe and subsequently analysing the change in the relative height of the dicrotic notch (ΔRhDN), on the arterial pulse waves, caused by acetylcholine (Ach) (see Klemsdal *et al.*, 1994). Afterwards, blood was taken for measurement of plasma levels of cholesterol and Vit E by standard methods and 8-epi-PGF2α by a method described by Nourooz-Zadeh *et al.*, (1995). Briefly, plasma (1ml) was hydrolysed with potassium hydroxide and 8-epi-PGF2α isolated by solid phase extraction using C18 and NH2 cartridges. The extracted isoprostane was derivatised to the pentafluorobenzylbromide ester and trimethylsilyl ether, and analysed by gas chromatography-mass spectrometry using negative ion chemical ionisation.

Dietary treatment of rabbits with cholesterol resulted in an enhanced production of 8-epi-PGF2α and a reduced ability of Ach to cause a change in RhDN (i.e. endothelial dysfunction) compared to animals given chow diet (Table 1). However, dietary Vit E (together with cholesterol) abolished the rise in plasma levels of 8-epi-PGF2α and significantly attenuated endothelial dysfunction.

This is the first study demonstrating increased formation of 8-epi-PGF2α in the cholesterol-fed rabbit. This isoprostane is raised in diseases such as type II diabetes (Gopaul *et al.*, 1995) where an enhanced production of reactive oxygen species is thought to play a role in the aetiology of the disease. Recent studies show that oxidant stress induces injury by peroxidation of cell membrane lipids which results in the formation of 8-epi-PGF2α (Morrow & Roberts, 1996). Since Vit E prevents the oxidation of low density lipoproteins (Matz *et al.*, 1994), the beneficial effects of Vit E observed in this study could be attributed to this antioxidant effect.

TABLE 1	Diet treatment groups		
Parameter	chow	1% cholesterol	1% cholesterol + 0.4% Vit E
8-epi-PGF2α (ng/ml)	0.03±0.01	0.12±0.04*	<10pg/ml
ΔRhDN to Ach (2μg/kg/min)	49.08±6.71	16.66±2.89*	39.76±4.96#
Cholesterol (mM)	0.37±0.04	44.17±6.41*	39.00±5.47*
Vit E (μM)	5±1	56±8*	414±52*#

Values (at 8 weeks) are given as mean ± s.e.mean (n=6). *$P<0.05$ *vs* chow & #$P<0.05$ *vs* 1% cholesterol by ANOVA (Bonferroni's test).

In summary, dietary Vit E prevents the enhanced generation of 8-epi-PGF2α and attenuates endothelial dysfunction in the cholesterol-fed rabbit.

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156P ATTENUATION OF CYTOKINE-STIMULATED FIBRINOGEN SYNTHESIS IN HEP G2 CELLS BY IL 1β AND CLOFIBRATE

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Raised fibrinogen levels have been shown to be a risk factor for cardiovascular and cerebrovascular diseases. The human cell line Hep G2 has been previously used as an *in vitro* model of fibrinogen synthesis and has been found to respond to cytokine stimulation (Dardik *et al* 1995).

Fibrate drugs reduce fibrinogen levels in Hep G2 cells stimulated with IL-6 (Overfield *et al* 1996). Here we present results to demonstrate the effects of IL-1β alone and IL-1β with IL-6 on fibrinogen synthesis in Hep G2 cells. The effect of clofibrate is also demonstrated.

Hep G2 cells were grown to confluence in medium (DMEM supplemented with FCS) and seeded on to 6 well plates ($1x10^6$ cells) then incubated with either IL1-β alone (25, 125, 250 iu/ml), IL-6 alone (12.5 iu/ml), or a mixture of IL-1β (25 iu/ml) and IL-6 (12.5, 32.3 iu/ml). In addition, clofibrate (10^{-4},10^{-6},10^{-8},10^{-10} M) was added to each set of plates. Cells were incubated in 5% CO_2 at 37°C for 24h followed by cytokine stimulation for 24h, or with cytokine and clofibrate for 24h. Trypan blue exclusion was used to assess cell viability. Supernatant was harvested and fibrinogen estimated by ELISA using a rabbit anti human-fibrinogen coating antibody and peroxidase-conjugated rabbit anti-human fibrinogen (Dakopatts).

Table 1 shows fibrinogen synthesis in Hep G2 cells after 24h (mean and standard error, n=3) with or without IL-1β (25 iu/ml), IL-6 (12.5, and 32.3 iu/ml) and clofibrate (10^{-8}M).

IL-1β did not have an effect on fibrinogen synthesis compared to unstimulated Hep G2 cells ($p = 0.51$) but significantly reduced IL-6 mediated stimulation ($p = 0.13$).

Clofibrate (10^{-8} M) showed a non-significant reduction on the level of fibrinogen produced in unstimulated cells ($p = 0.48$). In cells stimulated with with IL-6, clofibrate reduced fibrinogen synthesis to a similar extent to that caused by IL-1β although the effect was less marked ($p = 0.12$). This effect was also seen over a range of clofibrate concentrations. Fibrinogen levels were found to be similar when both IL-6 (12.5 iu/ml) and IL-1β (25 iu/ml) were present as well as clofibrate ($p = 0.48$).

These results indicate that clofibrate mimics the inhibitory effect of IL-1β on fibrinogen synthesis in Hep G2 cells and suggest a process whereby clofibrate may lower fibrinogen levels in cardiovascular risk patients.

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Table 1. Fibrinogen (μg x 10^6, cells n = 3)

Unstimulated	IL-1β only (25iu/ml)	IL-6 only (12.5iu/ml) (32.3iu/ml)	IL-6 (12.5iu/ml) & IL-1β (25iu/ml)	Clofibrate (10^{-8} M)	Clofibrate (10^{-8} M) & IL-6 (12.5iu/ml)	Clofibrate (10^{-8} M) & IL-6 (12.5iu/ml) & IL-1β (25iu/ml)
2.98 ± 0.14	2.42 ± 0.15	6.07 ± 1.1; 7.57 ±1.5	2.82 ± 0.63	2.43 ± 0.22	3.19 ± 0.009	3.20 ± 0.18

157P EFFECTS OF 7-NITROINDAZOLE AND L-NAME ON MK-801-INDUCED BEHAVIOURAL CHANGES AND IMMEDIATE EARLY GENE EXPRESSION IN THE MOUSE

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Several studies have examined the role of nitric oxide (NO) synthase inhibitors in cerebral ischaemia and other pathological conditions (O'Neill *et al.*, 1997). High concentrations of NO are toxic and increased NO· and O_2· interact to form the toxic peroxynitrite anion ($ONOO^-$). However, recent studies have shown that 7-nitroindazole (7-NI) also attenuates MK-801-induced explosive jumping in mice (Deutch *et al.*, 1996). Therefore in the present studies we have evaluated the effects of 7-NI (25-100 mg/kg s.c.) and L-NAME (12.5-50 mg/kg s.c.) MK-801-induced behavioural changes and c-fos immunoreactivity in the mouse.

Female BKTO mice (Bantin and Kingman, Hull, U.K.) were used in all experiments. MK-801 and L-NAME were dissolved in distilled water and 7-NI was dissolved in 25% β-cyclodextrin. Locomotor activity was measured in clear Perspex boxes (30x30x30cm) with 5 equally spaced horizontal photocell beams 5 cm above the floor and each beam break was recorded as a photocell count on a Compaq PC using software provided by Greenacre Instruments Ltd.

