103P PHARMACOLOGICAL IDENTIFICATION OF $mGlu_1$ AND $mGlu_5$ GROUP I METABOTROPIC RECEPTOR SUBTYPES ON NEONATAL RAT MOTONEURONES

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It is known that group I metabotropic glutamate (mGlu) receptors are present on neonatal rat motoneurones (Watkins and Collingridge, 1994). However, definitive evidence of which subtypes of group I receptors are present has not yet been presented. Here we report evidence that both known subtypes ($mGlu_1$ and $mGlu_5$) of group I mGlu receptors are present on neonatal rat motoneurones.

Recordings were made from ventral roots of hemisected spinal cords from neonatal rats (1-5 days old) bathed in medium containing tetrodotoxin (0.1μM). Depolarisations were produced by application of the selective group I agonists: (S)-3,5-dihydroxyphenylglycine (DHPG) and (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3R)-ACPD) (Watkins and Collingridge, 1994); and the $mGlu_5$ selective agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) (Doherty *et al.*, 1997).

Maintained application of 900μM CHPG produced a transient depolarisation of motoneurones that returned to baseline after 15 minutes. With CHPG continuously perfused, application of the selective group I agonist DHPG still produced a depolarisation of the motoneurones. Equi-effective concentrations of DHPG (6-12μM) to the depolarisation produced by 900μM CHPG were used in each experiment. Application of 3mM CHPG did not depolarise the motoneurones in the continuous presence of 900μM CHPG. Upon washout of the CHPG a larger depolarisation was observed after DHPG application, in addition re-application of 3mM CHPG produced a depolarisation of the motoneurones. These effects of DHPG and CHPG were observed in each of the three preparations used. We suggest that CHPG is causing a desensitisation of $mGlu_5$ receptors and that the residual depolarisation produced by DHPG is mediated *via* $mGlu_1$ receptors.

Application of 40μM (1S,3R)-ACPD produced a depolarisation of the motoneurones. In the presence of 30μM 7-hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester (CPCCOEt), a selective, non-competitive, $mGlu_1$ antagonist (Litschig *et al.*, 1999), the depolarisation produced by 1S,3R-ACPD was reduced in a time-dependent manner to a maximum reduction of 65% after 80 minutes. However, the effects of (1S,3R)-ACPD were never completely abolished by CPCCOEt (n=3). This suggests that the residual depolarisation produced by (1S,3R)-ACPD in the presence of CPCCOEt is mediated *via* $mGlu_5$ receptor activation and that CPCCOEt is reducing the (1S,3R)-ACPD response by antagonism of $mGlu_1$ receptors.

Concentration-response curves (CRCs) were constructed to DHPG, in the absence and presence of the non-competitive $mGlu_5$ antagonist 2-methyl-6-(phenylethynl)pyridine (MPEP) (Gasparini *et al.*, 1999). 10μM MPEP caused a non-parallel rightward shift in the CRC to DHPG (n=3). This suggests the presence of the $mGlu_5$ receptor subtypes on neonatal rat motoneurones. Together, these three sets of experiments strongly suggest the presence of both $mGlu_1$ and $mGlu_5$ receptor subtypes on neonatal rat motoneurones.

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104P THE EFFECT OF NON-NMDA GLUTAMATE RECEPTOR STIMULATION ON 5-HT OVERFLOW FROM THE SCN OF THE RAT

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The terminals of the serotonergic raphe-hypothalamic tract and the glutamatergic retino-hypothalamic tract converge in the ventro-lateral region of the suprachiasmatic nucleus (SCN), the site of the mammalian biological clock (Moga and Moore, 1997). We have investigated the effect of non-NMDA and metabotropic glutamate (GLU) receptor stimulation on 5-hydroxytrytamine (5-HT) overflow from the SCN at two zeitgeber times (ZT; ZT0 = lights on) in conscious rats by *in vivo* microdialysis. Male Wistar rats (240-280g), were housed under a 12:12 h light:dark cycle. Concentric microdialysis probes were implanted adjacent to the SCN. Probes were continuously perfused (1.2 μl/min) with aCSF containing 1μM citalopram. Drugs were infused via the probe over a 60 min period at ZT 6 and 18. 5-HT levels in three 15 min pre-intervention controls and eight subsequent dialysate samples were analysed by HPLC-ECD. AMPA (100 μM) infused into the SCN region failed to affect 5-HT overflow at either ZT 6 or ZT 18 whereas kainate (100 μM) increased 5-HT overflow at both ZT 6 (233±19.8%; $p<0.001$) and ZT 18 (198±45.5%; $p<0.05$). At ZT 6, the metabotropic GLU receptor agonist (1S,3R)-1-aminocycopentane-1,3-dicarboxylic acid (1S,3R-ACPD) had no effect (100 μM) or increased (1 mM; 183±31.7%; $p<0.05$) (Fig 1) 5-HT release from the SCN, an effect which was blocked by the antagonist (RS)-α-ethyl-4-carboxyphenylglycine (100 μM).1S,3R-ACPD (100 μM & 1 mM) had no effect on 5-HT release at ZT 18. These results demonstrate that in the SCN, metabotropic GLU receptor stimulation with 1S,3R-ACPD displays a diurnal variation. Whether the diurnal variation in response to metabotropic GLU receptor stimulation plays any role in regulating inputs to the circadian clock over 24 h remains to be elucidated.

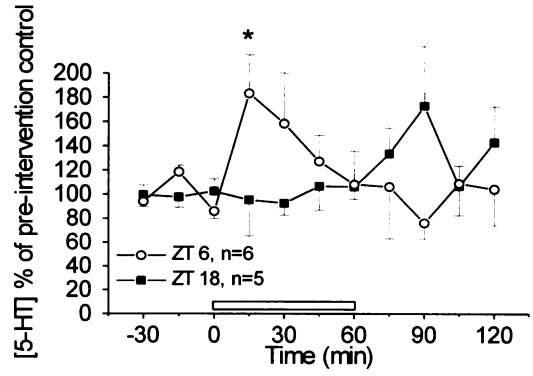

Figure 1. Effect of 1S,3R-ACPD (1mM) infusion (60 min; hollow bar) on 5-HT overflow from the SCN at ZT 6 and ZT 18. Time zero denotes ZT. * $p<0.05$; one way ANOVA with repeated measures with Student Newman Keuls post hoc test. Basal 5-HT levels were 36.3±8.89 and 28.1±5.34 fmol for ZT 6 and ZT 18, respectively.

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105P THE MECHANISM OF INHIBITION OF CLOMETHIAZOLE ON ISCHAEMIA-INDUCED GLUTAMATE RELEASE FROM CEREBRAL CORTEX *IN VIVO*

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We recently reported that incubating rat cortical slices in a hypoxic/aglycaemic (H/A) medium resulted in a significant enhancement of glutamate efflux that was inhibited by the GABAmimetic compound clomethiazole (CMZ) or GABA (Nelson *et al.*, 1999). We have examined further the pharmacology of this response to simulated ischaemia and also the effect of pentobarbitone on H/A-induced glutamate efflux.

Slices (350 μm^2) of cerebral cortex from rats (adult female Wistar, 200-250 g) were incubated in mesh baskets at 37°C in a normoxic HEPES buffered saline containing glucose (10 mM), or in H/A medium with no added glucose as described previously (Nelson *et al.*, 1999). Glutamate was measured fluorimetrically using the reduction of $NADP^+$ by glutamate dehydrogenase (Nicol *et al.*, 1996). Glutamate efflux is expressed as a % of the total tissue glutamate content. Significance was assessed by unpaired students t-test.

Incubation of the tissue in H/A conditions (30 min) produced a marked increase in glutamate efflux which was inhibited by CMZ (100 μM). The competitive $GABA_A$ receptor antagonist bicuculline (10 μM) abolished the effect of GABA on ischaemia-induced glutamate release (data not shown) but had no significant effect on CMZ-induced inhibition of glutamate release. In contrast, picrotoxin (100 μM), a $GABA_A$ chloride channel blocker, abolished this inhibitory effect of CMZ (Figure 1). Pentobarbitone at high concentration (300 μM) produced a modest inhibition of glutamate efflux ($p \leq 0.05$) which was blocked by bicuculline (Figure 1).

These data suggest that CMZ inhibits ischaemia-induced glutamate efflux *in vitro* by acting at the $GABA_A$ chloride channel site. and does not require the presence of endogenous GABA to produce its inhibitory effect. These data may have relevance to neuroprotection since CMZ, in contrast to pentobarbitone, is protective in several models of ischaemic stroke (see Green, 1998).

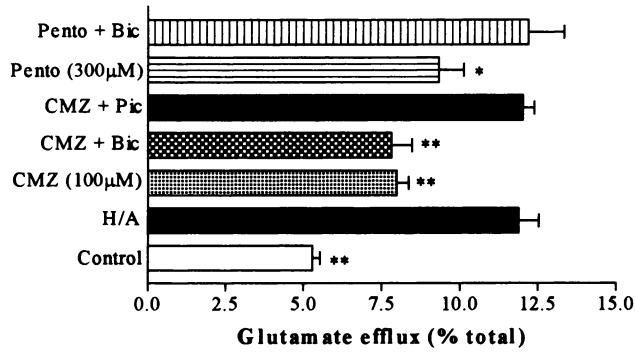

Figure 1. Effect of hypoxia/aglcaemia (H/A) on glutamate release from rat cortical slices and effect of CMZ, pentobarbitone (pento), bicuculline (bic, 10 μM) and picrotoxin (pic, 100 μM). Different from H/A *, $p \leq 0.05$. **, $p < 0.01$.

Supported in part by AstraZeneca R&D Södertälje.

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106P MULTIPLE METABOTROPIC GLUTAMATE RECEPTORS MODULATE EXCITATORY SYNAPTIC TRANSMISSION IN RAT STRIATAL CHOLINERGIC INTERNEURONES

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It is established that metabotropic glutamate receptors (mGluRs) can modulate neuronal inputs to striatal projection neurones (Lovinger & McCool 1995). However, it is not presently known whether these receptors modulate inputs to striatal interneurones. Thus, we have characterised synaptic currents in cholinergic interneurones and investigated their modulation by mGluRs.

200μm coronal brain slices containing the striatum were prepared from 13-16 day-old Wistar rats in physiological saline. Cholinergic interneurones were visually identified and preferentially targeted for patch-clamp recordings. The physiological saline contained (mM) 125.0 NaCl, 25.0 $NaHCO_3$, 10.0 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgCl_2$ and was bubbled with a 95%, 5% O_2/CO_2 gas mixture. Physiological saline was also used as the extracellular solution whilst the intracellular (pipette) solution comprised (mM) 120.0 Kgluconate, 10.0 NaCl, 2.0 $MgCl_2$, 0.5 K_2EGTA, 10.0 HEPES, 4.0 Na_2ATP, 0.3 Na_2GTP, pH 7.3. Synaptic currents were evoked using a concentric stimulating electrode. Stimuli were 20μs in duration and 5-10V in strength. To remove any contribution of $GABA_A$ receptors, currents were evoked in the presence of bicuculline (10μM).

Upon membrane breakthrough, neurones displayed electrophysiological characteristics similar to those previously attributed to cholinergic interneurones. Thus, the resting membrane potential immediately following formation of the whole cell configuration was -57.8 ± 1.2mV (n=42; mean ± S.E.M.) and the mean apparent input resistance was 214.0 ± 64.7MΩ (n=42).

In whole cell voltage clamp experiments at –60mV, intrastriatal stimulation evoked inward synaptic currents in 37 of 42 cells. Synaptic currents reached their peak within 2.5 ± 0.2ms of onset and were 113.5 ± 5.8pA in amplitude. These currents were completely abolished after addition of the ionotropic glutamate blockers dihydroxy-6-nitro-7-sulfamoyl-benzo (F)quinoxalone (NBQX; 5μM) and D-2-amino-5-phosphovalerate (D-AP5; 50μM) and were therefore termed excitatory post synaptic currents (EPSCs). Application of the non-selective mGluR agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD; 50μM) induced a depression of EPSCs evoked by intrastriatal stimulation (to 65.1 ± 9.3% of control; n=4; $p < 0.01$, Students t test) that was rapid in onset and reversed slowly (5-15mins). This depression of synaptic transmission could be mimicked by application of the selective group II mGluR agonist (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG IV; 10μM reduced responses to 28.6 ± 7.7% of control; n=7, $p < 0.005$) or the selective group III mGluR agonist L(+)-2-amino-4-phosphonobutyric acid (L-AP4; 50μM reduced responses to 35.0 ± 10.3% of control; n=3, $p < 0.005$). In contrast, the group I selective agonist (RS)-3,5-dihydroxyphenylglycine (DHPG; 25μM) was without effect on the evoked response (n=3). Consistent with these findings, the non-selective group II/group I mGluR antagonist (s)-α-methyl-4-carboxyphenylglycine (MCPG; 500μM) was seen to block the response to 1μM DCG IV (n=3) but was without effect on the depression of EPSCs induced by 10μM L-AP4 (n=3).

In conclusion, we have demonstrated that glutamatergic synaptic responses in striatal cholinergic interneurones are modulated by group II and group III metabotropic receptors.

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107P AGONIST ACTIVITY OF SULPHUR-CONTAINING EXCITATORY AMINO ACIDS AT PRESYNAPTIC METABOTROPIC GLUTAMATE ADRENOCEPTORS

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Group I metabotropic glutamate receptors ($mGlu_{1+5}$) are positively coupled to phospholipase C and the mobilisation of intracellular calcium. We have recently shown that presynaptic group I mGlu autoreceptors mediate a positive modulatory control on synaptic glutamate release in the rat forebrain both in vitro (Croucher et al., 1997; Thomas et al., 1999) and in vivo (Patel & Croucher, 1998). Recently, certain sulphur-containing amino acids (SCAAs) have also been shown to stimulate phosphoinositide hydrolysis (Porter & Roberts, 1993; Kingston et al., 1998). We now report that the SCAAs L-cysteic acid (CA) and L-cysteine sulphinic acid (CSA) also enhance neuronal glutamate release by activation of presynaptic group I mGlu receptors.

Glutamate release was studied using [^{3}H]D-aspartate ([^{3}H]D-asp) as a non-metabolisable marker for glutamate. Serial forebrain slices were cut and incubated as previously described (Patel & Croucher, 1997). After loading with [^{3}H]D-asp slices were superfused with oxygenated Krebs buffer and the influence of drugs on basal and electrically stimulated release of label was examined. Results are means of 3-7 independent observations. CSA, 1-100μM, dose-dependently enhanced electrically stimulated efflux (max. response 28.05-fold enhancement at 100μM; $P<0.01$) without influencing basal efflux. The broad spectrum mGlu receptor antagonist, (±)-α-methyl-4-carboxyphenylglycine ((±)-MCPG), 200μM, decreased the response to CSA, 10μM, by 78.4% ($P<0.05$ compared to CSA alone). However, the $mGlu_1$ receptor antagonist, (RS)-1-amino-indan-1,5-dicarboxylic acid (AIDA), 100-500μM, was inactive in this respect. CA, 3-100μM, also significantly potentiated electrically stimulated efflux with a maximum 6.1-fold enhancement seen following CA, 3μM ($P<0.01$). The somewhat lower responses seen at higher concentrations of CA were restored to maximal responses by the benzothiadiazide, cyclothiazide (10μM; $P<0.05$), suggesting the presence of mGlu receptor desensitization. As with CSA, (±)-MCPG, 200μM, significantly inhibited the response to CA, 3μM (79.0% reduction; $P<0.05$) whilst AIDA, 100-500μM, was without effect. Interestingly, CA, 1μM, caused a significant *reduction* in electrically stimulated efflux of label (to 39.1% of control values; $P<0.01$). The inhibition of this response by (±)-MCPG (200μM; $P<0.05$) suggests an action at inhibitory presynaptic group II mGlu receptors. The present results demonstrate a predominant positive modulatory action of SCAAs on synaptic glutamate release mediated by activation of presynaptic group I mGlu receptors, probably of the $mGlu_5$ subtype.

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108P WEAK ANTI-ABSENCE ACTION OF ETHOSUXIMIDE INFUSED DIRECTLY INTO THE VENTROBASAL THALAMIC COMPLEX IN A GENETIC RAT MODEL OF ABSENCE EPILEPSY

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Ethosuximide (ETX) is used clinically for its selective effect on absence seizures. Generation of the spike and wave discharges (SWD) that characterise absence epilepsy requires functional connectivity of thalamocortical circuitry. Low threshold Ca^{2+} potentials, generated by the low-voltage activated Ca^{2+} current, I_T, have been suggested to be involved in the activity of thalamic neurones during SWD, and the ability of ETX to reduce I_T in the same neurones has been interpreted as the mechanism by which this drug exerts its therapeutic action (Coulter et al., 1989). However, recent studies have shown no effect of ETX on I_T in thalamic neurones (Leresche et al., 1998) and the lack of low-threshold Ca^{2+} potentials in the activity of thalamocortical neurones in the ventrobasal thalamus (VB) during SWD (Pinault et al., 1998). To clarify whether ETX affects SWDs by an action in relay nuclei, we have now infused ETX directly into the ventrobasal thalamus of GAERS (Genetic Absence Epilepsy Rat from Strasbourg).

GAERS (306±13g) were anaesthetised with medetomidine/ketamine (0.5 & 75 mg/kg i.p. respectively) and implanted with a bipolar EEG electrode in the frontal cortex (AP, 2.2:L, 2.4; V, 2.6) and, bilaterally, with guide cannulae in the VB (AP, -2.1;L, 2.2; V, 5.0). The following day, the EEG signal was amplified, filtered and recorded (Neurolog NL 824/820/135/530) for a 30 min basal period. There were 5 experimental groups (n=4 each group); (a) ETX (1 nmol/side) infused directly into VB, (b) ETX (10 nmol/side) as in (a), (c) ETX (20 nmol/side) as in (a), (d) the $GABA_B$ antagonist, CGP 36742 (27 nmol/side) as in (a), and (e) systemic administration of ETX (100 mg/kg i.p.). Following drug administation, EEG was recorded for a further 2 x 30 min periods. SWD are expressed as the percentage of each 30 min time period. Drug effects are assessed by one-way ANOVA, with post-hoc comparison to basal values using Dunnett's test when significant ($p<0.05$) differences were found.

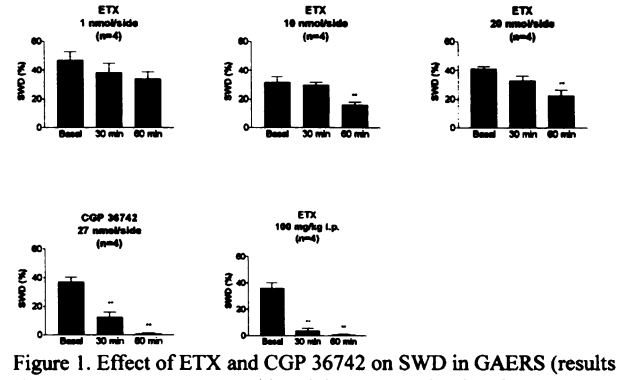

Figure 1. Effect of ETX and CGP 36742 on SWD in GAERS (results shown as mean ± s.e.mean, ** $p<0.01$ compared to basal).

Systemic administration of ETX (100 mg/kg i.p.) or direct infusion of CGP 36742 into VB (27 nmole/side) produced an immediate reduction in SWD, with almost complete cessation within 1h. In contrast, infusion of ETX into VB (1 - 20 nmol/side) had no effect on SWD within the first 30 min, and produced only a 50% reduction in SWD within 1h at the higher doses. These results suggest that ETX is not acting within the VB and that the delayed response may reflect diffusion of the drug to other thalamic areas. In addition, they support the suggestion that $GABA_B$ antagonists and ETX have different sites of action for their effects on SWDs.

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Pinault, D., Leresche, N., Charpier, S. et al. (1998) *J.Physiol.* 509,449-456.

109P BTS 72664: A BROAD SPECTRUM ANTICONVULSANT AS DEMONSTRATED IN A VARIETY OF RAT AND MOUSE EPILEPSY MODELS

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Epilepsy is due to imbalances in excitatory and inhibitory neurotransmission. Induction of tonic-clonic seizures in rodents using maximal electroshock is commonly used to model perturbations of excitatory neurotransmission, whereas induction of seizures with the $GABA_A$ receptor blocker, bicuculline, or the chloride channel blocker, picrotoxin, or another putative $GABA_A$ blocker, leptazol, provide models of inhibitory perturbations. In addition, GEPR-9 rats and DBA-2 mice provide audiogenic seizure models. The activity of the compound BTS 72664, (*R*)-7-[1-(4-chlorophenoxy)ethyl]-1,2,4-triazolo[1,5-*a*] pyrimidine, is investigated in this variety of seizure models, to reveal its potential as a broad spectrum anticonvulsant.

Animals (190g male Sprague-Dawley or Wistar rats in groups of 8, or GEPR-9 rats derived from the Institute of Psychiatry colony, 3-6 months old, of either sex in groups of 6-7; or in 25-30g Swiss CD-1 mice of either sex in groups of 10, or DBA-2 mice of both sexes, aged 25-29 days (9-13g), in groups of 10) were given BTS 72664 in 0.5% methylcellulose, p.o. Control animals received 0.5% methylcellulose vehicle. Maximal electroshock seizures were induced using a Basille 7801 ECT unit. A shock was applied with saline-moistened earclip electrodes using 80mA, 0.6ms pulses at 100Hz for 1s for rats; or 99mA, 0.4ms pulses at 50Hz for 1s for mice. Bicuculline was prepared in physiological saline and applied to groups of 8 female 100-120g Wistar rats (0.4mg/kg i.v. 2.5ml/kg) or mice (0.55mg/kg, 10ml/kg i.v.) via the tail vein. Various post-administration times were used in mice in these two models to determine the peak effect and duration of action times. Picrotoxin was prepared in saline and given at 2.6mg/kg s.c. to mice. Leptazol was prepared in saline and given at 85mg/kg s.c. to mice. Audiogenic seizures were induced in GEPR-9 rats or DBA-2 mice according to Jobe et al, (1973). Neurological toxicity was determined using rotarod and horizontal plane tests. All these tests were conducted 30min after BTS 72664 administration. The percentage reduction in the numbers of animals showing seizures, compared with the control group, for a range of drug doses, was used to calculate the ED_{50} value and 95% confidence interval (95% C.I.) for anticonvulsant activity. The percentage change in time spent on the rotarod or horizontal plane was used to calculate the TD_{50} (deficit in 50% animals) and 95% C.I.

BTS 72664 is anticonvulsant in all *in vivo* models of epilepsy tested. ED_{50} values and 95% C.I. are given in table 1. The peak effect time is 30 min against both maximal electroshock and bicuculline seizures in mice, with a duration of action of 4-6 hrs for electroshock or greater than 6 hrs for bicuculline seizures. Rotarod TD_{50} (95% C.I) was 82mg/kg p.o. (74-90) and horizontal plane was 99mg/kg p.o. (92-107).

Table 1: Efficacy of BTS 72664 against maximal electroshock, chemoconvulsant, and audiogenic seizures in rats and mice.

	ED_{50} rat (mg/kg p.o.)	95% C.I.	ED_{50} mouse (mg/kg p.o.)	95% C.I.
Electroshock	90	63-127	77	64-93
Bicuculline	5	3-8	9	6-16
Picrotoxin			60	38-94
Leptazol			14	7-26
DBA-2 mouse			9	6-14
GEPR-9 rat	30	23-36		

BTS 72664 is anticonvulsant in a range of animal models, and therefore has broad spectrum activity. Its TD_{50} for neurological deficits is up to 11 fold greater than it neuroprotective ED_{50}. Furthermore, the similarity of ED_{50} values in tests conducted in both rats and mice, reveals a lack of selectivity for these species.

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110P ON THE MECHANISM OF ACTION OF CHLORMETHIAZOLE IN THE RAT CORTEX *IN VITRO*

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Chlormethiazole has been used clinically in ethanol withdrawal (Majumdar, 1991), as an anticonvulsant, particularly against status epilepticus (Martin & Millac, 1994) and more recently has found favour as a potential treatment for stroke (Green, 1998). The present study investigated the mechanisms responsible for the anti-epileptic actions of chlormethiazole using the *in vitro* rat cortical slice and grease gap recordings.

Cortical slices from male, Sprague Dawley rats (80-120g) were placed across a grease barrier (Harrison & Simmonds, 1985) in a chamber continuously perfused (2-2.5 ml/minute) with oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF, contents, NaCl, 135 mM, KCl, 3 mM, NaH_2PO_4 1.25 mM, $CaCl_2$ 2 mM, glucose 5 mM, $NaHCO_3$ 26mM). The potential difference across the grease barrier was continuously displayed on a chart recorder. Exposure of the slices to this aCSF, lacking Mg^{2+} ions, led to the appearance of depolarising, spontaneous epileptiform events that were sensitive to blockade by known NMDA receptor antagonists. The slices also produced reproducible, depolarising responses to 1 minute applications every 20 minutes, of the glutamate agonists, L-Quisqualic acid, (Q, 3 μM) N-methyl D-aspartate, (N, 10 μM) and γ-amino butyric acid, (GABA, 1mM) applied through the perfusate in the presence and absence of chlormethiazole. During chlormethiazole application the frequency of the spontaneous epileptiform events was counted over a 20-30 minute period.

Chlormethiazole reduced the frequency of the spontaneous epileptiform events in a concentration dependent manner, IC_{50}= 230μM (n=6). At a similar concentration (300μM) chlormethiazole did not significantly alter Q or N responses (n=4). Our results therefore point to an additional mechanism of action of chlormethiazole. We tested the possibility that our findings could be explained by a potentiation of the action of GABA at the $GABA_A$ receptor. Firstly, with the addition of 50 μM N-methyl bicuculline (a $GABA_A$ receptor antagonist) chlormethiazole was less effective at reducing the spontaneously occurring epileptiform events, IC_{50} >1mM (n=5). Secondly, 300μM chlormethiazole potentiated the responses of the cortical slices to exogenously applied GABA to 161±14% of control (n=7, mean ± s.e.m.).

Our results support the idea that chlormethiazole exerts its actions predominantly via a potentiation of $GABA_A$ergic inhibition rather than through an antagonism of glutamate receptor activation.

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111P PREGNANOLONE-INDUCED INCREASES IN [^{3}H]-FLUNITRAZEPAM BINDING INVOLVE LOW AFFINITY BINDING SITES

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The neurosteroid pregnanolone increases the binding of [^{3}H]-flunitrazepam (FNZ) to $GABA_A$ receptors (Zhong & Simmonds, 1996). These studies at 4°C used a single concentration of 1 nM [^{3}H]-FNZ which approximates to the equilibrium dissociation constant (Speth *et al.* 1979). To characterise this effect of pregnanolone in more detail, we have determined its effects on the equilibrium dissociation constant (K_D) and the maximum (B_{max}) of high affinity [^{3}H]-FNZ binding, both at 4°C and at room temperature (21°C), and for recombinant $GABA_A$ receptors expressed in fibroblasts as well as for the native receptors of rat cerebral cortex.

Neuronal membranes were prepared from the cerebral cortex of male Wistar rats by the method of Zukin *et al.* (1974). Fibroblast membranes were similarly prepared from cultures of mouse fibroblasts transfected with human α1, β3, γ2 $GABA_A$ subunits (gift of Dr P Whiting, MS&D). High affinity [^{3}H]-FNZ binding was measured with concentrations of ligand between 0.1 and 15 nM. In a few experiments, concentrations up to 100 nM were used to identify a possible lower affinity component. Pregnanolone 3 μM was incubated with the membranes for 10 min at 37°C prior to a further 60 min incubation with [^{3}H]-FNZ at 4°C or 21°C. Specific binding was 90-95% of the total, as determined by displacement with 10 μM unlabelled FNZ. The high affinity binding data were fitted by a rectangular hyperbola from which K_D and B_{max} were determined (Prism). Statistical comparisons were made by ANOVA and the Tukey-Kramer test.

Table 1 shows that pregnanolone consistently increased B_{max} and also K_D ($P<0.05$). Such a combination of increased numbers of FNZ binding sites in the high affinity range, associated with an overall reduction in the measured affinity, could occur if pregnanolone had increased the affinity of a low affinity population of FNZ binding sites so that its binding curve overlapped that of the high affinity population to an increased extent. The existence of a low affinity population was confirmed by an increase in binding above the calculated high affinity B_{max} when the [^{3}H]-FNZ concentration was further increased, but we did not extend the concentration range sufficiently to make reliable estimates of K_D and B_{max} for the low affinity population.

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Zhong, Y. & Simmonds, M.A. (1996) *Neuropharmacology* 35, 1193-1198.
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TABLE 1.

[^{3}H]-FNZ binding		Cortex 4°C	Cortex 21°C	Fibroblast 21°C
Control	B_{max} (fmoles . mg protein^{-1})	488.9 ± 29.5 (4)	472.3 ± 33.5 (4)	336.8 ± 50.0 (7)
	K_D (nM)	1.70 ± 0.15 (4)	4.40 ± 0.28 (4)	6.05 ± 0.67 (7)
Pregnanolone 3 μM	B_{max} (fmoles . mg protein^{-1})	754.7 ± 72.9 (4)	851.2 ± 63.3 (4)	661.5 ± 64.1 (4)
	K_D (nM)	2.53 ± 0.13 (4)	7.52 ± 0.47 (4)	28.1 ± 3.97 (4)

112P EFFECTS OF CHOLESTEROL AND SOME ANALOGUES ON [^{3}H]-FNZ BINDING

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Cholesterol has been reported to be necessary for the functional activity of many membrane proteins (Liscum *et al.*, 1995). Cholesterol can modulate receptor function by two distinct mechanisms, by changes in the membrane fluidity and/or by highly specific molecular interactions (Gimpl *et al.*, 1997). Here we study the structural requirements of the cholesterol-receptor interactions on [^{3}H]-Flunitrazepam (FNZ) binding by pre-incubating neuronal membranes with various cholesterol analogues. The effect of these pre-incubations on the action of pregnanolone was also studied.

Neuronal membranes were prepared from cerebral cortex of male Wistar rats (250-300g) to yield a "buffy coat" fraction (Zukin *et al.*, 1974) which was well washed prior to storage at -20°C in wash buffer (5mM Tris base, 1mM EDTA, pH 7.4). Neuronal membranes were pre-incubated with 0.3mM sterol (cholesterol, epicholesterol, coprostanol or epicoprostanol) complexed with 3mM methyl-β-cyclodextrin as a carrier, in physiological salt solution, for 30min at 32°C. [^{3}H]-FNZ binding on cortical membranes was performed with final ligand concentrations over the range 0.1-21nM for 1h at room temperature. In experiments with pregnanolone 3μM, it was added alone to the pre-incubated membranes, for 10mins at 37°C before addition of [^{3}H]-FNZ and remained present during the binding incubation. Non-specific binding was determined with 10μM unlabelled Flunitrazpam and was in the region of 5-10%. Maximum binding (B_{max}) and the equilibrium dissociation constant (K_D) for [^{3}H]-FNZ were calculated from a rectangular hyperbola fitted to the data, and are expressed as mean ± S.E.M. Statistical analysis was by ANOVA + Tukey-Kramer test, with $P<0.05$ taken as significant.

Enriching membranes with cholesterol to 188% control did not significantly affect [^{3}H] FNZ binding. Pregnanolone caused similar significant B_{max} increases in cholesterol enriched membranes, from 636.5±79.96 (n=6) to 977.1±103.9 (n=7) fmol/mg protein in control membranes and from 491.6±56.8 (n=8) to 869±78.9 (n=5) fmol/mg protein in cholesterol enriched membranes. In contrast, pre-incubation with epicholesterol, the 3α-OH analogue of cholesterol, significantly reduced FNZ binding to 441.8±48.95 fmol/mg protein (n=5), and completely prevented the effect of 3μM pregnanolone, the FNZ B_{max} value being 411.8±88.69 fmol/mg protein (n=4). Pre-incubation with coprostanol, the ring-reduced (5β) analogue of cholesterol, had no significant effect on FNZ binding, the FNZ B_{max} value being 586.3±63.89 fmol/mg protein (n=8), but it did completely prevent the effect of pregnanolone, the FNZ B_{max} value being 495.7±82.19 fmol/mg protein (n=7). Epicoprostanol pre-incubation, however, caused a marked increase in FNZ B_{max} to 1024±78.9 fmol/mg protein (n=6). This elevated B_{max} was not further increased by pregnanolone 3μM, (883.1±36.36 fmol/mg protein, n=6). These effects of epicoprostanol were not seen if it was present only during the binding incubation without methyl-β-cyclodextrin. Significant increases in B_{max} were always accompanied by significant increases in kd, which has been interpreted as an increase in the affinity of low affinity FNZ binding sites (Simmonds & Haneef, this meeting).

The data suggest that epicoprostanol acts like pregnanolone whereas coprostanol and epicholesterol antagonise pregnanolone. Unlike pregnanolone, the cholesterol analogues require pre-incubation with methyl-β-cyclodextrin.

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Liscum, L., & Underwood, K.W. (1995) *J. Biol. Chem.* 15443-15446
Zukin, S.R., Young, A.B. & Snyder, S.H. (1974) *Proc. Natl Acad. Sci.* USA 71, 4802-4807.

113P EFFECT OF ANTIDEPRESSANT DRUGS ON GLUCOCORTICOID RECEPTOR TRANSLOCATION IN RAT HIPPOCAMPUS

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It has been repeatedly suggested that depression may be explained by a defective glucocorticoid feedback inhibition (Barden *et al.*, 1995). Glucocorticoids exert their negative feedback through binding to the glucocorticoid receptor (GR) in tissues of the hypothalamic-pituitary-adrenocortical axis (HPA) and in the hippocampus. After activation, the GR translocates to the nucleus and binds to the specific target DNA sequence for initiating changes in gene expression. In this study, we have examined the effect of *in vitro* and *in vivo* antidepressant treatment on GR translocation in the rat hippocampus.

Hippocampal primary cultures were prepared from 18 day-old Wistar rat fetuses and exposed to desipramine (DMI) or fluoxetine (FLU) (10 μM) for different times. In *in vivo* studies, male Wistar rats (170-180 g) received acute or chronic antidepressant treatment. In acute treatments, rats were killed 3 or 24 h after DMI (10 mg kg^{-1} ip) or FLU (5 mg kg^{-1} ip). In chronic treatments, rats received DMI (10 mg kg^{-1} ip) or FLU (5 mg kg^{-1} ip) for 21 consecutive days and were killed 24 h or 7 days after the last injection. The cytosolic depletion and nuclear translocation of GR was assessed by Western blot of cytosolic and nuclear extracts which were hybridized with an specific polyclonal antibody. The intensity of the hybridization signals was quantified by densitometric analysis and expressed as arbitrary units of optical density (OD).

Both DMI and FLU induced translocation of the GR from the cytosol to the nucleus in cultures of hippocampal neurons 2 h after exposure to the drugs. The effect of DMI was more sustained and was still apparent at the 24 h time point (Fig. 1). *In vivo*, GR translocation was found 3 h after acute drug administration and also 24 h after DMI (Table 1). Chronic treatment with either antidepressant drug did not induce, however, any effect on cytosolic depletion of the GR (not shown).

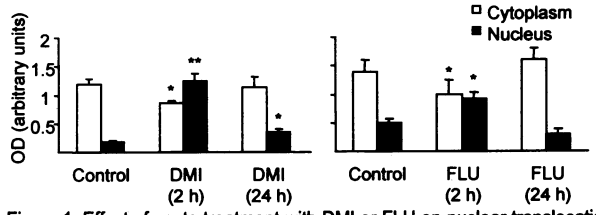

Figure 1. Effect of acute treatment with DMI or FLU on nuclear translocation of GR in cultured hippocampal neurons. Values are means±s.e.mean of 4-5 estimates. * P<0.05; ** P<0.01 vs control.

Table 1. Effect of acute treatment with DMI or FLU on nuclear translocation of GR in rat hippocampus

	OD (arbitrary units) 3 h	24 h
Control	1.40±0.08	1.40±0.08
Desipramine	1.94±0.20*	1.74±0.01*
Fluoxetine	1.80±0.04*	1.20±0.20

Values (means±s.e.mean of 5-6 rats) correspond to nuclear hybridization signals. *P<0.05 vs control.

The results show an early effect of antidepressant treatment on GR translocation in the rat hippocampus, both *in vitro* and *in vivo*, which may be perhaps of relevance at the time of interpreting other adaptive changes related to antidepressant action.

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Barden, N., Reul, J.M.H. & Holsboer, F. (1995) *Trends Neurosci.* **18**, 6-11.

114P EVIDENCE THAT CCR5 AND CXCR4 CHEMOKINE RECEPTORS ARE INVOLVED IN THE MECHANISMS OF HIV-1 GP120-INDUCED APOPTOSIS IN THE NEOCORTEX OF RAT

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Intracerebroventricular (i.c.v.) injection of gp120 causes apoptosis in the brain neocortex of rat suggesting that this may be implicated in neuronal loss described at *post-mortem* in the brains of patients suffering from AIDS dementia complex (see Bagetta *et al.*, 1999). It has been shown that gp120 binds to macrophages and T cells via the chemokine receptors CCR5 and CXCR4, respectively, which function as co-receptors of HIV in addition to CD4 and also are expressed by neurones and microglia (see Miller and Meucci, 1999).

The present experiments investigate the role CXCR4 and CCR5 receptors in the mechanisms of gp120-induced apoptosis. Male Wistar rats (250-280 g) received a single daily dose of gp120 (100ng i.c.v.) given alone or preceded (1h beforehand) by the β-chemokines (natural ligands for CCR5) RANTES, MIP1α or by the α-chemokine (natural ligand for CXCR4) SDF1α (0.25 and 2.5 pmoles given i.c.v. once daily), for 7 consecutive days; the injection was made using a 5 μl Hamilton syringe (2 μl volume of injection; 1μl min^{-1} rate). Apoptosis was assessed in coronal tissue sections (n=6 per brain) from the brain of rats sacrificed 24h after the last injection of gp120 by using the TUNEL method and haematoxylin and eosin staining (see Bagetta *et al.*, 1999). Quantitation of apoptotic cells (TUNEL$^+$ cells [1161 $\mu m^2]^{-1}$) in the brain neocortex was according to Bagetta *et al.* (1999).

Table 1 shows that a dose of 0.25 pmoles (i.c.v.) of the three chemokines tested abrogated apoptosis caused by gp120 whereas a higher dose (2.5 pmoles) of RANTES and SDF1α was ineffective. In fact, 2.5 pmoles of SDF1α *per se* produced apoptosis and this may be responsible for the lack of protection reported above.

Table1. α and βchemokines prevent gp120-induced apoptosis

Treatment (pmoles/i.c.v.)	N° of rats	TUNEL$^+$ cells (mean±s.e.m.) U	T
gp120 (100 ng, i.c.v.)	6	1.0±0.1	3.9±0.3
gp120+RANTES 0.25	3	0.2±0.1	2.2±0.1*
gp120+ RANTES 2.5	3	0.3±0.1	3.3±0.1
gp120+MIP1α 0.25	3	0.3±0.1	2.1±0.2*
gp120+ MIP1α 2.5	3	0.1±0.0	2.3±0.2*
gp120+SDF1α 0.25	3	0.0±0.0	2.2±0.1*
gp120+ SDF1α 2.5	4	0.7±0.2	4.1±0.3
SDF1α 2.5	4	0.4±0.1	1.6±0.3

U=untreated side; T=treated side. *denotes P<0.01 vs gp120 (ANOVA & Tukey-Kramer test).

In conclusion, we suggest that neuronal and microglial mechanisms downstream CCR5 and CXCR4 receptors stimulation may be responsible for apoptosis induced in the neocortex of rat by gp120 from the T tropic HIV-1 IIIB strain.

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Bagetta, G., *et al.* (1999) *Neuroscience* **7**, 1722-1724.
Miller, R.J. and Meucci, O. (1999) *TINS* **22**, 471-479.

115P EXOGENOUS NITRIC OXIDE POTENTIATES RESPONSES OF TRIGEMINAL NEURONES TO ELECTRICAL OR CHEMICAL STIMULATION IN A RAT MODEL OF MIGRAINE

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Endogenous nitric oxide (NO) is becoming increasingly implicated in the pathogenesis of migraine headache. Exogenous NO precipitates migraine attack in susceptible individuals (Thomsen *et al.*, 1994) and inhibitors of NO synthesis abort spontaneous migraine (Lassen *et al.*, 1997). Infusing glyceryl trinitrate (GTN) as an NO donor, we have used electrophysiological and c-*fos* immunostaining techniques to study the effects of exogenous NO on excitation of neurones in the nucleus trigeminalis caudalis (NTC) of the rat.

In all experiments, GTN or equivolumetric amounts of 0.9% saline were infused into urethane-anaesthetised (1.5g/kg) male Wistar rats (225-250g) via a femoral vein cannula. Extracellular recordings were made in the NTC from neurones responding to electrical stimulation (0.1Hz, 2msec, 1-10mA) of periorbital skin, or dura, following partial craniotomy. Responses of single units were monitored before, during and after a 30 min infusion of GTN (2μg/kg/min). Changes in neuronal excitability were assessed by recording the magnitude of reponses to repeated stimulation and by measuring activation threshold. The magnitude of NTC neurone responses (spikes/minute) to stimulation of periorbital afferents was potentiated significantly by GTN (166±20%, n=4) compared to pre-infusion levels or responses of neurones recorded during saline infusions (n=4; 2-way ANOVA, $P<0.001$). The mean threshold for activation of NTC neurones by stimulation of dural afferents was significantly reduced during/post-GTN (3.02±0.71mA, n=26) when compared to threshold measured during/post-saline infusion (4.51±0.33mA, n=26; Student's t-test, $P<0.005$).

In studies of the expression of protein product (FOS) of the immediate/early gene *c-fos*, rats were transcardially perfused with saline/paraformaldehyde 90 minutes after the end of GTN or saline infusion. The brainstem and upper cervical cord were removed for post-fixation, sectioning and staining to visualise FOS immunoreactivity (FOS-IR). Compared to saline-infused controls, FOS-IR was unchanged by GTN alone. Infraorbital subdermal injection of capsaicin, given immediately after saline infusion, induced a significant 215% increase in FOS-IR in ipsilateral NTC. Capsaicin-evoked FOS-IR was significantly increased by GTN: the number of FOS positive cells per section rose by 56% from 27±2 to 42±5 (Dunnett's test, $P<0.05$).