In antagonism studies mice (2 mice per box, 6 pairs per treatment group) were pre-treated with compound or vehicle and placed in the locomotor boxes. Thirty minutes later mice received either MK-801 (0.3 or 0.6 mg/kg s.c.) or vehicle and placed back in the boxes for a further 90 min. 24hr after behavioural testing the animals were given an overdose of anaesthetic and perfused transcardially with 0.9% saline followed by 10% buffered formalin. The brains were removed, processed and embedded in paraffin wax. 5 μm coronal brain sections were cut and immunostained using a rabbit polyclonal serum to c-fos. Immunoreactivity was visualised using the avidin-biotin method (Gass *et al.*, 1993). The number of immunoreactive cells in the several brain regions were counted (n = 4 animals per group).

Neither 7-NI (25-100 mg/kg s.c.) or L-NAME (12.5-50mg/kg s.c.) alone produced any effects on spontaneous locomotor activity. MK-801 (0.3 mg/kg s.c.) produced a significant increase in hyperactivity 30 to 90 min post-injection in all experiments ($P < 0.001$-0.05). L-NAME (25 and 50 mg/kg s.c.) and 7-NI (100mg/kg s.c.) blocked the MK-801-induced hyperactivity ($P<0.05$). The 0.3 mg/kg dose of MK-801 did not induce significant levels of c-fos immunoreactivity. The higher dose of MK-801 (0.6mg/kg s.c.) also produced a significant increase in hyperactivity 30 to 90 min post-injection in all experiments ($P < 0.001$-0.05). L-NAME (25 and 50 mg/kg s.c.) and 7-NI (100mg/kg s.c.) attenuated the MK-801-induced hyperactivity ($P<0.05$). This dose of MK-801 also produced an increase in the number of c-fos immunoreactive cells/grid (vehicle 73.6 ± 7.8, MK-801 130 ± 9.8, mean ± S.E.M., $P < 0.05$) in the posterior cingulate cortex. L-NAME failed to attenuate these effects, while the highest dose of 7-NI (100mg/kg s.c.) did attenuate the MK-801-induced c-fos immunoreactivity ($P < 0.05$).

These results indicate that nitric oxide plays a role in MK-801-induced behavioural effects, but has little effect on MK-801-induced immediate early gene expression in mice.

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158P EFFECTS OF THE mGlu RECEPTOR AGONIST (1S,3S)-ACPD ON GLUTAMATE, ASPARTATE AND GLYCINE RELEASE IN THE NUCLEUS ACCUMBENS AND STRIATUM OF THE RAT

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The metabotropic glutamate (mGlu) receptors are G protein-coupled receptors that can modulate cAMP levels, PI hydrolysis and Ca^{2+} mobilisation in the CNS (Roberts, 1995; Toms *et al.*, 1996). It has been proposed that presynaptic mGlu receptors may possess both inhibitory and facilitatory autoreceptor function. Here, we used microdialysis to investigate the effects of (1*S*,3*S*)-aminocyclopentane-1,3-dicarboxylate ((1*S*,3*S*)-ACPD; Pook *et al.*, 1992) on extracellular levels of aspartate (Asp), glutamate (Glu) and glycine (Gly) measured in the nucleus accumbens and caudate-putamen of freely-moving rats.

Male Wistar rats (250-300 g) were anaesthetised with chloral hydrate (0.4 g kg^{-1}, i.p.) and stereotaxically implanted with guide cannulae. At least 24 hr after surgery, microdialysis probes (310 μm o.d., 4 mm Hospal AN69 membrane) were placed into one nucleus accumbens core and the opposite caudate-putamen and perfused at 2 μl min^{-1} for at least 100 min prior to sampling with an artificial cerebrospinal fluid (in mM: NaCl 126.6, KCl 2.4, KH_2PO_4 0.49, $MgCl_2$ 1.28, $CaCl_2$ 1.1, $NaHCO_3$ 27.4, Na_2HPO_4 0.48, (+)-glucose 7.1; initial pH 7.4). Amino acids were assayed in 40 μl dialysate samples by *o*-phthaldialdehyde derivatisation, HPLC separation and fluorimetric detection (Kilpatrick & Mozley, 1986).

Respective mean contents of the four 'basal' samples (in pmol/40 μl) preceding the probe treatments in the nucleus accumbens and caudate-putamen were for Asp, 7.2 ± 0.6 and 14.1 ± 1.0, Glu, 37.1 ± 3.6 and 50.3 ± 3.1 and for Gly 18.6 ± 0.9 and 14.5 ± 0.4 (n=16-64). In nucleus accumbens, local (1*S*,3*S*)-ACPD (0.5 or 1.0 mM) reduced Asp levels to 81 ± 4 % and 48 ± 9% and Glu levels to 77 ± 6 % and 51 ± 11% of their respective basal values but raised those of Gly to maxima of 183 ± 14% and 221 ± 8% ($p<0.05$-0.01; n=4-5). All changes were assessed by 2-way ANOVA followed by Dunnett's test. In the caudate-putamen, similar responses were seen so that (1*S*,3*S*)-ACPD (0.5 and 1.0 mM) reduced Asp to 75 ± 8 and 55 ± 10 % and Glu to 79 ± 6 and 58 ± 8% of basal values but Gly levels were raised to maxima of 186 ± 12 and 213 ± 15% of basal ($p<0.05$-0.01; n=4-5). Given alone, the group III/II mGlu receptor antagonist, *(RS)*-α-cyclopropyl-4-phosphonophenylglycine (CPPG; Thomas *et al.*, 1996; 1 or 3 mM), had no action on basal levels of any amino acid in either area but it significantly inhibited all measured actions of (1*S*,3*S*)-ACPD. In each area, 1.0 mM infusions of L-2-amino-4-phosphonobutanoate or L-serine-*O*-phosphate, (selective group III mGlu agonists), caused similar reductions of Asp and Glu levels but did not alter Gly levels.

The falls in Asp and Glu levels evoked by (1*S*,3*S*)-ACPD may follow activation of group II or group III mGlu receptors and are consistent with a reduction of Ca^{2+} release and cAMP levels by this compound (Takahashi *et al.*, 1996). The observed rises in Gly levels may be mediated via interaction with presynaptic or glial group II or even group I mGlu receptors in both areas. These findings suggest an autoreceptor role for group II/III mGlu receptors and also raise the possibility of a mechanism for regulating Gly levels and hence NMDA receptor function.

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159P EVIDENCE FOR CYCLOTHIAZIDE-INSENSITIVE PRESYNAPTIC AMPA RECEPTORS IN THE RAT STRIATUM *IN VIVO*

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We have recently demonstrated the existence of presynaptic AMPA-type autoreceptors on glutamatergic terminals in rat forebrain slices *in vitro* (Patel & Croucher, 1995). These receptors provide a positive feedback control on synaptic glutamate release. In the present study we have used a rapid dual-label microdialysis method to investigate whether presynaptic AMPA receptors also play a role in the control of striatal neurotransmitter release *in vivo*.

Male Wistar rats (275-300g) were implanted bilaterally with dialysis monoprobes aimed at the corpus striatum, under Immobilon anaesthesia (0.23ml/kg). Preloading with [^{3}H]L-glutamate and [^{14}C]GABA, and subsequent dialysis with ACSF in the presence or absence of drugs, was performed as previously described (Young & Bradford, 1993). 15min samples (7.5µl/min) were collected and ^{3}H and ^{14}C contents were determined by liquid scintillation spectrometry. Responses to AMPA, in the presence or absence of the desensitization inhibitor cyclothiazide (CYZ) and the selective AMPA receptor antagonists NBQX and GYKI 52466, or kynurenic acid (KYN), a broader spectrum ionotropic glutamate receptor antagonist, are shown in Table1. None of the antagonists, nor CYZ, caused significant changes in efflux of label when given alone.

The present results provide further support for the concept that presynaptic AMPA receptors provide a mechanism of positive feedback control on synaptic glutamate release. The lack of effect of CYZ on AMPA-evoked responses in the striatum contrasts with the potentiation of responses previously seen in rat forebrain slices. Consistent with this, the competitive AMPA receptor antagonist NBQX (100µM), but not the non-competitive antagonist GYKI 52466 (100µM), markedly reduced AMPA (10µM)-evoked responses. The influence of AMPA receptor stimulation on the release of [^{14}C]GABA in the striatum supports a role for AMPA-type heteroreceptors in the central nervous system acting to enhance the release of other neurotransmitter substances. AMPA receptor-mediated positive feedback on synaptic glutamate release may play an important role in a range of physiological and pathological processes including synaptic plasticity, ischaemic brain damage and epileptogenesis. These receptors may therefore provide new targets for drug action.

Treatment(µM)	Maximum % increase over basal	
	[^{3}H]L-Glutamate	[^{14}C]GABA
AMPA, 0.01	17.0 ± 7.0 (6)	38.5 ± 12.0 (6)
0.1	24.4 ± 3.3 (6)**	38.8 ± 5.0 (6)**
1	26.1 ± 3.3 (7)**	54.0 ± 8.8 (7)**
10	38.0 ± 7.0 (8)**	66.8 ± 7.7 (8)**
100	42.0 ± 6.5 (7)**	46.0 ± 9.9 (7)**
AMPA, 10 (-Ca^{2+})	16.0 ± 2.0 (8)**†	33.1 ± 9.3 (8)**†
AMPA, 10 + CYZ, 10	33.6 ± 5.1 (7)**	55.4 ± 8.6 (7)**
AMPA, 100 + CYZ, 10	36.0 ± 7.0 (5)**	46.2 ± 7.6 (5)**
AMPA, 10 + KYN, 100	38.8 ± 1.9 (6)	65.5 ± 5.5 (6)
AMPA, 10 + KYN, 300	25.0 ± 1.3 (7)	66.0 ± 2.0 (7)
AMPA, 10 + KYN, 1000	9.5 ± 4.0 (6)††	46.8 ± 7.2 (6)†
AMPA, 10 + NBQX, 100	17.0 ± 2.6 (5)†	27.6 ± 3.6 (5)††
AMPA, 10 + GYKI 52466,100	32.2 ± 5.0 (5)	55.0 ± 6.6 (5)

Table 1. Values shown are mean ± s.e.mean max. % increase over basal (n values in parentheses). *P<0.05; **P<0.01 versus basal and †P<0.05; ††P<0.01 versus agonist alone (Student's t-test for independent groups).

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160P SELECTIVE REGIONAL INCREASE IN CORTICAL N-METHYL-D-ASPARTATE RECEPTOR GLYCINE SITES IN SCHIZOPHRENIA

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The glutamate hypothesis of schizophrenia postulates that impaired central glutamatergic transmission causes up-regulation of N-methyl-D-aspartate (NMDA) receptors on some cortical neurones. We have used radioligand binding to investigate whether differences could be detected between two NMDA modulatory sites in the cerebral cortex of drug-treated chronic schizophrenic and control subjects who were matched for age, gender and post-mortem interval (PMI; Table 1). PM human brain membranes were prepared from two regions of cerebral cortex from both hemispheres and radioligand binding experiments performed as described by Grimwood *et al.* (1992, 1996). Saturation analyses using the glycine site antagonist [^{3}H]L-689,560 revealed an increase in the maximum number of binding sites (B_{max}) in superior temporal cortex (Brodmann's area 22; BA22) from schizophrenic subjects whilst no differences were observed in pre-motor cortex (BA6) (Table 1). No differences in B_{max} were observed for either region using the glutamate site antagonist [^{3}H]CGP 39653 and no difference in affinity (K_d) was observed for either region using either radioligand (Table 1).

The differential change in NMDA sites occurs in temporal lobe, an area of brain linked to the neuropathology and symptoms of schizophrenia. The up-regulation of glycine sites is consistent with glutamate deficiency hypotheses of schizophrenia, in contrast to the normal glutamate site binding. Since the binding sites for [^{3}H]L-689,560, unlike the [^{3}H]CGP 39653 sites, occur on the NR1 subunit of the NMDA receptor complex (Grimwood *et al.*, 1995), the data may be explained by temporal cortex NMDA receptors having an altered subunit composition in schizophrenia (Akbarian *et al.*, 1996).

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Grimwood, S., Moseley, A.M., Carling, R.W. *et al.* (1992) *Mol. Pharmacol.* 41, 923-930.
Grimwood, S., Le Bourdellès, B. and Whiting, P.J. (1995) *J. Neurochem.* 64, 525-530.
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Table 1. Saturation analyses for [^{3}H]L-689,560 and [^{3}H]CGP 39653 binding to the NMDA receptor glycine and glutamate sites, respectively, in two cortical areas from control and drug-treated schizophrenic subjects.

	BA22					BA6				
Control	B_{max}	K_d (nM)	PMI (h)	Age (y)	Gender	B_{max}	K_d (nM)	PMI (h)	Age (y)	Gender
[^{3}H]L-689,560	2.53±0.15	1.86(1.69,2.04)	38.5±8.7	63.7±3.8	6M	2.93±0.25	2.59(2.15,3.12)	40.9±7.4	59.1±2.8	5M, 2F
[^{3}H]CGP 39653	2.25±0.30	11.3(8.74,14.7)	48.3±18.3	65.0±7.9	3M, 1F	2.31±0.21	6.76(5.83,7.83)	48.0±10.9	65.6±5.2	5M, 1F
Schizophrenic										
[^{3}H]L-689,560	3.23±0.20*	2.10(1.85,2.38)	25.2±7.3	52.8±9.7	5M, 1F	2.96±0.31	2.35(2.03,2.73)	27.6±6.4	49.1±6.6	7M
[^{3}H]CGP 39653	2.66±0.70	10.4(8.73,12.5)	35.3±10.7	65.3±8.5	4M	2.16±0.21	8.14(7.12,9.31)	28.0±5.7	63.3±6.9	5M, 1F

Data are the arithmetic mean ± s.e.mean, or geometric mean (-s.e.mean,+s.e.mean) ($n \geq 4$). B_{max} values are pmol/mg of protein. Gender refers to number of males (M) or females (F) in each group. *Data for schizophrenic tissue differed significantly to control, $P<0.05$, unpaired t test.

161P DIFFERENTIAL EFFECTS OF ROPINIROLE, BROMOCRIPTINE AND L-DOPA ON STRIATAL PREPROENKEPHALIN AND PREPROTACHYKININ mRNA EPXRESSION IN MPTP-TREATED COMMON MARMOSETS

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L-DOPA treatment of Parkinson's disease induces dyskinesia which is associated with an imbalance between striatal output pathways. In MPTP-treated common marmoset, L-DOPA, bromocriptine and ropinirole all improve motor function, but L-DOPA rapidly induces marked dyskinesia, whereas, bromocriptine induces mild dyskinesia and ropinirole produces only intermittent dyskinesia (Pearce et al., 1996). In the present study, we report the effect of these drugs on the striatal direct and indirect pathways by measuring the expression of striatal preproenkephalin A (PPE-A) and preprotachykinin (PPT) mRNA by in situ hybridisation immunohistochemistry.