These results in the anaesthetised rat, suggest that GTN alone may not activate trigeminovascular pathways. Nevertheless, exogenous NO sensitises the trigeminovascular system and potentiates the response to noxious stimulation of cutaneous sensory afferents or activation of dural afferents. This may underly some of the pathogenesis of migraine headache.

Lassen *et al.* 1997, Lancet **349**, 401-402.

Thomsen *et al.* 1994, Eur. J. Neurol. **1**, 73-80.

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116P AN INVESTIGATION OF THE HYPOTHESIS THAT NON-STEROIDAL ANTI-INFLAMMATORY DRUGS MODULATE NEURONAL LIGAND-GATED ION CHANNELS

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Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely consumed drugs worldwide. These agents enter the CNS (Bannwarth *et al.*, 1989) and are associated with adverse CNS effects including coma and convulsions (Smolinske *et al.*, 1990). These observations suggest that NSAIDs may have direct effects on neuronal function. Indeed we have recently demonstrated that the NSAID, mefenamic acid, is a highly subunit selective modulator of human recombinant $GABA_A$ receptors (Halliwell *et al.* 1999). This study investigates four NSAIDs, namely acetylsalicylic acid, ibuprofen, indomethacin and flufenamic acid for activity on native $GABA_A$, $5\text{-}HT_3$, nACh and $P2_x$ receptors expressed in the isolated rat vagus nerve.

Agonist-evoked responses were recorded from vagus nerves, excised from male Wistar rats (250-300g), using a standard extracellular recording technique. All drugs were perfused onto the nerve in a physiological salt solution. The contact time for agonists was 2.0-2.5 minutes. Control antagonists and NSAIDs were pre-incubated for at least 15 minutes before agonist responses were determined in their presence. Responses were measured at their peak amplitude and, in the presence of drugs, are expressed as a percentage of those in their absence (control). All recordings were made at ambient room temperature (20-23°C).

Application of GABA (3μM-3mM), 5-HT (100nM-30μM), 1,1-dimethyl-4-phenylpiperazinium (DMPP; 3μM-300μM) and α,β-methylene adenosine 5'-triphosphate (α,β MeATP; 1μM-300μM) to the vagus nerve evoked concentration-dependent depolarisations with EC_{50} values (geometric mean and 95% C.I.) of 45μM (38μM-54μM, n=22), 1 μM (0.94μM-1.1μM, n=36), 26μM (22μM-29μM, n=40) and 47μM (38 μM-50μM, n=19) respectively. Submaximal GABA (50μM), 5-HT (1μ M), DMPP (30μM) and α,β MeATP (30μM) evoked responses were inhibited by bicuculline (1μM), MDL-72222 (100nM), hexamethonium (1μM) and pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (10μM) to (mean±s.e.m) 61±5%, (n=8), 30±4% (n=9), 28±4% (n=9) and 32±6% of control responses, respectively. These data are consistent with activation of $GABA_A$, $5\text{-}HT_3$, nACh and $P2_x$ receptors in the vagus nerve.

[Agonist] (μM)	ASA	IBU	INDO	FFA
GABA (50)	111±4%	88±11%	73±6%	91±9%
5-HT (1)	85±1%	108±4%	106±4%	65±4%
DMPP (30)	77±2%	77±7%	83±9%	50±9%
α,β MeATP (30)	117±14%	84±4%	97±5%	59±7%

Table 1: The effects of acetylsalicylic acid (ASA), ibuprofen (IBU), indomethacin (INDO) and flufenamic acid (FFA) (all 100μM) on submaximal GABA, 5-HT, DMPP and α,β MeATP evoked-responses. The responses are expressed as % of control ±s.e.m., n= 3 - 6 for all.

Table 1, above, shows that the 4 NSAIDs tested had relatively little effect (up to 100μM) on the ligand-gated ion channels investigated. Concentration inhibition curves for NSAIDs that inhibited control responses by ≥ 25% at 100μM, however, revealed an IC_{50} of 260μM for indomethacin on GABA (50μM) evoked responses and, for flufenamic acid against 5-HT (1μM), DMPP (30μM) and α,β-MeATP (30μM) evoked responses 300μM, 96μM and 120μM, respectively.

In conclusion, these data indicate that the NSAIDs investigated in this study, at concentrations equal to or greater than those needed for cyclooxygenase inhibition (Gierse *et al.* 1995) have weak or no direct effect on a range of native neuronal ligand-gated ion channels.

Bannwarth B., *et al.* (1989) *Biomed. Pharmacother.*, 43, 121-126.
Gierse J.K., *et al.* (1995) *Biochem J.*, 305, 479-484.
Halliwell R.F., *et al.* (1999) *Eur. J. Neurosci.*, 11, 2897-2905.
Smolinske S.C., *et al.* (1990) *Drug Safety*, 5, 252-274.

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117P LONG-LASTING NEUROPROTECTIVE EFFECT OF CASPASE-1 INHIBITION BY Ac-YVAD.cmk: INVOLVEMENT OF APOPTOSIS AND INFLAMMATION

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Among the mechanisms which contribute to delayed progression of ischemic brain damage apoptosis and inflammatory processes play a central role (Dirnagl et al., 1999). The pro-inflammatory cytokine IL-1β originates through the activity of caspase-1, which is also an up-stream activator of the caspase cascade, a pathway leading to apoptotic cell death. For this reason, we evaluated the neuroprotective effects of an irreversible, caspase-1 selective inhibitor: Ac-YVAD.cmk. In addition, we analyzed the effects of drug treatment on apoptosis and on production and release of some inflammation mediators.

Focal cerebral ischemia was induced in male Sprague-Dawley rats (250g) by permanent, unilateral occlusion of the left middle cerebral artery (MCA). Rats (n=13-15/group) were given either the peptidic, caspase-1 inhibitor Ac-YVAD.cmk (300 ng/rat, or vehicle (0,6% DMSO) by intracerebroventricular (icv) injection, 10 min after MCA occlusion. No changes of arterial blood pressure and gases were observed after treatment. Infarct volumes were determined by morphometrical analysis, on paraffin-embedded sections (10μm) stained with cresyl violet, 24 hours and 6 days after ischaemia. Caspase-1 activity was measured applying a fluorogenic assay to cerebral cortex homogenates (n=7-19) supplemented with Ac-YVAD.amc (50 mM). Quantitation of apoptotic cells was assessed by monitoring histone-associated

DNA fragments, by an immunoassay kit for detection of free-nucleosome. Levels of cytokines were determined using commercially available enzyme-linked immuno-sorbent assays.

Treatment with Ac-YVAD.cmk significantly reduced ($p<0.05$) infarct volumes 24 h after occlusion (28% reduction in treated animals). At 6 days, reduction of infarct volumes was still the same (28% reduction in treated animals; $p<0.05$). Caspase-1 activity was almost completely inhibited [Δ caspase-1 activity (pmol/min.mg of protein) vehicle: 199.6 ± 40.5, Ac-YVAD.cmk: 6.9 ± 20.7; $p< 0.01$, n=9-17) at 24 hours. However, at 6 days activity levels were similar in control and treated animals. Twenty-four hours post-ischemia, the IL-1β increase was blunted by treatment with Ac-YVAD.cmk [Δ IL-1β (pg/ml.mg of protein) vehicle: 82.33 ± 20.05, Ac-YVAD.cmk: 11.49 ± 14.98; $p< 0.01$, n=10]. Quantitation of histone-associated DNA fragments in cortex homogenates of treated animals showed a relevant reduction of free nucleosomes release [Δ Abs at 405 nm/mg of protein vehicle: 1.7 ± 0.2, Ac-YVAD.cmk: 0.8 ±0.17; $p<0.01$, n=17-19] Cortical levels of TNF-α were elevated at 24 hours, but subsided by 6 days after ischemia. Treatment with Ac-YVAD.cmk induced a significant reduction of TNF-α levels in ipsilateral cortex at 24 hours [Δ TNF-α (pg/ml.mg of protein) vehicle: 67.30 ± 11.67, Ac-YVAD.cmk: 34.94 ± 6.93; $p< 0.05$, n=7-19] while the effect disappeared at 6 days. Other pro-inflammatory (MCP-1, MIP-2 and NO) or anti-inflammatory (IL-10) mediators were not affected by treatment. In conclusion, we show that caspase-1 inhibition leads to a long-lasting neuroprotective effect by the inhibition of both cell death via apoptosis and release of proinflammatory mediators.

Dirnagl et al.,1999, *Trends Neurosci.* 22(9):391-397

118P THE SELECTIVE NMDA NR1/2B ANTAGONIST PD 174494 POSSESSES ANTIHYPERALGESIC AND ANTIALLODYNIC ACTIONS IN INFLAMMATORY AND NEUROPATHIC MODELS OF PAIN

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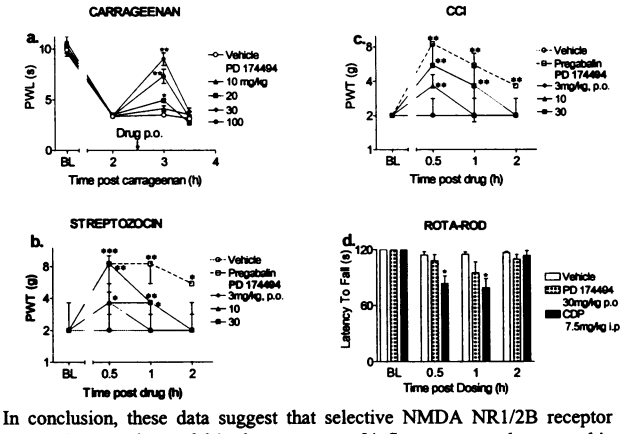
Preclinical data have shown that NMDA receptor antagonists may be useful for the treatment of pain. To date however, this class of compounds has demonstrated limited efficacy in the clinic due to intolerable side effects at effective doses. The NMDA receptor is composed of two subunits termed NR1 and NR2. There are a number of splice variants known to exist for both subunits allowing for the existence of distinct NMDA receptors. This has led to the search for selective NMDA receptor antagonists for specific NMDA receptor subtypes. PD 174494 (Co 101244) is a novel NMDA receptor ligand with high selectivity for the NR1/2B subunit combination (Zhou et al., 1999). We have examined the effect of this compound in rat models of inflammatory and neuropathic pain. Pregabalin is a novel compound related to gabapentin (Field et al., 1999) used in the present studies as a positive control.

Male Sprague Dawley rats (175-200g), obtained from Bantin and Kingman, (Hull, U.K.) were housed in groups of 6 under a 12 hour light/dark cycle (lights on at 07h 00min) with food and water *ad libitum*. All experiments were carried out by an observer blind to drug treatments. Carrageenan-induced thermal hyperalgesia was assessed using the rat plantar test (Ugo Basile, Italy) as previously described (Field et al., 1997). PD 174494 was administered p.o. 2.5h post carrageenan and paw withdrawal latencies (PWL) examined at 3 and 3.5h post carrageenan. Diabetes was induced by a single i.p. injection of streptozocin (50 mg/kg). Control animals received a similar administration of isotonic saline. For chronic constrictive injury (CCI) the sciatic nerve was ligated as previously described by Bennett and Xie, 1988. Static allodynia was evaluated as paw withdrawal threshold (PWT) by application of von Frey hairs as previously described (Field et al., 1999). PD 174494 was dissolved in PEG-200 (vehicle) and pregabalin was dissolved in saline. Drug administrations were made in a volume of 1ml/kg.

Following oral administration PD 174494 dose-dependently (10-100 mg/kg) blocked the maintenance of carrageenan-induced thermal hyperalgesia with a minimum effective dose (MED) of 20 mg/kg (Figure 1a). The dose of 100 mg/kg produced a complete blockade of the carrageenan-induced hyperalgesia. However, this antihyperalgesic action disappeared within 1h (Figure 1a). PD 174494 also dose-dependently (3-30mg/kg) blocked the maintenance of static allodynia in both the CCI and diabetic models with MEDs of 10 mg/kg (Figure 1b, c). The dose of 30 mg/kg produced an anti-allodynic action of a similar magnitude to that of the positive control pregabalin (30 mg/kg, p.o.) (Figure 1b, c). However, this action of PD 174494, unlike pregabalin, lasted around 2h in both models (Figure 1b, c). PD 174494 did not affect PWT in the contralateral paw in the CCI model (data not shown) and unlike chlordiazepoxide (CDP) it failed to produce sedation/ataxia in the rota-rod test at 30mg/kg (Figure 1d).

FIGURE 1.



In conclusion, these data suggest that selective NMDA NR1/2B receptor antagonists may be useful in the treatment of inflammatory and neuropathic pain. These selective ligands may be devoid of the limiting side effects associated with classical NMDA receptor antagonist. Further work is required to support this conclusion.

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119P UP-REGULATION OF PHOSPHACAN GENE EXPRESSION IN THE RAT LUMBAR SPINAL CORD FOLLOWING STREPTOZOTOCIN-INDUCED HYPERALGIA

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Neuropathic pain is a poorly understood and severely debilitating condition (Yaksh, 1999). In the present study we have attempted to identify changes in gene expression which are associated with this condition using subtractive hybridisation (Diatchenko *et al.*, 1996) in a rat model of diabetic neuropathy (Courteix *et al.*, 1993).

Adult male Sprague-Dawley rats were administered streptozotocin (STZ) (75 mg/kg, i.p.), whilst age-matched control animals were injected with saline. Static allodynia was measured using Von Frey hairs, animals were considered hyperalgesic if their paw withdrawal thresholds were found to be <4g. The lumbar spinal cord was rapidly dissected, snap frozen and total RNA was reverse transcribed into cDNA. The technique of suppression subtractive hybridisation was used to isolate genes differentially expressed in the lumbar spinal cord of streptozocin treated rats.

One of the genes found to be differentially expressed was the chondroitin sulfate proteoglycan phosphacan (Engel *et al.*, 1996). Phosphacan mRNA expression levels were analysed by quantitative RT-PCR using the TaqMan assay (Heid *et al.*, 1996). There was found to be a 2.5-fold ($\pm$ 0.21) increase in the expression of phosphacan mRNA in the STZ treated lumbar spinal cord, compared to untreated. To discount the possibility that the observed up-regulation in phosphacan expression was due to gliosis, semi-quantitative PCR was used to show that there was no significant increase glial fibriallary acidic protein gene expression between STZ treated and untreated lumbar spinal cord.

The anatomical distribution of the up-regulated phosphacan mRNA was then analysed by quantitative *in situ* hybridisation. A significant 2-fold increase (from 0.73 $\pm$ 0.009 to 1.54 $\pm$ 0.005 , $p<0.005$) in relative optical density levels was observed in all areas of the grey matter in the lumbar spinal cord of STZ treated animals compared to untreated animals.

In summary, we have shown that diabetic neuropathy is associated with a significant increase in phosphacan gene expression. Since phosphacan has been reported to perform an important role in neuronal migration and neuronal outgrowth (Milev *et al.*, 1994), this could be a measure of the central reorganisation and neural plasticity that gives rise to a 'central sensitisation' and resultant chronic pain.

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120P THE NOCICEPTIN RECEPTOR ANTAGONIST, [Nphe1]NC(1-13)NH2, REVERSES TOLERANCE TO SYSTEMIC MORPHINE-INDUCED ANALGESIA

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The mechanisms underlying the development of tolerance to the analgesic actions of opioids are not completely understood. Modifications of the cellular actions of opioids (desensitisation and receptor down-regulation) are implicated but do not fully explain opioid tolerance. Other mechanisms are therefore implicated; among these the actions of endogenous anti-opioid systems should be included. One of the best characterised anti-opioid systems is represented by nociceptin (NC) and its receptor (NCR). Previous in vitro and in vivo studies (Calo' et al., 1999) have indicated that [Nphe^1]NC(1-13)NH_2 acts as a selective and competitive NCR antagonist. In the present study we used this NCR antagonist in the mouse tail withdrawal assay (TW) to investigate whether the NC/NCR system is involved in tolerance to morphine analgesia.

Male Swiss mice weighing 25-30 g were used. Nociception was assessed using the TW assay with the water temperature set at 55°C; an experienced observer, blind to drug treatment, measured withdrawal latency times. A cut off time of 10 s was chosen to avoid tissue damage. Five mice were randomly assigned to each experimental group. TW latency was determined immediately before and at 5, 15, 30 and 60 min following treatment. Animals were injected with saline or morphine 30 mg/kg/day s.c. for 3 days. On the 4th day they received saline or [Nphe^1]NC(1-13)NH_2 30 nmol i.c.v (directly into the right lateral ventricle) soon after s.c. injection of morphine or saline. Data (mean $\pm$ s.e.mean) are expressed as percent of the maximal possible analgesic effect.

On day 1, morphine (30 mg/kg) induced a robust analgesic effect which peaked at 15 min (84 $\pm$ 5%, n=5) and lasted for at least 60 min. This effect progressively declined to 50 $\pm$ 10% on the 4th day of treatment (n=5, $p<0.05$ vs 1st day). [Nphe^1]NC(1-13)NH_2 applied at 30 nmol i.c.v. did not significantly modified *per se* TW latencies in naive or morphine tolerant mice. However, the analgesic action of morphine on day 4 was significantly enhanced in mice treated with the NCR antagonist (control 52 $\pm$ 8%, n=5; 30 nmol [Nphe^1]NC(1-13)NH_2 83 $\pm$ 4%, n=5, $p<0.05$ vs control).

NC signalling has already been implicated in the phenomenon of morphine tolerance. Indeed, it has been shown (Ueda et al., 1997) that mice lacking the NCR display reduced tolerance to the analgesic effect of morphine. Moreover, in the rat i.c.v. injected NC antibodies reverse morphine tolerance by around 50% (Tian et al., 1998). The present data, obtained by blocking endogenous NC signalling with a receptor antagonist, further substantiate the hypothesis that the NC/NCR system may play a crucial role in the development and/or expression of morphine tolerance.

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121P THE μ-OPIOID RECEPTOR RESPONSE IS UNALTERED IN ORL-1 KNOCKOUT MICE

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Nociceptin (NC) has been shown to exert a variety of biological effects some of which are similar to those of opioid peptides. Mutant mice lacking the ORL-1 receptor gene have been generated with the aim to study the physiological roles of the NC system. The present study examines whether the responses of opiate receptor subtypes are modified in mice lacking ORL-1 receptor. We carried out experiments in two different biological models: the isolated mouse *vas deferens*, which contains also μ, δ and κ opiate receptors, and the hot-plate assay. *Vas deferens* from male wild type (WT) and ORL-1 receptors knockout (KO) mice (35–45g) was used. Tissues were suspended in an organ bath containing modified oxygenated Krebs solution, under 0.3g tension and at 33°C. They were allowed to equilibrate for 30 min prior to electrical stimulation (pulse width 1 ms; voltage 200–300 mA; frequency 0.1 Hz). The electrically-evoked contractions were measured isometrically. Concentration-response curves to the selective μ agonist DAMGO (10^{-8} - 10^{-5}M) and NC (10^{-9} – 10^{-7}M) were obtained. Similar experiments were conducted in the presence of naloxone (10^{-8} – 10^{-7}M). Data were expressed as % inhibition of contraction. IC_{50} value was used to compare the effects of drugs. In *in vivo* experiments, WT and KO mice were tested using the hot-plate assay. The thermal response at 52.5 ± 0.1°C was assessed evaluating jumping latencies (cut-off 180 sec). Intrathecal injections (ith) were performed according to the method of Hylden and Wilcox (1980). In the WT *vas deferens*, NC was able to inhibit electrically-evoked contractions with an IC_{50} value of 6 nM (confidence limits: 4.5-8, n=10). The concentration-response curve of NC was not influenced by addition of naloxone (10^{-7}M). As expected, in the KO *vas deferens*, NC did not elicit any effect. In the WT and KO *vas deferens*, DAMGO induced a concentration-dependent inhibition of contractions with IC_{50} values of 154 nM (119-198, n=8) and 67 nM (36-135, n=8), respectively. In both groups this effect was antagonized by naloxone which shifted rightward the concentration-response curves to the agonist showing the same pA_2 of 8.7. Using the hot-plate test, no differences were found among baseline responses to thermal stimuli of KO and WT mice. NC (10 nmol/mouse, ith) produced a 2-fold increase of baseline latencies in WT mice, but it did not induce any changes in pain threshold of KO mice. Conversely, 30 min after administration of morphine (3 and 10 mg/Kg sc) the following test-latencies were found in KO mice: 138.8 ± 17.5 and 180 sec, respectively (vehicle: 87.9 ± 36) and in WT mice: 133.5 ± 13.3 and 180 sec, respectively (vehicle: 60.5 ± 22.5 sec). Morphine-induced analgesia lasted until 2 h after administration in both KO and WT mice. These findings confirm and extend previous observations (Nishi et al. 1997) that NC response is lacking in KO mice. Moreover, we provide evidence that the μ-opioid receptor response is similar in WT and KO mice. These data encourage the further evaluation of δ and κ opioid receptors in these models.

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122P LACK OF EFFECT OF OLEAMIDE ON EXTRACELLULAR DOPAMINE AND 5-HT IN THE STRIATUM AND ACCUMBENS OF THE RAT

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Oleamide (cis-9, 10 octadecenamide) is an endogenous fatty acid primary amide first isolated in the cerebral spinal fluid (CSF) of sleep deprived cats. Other fatty acid primary amides in addition to oleamide were identified as natural constituents of cat, rat, and human CSF suggesting a novel class of biological signalling molecule. Physiological sleep was induced in rats when given synthetic oleamide was given (i.p or i.c.v) and several chemical analogues have been shown to produce similar depressant activity (Cravatt 1995). Other studies have shown that oleamide potentiates 5-hydroxytryptamine (5-HT) agonists and that the effects of oleamide on $5HT_2$ receptor mediated behaviour were significantly inhibited by SR141716A, a cannabinoid antagonist suggesting involvement of CB_1 receptors in the actions of oleamide (Cheer 1999). Cannabinoid agonists have been shown to enhance extracellular dopamine in the nucleus accumbens, an effect abolished by cannabinoid antagonists (Tanda 1997). The present study used microdialysis to determine whether oleamide altered extracellular dopamine in a manner similar to cannabinoids and 5-HT in the accumbens and striatum of the rat.

Male Wistar rats (200-300g) were anaesthetised with halothane /N_2O/O_2 and microdialysis probes implanted into the striatum or the nucleus accumbens. The dialysis probes were continuously perfused with artificial CSF at 1.2μl/min. Dialysates were collected at 20 min intervals and after 120 min basal collection, oleamide (10mg/kg i.p) or vehicle was injected followed by 160 min collection. Samples were snap frozen and dialysates were subsequently analysed using HPLC with electrochemical detection for dopamine and 5-HT.

The results show that oleamide (10mg/kg) had no significant effect on extracellular dopamine and 5-HT in both the striatum (table.1) and the nucleus accumbens of the freely moving rat (table.2).

Table 1. % Change in striatum of basal levels post oleamide injection (n=6)

	1	2	3	4	5
Dopamine	119±25.3	146±40.9	141±37.6	93±12.0	109±31.0
5-HT	101±19.9	130±39.7	154±40.1	90±21.6	126±32.0

Table 2. % Change in nucleus accumbens of basal levels post oleamide Injection (n=6)

	1	2	3	4	5
Dopamine	109±15.3	106±24.9	111±15.6	119.3±19.0	113±17.6
5-HT	104±12.7	113±17.7	126±20.1	113±14.6	115±18.9

The present results suggests that behavioural effects of oleamide demonstrated in previous studies do not involve alterations to either dopamine or 5-HT in the striatum or the nucleus accumbens of the rat. Both GTPγS binding studies (Beckett 1999b) and C-fos immunoreactivity (Beckett 1999a) have shown oleamide to have no effect in striatal and accumbal areas and furthermore the pattern of regional activation produced by oleamide does not correlate with that of cannabinoids. In conclusion the presents results and other published data indicate that the effects of oleamide involve neither dopaminergic mechanisms nor cannabinoid receptors in the striatum or the nucleus accumbens.

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123P FACILITATORY EFFECT OF CALCITONIN GENE-RELATED PEPTIDE ON K^+-EVOKED RELEASE OF DOPAMINE IN THE RAT CAUDAL STRIATUM

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The role of calcitonin-gene related peptide (CGRP) in the processes of sensory transmission at central and peripheral sites is well recognised, but the function of CGRP in the brain is less clear. The peptide and its binding sites are densely invested in the nucleus accumbens and caudal striatum (Van Rossum *et al.*, 1997), suggesting a possible involvement in modulating dopaminergic function. Supporting this, central injection of CGRP enhances the cataleptic effect of haloperidol, while decreasing apomorphine-induced hyperlocomotion, however these actions occurred without effects on striatal contents of dopamine or DOPAC (Clementi et al., 1992). We have been investigating possible interactions between CGRP and dopamine systems, and here report on a stimulatory effect of αCGRP on K^+-evoked release of [^{3}H]-dopamine from slices of rat caudal striatum.

1.4mm diameter punches (one per side) of the striatum were cut from 400μm brain slices between 2.3 and 3.1mm posterior to bregma (male Hooded Lister rats 200-250g) and incubated for 30 min at room temperature in artificial cerebrospinal fluid (aCSF) containing the peptidase inhibitors captopril, bestatin and phosphoramidon (all 10μM), and 0.5μM [^{3}H]-dopamine (13Ci/mmol). Slices were transferred into individual chambers of a Brandel SF-20 superfusion system and after a 40-min wash period, aliquots were collected every 5 min (flow rate 0.55ml/min) for the following 60 min. One 5-min pulse of aCSF containing a 30mM excess of KCl was applied at 30 min to evoke the release of [^{3}H]-Dopamine, with αCGRP (rat) or vehicle (containing 0.045% BSA) normally added 5 min before, and during the high potassium pulse. In the experiments with tetrodotoxin (TTX), this agent was added for the duration of the experiment. Results were expressed in terms of percentage fractional rate (FR) of release. The amplitude of the peak of dopamine release in the presence of αCGRP was expressed as a percentage of that obtained in parallel in the presence of the vehicle. Each observation for concentration of test ligand was the mean from 3-4 separate experiments (4 replicates per experiment).

Prior to stimulation the basal FR of [^{3}H]-dopamine from slices of caudal striatum was stable at 0.54±0.03% (n=15); the exposure to 30mM K^+ caused a 2.16-fold increase to 1.17±0.11% (n=15). Up to 1μM of αCGRP had no effect on basal release, even after prolonged exposure, but caused a concentration-dependent increase in the K^+-evoked release of [^{3}H]-dopamine (fitted EC_{50} 215.3nM, 95% c.l. 193nM, 2.4μM, Graphpad Prism 3.0). The maximum effect was obtained with 1μM αCGRP when the FR was increased 3.75-fold over basal, representing a 73±5% increase in the evoked release over that for 30mM K^+ alone.

In experiments to test the specificity of the action of αCGRP in facilitating the K^+-evoked release, the antagonist hCGRP(8-37) was administered at 3μM at the same time as 1μM αCGRP. The stimulatory effect of αCGRP on K^+-evoked release of [^{3}H]-dopamine (57.3± 16.9 % increase over K^+ alone) was blocked by hCGRP(8-37), with the peak of release not significantly different from that obtained in parallel with K^+ alone (91.46 ± 8.7% of vehicle). hCGRP(8-37) 3μM by itself did not cause any significant change in the evoked release of [^{3}H]-dopamine. When TTX (1μM) was added the FR for dopamine did not increase over basal at any time after the changeover to αCGRP 1μM + 30mM K^+ (FR 0.52 ± 0.12% for basal *versus* 0.71 ± 0.15% for αCGRP + K^+ + TTX, n=3). This result suggests an indirect neuronal effect on dopamine release is dominant in the caudal striatal slice, as has been reported for the nucleus accumbens (Jacocks & Cox, 1992).

In conclusion, the results point to a novel modulatory role for αCGRP to facilitate dopaminergic transmission in the rat caudal striatum, by an action possibly involving release of an excitatory transmitter. The functional consequences of this effect remain to be determined.

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124P CO-OPERATION BETWEEN NICOTINIC AChR AND MUSCARINIC AChR IN THE MODULATION OF [^{3}H]DOPAMINE RELEASE FROM RAT STRIATAL NERVE TERMINALS

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Nicotinic and muscarinic subclasses of acetylcholine receptors (AChR) are widely distributed throughout the brain. Neuronal nicotinic AChR are a family of ligand gated ion channels, while muscarinic AChR belong to the family of G protein coupled receptors. In rat striatal preparations in vitro dopamine release can be evoked by stimulation of both nicotinic and muscarinic AChR (Raiteri et al., 1984; Wonnacott, 1997). This raises the possibility of co-operation ("cross-talk") between them. Here we have demonstrated that co-application of the nicotinic agonist anatoxin-a (AnTx) and the muscarinic agonist oxotremorine M (Oxo-M) resulted in the potentiation of [^{3}H]dopamine ([^{3}H]DA) release from rat striatal synaptosomes, and this may be mediated via activation of protein kinase C (PKC).

All experiments were performed on male Spague-Dawley rats (average weight 250g) obtained from the University of Bath Animal House breeding colony. For preparation of synaptosomes, rats were killed by cervical dislocation and brain striata were rapidly dissected. Preparation of synaptosomes and all superfusion procedures were carried out essentially as described previously (Soliakov et al., 1995). After loading with [^{3}H]DA, synaptosomes were washed for 20 min with Krebs-bicarbonate buffer, followed by a further 10 min with normal buffer or buffer containing antagonist. All inhibitors were applied 10 min before the stimulation with AnTx (1μM, 40 s) and/or Oxo-M. (100μM, 40 s) and mantained in the medium until the end of experiment. ^{86}Rb efflux experiments were performed in accordence to the procedure of Marks et al (1993). Results are presented as a percentage of control response (evoked with 1μM AnTx in the absence of any drugs), which was taken as 100%. For statistical analysis, paired Student's t-test was used

Oxo-M (100μM) potentiated AnTx-evoked [^{3}H]DA release (34.6±2.3% above control response to AnTx, $P<0.05$, n=4) when both agonists were applied simultaneously. No increase in the [^{3}H]DA release over baseline was observed when Oxo-M was applied alone. The inability of Oxo-M to activate nicotinic AChR was supported also by the absence of any increase in ^{86}Rb efflux from rat synaptosomes, following stimulation with Oxo-M (100μM) alone. The potentiation of AnTx-evoked [^{3}H]DA release by Oxo-M was reduced to the control level by the nonspecific mucarinic agonist atropine (10μM) and pirenzipine (300nM), a specific antagonist of M1 muscarinic AChR. In contrast, AFDX116 (100nM), more specific at the M2 type of muscarinic AChR, was not effective in inhibiting the potentiation. No inhibitory action of pirenzipine on AnTx-evoked release of [^{3}H]DA was observed, suggesting that Oxo-M potentiated the AnTx-evoked release of [^{3}H]DA via the M1 muscarinic AChR. On the other hand, [^{3}H]DA release evoked by AnTx alone or by co-application of both agonists simultaneously, was sensitive to inhibition by the specific PKC antagonist Ro 31-8220 (1μM), giving a similar reduction in both cases to 50-60% of control response.

Thus, our results suggest the exsistence of "cross-talk" between nicotinic and muscarinic AChR in the modulation of [^{3}H]DA release from rat striatal synaptosomes. The mechanism of this a co-operation may work via activation of PKC-dependent pathway(s) which eventually lead to the elevation of dopamine release.

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125P ADENOSINE MODULATES EXCITATORY SYNAPTIC TRANSMISSION IN THE DORSAL HORN OF THE ADULT SPINAL CORD VIA AN A_1 RECEPTOR-MEDIATED EFFECT

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Adenosine is known to exert multiple influences on pain transmission at the level of the spinal cord (Sawynok, 1998). Despite the potential importance of this system, little is known about the electrophysiological effects of adenosine and its analogues within the adult spinal cord. In view of this, we have recently shown that adenosine can hyperpolarise dorsal horn neurones through an A_1 receptor mediated effect (Patel *et al.*, in press). In the present study we have examined the effects of adenosine and an A_1 receptor agonist on synaptic transmission in dorsal horn neurones of the adult spinal cord.

Longitudinal spinal cord slices (150μm) were prepared from male Wistar rats (250-400g). Slices were superfused (2.5mls. min^{-1}: 32^0C) with artificial cerebrospinal fluid solution comprising (mM) 125.0 NaCl, 25.0 $NaHCO_3$, 10.0 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgCl_2$ bubbled with a 95%, 5% O_2/CO_2. Whole-cell patch clamp recordings were made from visually identified dorsal horn neurones using electrodes filled with (mM) 120.0 Kgluconate, 10.0 NaCl, 2.0 $MgCl_2$, 0.5 K_2EGTA, 10.0 HEPES, 4.0 Na_2ATP, 0.3 Na_2GTP, pH 7.2 and held at –60mV. An evoked synaptic response was attained by stimulation of the dorsal root entry zone (2-12V; 20-200μsec: 30sec intervals) using a concentric electrode placed in the dorsal root entry zone. Data are presented as mean ± SE and a Students *t* test used to determine significance.

Stimulation of the dorsal root entry zone evoked a transient inward current of 302 ± 23.7 pA that was completely abolished by the application of the excitatory amino acid antagonist NBQX (10μM; n=3). On the basis of this we term this inward current an excitatory postsynaptic current (EPSC). Bath application of adenosine (10μM) induced an outward current of 29.5 ± 5.8pA (n=5) and inhibited the EPSC by 76% (from -269.5 ± 18.5 to –64.8 ± 10.5pA; $P<0.005$, n=5). The effects of adenosine were fully reversible on washout and were completely inhibited by pre-incubation with the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DCPCX; 500nM, n=4). The effects of adenosine were mimicked by the A_1 receptor agonist R-phenylisopropyl adenosine (RPIA) in a concentration dependant manner; thus 200nM inhibited the EPSC by 48.8 ± 7.6% (n=7), 500nM by 66 ± 4.1% (n=3) and 1μM by 85.2 ± 7.5% (n=4). Like adenosine the effects of RPIA were fully reversible on washout and were completely inhibited by pre-incubation with DCPCX (500nM, n=4). In addition bath application of adenosine (50μM) or RPIA (1μM) decreased the frequency of spontaneous miniature EPSCs from 1.9 ± 0.8 to 0.9 ± 0.4Hz (n=3) and 2.9 ± 0.6 to 0.8 ± 0.3Hz (n=3) respectively without significantly affecting amplitude.

In conclusion, we have demonstrated that adenosine can modulate synaptic transmission in the dorsal horn of the adult spinal cord via A_1 receptor mediated effect.

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126P ADENOSINE MODULATES THE EXCITATORY ACTION OF VIP ON SYNAPTIC TRANSMISSION IN THE CA1 AREA OF THE HIPPOCAMPUS

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Vasoactive intestinal peptide (VIP) is a 28 a.a. peptide, which in the rat hippocampus, has been essentially associated to the GABAergic interneurones (Acsády *et al*, 1996). We have shown that VIP (0.3-30nM) has an excitatory action on GABA release from hippocampal nerve terminals, and that this effect is strongly dependent on A_{2A} adenosine receptor activation by endogenous adenosine (Cunha-Reis *et al.*, 1999). In the present work we investigated the action of VIP on synaptic transmission in the CA1 area of the hippocampus and whether the effect of this neuropeptide is modulated by tonic A_{2A} and A_1 receptor activation by endogenous adenosine.

Field-excitatory post-synaptic potentials (fEPSPs) were recorded from the CA1 area of hippocampal slices of male Wistar rats (5-6 weeks old). The slices were kept under continuous perfusion (4 ml/min) with gassed (95% O_2 / 5% CO_2) Krebs solution (mM: NaCl 124; KCl 3; NaH_2PO_4 1.25; $NaHCO_3$ 26; $MgSO_4$ 1; $CaCl_2$ 2; glucose 10). Responses were evoked by stimulation (rectangular 0.1 ms pulses, once every 15 s) of the Schaffer collateral/comissural fibres through a concentric bipolar electrode. fEPSPs were recorded through a microelectrode (4M NaCl, 3-5MΩ) placed at the *stratum radiatum*. The involvement of adenosine in the effect of VIP was tested in the presence of adenosine deaminase (ADA, 1U/ml), an enzyme that removes adenosine from the media by its conversion to inosine, a compound that is inactive in synaptic transmission. The influence of A_{2A} or A_1 receptor activation on the effect of VIP was evaluated by testing the effect of VIP in the presence of the A_{2A} receptor antagonist, ZM 241385 (20nM) or the A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 10nM), respectively. Significance of the effects of VIP was calculated by the Student's *t* test.

VIP (0.3-100 nM) caused a biphasic increase in fEPSP slope with maximum excitatory effect (25.9±2.2%, $P<0.05$, n=7) observed with 1 nM VIP. In the presence of ADA (1U/ml) this excitatory effect of 1 nM VIP on fEPSP slope was reversed into an inhibitory action (-14.5±5.8%, $P<0.05$, n=5). ZM 241385 (20nM) attenuated the excitatory effect of 1nM VIP, which under these conditions increased fEPSP slope by only 6.0±1.2% ($P<0,05$, n=3). DPCPX (10 nM) prevented the excitatory effect of 1nM VIP (% change: 5.0±2.8%, $P>0,05$, n=4).

It is concluded that at low nanomolar concentrations VIP facilitates synaptic transmission, and that this effect is dependent on activation of both A_1 and A_{2A} receptors by endogenous adenosine.

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127P IMMUNOHISTOCHEMICAL LOCALISATION OF THE ADENOSINE A1 RECEPTOR IN HUMAN BRAIN, SPINAL CORD AND TRIGEMINAL NEURALGIA

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Extracellular adenosine has a variety of actions throughout the body (Jarvis and Williams, 1990). It influences the transmission of pain stimuli at a number of sites in the central nervous system and the periphery (Sawynok, 1998). Adenosine exerts its actions by binding to specific, high affinity receptors that couple to G proteins. Of the four cloned adenosine receptor types (A_1, A_{2a}, A_{2b}, A_3), the A1 receptor appears to mediate the antinociceptive effects of adenosine (Sawynok, 1998). Little is known about the cellular localisation of the A_1 receptor protein in any human tissue. Therefore, we have developed two polyclonal antibodies and used these to assess the localisation of the A_1 receptor protein in human brain regions, spinal cord and trigeminal ganglia.

Human post mortem material was obtained from three individuals, none of whom showed any neurological disorders. Post mortem delay was 27, 30 and 50h. To raise specific antibodies, peptides were made corresponding to the following sequences: CLSAVERAWAA (antibody 64, second extracellular loop) and CIDEDLPEERPDD (antibody 72, intracellular carboxy-terminus). Peptides were coupled to keyhole limpet hemocyanine and the conjugates were used for immunisation of two rabbits each following standard protocols. Serum was obtained from regular bleeds. Immunohistochemistry and Western blotting was carried out as described previously (Schindler *et al.*, 1997). Control experiments carried out included the omission of primary antibody, the use of pre-immune serum and preadsorption of the antibodies with the antigenic peptides at 100μM overnight, prior to immunohistochemistry.

The specificity of the polyclonal antibodies was demonstrated by Western blotting. When probing blots, containing membranes from cell lines expressing the human recombinant A_1 receptor or from human cortex, both antisera detected a broad band with an apparent molecular weight of 36-40kD. No such band was apparent when using recombinant A_{2a}, A_{2b} or A_3 receptor expressing cell lines.

Immunohistochemistry, in the human cerebral cortex, showed the presence of A_1 receptor immunoreactivity (A_1-ir) in pyramidal cells, where cell bodies as well as apical and ascending dendrites were labelled. Immunoreactive pyramidal cells were present in layers II-VI, but were most prominent in layer V. In the hippocampus, strongest A_1-ir was seen in CA3 and CA4, where pyramidal cells were labelled, whilst the dentate gyrus showed no specific staining. In the cerebellum, Purkinje cell bodies and their dendritic tree were clearly labelled. Furthermore, occasionally punctate staining surrounding the Purkinje cells was observed. Diffuse staining was detected in the granular layer. In the human spinal cord, weak diffuse staining was observed in the dorsal horn, whereas in the ventral horn clearly labelled large cell bodies of motorneurones were found. In human trigeminal ganglia, A_1-ir was present in a large number of neurones, showing cytoplasmatic and membrane-bound staining. All signals were absent in control experiments.

In this study, we present for the first time the localisation of the A_1 receptor protein in human brain regions, where it shows a widespread distribution. Furthermore, we have provided immunohistochemical evidence for the presence of the A_1 receptor on cells in the trigeminal ganglia and the spinal cord, which may provide an anatomical basis for some analgesic effects mediated by the A_1 receptor.

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128P HISTAMINE DEPOLARISES CHOLINERGIC INTERNEURONES IN THE RAT STRIATUM THROUGH AN H_1 RECEPTOR-MEDIATED INWARD CURRENT

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It is known that histamine can stimulate the release of acetylcholine from the rat striatum *in vivo* (Prast *et al.* 1997). In the present study we have tested for the functional presence of histamine receptors in identified striatal cholinergic neurones.

200μm coronal brain slices containing the striatum were prepared from 13-21 day-old Wistar rats in physiological saline. Cholinergic interneurones were visually identified using infra-red video microscopy and preferentially targeted for whole cell patch clamp recordings. The physiological saline contained (mM) 125.0 NaCl, 25.0 $NaHCO_3$, 10.0 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgCl_2$ and was bubbled with a 95%, 5% O_2/CO_2 gas mixture. Physiological saline was also used as the extracellular solution whilst the intracellular (pipette) solution comprised (mM) 120.0 Kgluconate, 10.0 NaCl, 2.0 $MgCl_2$, 0.5 K_2EGTA, 10.0 HEPES, 4.0 Na_2ATP, 0.3 Na_2GTP, pH 7.3.