Adult common marmosets (n=16, 280-360 g, Callithrix jacchus, either sex) were treated with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropridine hydrochloride) 2 mg/kg (s.c.) once daily for 5 days. Animals were divided into 4 groups and received 10% sucrose solution, bromocriptine (0.5 mg/kg, p.o.), L-DOPA plus carbidopa (12.5 mg/kg plus 12.5 mg/kg, p.o.) or ropinirole (0.3-0.5 mg/kg, p.o.) once daily for 4 weeks. A further 4 animals were used as normal controls. At the end of the study, animals were killed, brains were removed and flash-frozen. Coronal sections (20 µm) were incubated with ^{35}S-labelled oligonucleotide probes for human PPE-A and PPT (Jolkkonen et al., 1995). Non-specific hybridisation was carried out with excess amount (100x) of unlabelled oligonucleotide. Quantitative evaluation of autoradiograms was undertaken by computerised densitometry (MCID, Imaging Research Inc.) and results were analysed by one way ANOVA followed by post hoc Dunnett's test.

PPE-A mRNA expression was increased in both caudate and putamen in MPTP-treated animals. L-DOPA did not normalise increased levels of PPE-A mRNA on either the caudate or putamen (Table 1). Ropinirole decreased the elevation in PPE-A mRNA in the caudate and putamen when compared with normal controls. Bromocriptine attenuated the elevation in PPE-A mRNA in caudate nucleus but not in the putamen compared with MPTP-treated marmosets. MPTP-treatment significantly decreased PPT mRNA levels in both caudate and putamen. Bromocriptine and ropinirole did not attenuate the decrease in PPT mRNA. However, L-DOPA reversed the decrease in PPT mRNA expression in both caudate and putamen when compared with normal controls.

The present study confirms that chronic L-DOPA treatment normalises the decrease in PPT mRNA (direct pathway) but fails to attenuate the elevated PPE-A mRNA (indirect pathway) following MPTP-treatment (Herrero et al., 1995, Jolkkonen et al., 1995, Zeng et al., 1995). The patterns of alteration in striatal PPE-A and PPT mRNA, in the present study, produced by L-DOPA bromocriptine and ropinirole reflect differential improvement of locomotor activity and appearance of dyskinesia.

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Table 1: PPE mRNA expression (nCi/g) relative to ^{14}C standards (Amersham) in the striatum of MPTP-treated marmosets.

	Normal	MPTP	MPTP+L-DOPA	MPTP+Bromocriptine	MPTP+Ropinirole
Caudate	130.2±17.6	221.0±47.6*#	236.9±28.3**	172.6±9.0	158.3±18.3
Putamen	130.0±7.2	213.3±24.5*	222.9±66.8*	175.4±11.0	144.3±14.0

*P<0.01 vs normal marmoset and MPTP+ropinirole;#P<0.05 vs MPTP+bromocriptine
**P<0.01 vs normal marmoset, MPTP+bromocriptine and MPTP+ropinirole

162P INTRAVENTRICULAR ADMINISTRATION OF GLIAL-CELL-LINE-DERIVED NEUROTROPHIC FACTOR IN THE MPTP-TREATED COMMON MARMOSET

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Parkinson's disease (PD) results from progressive degeneration of dopamine producing neurons of the substantia nigra pars compacta. Glial-cell-line-derived neurotrophic factor (GDNF) exerts neuroprotective effects and prevents nigral dopamine neurone degeneration in rats treated with 6-OHDA (Shults *et al.*, 1996). Similarly, in rhesus monkeys with an unilateral MPTP lesion of substantia nigra, GDNF appeared to protect against nigral degeneration (Gash *et al.*, 1996). We now report on the ability of GDNF to decrease motor deficits induced by subcutaneous injection of MPTP in common marmosets and the subsequent bilateral degeneration of the substantia nigra.

GDNF was administered to MPTP-treated adult common marmosets (*Callithrix jacchus*) of either sex (n=16) weighing 300-450g. The animals were divided into four groups and received intraventricular injections of GDNF 10µg, 100µg and 500µg or vehicle at weeks 9 and 13. Assessments were made by means of automated activity cages equipped with infrared beams and photocells which counted locomotor activity. Behaviour was assessed by observers who scored animal disability against a grading scale ranging from 0 (normal) to 21 (disabled) (Pearce *et al*, 1996) spreading over the following different parameters. All animals showed imbalance, tremor, rigidity and poor fur condition. However alertness, checking movements and posture were behaviours that eventually set the groups apart.

MPTP treatment alone caused a marked decrease in locomotor activity and the onset of motor disability. Intraventricular injection of GDNF 10 µg had no effect on the behaviour of the animals. However, after administration of 100 and 500 µg of GDNF there was an increase in locomotor activity compared to MPTP-treated animals and a reduction in disability scores compared to the pre-GDNF period (Figure 1). The difference was significant at the 500 µg level (Mann-Whitney *U* test).

We conclude that GDNF administration may improve motor disability following MPTP treatment and that this may reflect an action of GDNF on nigral dopamine neurons.

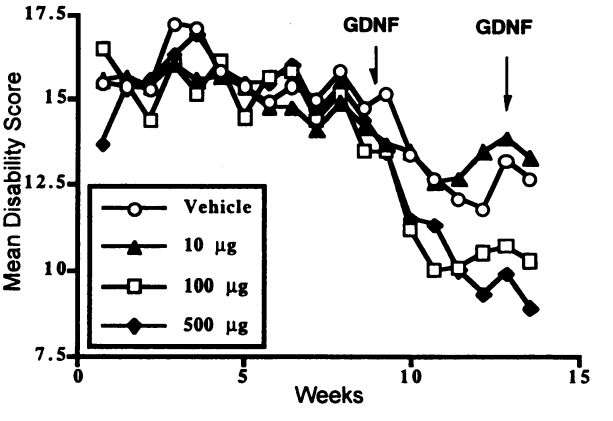

Figure 1 Mean disability scores in MPTP-treated common marmosets (n=4 in each group) receiving vehicle or GDNF.

Gash D.M., Zhang Z., Ovadia A., *et al.* (1996) *Nature* **380(6571)**:252-5
Pearce R.K.B., Collins P., Jenner P., *et al.* (1996) *Synapse* **23**:192-200
Shults C.W., Kimber T., Martin D. (1996) *Neuroreport* **7(2)**:627-31

163P HAEM OXYGENASE EXPRESSION IN THE SUBSTANTIA NIGRA OF RAT AND MARMOSET

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The cause of nigral cell loss in Parkinson's disease remains unknown, but a genetic susceptibility coupled to toxin exposure may be involved. Alterations in cytochrome P450 may be one susceptibility factor since it metabolises neurotoxins, such as MPTP or tetrahydroisoquinolines. Indeed, specific P450 isoforms are found within the rat substantia nigra and globus pallidus (Riedl *et al.*,1996). Activity of P450 may be regulated by endogenous carbon monoxide (CO) formed by the action of constitutive haem oxygenase-2 (HO-2) or inducible haem oxygenase-1 (HO-1). We now investigate the distribution of HO-1 and HO-2 within rat and marmoset basal ganglia.

Adult male Wistar rats (180-220g; n=6) and male common marmosets (*Callithrix jacchus*; 290-360g; n=2) were anaesthetised with sodium pentobarbitone (100 mg/kg or 200 mg/kg i.p., respectively) 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 in PBS, cryoprotected in 30% sucrose before being 'snap' frozen in isopentane. Contiguous brain sections (30 µm) were sliced from the rostral end of the striatum to the caudal limit of the substantia nigra in rat and throughout substantia nigra in the marmoset. Free-floating sections were incubated overnight with rabbit anti-rat HO-1 or HO-2 (1:750 - 1:1000, Stressgen Biotech. Corp., Victoria, Canada). Second layer antibodies consisted of goat anti-rabbit IgG amplified by the ABC method.

HO-2 protein was found to be intensely expressed throughout rat basal ganglia, including the striatum and substantia nigra (Table 1). In the marmoset, HO-2 was expressed within the substantia nigra pars compacta, but only sparsely in the pars reticulata. The inducible HO-1 isoform was not detected in any of the rat basal ganglia structures investigated or within marmoset nigra. However HO-1 immunoreactivity was observed in rat hippocampus and spleen, these tissues being used as positive controls.

Table 1. HO-1 & HO-2 Immunoreactivity in Rat Basal Ganglia

	HO-1	HO-2
Striatum	-	++
Globus Pallidus	-	+++
Subthalamic Nucleus	-	+++
Substantia Nigra pars compacta	-	+++
Substantia Nigra pars reticulata	-	++

(- absent; + mild; ++ moderate; +++ intense)

These results confirm and extend the findings of Ewing & Maines (1992) of HO-2 expression in rat brain. This is the first description of HO-2 expression exclusively within the zona compacta of substantia nigra of the common marmoset. These results suggest CO could regulate P450 enzymes present in basal ganglia.

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Riedl, A.G., Watts, P. M., Edwards, R. J. *et al.* (1996) *Brain Research* 743, 324-328.

164P CHRONIC GLUTATHIONE DEPLETION DOES NOT INDUCE MPTP TOXICITY IN THE RAT

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The cause of nigral cell death in Parkinson's disease remains unknown, but may involve oxidative stress. In particular loss of reduced glutathione (GSH) from substantia nigra (SN) is the earliest biochemical marker of oxidative stress. However, in rats GSH depletion induced by L-buthionine sulfoximine (BSO) does not cause nigral cell loss (Toffa *et al.*, 1997) but potentiates the toxicity of 6-OHDA (Seaton *et al.*, 1996) and MPP^+ (Wüllner *et al.*, 1996). We now investigate whether GSH depletion can produce nigral cell loss following treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment of rats, which are normally resistant to its toxicity.

Male wistar rats were cannulated into the left ventricle (lambda A +0.64, L +0.20, V +0.40) and treated with either BSO (160mg/ml in 0.9% saline; 0.96mg/day) or 0.9% saline using Alzet osmotic pumps (model 2004). On days 14 to 17 of the infusion period, animals were injected with MPTP (30mg/kg i.p.) or 0.9% saline. Animals were killed on day 25 and the brains removed and divided at the optic chiasma. The posterior part was fixed in 4% formaldehyde for tyrosine hydroxylase (TH) immunohistochemistry. The anterior half was frozen in isopentane. GSH analysis was performed on striatum using HPLC (Reed *et al.*, 1980) and striatal sections (12µm) were cut for [^{3}H]-mazindol binding (Rose *et al.*, 1993) using the frozen part. No differences were seen between the left and right sides of the brain (one-way ANOVA) and in all further analysis the hemispheres have been pooled.

No changes were observed in general behaviour as a result of either BSO infusion or MPTP treatment. Striatal GSH levels were reduced in both BSO-treated groups but its loss was not potentiated by MPTP (Dunn's t-test). Specific [^{3}H]-mazindol binding in the striatum was not altered either by BSO or MPTP treatment, alone or in combination (Table 1). There was no difference in TH immunostaining as a result of BSO or MPTP treatment

These results indicate that GSH depletion does not render the nigro-striatal tract of the rat susceptible to the toxic actions of MPTP.

Table 1. Changes in striatal GSH levels and specific [^{3}H]-mazindol binding.

Treatment	GSH (nmol/mg)	[^{3}H]-Mazindol binding (fmol/mg)
Saline + Saline	1.74 ± 0.09	256.7 ± 12.4
Saline + MPTP	1.92 ± 0.10	269.9 ± 3.2
BSO + Saline	0.90 ± 0.21*	218.7 ± 14.6
BSO + MPTP	1.03 ± 0.13**	281.1 ± 15.0

* $p<0.05$ ** $p<0.01$ by comparison to Saline + wSaline group (Dunn's t-test); n=5 per group

Reed, D.J., Babson, J.R., Beatty, P.W. *et* al (1980) *Anal.* Biochem 106:55-62.
Rose, S., Jenner, P., Green, A.R. (1993) *Neurodegeneration* 2:267-273.
Seaton, T.A., Jenner, P., Marsden, C.D. (1996) *J. Neural Trans* 103:315-329.
Toffa, S.E.K., Kunikowska, G.M. Zeng, B-Y. *et al.* (1997) *J. Neural Trans.* 104:67-75.
Wüllner, U., Löschmann, P-A., Schulz, J.B. (1996) *NeuroReport* 7:921-923.

165P INHIBITION OF THE NICOTINIC STIMULATION OF DOPAMINE RELEASE FROM THE RAT STRIATUM *IN VITRO* BY α-CONOTOXIN-MII, AN ANTAGONIST SELECTIVE FOR THE α3β2 NICOTINIC RECEPTOR SUBTYPE

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Presynaptic nicotinic acetylcholine receptors (AChRs) modulate the release of dopamine (DA) from rat striatum (Wonnacott, 1997). Neuronal nicotinic AChRs are a family of pentameric ligand gated ion channels, and the subtype(s) modulating dopamine release is not known. Recently, α-conotoxin-MII (α-CTx-MII) was described as a potent and selective antagonist at a subtype composed of α3 and β2 subunits (Cartier *et al.*, 1996). We have synthesised this peptide toxin to examine the involvement of the α3β2 subtype in the nicotinic modulation of [^{3}H]-DA release from striatal slices and synaptosomes.

P2 synaptosomes and slices (0.3 mm) were prepared from the striata of male Sprague-Dawley rats (240-250 g), loaded with [^{3}H]-DA and perfused as previously described (Marshall *et al.*, 1996). After 20 min washing with Krebs bicarbonate buffer, and a further 10 min with normal buffer or buffer containing antagonist (α-CTx-MII, 1-112 nM, or mecamylamine, 10μM), the potent nicotinic agonist (±)-anatoxin-a was applied for 40 s. Two min fractions were collected and counted for radioactivity. Data were analysed using SigmaPlot™ for Windows, and responses were compared using Student's paired *t*-test.

In striatal synaptosomes, α-CTx-MII dose-dependently inhibited [^{3}H]-DA release elicited by 1 μM (±)-anatoxin-a, with an IC_{50} value of 22.1 nM (Figure 1). Maximum inhibition of 46.2 ± 6.6 % (n=6, $p<0.01$) was achieved with 112nM α-CTx-MII. At this concentration of α-CTx MII, the α3β2 subtype of nicotinic AChR expressed in *Xenopus* oocytes is completely blocked whereas other pairwise combinations of subunits are not inhibited (Cartier *et al.*, 1996). The non-selective nicotinic AChR antagonist mecamylamine inhibited (±)-anatoxin-a-evoked [^{3}H]-DA release by 80.2 ± 3.8 % (n=4). At a higher agonist concentration (25 μM (±)-anatoxin-a) the inhibition curve for α-CTx-MII was displaced to the right, suggesting a competitive mode of action.

In slices (Figure 1), [^{3}H]-DA release evoked by 1 μM (±)-anatoxin-a was also partially inhibited by α-CTx-MII, with a sensitivity similar to that seen in synaptosomes (IC_{50}=16.9 nM). However, the maximum inhibition in slices was less (24.4 ± 4.9 %, n=5, $p<0.01$), although 10 μM mecamylamine inhibited [^{3}H]-DA release by 88.0 ± 2.1 % (n=5).