In whole-cell current clamp recordings, bath application of 10μM histamine depolarised all neurones tested by 12.5 ± 1.2mV (n=8; mean ± S.E.M.). This depolarisation was associated with an increase in input resistance in all neurones tested (from 161.3 ± 11.3MΩ to 259.7 ± 18.2MΩ). The effects of histamine on membrane potential and input resistance were maintained when 1μM tetrodotoxin (TTX) was added to the bathing solution (11.7 ± 1.5mV depolarisation; n=3) but could be significantly inhibited by bath addition of the H_1 antagonist triprolidine (1μM reduced the depolarisation by 80.2 ± 2.3%; $p<0.005$; Students *t* test; n=4).

In whole-cell voltage clamp recordings, histamine induced an inward current that was dose dependent in nature. Thus 10nM was without measurable effect (n=3), 1μM caused an inward current of 19.2 ± 7.2pA (n=5) and 10μM caused an inward current of 87.9 ± 16.1pA (n=11). The inward current was unaffected by the removal of extracellular Ca^{2+} with the addition of 10mM $MgCl_2$, 5μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F)quinoxalone (NBQX) and 50μM D-2-amino-5-phosphovalerate (95.3 ± 18.4pA (n=3)). Using voltage ramps from -140 to -60mV we were able to determine a reversal potential for the histamine induced current close to E_K in some cells (-88.7 ± 4.0mV; n=5) but in others we were unable to measure a consistent reversal potential. To investigate whether the inward current elicited by 10μM histamine was carried by a potassium conductance, 2mM barium was added to the bathing solution. Under these conditions the response was reduced to 39.2 + 12.4% of its control value (n=5; $p<0.005$). To test for the possible involvement of sodium ions in the generation of this current, extracellular NaCl was replaced with Tris HCl. Under these conditions, the amplitude of the histamine-induced current was reduced to 41.1 ± 6.9 % its control value (n=5, $p<0.005$).

In conclusion, we have demonstrated that histamine depolarises cholinergic interneurones in the rat striatum through activation of an H_1 receptor mediated inward current that may have multiple ionic components.

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129P MODULATION OF MUSCARINIC mRNA (m1, m2 AND m3) AFTER ACUTE AND CHRONIC CLOZAPINE AND HALOPERIDOL TREATMENT

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The atypical antipsychotic (APD) clozapine (CLOZ) is effective at treating schizophrenic symptoms without the induction of extrapyramidal side effects. Clozapine has affinity for many receptors, including the D_2, 5-HT_{2A}, and the muscarinic M_1, M_2 and M_3 receptors. However, the mechanism of its APD action remains unclear The present study investigates the effect of acute and chronic CLOZ and haloperidol (HAL) treatment upon M_1, M_2, and M_3 receptor mRNA.

Osmotic minipumps were implanted (s.c.) at day 1 in male hooded Long Evans rats (176-248g) under halothane anaesthesia. Acute APD and vehicle (VEH) treated animals were implanted with pumps containing VEH (3% acetic acid/water). For chronic APD treatment, pumps were filled with either CLOZ or HAL to give daily doses of 20 $mgkg^{-1}$ or 1 $mgkg^{-1}$ respectively. At day 22 all VEH and chronic APD treated animals were injected with VEH ($1mlkg^{-1}$; i.p.). Acute APD treated animals were injected with either CLOZ ($20mgkg^{-1}$) or HAL ($1mgkg^{-1}$) in volumes of $1mlkg^{-1}$. All animals were killed 45 min later. The brains were removed, rapidly frozen and stored at -70°C. Tissue was sectioned (20μm), and sections were prepared for *in-situ* hybridisation (ISH) as previously described (Wisden and Morris, 1994). ISH was performed using m1, m2 and m3 mRNA probes which were based on sequences used by Weiner et al., (1990). The m1 GenBank accession (Ac) no. M160406 bases 830-874; m2 bases 246-290 (Gocayne et al., 1987) and the m3 oligo Ac. No. M16407, 47-91 (Bonner at al., 1987). Oligo.'s were labelled using [^{35}S]dATP. They were exposed to Kodak Biomax MR film for 1 month then analysed using an MCID imaging system. Statistical significance was assessed using one-way ANOVA followed by Student Newman-Keuls multiple range test where appropriate. Significance was defined at $p < 0.05$.

Chronic CLOZ treatment significantly decreased hippocampal m1 mRNA in the CA1 field by 20%, whereas chronic HAL produced an increase of 25% in the CA2 field compared to control (table 1). Acute HAL and CLOZ increased m1 mRNA in the dorsolateral striatum by approximately 130% of control. No changes in m2 and m3 receptor mRNA were observed after the treatment regimes, with the exception of acute HAL which significantly increased m2 mRNA in the cingulate cortex by ~182%; (0.0331±0.0164,VEH; 0.0932±0.0181* acute HAL; n = 4-6).

CLOZ, a muscarinic antagonist, produces alterations in m1, and generally no effect on m2 or m3 mRNA's, whereas HAL, an antipsychotic with no muscarinic properties also produces changes in m1 and m2 mRNA probably indirectly via D_2 receptor antagonism. Changes in these mRNAs may have implications for the differing effects of these drugs on cognition.

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Table 1 m1 mRNA data is relative optical density (ROD). All data are expressed as mean ± sem (n=4-6 per group). * p<0.05 Vs vehicle treated animals.

	Vehicle	Clozapine		Haloperidol	
Brain region		Acute	Chronic	Acute	Chronic
Dorsolateral striatum	0.0258 ± 0.0036	0.0582 ± 0.0050 *	0.0331 ± 0.0060	0.0677 ± 0.0082*	0.0467 ± 0.0101
Hippocampus (CA1)	0.3048±0.0186	0.2941 ± 0.0115	0.2436 ± 0.023 *	0.3201 ± 0.0082	0.3384 ± 0.0104
Hippocampus (CA2)	0.2499 ± 0.0146	0.2619 ± 0.0171	0.2092 ± 0.0157	0.2801 ± 0.0119	0.3118 ± 0.0171*

130P TACRINE BLOCK OF MUSCLE NICOTINIC ACETYLCHOLINE RECEPTORS

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Tacrine (1,2,3,4-tetrahydro-9-aminoacridine) is an acetylcholinesterase inhibitor used to treat Alzheimer's disease. However, at higher concentrations than those used clinically, tacrine can also block nicotinic acetylcholine receptors (AChRs) (Clarke et al., 1994; Sugawara et al., 1997) but the mechanism of this block is unclear.

To determine how tacrine inhibits adult muscle AChRs we transiently expressed human α, β, δ and ε subunits in 293 human embryonic kidney (HEK) cells and recorded single channel responses to acetylcholine (ACh) using the patch-clamp in cell attached mode (Prince & Sine, 1998). Data were compared using Student's t-test.

At 100 μM ACh, clusters of channel activity corresponding to the activation of single AChRs were readily discernible. Within clusters, the receptor had a mean channel open-time of 1.03 ± 0.1 ms (n=4) and a high probability of being open (0.92 ± 0.02). Addition of tacrine to the patch pipette (1-100 μM) caused a concentration-dependent decrease in the probability of the channel opening within clusters (IC_{50} 4.3 ± 0.2 μM, Hill coefficient: 1.3 ± 0.07, n=3). Tacrine had two main effects on channel kinetics. First, there was a concentration- and voltage-dependent decrease in the mean channel open-time (3 μM tacrine: 0.67 ± 0.01 ms, n=3, $p<0.05$ vs zero tacrine; 10 μM tacrine: 0.5 ± 0.02 ms, n=3, $p<0.01$ vs zero tacrine, $p<0.01$ vs 3 μM tacrine). Second, an additional class of closings was observed in the closed-time histogram. The duration of these closings increased with tacrine concentration (3 μM tacrine: 1.6 ± 0.4 ms, n=3; 10 μM tacrine: 4.6 ± 1 ms, n=3, $p<0.05$ vs 3 μM tacrine).

The effects of tacrine on mean channel open-time are consistent with a site of interaction within the receptor channel. However, a simple, open-channel block mechanism predicts that tacrine-induced channel closings should be *independent of concentration*. Thus, tacrine probably binds within the AChR channel, but its binding does not follow the classic mechanism for open channel block.

Tacrine block of human AChRs has similar characteristics to block of *Ascaris* muscle AChRs by the anthelmintic morantel (Evans & Martin, 1996). Like tacrine, morantel produces closings that increase in duration with concentration. The concentration dependence of block closings produced by morantel was explained by expanding the scheme for channel block to incorporate two binding sites for morantel within the channel. We hypothesise that the unusual kinetics of tacrine at the human adult muscle AChR may likewise be due to the presence of multiple recognition sites within the channel domain.

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131P EVIDENCE FOR A DUAL INFLUENCE OF NICOTINIC ACETYLCHOLINE RECEPTORS ON HIPPOCAMPAL SEROTONIN RELEASE

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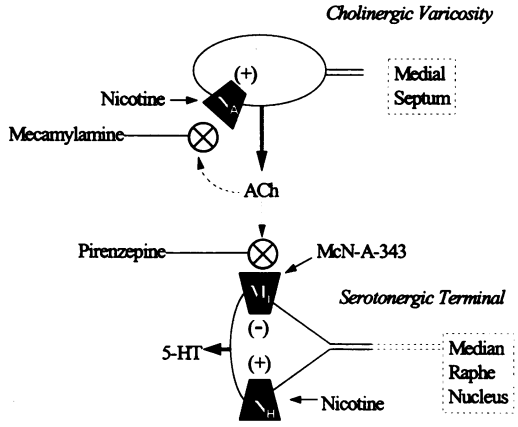

Figure 1: Proposed mechanism by which nicotine and mecamylamine modulate dorsal hippocampal [^{3}H]-5-HT release. N_A, nicotinic autoreceptor; ACh, acetylcholine; M_1, M_1 muscarinic receptor; N_H, nicotinic heteroreceptor; (+), indicates a stimulatory effect; (-), indicates an inhibitory effect; ⊗, indicates receptor blockade.

Nicotine (50-500 μM) increased the release of [^{3}H]-5-HT from isolated superfused rat dorsal hippocampal slices in a concentration-dependent manner ($p<0.001$). This effect of nicotine (250 μM) was significantly reduced (from 54.9 ± 2.2 % to 39.7 ± 4.3 %; $p<0.05$) by a low concentration of the specific nicotinic receptor antagonist, mecamylamine (0.5 μM), indicating that a nicotinic acetylcholine receptor mediates the ability of nicotine to stimulate 5-HT release. This nicotinic receptor (N_H) is probably located presynaptically on serotonergic terminals within the dorsal hippocampus because tetrodotoxin (3 μM) failed to block the effect of nicotine on 5-HT release (see Fig. 1). Further, glycine (20 mM) significantly potentiated nicotine (250 μM) stimulated 5-HT release in a strychnine-sensitive manner (from 40.8 ± 2.9 % to 81.4 ± 11.6 %; $p<0.05$). Paradoxically, higher concentrations of mecamylamine (1-50 μM) also increased the release of 5-HT ($p<0.05$), suggesting the presence of a tonically active nicotinic receptor-mediated inhibitory input to 5-HT neurones. This effect of mecamylamine (50 μM) was reduced by the muscarinic M_1 receptor agonist, McN-A-343 (100 μM) (from 69.5 ± 5.9 % to 33.9 ± 6.1 %; $p<0.05$) and by glycine (20 mM), acting at a strychnine-insensitive receptor (from 51.7 ± 11.7 % to 23.6 ± 3.1 %; $p<0.01$). Furthermore, exposure of the hippocampal slices to the M_1 receptor antagonist, pirenzepine (0.005-1 μM) increased the release of 5-HT ($p<0.001$). Since hippocampal 5-HT neurones are known to possess M_1 receptors, our results suggest that their (inhibitory) cholinergic input arises from neurones which themselves possess nicotinic receptors (N_A) (see Fig. 1).
All statistical analyses were made by Kruskal-Wallis one-way ANOVA, with Mann-Whitney U-tests for comparisons between individual groups.

132P [^{3}H]SB-269970 SELECTIVELY RADIOLABELS 5-HT$_7$ RECEPTORS IN MOUSE, RAT AND PIG BRAIN MEMBRANES

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5-HT$_7$ receptors have been shown to be present in a number of regions of rat and guinea pig brain including cortical, thalamic and limbic areas (e.g. To *et al.*, 1995). 5-HT$_7$ receptor binding studies in both recombinant systems and brain tissue have previously used the non-selective agonist radioligands [^{3}H]-5-HT or [^{3}H]-5-CT. Recently, [^{3}H]-SB-269970 has been reported to be a selective, high affinity antagonist radioligand at both human recombinant (Price *et al.*, 1999) and guinea pig brain (Atkinson *et al.*, 1999) 5-HT$_7$ receptors. In the present study, [^{3}H]-SB-269970 has been used to characterise 5-HT$_7$ receptors in brain tissues from a number of other species, namely mouse, rat and pig. The profile of binding of [^{3}H]-SB-269970 to brain homogenates from these species has been compared with the profile previously reported for human recombinant 5-HT$_{7(a)}$ receptors and guinea pig brain.

Radioligand binding to membranes prepared from mouse forebrain (whole brain minus cerebellum/brainstem), rat cerebral cortex and pig cerebral cortex was carried out as described by Boyland *et al.*, (1996), using 0.1-10nM [^{3}H]-SB-269970 for saturation studies and 1nM [^{3}H]-SB-269970 for drug competition studies. 10μM 5-HT was used to define non-specific binding.

[^{3}H]-SB-269970 bound saturably to a single population of receptors in the mouse, rat and pig brain membranes, and specific binding at 1nM represented 50-60% of total binding. K_d values for [^{3}H]-SB-269970 binding were, in each case, similar to those reported previously for binding to guinea pig cerebral cortex and human recombinant 5-HT$_{7(a)}$ receptors (Table 1). However, B_{max} values for [^{3}H]-SB-269970 binding to the mouse, rat and pig brain membranes were 4-6 fold lower than that reported for guinea pig cortex membranes (Table 1). For each species, the profile of inhibition of [^{3}H]-SB-269970 binding, defined using a range of 5-HT$_7$ receptor agonists and antagonists, correlated well with that for [^{3}H]-SB-269970 binding to the human recombinant 5-HT$_{7(a)}$ receptor (correlation coefficients were 0.96, 0.92, 0.93 for mouse, rat and pig, respectively). Hill slopes for drug inhibition of [^{3}H]-SB-269970 binding to mouse, rat and pig tissues were not significantly different from 1 (data not shown), consistent with binding to a single population of receptors in these tissues.

Table 1. K_d and B_{max} values for [^{3}H]-SB-269970 binding to human recombinant 5-HT$_{7(a)}$ receptors and brain tissue from different species

	K_d (nM)	B_{max} (fmoles mg^{-1} protein)
h5-HT $_{7(a)}$ /HEK293	1.3 ± 0.1	5800 ± 380
Guinea pig cortex	1.7 ± 0.3	125 ± 8.2
Mouse forebrain	0.9 ± 0.2	20 ± 3.3
Rat cortex	0.9 ± 0.1	30 ± 2.1
Pig cortex	1.0 ± 0.3	31 ± 7.8

Data represent the mean (± s.e.m) of at least 3 experiments

In summary, [^{3}H]-SB-269970 has been used to selectively label 5-HT$_7$ receptors in mouse, rat and pig brain. The pharmacological profile of [^{3}H]-SB-269970 binding is consistent across species. However, the density of 5-HT$_7$ receptors in mouse, rat and pig brain tissue appears low compared to that in guinea pig cortex.

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133P DOES PHENTERMINE POTENTIATE THE REDUCTION IN 5-HT REUPTAKE SITES PRODUCED BY *d*-FENFLURAMINE IN RAT BRAIN?

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The combination of fenfluramine and phentermine 'fen/phen' was widely used clinically for weight loss until the withdrawal of the fenfluramines. In man and rats, 'fen/phen' produces a greater reduction in body weight than either drug alone (Weintraub, 1992; Lew *et al.*, 1997). *d*-Fenfluramine is known to cause long-term loss of axonal 5-HT and 5-HT transporters (Schuster *et al.*, 1986; McCann *et al.*, 1994). We have determined the effects of pharmacologically equivalent doses of *d*-fenfluramine and phentermine on rat brain 5-HT reuptake sites using quantitative autoradiography.

Male CD rats (80-100g) were given *d*-fenfluramine (d-fen; 3mg kg^{-1} po) or phentermine (phen; 13.2mg kg^{-1} po) alone and in combination, twice daily for 4 days. Doses were 3 times their ED_{50} values to reduce food intake at 2h (*d*-fen 1mg kg^{-1}; phen 4.4mg kg^{-1}) determined as described by Jackson *et al.* (1997). Controls received appropriate vehicle. Following a 14 day drug-free period, whole brains were removed, divided coronally into 3 sections and frozen in iso-pentane cooled to -35°C. Coronal sections (16μM) were prepared, thaw-mounted onto gelatinised slides and processed for autoradiographic localisation of 5-HT uptake sites using [^{3}H]citalopram. Non-specific binding was defined by 1μM fluoxetine. Sections were apposed to tritium sensitive film for 8 weeks alongside commmercially available standards, allowing conversion of autoradiogram optical density to fmoles bound mg tissue equivalent^{-1}.

As in previous studies, *d*-fen reduced binding to 5-HT reuptake sites in all brain regions studied, except the dorsal raphe (Table 1; Schuster *et al.*, 1986; McCann *et al.*, 1994). In contrast, phen had no effect (Table 1). Fen/phen, at high dose, has been reported to decrease the number of striatal 5-HT reuptake sites, labelled with [^{125}I]RTI-55, compared to fen alone (Lew *et al.*, 1997). In our study, d-fen/phen reduced the density of 5-HT reuptake sites versus vehicle, however, no differences were observed when compared to d-fen alone, except a small decrease in frontal cortex (Table 1). Thus, *d*-fenfluramine-induced 5-HT reuptake site depletions are not generally potentiated by phentermine given at pharmacologically relevant doses.

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Table 1. Specific binding of [^{3}H]citalopram in rat brain following administration of *d*-fenfluramine and phentermine alone and in combination.

	Control	Phentermine	*d*-Fenfluramine	Phen + *d*-Fen
Frontal cortex	77 ± 3	77 ± 4	48 ± 4 ***	36 ± 5 *** ◆
Nucleus accumbens	67 ± 4	72 ± 4	46 ± 3 ***	40 ± 3 ***
Caudate putamen	36 ± 3	34 ± 2	29 ± 3 *	28 ± 2 *
Dorsal hypothalamus	152 ± 5	157 ± 5	117 ± 9 ***	110 ± 9 ***
Hippocampus (CA3)	131 ± 5	134 ± 2	76 ± 6 ***	87 ± 6 ***
Dorsal Raphe	241 ± 10	249 ± 11	245 ± 16	250 ± 17

Values are mean ± s.e.mean in fmoles of [^{3}H]citalopram bound mg tissue equivalent^{-1} (n=6). *p<0.05, ***p<0.001 control vs treatment; ◆p<0.05, Phen + *d*-Fen vs *d*-Fen, by a mixed linear model with treatment as a fixed factor and animal and assay as random factors with multiple t-test.

134P BIPHASIC EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR GENE IN RAT HIPPOCAMPUS FOLLOWING FLUOXETINE TREATMENT

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Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), used clinically as an antidepressant. Chronic administration of fluoxetine in rats increases hippocampal Brain-derived Neurotrophic Factor (BDNF) mRNA expression (Nibuya *et al*, 1996). In contrast, we have recently shown that single administrations of fluoxetine decrease BDNF gene expression in rat hippocampus via a 5-HT-dependent mechanism (Coppell *et al.*, 1999). Similarly, acute increases in brain 5-HT levels by tranylcypromine with l-tryptophan decrease BDNF gene expression in hippocampus (Zetterström *et al.*, 1999). To clarify these differences we compared the effects of time and of single and repeated administration of fluoxetine on BDNF gene expression in rat hippocampus.

Male Sprague-Dawley rats (250-274g) were injected twice daily with fluoxetine (10mg/kg ip) or 0.9% saline vehicle (1ml/kg) for 14d ('chronic'), or given drug by single injection ('acute'). Rats were sacrificed 4h or 24h after the final injection and transcardially perfused with saline under pentobarbitone anaesthesia. Brains were processed for *in-situ* hybridisation using [^{35}S]-dATP-labelled oligonucleotide probe complementary to rat BDNF cDNA (bases 642-686). Relative abundance of BDNF mRNA was determined by quantitative densitometry of autoradiograms. Statistical analysis was performed on values normalised to control using Dunnett's t-test, or where more than one treatment group existed normalised data was ranked and analysed using the Mann-Whitney U-test.

4h after a single injection, fluoxetine (n=17; 3 treatment groups) significantly reduced BDNF mRNA levels in dentate gyrus (DG), CA1 and CA3 by 33.8%, 19.5% and 19.9% respectively compared to saline-treated vehicle controls (n=15). At 24h following a single injection (n=6), BDNF mRNA levels were not significantly different from the 24h saline-injected group (n=6). See Table 1. 4h after chronic fluoxetine (n=8), BDNF mRNA levels were significantly downregulated in DG by 31.4% compared to vehicle controls (n=4). In contrast, 24h following chronic fluoxetine injections (n=12; 2 treatment groups), BDNF mRNA expression displayed a significant upregulation in DG by 26.7% over vehicle controls (n=11). See Table 1.