These data suggest that striatal dopamine release may be regulated by two populations of presynaptic nicotinic AChRs, one of which is comprised of α3 and β2 subunits. The lower maximum inhibition observed in the slice preparation suggests the contribution of an additional, indirect modulation (e.g. via nicotinic AChR on glutamate terminals) which is preserved in this more integrated preparation.

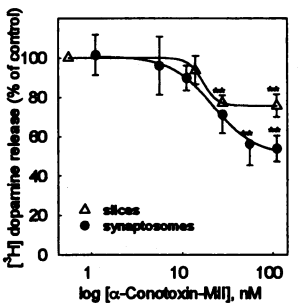

Figure 1. Effect of α-CTx-MII on 1 μM (±)-anatoxin-a evoked striatal [^{3}H]-DA release (** $p<0.01$ in comparison to corresponding controls).

Supported by MRC. We are grateful to Sue Philips and Richard Kinsman for synthesis of the α-CTx-MII.

Cartier, G.E., Yoshikami, D.J., Gray, W.R. *et al.* (1996) *J.Biol.Chem.* 271, 7522-7528.
Marshall, D.L., Soliakov, L., Redfern, P. *et al.* (1996) *Neuropharmacology* 35, 1531-1536
Wonnacott, S. (1997) *TINS* 20, 92-98

166P MODULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR BINDING TO ADULT AND AGED RAT BRAIN BY GALANIN

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The 29 amino acid neuroactive peptide galanin has a widespread mammalian CNS distribution, is known to co-localise with a number of neurotransmitters including acetylcholine and has previously been shown to inhibit cholinergic function by modulating both pre- and post-synaptic muscarinic receptors (Patel and Hutson, 1994). However, neuronal function is also modulated by nicotinic receptors (nAChR) which exist as multiple subtypes throughout the CNS. Therefore in the present study we have examined the effects of galanin on nAChR binding using radioligands with preferential selectivity for two subtypes, α4-β2 and α7.

Binding of [^{3}H]nicotine and [^{125}I] α-bungarotoxin (α-BGTx) which preferentially label the α4-β2 and α7 subtypes respectively, was determined in young adult male SD rats (220 - 280g) or aged rats (24 month; 400 - 600g). The effects of pretreating membranes for 10 min at 30 °C with galanin (10, 100, 1000nM,Fuxe et al, 1988) was assessed in whole brain and/ or thalamus preparations from these animals.

The results (table 1) indicate that in adult rats, galanin concentration - dependently reduced the affinity of [^{3}H]nicotine binding in whole brain and thalamus without affecting the B_{max}. [^{125}I]α-BGTx binding in whole brain was unaffected by galanin. In aged rats there was a significant reduction in affinity of [^{3}H]nicotine binding in rat whole brain [K_D = 6.3 (5.1;7.8) nM, n = 4, P < 0.01] and a concommitant increase in maximum number of binding sites (98 ± 0.86 fmol/mg P < 0.01) compared with adult rats. However, the modulatory effect of galanin on nAChR affinity was no longer observed [K_D = 6.4 (5.5;7.3) nM, n = 4]. [^{125}I]α-BGTx binding to whole brain in aged rats was not significantly different from adult rats [K_D = 0.31(0.25;0.39) nM: B_{max} = 21 ± 2.3 fmol/mg, n = 4].

Table 1. [^{3}H]nicotine binding to adult rat brain membranes.

[^{3}H]nicotine	whole brain		thalamus	
	K_D	B_{max}	K_D	B_{max}
Control	2.3 (1.8;2.9)	56 ± 5.0	3.0 (2.5;3.6)	60 ± 12
+ galanin				
10 nM	2.4 (2.1;2.7)	60 ± 13	3.8 (2.7;5.2)	78 ± 17
100 nM	3.7 (3.2;4.2)*	87 ± 12	6.6 (5.0;8.4)	98 ± 22
1000 nM	4.5 (4.1;4.9)**	60 ± 7.4	5.2 (4.6;5.9)*	99 ± 21
[^{125}I]α-BGTx				
Control	0.30 (0.19;0.47)	25 ± 4.7	N.D.	N.D.
+ galanin				
1000 nM	0.39 (0.25;0.59)	27 ± 5.1	N.D.	N.D.

K_D values (nM) are geometric means (numbers in parentheses indicate low and high errors of this mean, n = 3 - 7, (**P < 0.01; * P < 0.05 vs control; ANOVA followed by Dunnett's test). B_{max} values (fmol/mg) are expressed as arithmetic means ± s.e.m. (N.D. = not determined).

The results suggest that galanin selectively reduces the affinity of [^{3}H]nicotine binding in adult but not aged rat brain, whereas [^{125}I] α-BGTx binding was unaffected by galanin in adult or aged rat brain.

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Patel, S. and Hutson, P.H. (1994), *Eur. J. Pharmacol.* 255, 25.

167P EFFECTS OF CORTICOSTERONE TREATMENT ON THE ETHANOL PREFERENCE OF THE LACG (ALCOHOL AVOIDING) STRAIN OF MICE

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The relationship between stress and alcohol consumption in humans has been much discussed, but the mechanisms are unclear and there appears to be no correlation between circulating cortisol levels and alcohol consumption in humans. Experimental studies have shown effects of corticosterone on alcohol intake but prolonged effects have been little studied. In the present study, voluntary ethanol intake of the LACG strain of mice was measured during, and after, daily injections of corticosterone. The inbred LACG strain has been bred at Bristol University for over 20 years and shows a low voluntary preference for alcohol solutions (Connelly et al., 1983).

Groups of 8-10 male LACG mice (25-30g, originally from the Bristol colony, now established at Durham University) were used. The animals were single-housed with ad lib access to water, food and 8% ethanol. On days 1 to 7, the treatment groups were given daily i.p. injections of corticosterone (2 or 20 mg/kg) or Tween (0.5%) vehicle. On days 8 to 14 all animals were given an i.p. injection of the Tween vehicle. The volume of each fluid drunk was monitored every day at the time of the injections and the amount of 8% ethanol drunk as a proportion of total daily fluid intake was calculated. Statistical analysis was by 2-way repeated measures ANOVA.

The results in Table 1 demonstrate that over the first 3 days of injections, the ethanol intake increased in the group given 2 mg/kg corticosterone ($P < 0.05$); this change was no longer seen on days 4 - 7. There was no change in preference seen in the group given 20 mg/kg corticosterone. However, the results in Table 2 show that at the end of the 20 mg/kg corticosterone treatment, there was an increase in ethanol intake. When intake during the 3 days after the corticosterone treatment, during which the animals received vehicle injections, was compared with that during the last three days of corticosterone injections, the increase in preference was statistically significant ($P < 0.05$). This difference was not observed in the control group ($P > 0.1$). No differences were seen in total fluid intake of the mice.

These results may indicate that it is not just corticosterone concentrations that determine the ethanol preference in these mice. It is possible that adaptations occur to corticosterone, the effects of which are seen later than the changes in corticosterone levels.

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Table 1: Effect of corticosterone injections on ethanol preference

Treatment	Day 1	Day 2	Day 3
Control	0.36 ± 0.01	0.24 ± 0.03	0.19±0.03
Cort 2 mg/kg *	0.49 ± 0.05	0.38 ± 0.06	0.36±0.08
Cort 20 mg/kg	0.40 ± 0.03	0.23 ± 0.01	0.20±0.02

cort = corticosterone; results are mean ± s.e.m. of ethanol consumption as ratio of total fluid intake.
* $P < 0.05$ corticosterone 2 mg/kg cf controls
† $P < 0.05$ vehicle injections cf corticosterone 20 mg/kg

Table 2: Effect on ethanol preference of replacing corticosterone injections (days 5-7) with vehicle injections (days 8-10).