Table 1 Hippocampal BDNF gene expression 4h or 24h after acute or chronic administration of fluoxetine expressed as % of vehicle controls (Mean ± s.e.mean)

	BDNF mRNA expression as % of Control			
	Acute 4h	Acute 24h	Chronic 4h	Chronic 24h
DG	66.2±3.7§	95.4±11.0	68.6±4.2*	126.7±6.1§
CA1	80.5±3.2§	94.7±10.4	77.8±5.0	109.1±3.4
CA3	80.1±3.2§	102.2±9.8	93.9±6.3	92.6±3.1

*p<0.05 vs control, Dunnett's t-test. §p<0.01 vs control, Mann-Whitney U-test.

In summary the present study has demonstrated that fluoxetine downregulates DG BDNF mRNA expression at 4h after both acute and chronic injections. However at 24h there appears to exist a difference, in that an acute fluoxetine treatment causes no change from control whereas chronic fluoxetine injections produce significant upregulation of BDNF mRNA expression in DG. This suggests the development of some neuroadaptive change in the chronic situation.

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135P BTS 72664: LIGAND BINDING AND ENZYME ACTIVITY SHOW NO SPECIFIC AFFINITY; [^{86}Rb] EFFLUX FROM DEPOLARISED SYNAPTOSOMES INDICATES A MEMBRANE DEPOLARISATION EFFECT

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BTS 72664, (*R*)-7-[1-(4-chlorophenoxy)ethyl]-1,2,4-triazolo[1,5-*a*] pyrimidine, has broad spectrum anticonvulsant activity in rodent epilepsy models of excitatory perturbations (maximal electroshock) and of inhibitory perturbations (bicuculline, picrotoxin, leptazol; Thompson et al, 2000). Here, ligand binding at voltage, calcium and ATP-activated potassium channels, voltage-activated sodium channels, and NMDA receptors, is used to investigate possible interactions with excitatory mechanisms, while $GABA_A$ receptor ligand binding, and glutamic acid decarboxylase (GAD) and GABA transaminase (GABA-T) enzyme assays are used to investigate possible inhibitory interactions. Efflux of rubidium [^{86}Rb] from preloaded synaptosomes is used to further investigate actions at potassium channels. From these and *in vivo* studies, a putative mode of action can be deduced.

Synaptosomal membranes were prepared from male 150-170g Sprague-Dawley or 200-250g Wistar rats by standard methods. The final washed membrane pellet was resuspended in buffer and used for Skatron filter-based binding using the ligands: [^{3}H]-batrachotoxinin-20α-benzoate, [^{125}I]-apamin, [^{125}I]-charybdotoxin, [^{125}I]-α-dendrotoxin, [^{3}H]-glibenclamide, [^{3}H]-CGS-19755, [^{3}H]-SR95531 and [^{3}H]-flunitrazepam. After determination of the K_D for each ligand, displacement of the ligand at its K_D by BTS 72664 at either a range of concentrations, or 100μM (dissolved in 0.5% ethanol or dimethylsulphoxide) was tested at room temperature, 4°C or 37°C, at times ranging from 15 min to 2 hrs. Crude, Politron-homogenised, 17-35,000g supernatant enzyme extracts were prepared from brains of male 28-30g CD-1 mice. GAD activity was determined by release of $^{14}CO_2$ from [^{14}C]glutamic acid (Blinderman et. al., 1978). GABA-T activity was determined by formation of succinic semialdehyde from GABA (Gonzalez et al., 1983). ^{86}Rb efflux from synaptosomes was determined according to Bartschat and Blaustein (1985), in the presence or absence of 1mM Ca^{++}, and either normal (5mM) or depolarising (50mM) concentrations of K^+, with BTS 72664 added at 300μM.

Percentage displacements or K_is for BTS 72664 are given in Table 1. BTS 72664 did not alter GAD or GABA-T activity at 100μM. However, it decreased the rate of ^{86}Rb efflux from synaptosomes in the presence of 1mM Ca^{++} and 50mM K^+ by 50% ($p<0.001$).

Table 1: BTS 72664 affinity at a range of ion channels and receptors.

Target	Ligand	NS Binding	K_D	% displaced or K_i
Na	batrachotoxin	aconitine 200μM	43.6±7.2nM	350±78μM
SK_{Ca}	apamin	apamin 0.5μM	16.0±2.9pM	12±3%
BK_{Ca}	charybdotoxin	charyb 10nM	180±11pM	-1±13%
K_v	α-dendrotoxin	α-dendro 0.5μM	400±40pM	3±1%
K_{ATP}	glibenclamide	glib 0.1μM	0.19±0.01nM	-38%
NMDA	CGS-19755	NMDA 100μM	5nM	28%
$GABA_A$	SR-95531	GABA 100μM	29±4.5nM	9±5%
$GABA_A$	flunitrazepam	clonazepam 2μM	1.7±0.08nM	>10μM

These data indicate that BTS 72664 does not have any marked affinity at the range of targets tested to account for its broad spectrum anticonvulsant profile. Its ability to reduce ^{86}Rb efflux suggests it has K^+ channel blocking activity, but this is not consistent with its anticonvulsant profile. A more likely explanation is that BTS 72664 counteracts depolarisation by acting as a Cl^- channel opener, congruent with its ability to prevent picrotoxin and bicuculline induced seizures.

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136P EVIDENCE THAT POLY(ADP)RIBOSE POLYMERASE IS INVOLVED IN APOPTOSIS INDUCED BY MONOCULAR DEPRIVATION (MD) IN THE LATERAL GENICULATE NUCLEUS (LGN) OF NEW-BORN MICE

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We have recently reported that MD during post-natal life induces apoptosis in the LGN of rats and this is accompanied by a significant increase in the LGN content of citrulline, the co-product of nitric oxide (NO) synthesis (Nucci *et al.*, 1998; 1999). Systemic treatment with either glutamate receptor antagonists, or inhibitor of NO-synthase abolished accumulation of citrulline, and prevented apoptosis, suggesting an excitotoxic, NO-mediated, mechanism of apoptosis in the LGN of rats (Nucci *et al.*, 1998; 1999). Here we now describe original data indicating that MD-evoked apoptosis involves poly(ADP)ribose polymerase (PARP), a nuclear enzyme activated to participate in DNA repair that can cause cell death by excessive depletion of cellular energy. PARP -/- mice (C57bl/6 x 129 background) were generated and donated by Z.Q. Wang (IARC, Lyons, France) (Wang *et al.*, 1995). They were crossed back to C57Bl/6 wild-type (wt) mice through 2 cycles of heterozygous matings, and for the experiments 14-day-old wt or -/- offspring pups were used after typing by PCR. The right eyelids of -/- or wt newborn mice (n=6 per group) were sutured for 2 and 7 days. Age-matched, non-deprived mice (n=6 per group) were used as controls. Serial brain coronal sections (15μm) were processed for *in situ* detection of DNA fragmentation according to the TUNEL technique (Gavrieli *et al.*, 1992). Morphological characteristics of adjacent brain sections were assessed under light microscopy using haematoxylin and eosin (H&E) staining. DNA fragmentation (TUNEL positive cells) was observed in areas of the sections (n=6 per brain) corresponding to (Pellegrino *et al.*, 1981) the LGN of wt mice deprived for 2 and 7 days (Tab. 1). Nuclear chromatin marginalization and condensation, typical features of apoptosis, were observed in adjacent H&E stained sections. TUNEL positive cells were never observed in the LGN of wt controls and PARP -/- mice (Tab. 1).

Table 1: MD-induced apoptosis is prevented in PARP -/- mice

	PARP +/+	PARP -/-
Control	0.0±0.0	0.0±0.0
MD for 2 days	2.7±0.2	0.9±0.2*
MD for 7 days	3.5±0.3	0.3±1.2*

Data are means±sem. *: $p<0.05$ vs wt (Student's "t" test).

In conclusion, our present and previous data suggest that MD during development causes excessive stimulation of glutamate receptors, elevates NO and produced DNA fragmentation; the latter event is known to activate PARP which in turn may cause apoptosis in the LGN of mice.

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137P NEUROPROTECTIVE EFFECTS OF THE NEURONAL CALCIUM CHANNEL BLOCKER LY 393615 AGAINST CEREBRAL HYPOXIC-HYPOGLYCAEMIC INSULT *IN VITRO*

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LY 393615 (N-Butyl-[5,5-bis-(4-fluorophenyl)tetrahydrofuran-2-yl]methylamine hydrochloride) is a novel broad-spectrum neuronal calcium channel blocker with neuroprotective properties in various *in vivo* models of cerebral ischaemia (Thomas et al, 1999). In this study, we examined its efficacy in an *in vitro* model of cerebral ischaemia.

Coronal rat brain slices (350 μm thick) at the level of the neostriatum were incubated in oxygenated artificial cerebro-spinal fluid (aCSF) at 34°C until required. Slices were then subjected to "ischaemia" by transfer to deoxygenated, hypoglycaemic aCSF for 10 minutes followed by a further 30 minutes of reincubation in oxygenated aCSF. Control slices were maintained in oxygenated aCSF and in some cases LY 393615 (10 μM) was included in the aCSF throughout.

Slices were then stained for 30 minutes at 37°C with 2,3,5-triphenyl tetrazolium chloride (TTC, a colorimetric index of mitochondrial enzyme activity), fixed in 10% formalin and transferred to coverslips. Stained slices were scanned using a desktop scanner and the images were converted to greyscale. Intensity of striatal and cortical TTC staining was analysed by Osiris™ software (Mathews et al, 1999). Statistical comparisons between groups were made by One Way ANOVA with posthoc application of the Student-Newman-Keuls test.

Control slices mostly stained a deep pink, the intensity of which was readily determined densitometrically. Ischaemia (10 min) caused a significant ($P < 0.05$) reduction in staining intensity in both neostriatum and cerebral cortex (Table 1). LY 393615 had no significant effect on staining intensity in control slices but prevented the reduction in staining induced by "ischaemia" in both striatum ($P < 0.01$) and cortex ($P < 0.001$).

Table 1: Effect of LY 393615 (10 μM) on ischaemia-induced reduction in TTC staining (expressed as percent greyscale).

	Neostriatum	Cerebral cortex
Control	45.3 ± 5.2	47.2 ± 4.0
Ischaemia	34.4 ± 4.1[a,d]	36.0 ± 3.3[a,d]
Control + LY 393615	56.6 ± 2.4	53.8 ± 2.3
Ischaemia + LY 393615	56.1 ± 2.1[b]	52.2 ± 2.8[c]

Means ± s.e.m (n=11/15). [a]P<0.05 vs Control, [b]P<0.01, [c]P < 0.001 vs Ischaemia, [d]P < 0.001 vs Control + LY 393615.

We have previously reported the use of TTC staining as a simple means of quantifying *in vitro* "ischaemic" injury in brain slices (Mathews et al, 1999) and neuroprotection. LY 393615, at this concentration, has been shown to reduce synaptic transmission in brain slices (Thomas et al, 1999). The present data demonstrate that LY 393615 also shows neuroprotective properties *in vitro*, perhaps by reducing transmitter release.

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138P A SLOWLY DEVELOPING FACILITATION OF NEURONES IN RAT LUMBAR SPINAL SUPERFICIAL DORSAL HORN *IN VITRO* PRODUCED BY NEUROTENSIN IS BLOCKED BY SR48962

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We have previously shown that NT caused a range of different actions on neurones in the superficial dorsal horn of the spinal cord (Liu and Morris, 1999). In the present study, these effects were investigated further using a non-peptide neurotensin receptor antagonist, SR48962 (Gully et al., 1993).

Neonatal rats (12-18 days old) were anaesthetised with ether and decapitated. A parasagittal slice was prepared from the lumbar spinal cord with dorsal roots, dorsal root ganglia and the peripheral nerves. The slice was arranged in a multicompartment bath and perfused with a gassed (95% O_2, 5% CO_2) artificial cerebral spinal fluid at room temperature. Intracellular recordings were made with sharp microelectrodes. Stimuli (80 mV, 0.4 ms) which recruit both A- and C- fibres, were applied to the peripheral nerves via two pairs of platinum electrodes.

Superfusion of 1-2 μM NT, for 2-3 minutes, induced slowly developing and long lasting (15-30 min duration) depolarisations in 19/24 cells (mean peak amplitude: 5.5 ± 0.7 mV) accompanied by increased neuronal excitability including increased input resistance and reduced rheobase currents. In 17 cells these depolarisations were accompanied by an increase in spontaneous EPSPs (sEPSPs) and in some cases spontaneous IPSPs (sIPSPs) (n=4). In 2 neurones, although application of NT (1-2 μM) did not produce any depolarisation, a slowly developing increase in sEPSPs occurred. These depolarisations and increases in synaptic activity either initiated actions potentials in silent (n=9) or increased the number of action potentials in spontaneously active (n=10) neurones. More detailed analysis revealed that the mean amplitude of sEPSPs was initially reduced in the first 2-5 min after the start of NT application (-17.2 ± 2.1%, $P < 0.01$, n=15/19), then this was followed by an increase (+ 41.5 ± 11.9%, $P < 0.05$) 6-10 minutes after stopping application of NT. The sEPSPs amplitude typically returned to control as the depolarisation returned to baseline levels. The frequencies of sEPSPs were significantly increased in all 17 neurones by 1-2 μM NT (34.7 ± 5.1%, $P < 0.01$), but in 9 cells the increased frequency developed only 6-10 min after stopping NT perfusion. In the 4 cells showing increases in both amplitude and frequency of sIPSPs, this effect started to develop 6-10 minutes after stopping NT perfusion.

Bath application of SR48962 alone (5 μM, n=5; 10 μM, n=3) had no effect on the resting membrane potential, the evoked EPSPs and the frequency and amplitude of sEPSPs. In the presence of SR48962 (5-10 μM), NT-induced depolarisation was significantly reduced in mean peak amplitude and duration by 73.6 ± 11.1% ($P < 0.01$; n=8) and 69.8% ± 18.6 % ($P < 0.05$; n=8) respectively. SR48962 (5-10 μM) also completely blocked NT-induced increases in input resistance and the number of evoked action potential in 3 cells. However, SR48962 up to 10 μM had little effect on the NT-induced increase in sEPSPs (n=6) or sIPSPs (n=2). As reported previously, NT caused small reductions in evoked EPSPs (Liu and Morris, 1999). Whilst in contrast, attenuation of duration of the evoked EPSPs induced by 1-2 μM NT was significantly reduced in the presence of 5-10 μM SR48962 (from 53.0 ± 9.9% to 9.2 ± 3.8%, $P < 0.05$; n=5).

These results suggest that the actions of neurotensin in the superficial dorsal horn may be mediated by multiple receptors. The direct depolarisation and inhibition of primary afferent evoked responses may be mediated by a different receptor type to that mediating the increases in synaptic transmission.

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139P BEHAVIOURAL EFFECTS OF 3,5-DIMETHYLADAMANTAN-1-AMINOHYDROCHLORIDE AND 1-AMINO-3,5-DIMETHYLADAMANTANE IN TWO ANIMAL MODELS OF ANALGESIA

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N-Methyl-D-Asparate (NMDA) receptor antagonists reduce nociception in clinical and pre-clinical models (e.g., Wong et al., 1995; Parsons et al., 1999). In the present study the anti-nociceptive properties of 2 NMDA receptor antagonists, Cerestat (3,5-dimethyladamantan-1-aminehydrochloride) and Memantine, (1-amino-3,5-dimethyladamantane), were assessed in 2 animal models of analgesia.
In the mouse writhing model, female Tuck mice (21-31g; n=10) were injected s.c. with test compound, morphine (MOR) or vehicle (VEH). Thirty min (compound or VEH) or 20 min (MOR) later mice were given an i.p. injection of an irritant (phenyl-p-benzoquinone, 2mg/kg, 10 ml/kg). For the next 10 min the number of abdominal writhes were recorded. In the formalin test, male Sprague Dawley rats (310-488g; n=8) were given a s.c. injection of test compound, MOR or VEH. Thirty min (test compound or VEH) or 20 min (MOR) later rats were given a 50µl sub-plantar injection of 5% formalin. Immediately after the second injection rats were rated on a 0-3 scale for behaviours directed at the injected paw (see Dubuisson & Dennis, 1977). Mean scores (per min) were recorded for the period 16-30 min after formalin administration.
Writhing was significantly reduced by Cerestat (1.5 mg/kg) and MOR [Fs ≥ 8.10, ps<0.05] (see Table 1) but was unaffected by memantine. In the formalin test significant analgesia was seen following Cerestat (1.0 & 1.5 mg/kg), Memantine (30 mg/kg) and MOR [H = ≥ 18.45, p <0.005]. The analgesic effect of memantine is consistent with previous findings (e.g., Davidson & Carlton, 1998), although activity was restricted to the formalin test. In addition these studies provide the first evidence that Cerestat is anti-nociceptive in both the writhing and formalin tests.

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Table 1: The effects of Cerestat (mg/kg), Memantine (mg/kg) and MOR (mg/kg) in 2 animal models of analgesia.

Experiments 1 & 2: Writhing				Experiments 3 & 4: Paw lick			
Cerestat		Memantine		Cerestat		Memantine	
VEH	31.9±6.1	VEH	22.9±4.3	VEH	2.2±0.1	VEH	2.3±0.1
0.5	31.2±3.6	0.3	16.4±4.6	0.5	1.5±0.4	5	2.1±0.1
1.0	19.6±4.3	3.0	21.8±5.0	1.0	0.2±0.1†	10	1.3±0.3†
1.5	9.4±3.8*	30.0	11.9±3.5	1.5	0.05±0.03†	20	1.3±0.2†
MOR 1.0	4.9±3.3*	MOR 1.0	1.2±0.6*	MOR 7.5	0.05±0.05†	MOR 7.5	0.3±0.2†

Data presented are mean ± s.e.mean. *P<0.05 versus vehicle-control group (one-way ANOVA followed by a post-hoc Dunnett's test).
† P<0.01 versus vehicle-control group (one-way Kruskall Wallis ANOVA followed by post-hoc Mann Whitney U-tests).

140P SPINAL ADMINISTRATION OF THE ENDOCANNABINOID ANANDAMIDE INHIBITS C-FIBRE EVOKED POST-DISCHARGE RESPONSES OF DORSAL HORN NEURONES IN CARRAGEENAN INFLAMED RATS

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Introduced by D.A. Kendall

Evidence suggests that the antinociceptive effectiveness of endocannabinoids is dependent on the type of pain-state employed. Behavioural studies have shown an increased antinociceptive potency of the endocannabinoid, anandamide, in animals with peripheral carrageenan inflammation compared to controls (Richardson *et al.*, 1998). Here *in vivo* electrophysiology was used to investigate the effect of spinal anandamide on noxious and innocuous evoked responses of dorsal horn neurones of rats with carrageenan inflammation of the hind paw, compared to controls.

Extracellular recordings of convergent dorsal horn neurones were made in halothane anaesthetised (1.5% in 66% N_2O/33% O_2) Sprague Dawley rats (Chapman *et al.*, 1994). Neuronal responses to transcutaneous electrical stimulation (at 3 x C-fibre threshold, trains of 16 stimuli at 0.5 Hz) of the receptive field were recorded and post-stimulus histograms were constructed. Responses were separated and quantified on the basis of post stimulus latencies: Aβ-fibre 0-20ms; Aδ-fibre 20-90ms; C-fibre 90-300ms and post discharge 300-800ms. The last three recordings were taken as controls and anandamide (0.1-50µg/50µl, $5.8x10^{-6}$-$2.9x10^{-3}$M) was applied to the spinal cord. Drug effects were followed every 10 min for 40 min per dose. In a separate group of rats, λ-carrageenan (100µl, 2% in saline) was injected into the plantar region of a hind paw 3h before anandamide dose-response data was obtained. Data are presented as mean maximal effects; statistical analysis was performed with ANOVA.

The mean depth of neurones studied in non-inflamed rats was 804±75µm (mean ± s.e.mean; n=8). C-fibre threshold and latency of neuronal responses were 1.7±0.1mA and 168±6 ms, respectively. Only the top dose of anandamide studied (50µg/50µl) reduced the post-discharge response of neurones to 77±19% (n=5) of control (control value: 300±65 action potentials). The top dose of anandamide studied had no effect on Aβ-(92±7%), Aδ- (90±11%) or C-fibre (99±6%) evoked responses (n=3-5).

The mean depth of neurones studied in carrageenan treated rats was 874±58µm (n=8). C-fibre threshold and latency of neuronal responses were 1.9±0.2mA and 195±20 ms, respectively. Control Aβ-fibre, Aδ-fibre, C-fibre and post-discharge responses were 65±9, 74±18, 317±32 and 187±20 action potentials, respectively. In carrageenan inflamed rats, anandamide (0.1-50µg/50µl) inhibited the post-discharge response of neurones (100±17%, 72±10%, 65±9% and 57±10% ($p<0.05$) of controls, respectively; n=7-8). Aβ- (97±13%), Aδ- (75±12%) and C-fibre (94±6%) evoked responses of neurones of carrageenan inflamed rats were not significantly influenced by spinal anandamide (n=6-7).

Spinal anandamide inhibited the post-discharge response of dorsal horn neurones in a model of inflammatory pain. This finding further highlights the ability of spinal cannabinoids to modulate C-fibre driven hyperexcitability of spinal neurones. These results confirm and extend previous behavioural studies indicating a novel antinociceptive effect of spinal exogenous anandamide following peripheral inflammation.

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141P COMPARISON OF THE EFFECTS OF ANANDAMIDE AND METHANANDAMIDE IN BIOASSAYS

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Since the endogenous cannabinoid, anandamide, is rapidly metabolised, it has been considered that use of stable analogues, such as methanandamide, might give a better index of potency in bioassays (see Pertwee, 1997). To determine if methanadamide is consistently more potent than anandamide, we compared the effects of the two compounds with respect to [^{35}S]-GTPγS binding to rat brain membranes, vasodilatation of the isolated, preconstricted, mesenteric vascular bed, and regional haemodynamics in conscious rats.

Measurements of [^{35}S]-GTPγS binding to membranes prepared from male, Wistar rat cerebral cortex and hippocampus were made as described by Traynor & Nahorski (1995). Mesenteric vascular beds were isolated from male Wistar rats (250 - 300g), killed by exposure to CO_2 followed by decapitation. The preparations were perfused with oxygenated Krebs' solution at 5 ml min^{-1} (Ralevic *et al.*, 1995) and preconstricted with methoxamine (0.3-60 μM). For *in vivo* studies, male, Long Evans rats (350 - 450g) were anaesthetised (sodium methohexitone, 60mg kg^{-1} i.p., supplemented as required) and had pulsed Doppler probes and intravascular catheters implanted to monitor changes in renal, mesenteric and hindquarters haemodynamics, and for i.v. substance administration and recording blood pressure and heart rate (Gardiner *et al.*, 1995). All data are presented as mean ± s.e. mean.

The increase in specific [^{35}S]-GTPγS binding in the presence of a maximally effective concentration of anandamide (912±29 dpm over basal) was significantly ($P<0.01$; Student's t test) greater than in the presence of methanandamide (288±29 dpm; n=3 for both). However, methanandamide was more potent than anandamide (EC_{50} 0.9±0.1 and 10.5± 0.1 μM, respectively; $P<0.01$; Student's t test).

Both anandamide and methanandamide elicited dose-dependent vasorelaxation of the isolated mesenteric arterial beds, but anandamide was more potent. The ED_{30} values for anandamide and methanandamide were 30.4±10.2 nmol (n=6) and 249.4±84.8 nmol (n=9), respectively ($P<0.05$; Student's t test).

Rats (n=9) given bolus i.v. doses of anandamide (2-8μmol kg^{-1}) showed dose-dependent pressor and vasoconstrictor effects (maximum Δ : blood pressure, 43±3 mmHg; vascular conductance : renal, −49±3%; mesenteric, −78±6%; hindquarters, −75±4%), with marked bradycardia (−189±28 beats min^{-1}) at the highest dose tested. In contrast, methanandamide in doses up to 24μmol kg^{-1} had no consistent cardiovascular effects (n=3).

In summary, at cannabinoid receptors in brain membranes, methanandamide is more potent, albeit less efficacious, than anandamide in causing [^{35}S]-GTPγS binding. In contrast, anandamide is more potent than methanandamide at eliciting cardiovascular responses in the isolated mesenteric arterial bed and in conscious rats. Vanilloid receptors mediate vasodilatation to anandamide and methanandamide in rat isolated mesenteric arteries (Zygmunt *et al.* 1999; Ralevic *et al.* 1999) These data suggest that there is a different order of potency of anandamide and methanandamide at cannabinoid and vanilloid receptors.

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142P THE EFFECTS OF DANTROLENE IN AN ANIMAL MODEL OF MALIGNANT HYPERTHERMIA

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Malignant Hyperthermia (MH) is a main cause of death during general anaesthesia, and susceptible individuals have an inherited defective regulation of calcium flux in their skeletal muscles. Research has been hampered by the lack of a convenient animal model. We recently described (Gonzalez et al, 1998) spontaneous myopathy and a fatal syndrome of generalised muscle rigidity, triggered by halothane, in the hooded Lister outbred strain of rat (from Harlan, Bicester, UK). We have never observed this syndrome in hooded Lister rats from other suppliers. The syndrome is characterised by generalised, progressive and severe muscle rigidity, occurring first in the hind-limbs, and long-lasting hyperthermia. The animals are pyrexic and rigid prior to death, but become flaccid after death, i.e. they do not develop normal rigor mortis. This indicates that a hypermetabolic process, triggered by the anaesthetic, produced biochemical changes in the muscle. Histological examination of skeletal muscle revealed severe abnormalities indicating chronic underlying myopathy. We have now found similar changes in heart muscle, but greatly reduced in severity. Thus, in heart muscle, we have observed regions of hypercellularity with mild monocytic infiltration. The pathological changes have been restricted to muscle tissue and no abnormalities have been found in brain tissue. Standard resuscitation procedures of discontinuing halothane, cardiac massage and artificial ventilation proved ineffective in untreated rats (n=6). Therefore, we examined the effects of dantrolene (2.5 mg/kg s.c.), the only clinical antidote for MH, injected less than 2 min (n=3) and greater than 15 min (n=1), after the onset of muscle rigidity was observed in rats.

Table 1. Effects of dantrolene treatment on symptoms of MH syndrome

	Untreated	Dantrolene	
		≤ 2 min	>15 min
No. Regaining Consciousness	0/6	3/3*	0/1
No. Moving Around Cage	0/6	3/3*	0/1

*$P<0.05$ Fisher Exact Probability Test.

Although dantrolene treatment reversed the MH syndrome as far as regaining consciousness, all rats eventually died. The failure to prevent death is likely to be due to the slow onset of drug action because of the subcutaneous route of administration. We believe the responses to dantrolene strengthens the evidence that these rats provide a new and extremely useful animal model to study the fatal disorder of MH. The availability of a rat phenotype for MH will greatly facilitate genetic and biochemical studies of this disorder.

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143P THERMOGENIC EFFECT OF CHRONIC SIBUTRAMINE TREATMENT IN FEMALE WISTAR RATS

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Regulation of body weight and composition, through energy balance, is dependent on controlling both food intake and energy expenditure. Sibutramine is a novel 5-hydroxytryptamine (5-HT) and noradrenaline (NA) re-uptake inhibitor which has recently been introduced to manage obesity. Acute administration of sibutramine reduces food intake and stimulates thermogenesis in rats (Connoley *et al.*, 1999; Heal *et al., 1998*). On chronic administration, tolerance develops to the effects of sibutramine on food intake, although the weight-reducing properties of sibutramine remain (Connoley *et al.*, 1995). This study investigates the effects of repeated administration of sibutramine on energy expenditure.

Oxygen consumption (VO_2), in female Wistar rats (259-342g), was measured using an improved version of a closed-circuit calorimeter (Stock, 1975). Animals (n=5-8) were dosed with either sibutramine (10mg/kg po) or vehicle (deionised water) once daily for 5 days. VO_2 was measured by recording a 2 h baseline before dosing followed by a 4 h post-dose measurement. Body weight and food consumption were also recorded each day. Data analysed was by two-way analysis of covariance.

Table 1 Effects of chronic sibutramine treatment on baseline oxygen consumption.

Day	Control Mean	(s.e.mean)	Sibutramine Mean	(s.e.mean)	Diff from control	p
2	15.9	(0.8)	17.8	(1.0)	1.9	0.16
3	15.5	(0.6)	17.3	(0.7)	1.8	0.08
4	15.1	(0.7)	20.4	(2.1)	5.3	0.03
5	15.4	(0.5)	20.5	(1.3)	5.1	0.004

Values are mean VO_2 (O_2 mls/min/$kg^{0.75}$).

Table 2 Effects of chronic sibutramine treatment on post-dose oxygen consumption.

Day	Control Mean	(s.e.mean)	Sibutramine Mean	(s.e.mean)	Diff from control	p
1	15.7	(1.1)	20.4	(1.1)	4.7	0.02
2	14.7	(1.0)	21.0	(2.7)	6.3	0.05
3	15.0	(0.8)	18.5	(1.4)	3.5	0.05
4	14.5	(0.7)	17.5	(1.2)	3.0	0.05
5	14.5	(0.6)	17.8	(1.3)	3.3	0.04

Values are mean VO_2 (O_2 mls/min/$kg^{0.75}$).

Tables 1 and 2 demonstrate the changes seen in baseline and post-dose VO_2 measurements following sibutramine treatment. Food consumption was significantly reduced by 70% ($p<0.001$) after day 1. This remained significantly reduced over the next 4 doses (59, 68, 54 and 19%, respectively). Body weight was also significantly lowered with a 5% reduction ($p<0.001$) seen after sibutramine's first dose. This reduction in body weight increased over the subsequent 4 days with a 10% reduction being seen on day 4.

These results show that sibutramine's thermogenic effect can be demonstrated after repeated dosing in a closed-circuit calorimeter. The increase in the 2 h baseline is of great interest because it provides evidence that basal metabolic rate is increased following multiple doses of sibutramine and may contribute to the overall weight loss observed with this agent.

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144P DOPAMINERGIC TRANSMISSION IN THE RAT NUCLEUS ACCUMBENS IS NOT INVOLVED IN SIBUTRAMINE'S REDUCTION IN FOOD INTAKE: AN *IN VIVO* MICRODIALYSIS STUDY

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Sibutramine is a novel antiobesity drug which functions via combined uptake inhibition of 5-HT and noradrenaline (Stock, 1997). In the present study, we have investigated the effects of sibutramine and the weight-modifying drugs, phentermine and *d*-amphetamine, at their respective 2 h ED_{50} values for inhibition of food intake in rats, on extracellular dopamine (DA) in the nucleus accumbens of freely-moving rats using *in vivo* microdialysis.

Male, CD rats (250 - 350 g; Charles River) were anaesthetised with isoflurane in an O_2/N_2O mixture and a concentric microdialysis probe (2 mm tip, Hospal AN 69 membrane) was stereotaxically implanted into the nucleus accumbens (A +2.2 mm, L -1.5 mm relative to bregma; V -8.0 mm from the skull surface; Paxinos and Watson, 1986). Following surgery, rats were returned to a home cage and allowed to recover for at least 16 h with food and water available *ad libitum*. Probes were continuously perfused with an artificial cerebrospinal fluid at a rate of 1.2 µl min^{-1} and samples collected every 20 min. Five 'basal' samples were taken prior to a single i.p. injection of drug (dose expressed as free base) or saline. Dialysate DA was determined by reverse-phase HPLC with electrochemical detection. Values are mean ± s.e.mean (n = 8 - 11) and statistical comparisons were made between treatment groups and controls by ANCOVA with *post hoc* multiple t-tests.

Sibutramine (2.0 mg kg^{-1}) had no effect on basal extracellular DA levels (0.75 ± 0.09 fmol 20 μl^{-1}; Figure 1). In contrast, phentermine (1.3 mg kg^{-1}), evoked a significant ($p<0.001$) increase in DA above basal levels (0.78 ± 0.10 fmol 20 μl^{-1}) for up to 120 min post-injection (Figure 1). *d*-Amphetamine (0.5 mg kg^{-1}), also evoked a significant ($p<0.001$) elevation in basal DA levels (0.70 ± 0.06 fmol 20 μl^{-1}) for up to 120 min post-injection (Figure 1).

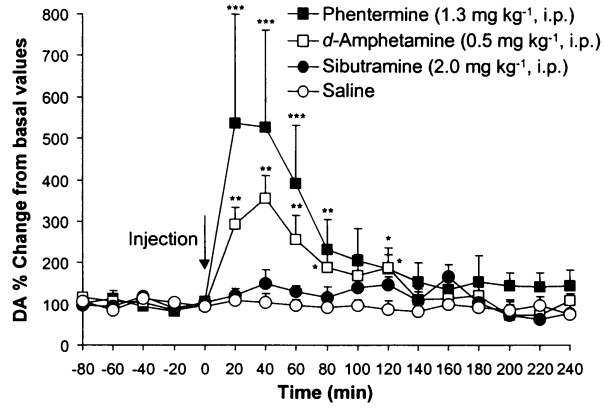

Figure 1. Effects of sibutramine, phentermine and *d*-amphetamine on DA levels in rat nucleus accumbens. *p<0.05; **p<0.01; ***p<0.001 compared to saline-treated controls.

The present findings demonstrate that sibutramine, at its 2 h ED_{50} value to reduce food intake in rats, had no effect on extracellular limbic DA efflux. This confirms that dopaminergic mechanisms are not involved in sibutramine's hypophagic action (Stricker-Krongrad *et al.*, 1996). In contrast, phentermine and *d*-amphetamine, at their functionally equivalent doses, both evoked rapid, significant increases in limbic DA levels, in a manner consistent with a DA-releasing mode of action.

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145P EFFECTS OF SIBUTRAMINE, PHENTERMINE AND *D*-AMPHETAMINE ON EXTRACELLULAR DOPAMINE LEVELS IN THE NUCLEUS ACCUMBENS AND ON THE LOCOMOTOR ACTIVITY OF RATS

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We have previously shown that the limbic dopaminergic system is not involved in the reduction of food intake by sibutramine, a novel antiobesity drug (Rowley *et al.*, 2000). Here we have investigated the effects of sibutramine and the appetite suppressants, phentermine and *d*-amphetamine, at 3 *x* their respective 2 h ED_{50} values, on (i) dopamine (DA) efflux in the nucleus accumbens of freely-moving rats using *in vivo* microdialysis and (ii) rat locomotor activity.

Male, CD rats (250-350 g; Charles River) were used. *Microdialysis*: rats were anaesthetised with an isoflurane/O_2/N_2O mixture and a concentric microdialysis probe (2 mm Hospal AN 69 membrane) was implanted into the nucleus accumbens (A +2.2 mm, L -1.5 mm relative to bregma; V -8.0 mm from the skull surface; Paxinos and Watson, 1986). Rats were allowed to recover for at least 16 h with food and water available *ad libitum* and probes were continuously perfused with an artificial cerebrospinal fluid at a rate of 1.2 µl min^{-1}. Five 'basal' samples were taken (20 min intervals; 0.71 ± 0.04 fmol 20 $µl^{-1}$) prior to an i.p. injection of drug (free base) or saline. Dialysate DA was determined by reverse-phase HPLC with electrochemical detection. Statistical comparisons were made using ANCOVA with *post hoc* multiple t-tests. *Locomotor activity*: rats were housed individually for 16 h prior to the experiment and then placed in activity test cages and allowed to acclimatise for a further 1 h prior to an i.p. injection of drug or saline. Locomotor activity was measured continuously in 10 min blocks using infra-red detectors for 3 h post-treatment. Square root transformed data were analysed by ANOVA with *post hoc* least significant difference test.

Basal DA levels were increased by sibutramine for up to 160 min (Table 1), but the drug had no effect on locomotor activity (total counts over 180 min: sibutramine 462.8 ± 102.7 and saline 426.3 ± 78.6). Phentermine elevated DA levels for up to 140 min and locomotor activity for up to 100 min (1938.0 ± 486.3 total counts), whilst *d*-amphetamine increased DA efflux and locomotion (4393.1 ± 898.8 total counts) for up to 180 and 160 min, respectively (Table 1).

These data demonstrate that, in contrast to the large rises in DA efflux evoked by both phentermine and *d*-amphetamine, the modest increase in extracellular DA levels evoked by sibutramine was not of sufficient magnitude or duration to stimulate locomotor activity. Since increased locomotor activity is linked to psychostimulant drugs of abuse and their ability to cause substantial DA release (Kelly *et al.*, 1975), these findings provide further evidence that sibutramine lacks abuse potential.

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Table 1. Effects of sibutramine and other weight-reducing drugs on extracellular DA levels in the nucleus accumbens and locomotor activity.

	Extracellular DA levels		Locomotor activity counts	
Drug dose (mg kg^{-1}, i.p.)	Maximum % change	Time of peak effect	Maximum % change	Time of peak effect
Sibutramine (6.0)	+217.0 ± 69.6***	60 min	NS	NS
Phentermine (3.9)	+764.7 ± 324.4***	40 min	+1529.6 ± 342.9***	40 min
d-Amphetamine (1.5)	+630.1 ± 294.0***	40 min	+2915.4 ± 451.6***	40 min

Values are mean ± s.e.mean (n = 8 - 11); NS = no significant change; ***p<0.001 compared to saline-treated controls.

146P EFFECT OF 2-NAP ON FOOD INTAKE IN RATS: IMPLICATIONS FOR THE CHOLECYSTOKININ-SATIETY HYPOTHESIS

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The CCK-satiety hypothesis states that endogenous CCK released from the small intestine during a meal acts in a negative feedback manner to induce a state of satiety (Gibbs et al., 1973). As CCK cannot cross the blood brain barrier (BBB), it has been proposed that it must act at a peripheral site to elicit its hypophagic actions.If the hypothesis is not to be rejected, then a specific peripherally acting antagonist of CCK should increase food intake by blocking the "satiety effect" of endogenously released peripheral CCK. The present study was carried out to test the hypothesis by investigating the effects of the CCK_A receptor antagonist 2-naphthalene sulphanyl-L-aspartyl-2-(phenethyl) amide (2-NAP), which is unlikely to cross the BBB (see Ebenezer & Baldwin, 1995), on food intake in rats.

Male Wistar rats (b.wt. 250-350g) were used in these experiments. Experiment 1. Solid (Expt.1a&b) and liquid (Expt.1c&d) food intake was measured, as described previously (Ebenezer, 1995; Ebenezer and Baldwin, 1995), under the following conditions: (a) in 5h fasted rats (n=8) that were presented with pelleted food (b) in 20h food-deprived rats (n=8) that were given a 30 min oral food preload 60 min prior to presentation of pelleted food, (c) in 22h food deprived rats (n=18) that were presented with 2%$^w/_v$ (n=6), 6%$^w/_v$ (n=6) and 12%$^w/_v$ (n=6) solutions of sucrose, (d) in 19h food-deprived rats (n=8) that were given a 30 min oral preload of 20 ml of a palatable liquid diet (500 ml contain 50g sucrose, 40g Horlicks [SmithKlineBeechams], 250 ml milk and water) 60 min prior to presentation of the same liquid diet. The rats were injected i.p. with saline (vehicle) or 2-NAP (in doses ranging from 1 - 8 mg kg^{-1}) immediately (Expt. 1a&c) or 30 min (Expt. 1b&d) prior to each session. In all experiments, a repeated measures design was used with each rat receiving all treatments; 3- 4 days separated successive drug trials. Experiment 2: The rats (n=8) received each of the following pairs of treatments (successive i.p. injections were given 30 min apart): saline+saline; saline+CCK (4 µg kg^{-1}); 2-NAP (2 mg kg^{-1})+saline; 2NAP (2 mg kg^{-1})+CCK (4 µg kg^{-1}). Both injections were given i.p. and 30 min separated the 2 injections. Food intake was measured for 30 min immediately after the second injection. In both experiments the results were analysed by repeated measures ANOVA and post hoc Tukey test.

2-NAP (1 - 8 mg kg^{-1}) had no significant effects on solid or liquid food intake in rats that were food-deprived or given an oral preload (Experiments 1a-d). For example, in rats that were deprived of food for 5h, mean food intake (g) ± s.e.mean at 120 min was 3.1±0.5g for saline and 2.8±0.7g, 2.5±0.72g, 2.5±0.7 and 3.1±0.6g for 2NAP 1, 2, 4 and 8 mg kg^{-1}, respectively. CCK-8S (4 µg kg^{-1}) significantly reduced food intake (P<0.01 at 30 min; Experiment 2), which was abolished by pretreatment with 2NAP (2 mg kg^{-1}) [mean food intake (g) ± s.e.mean at 30 min: saline+saline 6.8 ± 0.4g; saline+CCK-8S 2.1 ± 0.5g; 2-NAP+saline 6.6 ± 0.4; 2-NAP+CCK-8S 5.3 ± 0.7]. The present findings that 2-NAP does not increase food intake on its own but abolishes the suppressant effect of exogenous peripheral CCK, confirms and extends previous results (Ebenezer & Baldwin, 1995) and suggests that endogenously released peripheral CCK is not a satiety factor in rats.

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147P A COMPARISON OF THE DISTRIBUTION OF OREXIN A AND THE OREXIN RECEPTORS, OX1 AND OX2, IN LEAN AND OBESE ZUCKER RATS

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Recently two novel neuropeptides, orexin A and B, were discovered which are proteolytically cleaved from the same precursor prepro-orexin. They bind to two G-protein coupled receptors, OX1 and OX2 (Sakurai *et al.*, 1998). Down-regulation of the prepro-orexin gene in *ob/ob* and *db/db* mice implies a link with the leptin system, which has an integral role in the regulation of energy homeostasis (Friedman and Halaas, 1998; Yamamoto *et al.*, 1999). We have now compared the densities and distribution of orexin A, OX1 and OX2 receptors in lean and genetically obese Zucker rats, which have a mutation in the leptin receptor, using immunocytochemistry.