	Cort injections			Vehicle injections		
Treatment	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control	0.27 ± 0.05	0.17 ± 0.03	0.18 ± 0.04	0.22 ± 0.05	0.29 ± 0.05	0.24 ± 0.05
Corticosterone 2 mg/kg	0.36 ± 0.08	0.25 ± 0.06	0.25 ± 0.07	0.27 ± 0.06	0.33 ± 0.07	0.33 ± 0.08
Corticosterone 20 mg/kg †	0.20 ± 0.02	0.18 ± 0.02	0.13 ± 0.02	0.23 ± 0.05	0.34 ± 0.06	0.32 ± 0.08

168P LACK OF EFFECT OF SELECTIVE IMIDAZOLINE I_2 LIGANDS IN FORCED SWIM TEST IN MICE

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Evidence suggests that compounds originally characterised around the imidazoline structural class may define a novel receptor mechanism, modulating adrenergic activity and involved in the mediation of depressive symptoms (Nutt et al, 1995). We tested compounds with in vitro selectivity for the imidazoline receptor site in the mouse forced swim test, (FST) a putative model of depression. BU224 and BU239 have previously been described as high affinity ligands for I_2 sites (Lione et al, 1996). BDF8082 (4-chloro-2-(2-imidazoline)-isondoline) is a novel compound with nM affinity for I_2 receptors.

For the FST, female BKTO mice (Bantin and Kingman, Hull) were housed under standard conditions in groups of 15. Animals were kept in the holding facility for two weeks after arrival before experimental use. Mice were 25-35g at time of use. Immobility was measured in 11 beakers filled with 600ml of water (23°C). Time spent immobile was measured with a stopwatch. The animals were placed in the beakers for 5 mins. Immobility was measured for the final four minutes. All animals swam for the first minute irrespective of treatment. Data were analysed by GLM ANOVA using SAS statistical package.($n \geq 6$) Significant differences were determined by post hoc tests (Least Square Means) following significant ANOVA. α_2 binding was performed in using tissues from Wistar male rats (300-350g) using the methods described by Ernsberger et al (1986). α_2 binding was determined by displacement of [^{125}I]clonidine (0.5nM). I_2 binding was measured as displacement of [^{3}H]-idazoxan and with H-3-BFI in rat liver homogenates as described by Tesson et al (1991) (n = 2-6). Clonidine (0.12-0.5mg/kg s.c.) induces a dose-dependent reduction in the time spent immobile (Table 1). The minimum effective dose (M.E.D.) was 0.25mg/kg. At higher doses clonidine appeared to have sedating properties. Neither yohimbine nor idazoxan had any effect on immobility in the FST alone but both reversed the effect of clonidine (data not shown). The min E.D. were 5mg/kg and 0.025mg/kg resp. None of the compounds with selective affinity for I_2 sites BDF8082, BU224, BU239 (2.5-10mg/kg) affected time spent swimming in the test, nor did they alter the response to imipramine (3mg/kg) (data not shown).

TABLE 1. Effect of compounds on α_2 and I_2 binding and on activity in the forced swim test in mice.

COMPOUND	I_2 Ki nM	α_2 Ki nM	FST MED mg/kg	Sig Level vs veh
Clonidine	>5000	4	0.25	0.001
Yohimbine	>5000	49	>5.0	ns
Idazoxan	22	35	>0.125	ns
BU224	4	5000	>10	ns
BU239	24	>5000	>10	ns
BDF8082	2	8500	>10	ns

Clonidine decreases immobility in the FST whereas the I_2 selective compounds BU224, BU239 and BDF8082 were without effect. These results indicate that selective I_2 affinity is not associated with antidepressant-like activity in the FST test in mice at the doses tested.

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Lione, L.A., Nutt, D and Hudson A.L. (1996) J. Psychopharm.10, 3, Suppl. A19.
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169P ANTAGONISM BY OPIOIDS OF COMPOUND 48/80-INDUCED SCRATCHING IN MICE

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Pain and itch are probably independent sensory modalities. The pharmacological bases of itch - an orphan symptom - remain ill-defined. Scratch behavior is specific for itching and differentiates itch from pain. Kuraishi *et al.* (1995) recently described an animal model of itch which involves Compound 48/80-induced scratching in mice. We have standardized this model and studied the effects of morphine (mu receptor directed), enadoline (kappa), ICI 204448 (peripheral kappa), SNC 80 (delta) and saline on scratching over 30 min caused by 50 µg (100 µl) of Compound 48/80 injected s.c. into the back of the neck of albino Swiss male mice (25-27 g; n=7-10). Antiscratch-50 values were obtained by nonlinear regression analysis (KaleidaGraph) for morphine (0.25-2 mg/kg s.c. given 10 min before Compound 48/80), enadoline (2.5-20 µg/kg s.c. at -5 min), ICI 204448 (2.5-10 mg/kg s.c. at -20 min) and SNC 80 (0.25-25 mg/kg s.c. at -30 min) (Table 1).

Kappa opioids are active against bombesin (another scratch-inducing agent) in rats and nalbuphine can ameliorate pruritus caused by spinally administered opioids in humans (Gmerek & Cowan, 1988). We have therefore focused on the role of kappa receptors in mediating the sensation of itch. Kappa receptors seem to be involved in the present model since pretreatment of mice with a behaviourally neutral dose of norbinaltorphimine (20 mg/kg s.c. at -15 h) antagonized the antiscratch activity of enadoline (0.01 mg/kg) (76±4% to 37±12%, s.e. mean). Also, our demonstration of activity for ICI 204448 against Compound 48/80 (in contrast to the bombesin model) affords another endpoint for structure-activity studies with peripheral kappa agonists.

Table 1. Antiscratch-50 values (and 95% confidence limits) of opioids against Compound 48/80 in mice.

Enadoline	0.004 (0.002-0.005) mg/kg
Morphine	0.38 (0.24-0.52) mg/kg
SNC 80	2.73 (1.43-4.03) mg/kg
ICI 204448	2.82 (1.38-4.26) mg/kg

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Kuraishi, Y., Nagasawa, T., Hayashi, K. *et al.* (1995) *Eur. J. Pharmacol.* 275, 229-233

170P NO EVIDENCE OF MELATONIN-MEDIATED ALTERATIONS IN cAMP LEVELS IN RAT SUPRACHIASMATIC NUCLEI (SCN)

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Nocturnal secretion of pineal melatonin modulates circadian rhythms through actions at high affinity melatonin receptors in the SCN, the primary mammalian circadian pacemaker. The SCN are responsible for the generation and entrainment of physiological and behavioural rhythms, including that of melatonin secretion itself. Although melatonin receptors couple to an inhibitory G-protein ($G_{i\alpha}$) to decrease adenylyl cyclase activity and cAMP production in pituitary, cortex and retina, or in cells transfected with melatonin receptor cDNA (reviewed in Morgan *et al.*, 1994), the transduction mechanism in the SCN has not yet been determined. This study addresses whether melatonin affects basal or forskolin-stimulated cAMP levels in rat SCN tissue.

Experiments were performed when melatonin most effectively modifies SCN function, near the light-to-dark transition. Hypothalamic slices (500 µm), containing the SCN, were obtained from adult Male Lister-Hooded rats and dissected to isolate individual SCN. Following pre-incubation (>60 min) in standard Krebs buffer (37°C), test compounds were added. Slices were inactivated by boiling in hypotonic solution (50 mM Tris, 4 mM EDTA) for 5-10 min. Samples were homogenized and cAMP measured ([^{3}H]cAMP kit; Amersham, UK). Protein concentrations were determined by the method of Bio-Rad. Data are expressed as mean ± s.e.mean pmol cAMP.mg protein^{-1}. Statistical differences between data groups were determined using a Student's unpaired t-test.

The mean basal concentration of cAMP in SCN was 54.4 ± 12.0 pmol.mg protein^{-1} (n=14 SCN). Application of melatonin (10 nM, 1 h incubation) did not significantly alter basal cAMP levels ($P>0.05$, n=4). Forskolin induced a concentration-dependent elevation in tissue cAMP levels; maximum responses (578 ± 82 pmol.mg protein^{-1}, n=4) were achieved within 10 min using 330 µM forskolin (half maximal response at 94 µM). Melatonin did not modulate forskolin-stimulated cAMP levels when tested (a) at a single concentration (10 nM) against the full concentration range (3-330 µM) of forskolin, (b) for a range of pre-incubation periods (15-60 min) prior to the addition of 10 µM forskolin, or (c) at a range of concentrations (10 pM-1 µM) for 15 min pre-incubation before the addition of 30 µM forskolin. Furthermore, pre-incubation (1 h) with the phosphodiesterase inhibitor IBMX (1 mM) did not reveal any significant ($P>0.05$) modulation of forskolin-stimulated cAMP levels (10 µM; 427 ± 48 pmol.mg protein^{-1}, n=4) by melatonin (10 nM, 1 h; 351 ± 22 pmol.mg protein^{-1}, n=4).

Within the limitations of sensitivity of the assay, we found no evidence that melatonin modulates basal or forskolin-stimulated cAMP levels in rat SCN. The possibility that melatonin receptors are coupled to other signal transduction pathways in the SCN remains to be determined.

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171P MELATONIN MEL_{1A} RECEPTOR-MEDIATED RESPONSES AS MEASURED BY MICROPHYSIOMETRY

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Many of the actions of melatonin are believed to be mediated through high affinity MEL_1 receptors which are coupled through $G_{i\alpha}$ to inhibition of adenylyl cyclase (reviewed in Reppert *et al.*, 1996). Expression cloning has revealed three MEL_1 receptor subtypes, two of which have been identified in human, termed MEL_{1A} and MEL_{1B} (Reppert *et al.*, 1996). This study examined the functional characteristics of activation of MEL_{1A} receptors using a microphysiometer (Molecular Devices Corp., USA), a system able to detect changes in pH when a cell excretes acidic metabolites to the external environment (proton extrusion).

CHO cells, stably transfected with the human melatonin MEL_{1A} receptor, were seeded (10^6 cells.ml^{-1}) in specialized chambers 14-18 h before experimentation. Chambers were maintained at 37°C and perfused (100 $\mu l.min^{-1}$) with minimum essential medium Eagle (pH=7.4). Drug solutions were contained in the perfusate (contact time ~1 min) and cumulative concentration response curves were generated. Drug-induced responses were expressed as a percentage of the basal (pre-drug) acidification rate and EC_{50} values were calculated using ALLFIT. Data represent mean ± s.e.mean of 3-6 experiments.

The basal acidification rates in these cell were between 50-300 $\mu V.s^{-1}$ (equivalent to ~50-300 milli-pH units.min^{-1}). Application of melatonin produced two, concentration-dependent, effects on acidification rate; low concentrations increased acidification rate (EC_{50}=28.6 ± 3.5 pM, 22.2 ± 2.1% maximal increase, n=19), while higher concentrations of melatonin decreased the rate of proton extrusion (EC_{50}=2.8 ± 0.5 nM, 41.7 ± 7.0% maximal decrease, n=11). Cells devoid of the transfected MEL_{1A} receptor were unresponsive. Both effects were antagonized by the MEL_1-selective receptor antagonist luzindole (1-10 μM), which blocked both the increase (pA_2=6.9 ± 0.1, n=4) and decrease (pA_2=6.3 ± 0.1, n=9) in acidification rate. Pre-treatment with pertussis toxin (PTX; 200 $ng.ml^{-1}$, 20-21 h) completely abolished both the melatonin-evoked increase (2.6 ± 2.0% decrease, n=4) and decrease (2.1 ± 1.2% decrease, n=4) in acidification rate compared to control values.

Activation of MEL_{1A} receptors both increased and decreased extracellular acidification rate with picomolar and nanomolar potencies, respectively. Both responses were antagonized by luzindole and were abolished by treatment with PTX. The higher potency response is in agreement with that for MEL_{1A}-mediated inhibition of cAMP (Reppert *et al.*, 1996). The lower potency response raises the possibility that alternative signalling mechanisms may exist, possibly mediated via the $G_{i\beta\gamma}$-subunit.

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172P THE EFFECTS OF AN nNOS INHIBITOR 1-(2-TRIFLUOROMETHYLPHENYL)IMIDAZOLE (TRIM) ON SAPHENOUS NERVE-INDUCED PLASMA EXTRAVASATION IN THE RAT PAW

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1-(2-trifluoromethylphenyl)imidazole (TRIM) is a selective inhibitor of neuronal nitric oxide synthase (nNOS) both *in vitro* and *in vivo* (Handy *et al.*, 1995). Nitric oxide (NO) has been implicated in the pathogenesis of diseases such as arthritis, stroke and chronic inflammation (see Gross & Wolin, 1995 for review), and a role for nitric oxide in neurogenic inflammation has been suggested (Kajekar *et al.* 1995). NO has been shown not to play a vasorelaxant role in neurogenic vasodilation, but may contribute to the release of neuropeptides from sensory nerve terminals following stimulation. We have examined the effects of TRIM on plasma extravasation induced by stimulation of the saphenous nerve.

Male Wistar rats (200-250g), anaesthetised with sodium pentobarbitone (50$mgkg^{-1}$, i.p.), were prepared for electrical stimulation of the saphenous nerve of one hind leg, with the other hind leg serving as sham control. The nerve was stimulated at 10V, 1ms, 2Hz for 0-5 min, and accumulation measured 0-30 min. Plasma extravasation was assessed in skin by the extravascular accumulation of i.v. ^{125}I-albumin. Statistics were by one way ANOVA and Bonferroni's modified t-test.

The results (Table 1) show that TRIM (50 mg kg^{-1}, i.p.) significantly inhibited plasma extravasation induced by saphenous nerve stimulation. This effect was significantly, but not totally reversed by i.v. injection of L-Arginine (100 mg kg^{-1}). 2-Trifluoromethylphenol (TRIMPOH), which is chemically related to TRIM, but has no activity as an nNOS inhibitor (Handy *et al.*, 1996), did not reduce plasma extravasation.

Therefore, selective nNOS inhibitors may potentially be of use in the treatment of conditions involving a component of neurogenic inflammation.

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Table 1. Plasma extravasation induced by electrical stimulation of the saphenous nerve. Results are expressed as μl plasma extravasated per 100mg tissue. Mean ± s.e.mean., n = 5-7, **P<0.01 compared to vehicle (study A), *P<0.05 compared to TRIM (study B).

	STUDY A			STUDY B			
	Vehicle	TRIM	TRIMPOH	Vehicle	L-Arg + vehicle	TRIM	TRIM + L-Arg
Plasma extravasated (μl):							
Stimulated paw	24.6 ± 2.6	12.8 ± 1.9**	29.7 ± 1.9	34.6 ± 0.9	34.7± 1.2	18.6 ±1.9	24.1 ± 1.1*
Sham control paw	2.7 ± 0.5	2.0 ± 0.8	2.5 ± 0.3	2.9 ± 0.4	3.6 ± 0.4	2.9 ± 0.3	3.1 ± 0.4