Polyclonal antibodies against unique, ten amino acid, synthetic peptide sequences from the N-terminal regions of each receptor were raised in female New Zealand white rabbits. The antiserum was purified by caprylic acid/ammonium sulphate precipitation. Rabbit antiserum against orexin A was obtained commercially (Phoenix Pharmaceuticals, USA). Male Zucker rats (4 lean, 300-330g; 4 obese, 340-400g) were perfused transcardially. Brains were post-fixed for 24h at 4°C in phosphate buffered 4% paraformaldehyde then stored in 0.9% phosphate buffered saline. A vibratome was used to cut 50µm coronal sections in five areas from the hypothalamic and dorsal raphe areas. Sections were blocked with 5% goat serum (1h at 25°C) for orexin A or 20% swine serum (30min at 25°C) for OX1/OX2, then incubated with rabbit anti-orexin A IgG (1:5000, 48h at 4°C) or rabbit anti-OX1/anti-OX2 (1:1000, 24h at 4°C). Positive immunolabelling was visualised using a peroxidase-linked streptavidin-biotin system.

Immunolocalisation of orexin A was appropriate for a neuropeptide with positive immunoreactivity in long varicose processes distributed throughout the areas sampled. Cell body staining was observed in the lateral hypothalamic areas. The overall pattern of staining agrees with data for Wistar rats (Table 1; Nambu *et al.*, 1999). OX1 staining was confined to cell bodies but faint axonal immunoreactivity was also observed in the cingulate cortex. The areas most consistently labelled were: thalamus, particularly the paraventricular (PVT), anterior, medial ventral and lateral regions, motor cortex, S1/S2 cortex, cingulate gyrus, insular cortex, piriform cortex, preoptic, striatal, hypothalamic, raphe and hippocampal nuclei (Table 1). OX2 distribution was similar to OX1 receptor (Table 1). Subjective grading of the immunolabelling signal revealed no overall differences between lean and obese Zucker rats (Table 1). Thus, it would appear that the orexin system does not play a role in the obese Zucker phenotype.

Table 1. Orexin A, OX1 and OX2 in lean and obese Zucker rats

Brain Region	Orexin A		OX1		OX2	
	L	O	L	O	L	O
PVT	+++	+++	+++	+++	+++	+++
Motor cortex	++	+	++	++	++	++
S1/S2 cortex	+	+	++	++	++	++
Amygdala	++	+	++	++	++	++
Striatum	+	+	++	++	++	++
Hypothalamus	+++	++	++	++	++	+
Dorsal raphe	+	++	++	++	-	++
Hippocampus	-	+	+++	+++	++	++
Preoptic nucleus	++	++	++	++	+	++

L = Lean, O = Obese. Immunolabelling graded subjectively by an independent assessor based upon staining intensity using a four point scale: +++ highest density, ++ moderate density, + low but above background, - indistinguishable from background.

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148P EFFECT OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) ON FOOD INTAKE IN MALE RATS

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We have previously demonstrated that systemic administration of CGRP produces a short lasting decrease in food intake in female rats without affecting water intake (Price et al., 1998). In the present study we investigated (i) the effects of ip injection of rat (r)CGRP on food intake in fasted male rats (ii) whether exogenous rCGRP decreases food intake by an aversive mechanism of action, and (iii) whether rCGRP elicits its hypophagic effect by releasing the "putative satiety" hormone cholecystokinin (CCK) (Baldwin et al, 1998).

Experiment 1. Male Wistar rats (n=8; b. wt. 310 – 410g) were fasted for 22h, then injected i.p. with either saline (sal) or rCGRP (2, 4, 8 and 16 µg kg^{-1}) and placed in experimental cages where they had free access to food and water. Food consumption was measured as described previously (Ebenezer, 1990). Each animal received all doses of drug and the results were analysed by repeated measures ANOVA; 3- 5 days separated successive trials. Experiment 2. Details of the taste aversion experimental design have been reported previously (Ebenezer et al., 1993). Briefly, male Wistar rats (n=20; b. wt 300 – 420g) were divided into four equal treatment groups and deprived of water for 16h a day. They were given 3 daily training when they were presented with two drinking bottles containing either sucrose solution (12%$^{w}/_{v}$) or water. On the fourth day they were presented with the sucrose solution for 15 min and then injected ip with either saline, CGRP (10 or 20 µg kg^{-1}); or lithium chloride (LiCl; 100 mg kg^{-1}). 24h later the rats were presented with both water and sucrose solution for 15 min, and consumption of both liquids measured. Expt. 3. Male Wistar rats (n=8, b.wt. 300 - 380g) were fasted for 22h, and injected with either sal followed by sal, sal followed by rCGRP (10 µg kg^{-1}), the CCK_A receptor antagonist 2-naphthalene sulphanyl-L-aspartyl 2-(phenethyl) amide (2-NAP) (5 mg kg^{-1}) followed by sal, or 2-NAP (5 mg kg^{-1}) followed by rCGRP (10 µg kg^{-1}). Both injections were given i.p; 30 min separated the 2 injections. Immediately after the second injection, the rats were placed separately in experimental cages with access to food and food consumption was measured after 30 min. Each animal receiving all four treatments in random order and the results analysed by ANOVA and post-hoc Tukey test.

rCGRP (2 –16 µg kg^{-1}) suppressed food intake in a dose-related manner (Experiment 1). Thus, 15 min after administration, mean food intake (g) ± s.e. mean was as follows: sal 6.8±1.2g and rCGRP (2, 4, 8 and 16 µg kg^{-1}) 5.7±0.7g, 5.2±0.3g, 4.6±0.6g ($p<0.01$) and 3.5±0.3g ($p<0.01$), respectively. The results obtained in Experiment 2 show that the rats treated with rCGRP (10 and 20 µg kg^{-1}) did not display taste aversion to the sucrose solution, but rats treated with the known aversive agent LiCl did [Mean sucrose consumption (ml) ± s.e. mean on the test day was as follows: sal 14.2±1.6 ml; CGRP (10 µg kg^{-1}) 16.4±1.3ml (ns); CGRP (20 µg kg^{-1}) 15.9±0.5 ml (ns); LiCl (100 mg kg^{-1}) 0±0 ml ($p<0.01$)]. Pretreatment with the preipherally acting CCK receptor antagonist 2-NAP (Baldwin et al., 1998) did not attenuate the inhibitory effect of rCGRP (10 µg kg^{-1}) on food intake (Expt. 3).[Mean food intake(g) ± s.e. mean was as follows: sal-sal 9.8±0.6g; 2-NAP-sal 9.7±0.5g; sal-rCGRP 6.3±0.5g ($p<0.01$); 2-NAP-rCGRP 6.3±0.5g ($p<0.01$)].

The results indicate that rCGRP inhibits food intake in rats by a non-aversive mechanism of action, and lends support to the suggestion that CGRP may act as a peripheral satiety factor (see Price et al., 1998). Furthermore, it is unlikely that CGRP elicits its hypophagic action secondary to the release of CCK.

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149P THE EFFECTS OF THE BENZODIAZEPINE INVERSE AGONIST 3-METHOXYCARBONYL)-AMINO-β-CARBOLINE (β-CMC) ON FOOD AND WATER INTAKE IN RATS

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Benzodiazepine (BDZ) inverse agonists act on BDZ receptors to produce effects that are opposite to those of the BDZs and are thus anxiogenic and proconvulsant (Cooper et al., 1989). There is also evidence to suggest that these drugs suppress food intake in hungry animals (Cooper et al., 1989; Ebenezer et al., 1997). β-CMC is a BDZ inverse agonist whose main pharmacological properties were first described a decade ago (Nutt & Lister, 1989). However, its effects on ingestive behaviour have not been reported. The present study was therefore undertaken to investigate the effects of β-CMC on food and water intake in the rat.

Experiment 1. Male Wistar rats (n=8; b.wt. 290 – 320g) were fasted for 22h and injected i.p. with either vehicle (physiological saline and 2 drops of Tween'80) or β-CMC (2, 4 or 8 mg kg^{-1}). Fifteen min later the rats were placed separately in experimental cages with free access to food and consumption measured for 120 min. Water was available ad libitum throughout. Each animal receiving all treatments in random order; 3-4 days separated successive drug trials. The data were analysed by ANOVA and post-hoc Tukey test.
Experiment 2. Male Wistar rats (n=8; b. wt. 300 – 350g) were deprived of water for 16h and injected i.p. with either vehicle or β-CMC (8 mg kg^{-1}). 15 min before presentation of water. Water intake was measured for 120 min as described previously (Ebenezer et al., 1992). A cross-over design was used and the data analysed using the paired t-test. Four days separated successive trials.
The results obtained in Experiment 1 show that β-CMC decreases food intake in a dose-dependant manner (Figure1). The 8 mg kg^{-1} dose significantly reduced cumulative food intake compared with control values at the 15, 30 and 60 min measurement intervals ($p<0.01$ in each case). The lower doses did not significantly reduce food intake. None of the doses of β-CMC produced overt abnormal behaviours. The results of Experiment 2 show that β-CMC (8 mg kg^{-1}) had no effects on water consumption in 16h water-deprived rats. Thus, for example, at the 30 min measurement interval, cumulative water intake was 12.9±1.1 ml for vehicle and 12.2±1.0 ml for β-CMC (8 mg kg^{-1}).
The results of this study confirm and extend previous studies with other BDZ inverse agonists (Cooper et al., 1989; Ebenezer et al., 1997) and show that i.p. administration of a low dose of β-CMC (8 mg kg^{-1})decreases food intake in rats. Furthermore, the observation that β-CMC has no effect on water intake suggests that the inhibitory action of the BDZ inverse agonist on food intake is behaviourally specific.

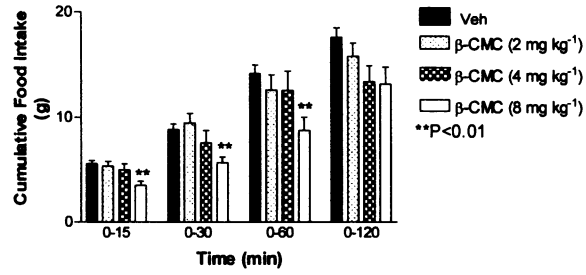

Figure 1. Effects of β-CMC (2 - 8 mg kg^{-1}) on food intake in rats.

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150P MODULATION OF RESTRAINT STRESS-INDUCED C-FOS IMMUNOREACTIVITY BY THE 5-HT$_7$ ANTAGONIST SB-258719 IN THE RAT BRAIN

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Until recently, the absence of selective ligands for the 5-hydroxtryptamine$_7$ (5-HT$_7$) receptor has hindered definition of the CNS function of this receptor. In this study, by using c-fos immunohistochemistry as a marker of neuronal activity, we have characterised the effect of the 5-HT$_7$-receptor antagonist SB-258719 ((R)-3,*N*-Dimethyl-*N*-[1-methyl-3-(4-methyl-piperidin-1yl)propyl] benzenesulfonamide, Forbes *et al.*, 1998) in rats housed in their home cage environment or subject to restraint stress. We have identified discrete regional c-fos expression associated with both stress and 5HT$_7$ receptor function.

Four groups of male Lister hooded rats (320-450g, n=16) were given SB-258719 (10mg kg^{-1}) or saline (1ml kg^{-1} i.p.) and returned to their home cage either for 90 min or for 15 min prior to restraint in a perspex tube (25cm x 6cm diameter) for 75 min. All rats were then anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.) and transcardially perfused with saline (0.9%, 180ml) followed by 4% paraformaldehyde (PFA) in 0.1M phosphate buffer (180ml). Brains were removed, post-fixed overnight in PFA at 4°C and 100μm vibratome coronal slices processed for Fos-like immunoreactivity (F-LI) by immunocytochemistry using a Vectastain ABC kit. The number of Fos-immunoreactive nuclei in a 400x400μm region of selected brain nuclei were counted (mean ± s.e.mean) and analysed by one and two-way ANOVA.

The pattern of F-LI was comparable in rats housed in their home cage irrespective of whether they were pretreated with SB-258719 or saline (Table 1). Restraint stress induced a two to three fold increase in F-LI in the paraventricular hypothalamus (PVA) and central amygdala irrespective of SB-258719 treatment (although the increase in the PVA just failed to reach significance with SB-258719). The F-LI in the suprachiasmatic nucleus (SCN) was only increased significantly by the combination of the 5-HT$_7$ antagonist and stress. A similar pattern was observed in the central amygdala where the stress-induced increase in F-LI reached a greater level of significance in the presence of SB-258719 (Table 1).

Table1. Distribution of F-LI in selected nuclei (n.) of amygdala (Am.), hypothalamus (Hyp.) and periaqueductal grey (PAG).

	HOME CAGE		RESTRAINT STRESS	
Brain Region	**Saline**	**SB-258719**	**Saline**	**SB-258719**
Basolateral Am.	36±13	39±5	39±12	41±12
Central n. Am.	46±13	42±3	85±9*	113±15**
Medial n. Am.	41±7	52±13	85±17	80±16
Dorsomedial Hyp.	76±23	94±22	147±24	184±50
Lateral n. Hyp.	27±8	37±14	66±16	64±10
PVA.	107±35	81±15	233±52*	240±50
SCN.	35±13	50±21	82±14	173±14**††
Lateral PAG	43±5	64±11	75±7	56±5
Dorsal PAG	43±5	65±11	65±11	76±28

(mean ± s.e.mean, n=4 each group) *$p<0.05$ and **$p<0.01$ compared with home cage, same drug treatment. †$p<0.05$ and ††$p<0.01$ compared with saline, same environment, Student-Newman-Keuls post-hoc.

As expected, stress increased F-LI in certain amygdaloid and hypothalamic nuclei, although not all reached significance. SB-258719 had no effect on the stress-induced increase in F-LI in the PVA, suggesting that the 5-HT$_7$ receptor does not regulate stress-induced corticosterone secretion. The significant effect of SB-258719 in the SCN may support the proposed involvement of the 5-HT$_7$ receptor in biological rhythms (Lovenberg et al., 1993) given the key role of this area in the mediation of circadian periodicity. Overall, the data indicate a role for the 5-HT$_7$ receptor in the response to stress and may further suggest a role for the receptor in conditions involving disruption of circadian rhythms such as sleep disorders.

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151P THE SUPPRESSANT EFFECT OF THE 5-HT_{1A} AGONIST IPSAPIRONE ON FOOD INTAKE IN FOOD DEPRIVED RATS IS MEDIATED BY 5-HT_{1A} RECEPTORS

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It has been previously shown that while $5HT_{1A}$ receptor agonists, such as 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT), buspirone and gepirone, increase food intake in non-deprived rats by an action at $5HT_{1A}$ autoreceptors (see Gilbert & Dourish., 1987), they decrease feeding in food-deprived animals (Ebenezer, 1992; 1996). However, it is not known whether these agents suppress food intake in hungry animals by an action at $5HT_{1A}$ receptors. The present study was therefore undertaken to (i) investigate the effects the $5HT_{1A}$ agonist ipsapirone on food intake in food deprived rats and (ii) determine whether the hypophagic effect is mediated by an action of the drug at $5HT_{1A}$ receptors.

Expt.1. Male Wistar rats (b. wt. 330 – 400g) were fasted for 22h and injected s.c. with saline (n=5) or ipsapirone (2.5, 5 and 10 mg kg^{-1}; n=5 for each dose) and placed separately in experimental cages with free access to food and water. Food intake was measured. as described previously (Ebenezer, 1992). Expt.2. Male Wistar rats (n=20, b.wt. 260 - 300g) were fasted for 22h, and injected with either saline followed by saline (n=5), saline followed by isapirone (5 mg kg^{-1}; n=5), the $5HT_{1A}$ receptor antagonist WAY 100635 (0.3 mg kg^{-1}) followed by saline (n=5), WAY 100635 (0.3 mg kg^{-1}) followed by isapirone (5 µg kg^{-1}; n=5). Both injections were given s.c.; 30 min separated the 2 injections. Immediately after the second injection, the rats were placed separately in experimental cages with access to food. Food consumption was measured after 30 min. The results obtained in Expt. 1 and 2 were analysed by ANOVA.

Ipsapirone (2.5 - 10 mg kg^{-1}) produced a dose-related decrease in food intake (Expt. 1). The 2.5 mg kg^{-1} dose only suppressed feeding during the first 15 min after administration, while the higher doses inhibited cumulative food intake during the first 60 min. Thus, for example, mean food intake ± s.e. mean (g) at 15 min were: saline 5.3±0.6g; isapirone (2.5, 5 and 10 mg kg^{-1}) 3.7±0.3g ($p<0.05$), 2.5±0.4g ($p<0.01$) and 2±0.4g ($p<0.01$), respectively. Pretreatment with the selective $5HT_{1A}$ agonist WAY 100635 (0.3 mg kg^{-1}) completely abolished the hypophagic effect of the 5 mg kg^{-1} dose of ipsapirone (Expt. 2; see Figure 1).

The results of this study confirm and extend previous findings with other $5HT_{1A}$ agonists (Ebenezer, 1992; 1996) and show that ipsapirone has acute dose-dependent depressant effects on food intake in fasted rats. Furthermore the results suggest that the hypophagic effect of ipsapirone is mediated by $5HT_{1A}$ receptors.

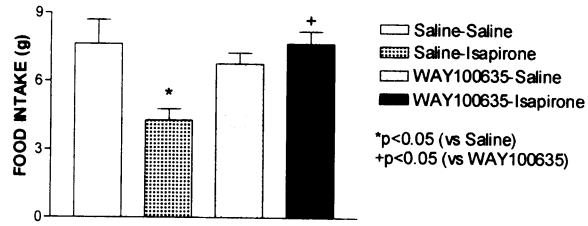

Figure 1. Effect of WAY100635 (0.3 mg kg^{-1}) on the hypophagic effect of ipsapirone (5 mg kg^{-1}) in 22h food-deprived rats. Food intake was measure for 30 min.

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152P INTERACTION OF 5-HT_{2A} AND 5-HT_{2C} RECEPTORS IN THE MEDIATION OF HEAD SHAKE BEHAVIOUR IN RATS

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Despite having only 3-fold functional selectivity for the 5-HT_{2C} over the 5-HT_{2A} receptor, the 5-$HT_{2A/2B/2C}$ receptor agonist, Ro 60-0175 (Ro), fails to elicit 5-HT_{2A} receptor mediated head shakes in rats (Martin et al., 1998). This is not due to poor bioavailability as Ro elicits 5-HT_{2C} receptor mediated hypophagia at doses of 1 mg/kg s.c. (Kennett et al., 1999). The existence of an interaction between 5-HT_{2A} and 5-$HT_{2C/2B}$ receptors was therefore investigated.

Male hooded Lister rats (180-200 g) were held on a 12 h light/dark cycle with free access to food and water. On the test day, they were injected in treatment groups of 6 either p.o. 1 h pre-test with the 5-HT_{2B} receptor antagonist, SB-215505 (Kennett et al., 1998), or i.p. 40 min pre-test with either the 5-HT_{2C} receptor antagonist, SB-242084 (Kennett et al., 1997) and/or one of two 5-HT_{2A} receptor antagonists, MDL 100907 or ketanserin (Schreiber et al., 1995). Rats were then given Ro s.c. 30 min pre-test and head shakes counted for 20 min by an observer blinded to treatments. Data were analysed by 1 way ANOVA and Dunnett's tests and given as means ± s.e.mean.

Ro 10 mg/kg s.c. only elicited head shakes when given with SB-242084 (3 mg kg-1 i.p.) (Table 1). Head shakes induced by Ro (5 mg kg-1 s.c.) and SB-242084 (3 mg kg-1 i.p.) were antagonised by MDL 100907 (Table 1) and ketanserin 0.2 and 0.5 mg kg-1 s.c. (Number of head shakes: Ro + SB-242084 9.2 ± 1.1, Ro + SB-242084 + Ketanserin 0.2 3.3 ± 1.2 $P<0.05$, 0.5 2.2 ± 0.4 $p<0.01$) but were unaltered by the 5-HT_{2B} receptor antagonist, SB-215505 3 mg kg-1 p.o. (Ro + SB-242084 13.0 ± 3.5, Ro + SB-242084 + SB-215505 10.4 ± 1.3 ns). None of the antagonists affected basal head shakes when given alone.

Thus, activation of 5-HT_{2C}, but not 5-HT_{2B} receptors, can suppress 5-HT_{2A} receptor mediated head shakes which may explain why Ro has been described as a selective 5-HT_{2C} receptor agonist in vivo. Indeed, the current results suggest Ro has only modest selectivity for the 5-HT_{2C} over the 5-HT_{2A} receptor in the rat when compared to its potency to elicit 5-HT_{2C} receptor mediated hypocolomotion (Kennett et al., 1999).

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Table 1: Effect of SB-242084 and MDL 100907 on Ro 60-0175-induced head shakes

	Dose of Ro 60 0175 (mg kg-1 s.c. 30 min pre-test)				
Pre-treatment (mg kg-1 40 min pre-test)	Saline	1	3	5	10
Vehicle	2.0 ± 1.4				8.3 ± 1.5
SB-242084 3 i.p.	1.8 ± 1.2	4.5 ± 2.6	11.8 ± 5.2*		21.3 ± 1.9**++
SB-242084 3 i.p.	1.6 ± 0.6			12.0 ± 3.1**	
SB-242084 3 i.p. + MDL 100907 0.1 s.c.	1.8 ± 1.0			0.6 ± 0.6++	

Different from saline control * $p<0.05$, ** $p<0.01$, or from Ro 60-0175, 5 or 10 mg/kg treated group ++ $P<0.01$ by Dunnett's test and ANOVA.

153P INVESTIGATION OF THE CONTRIBUTION OF 5-HT_{2B} RECEPTOR ACTIVATION TO THE ACTIVITY OF Ro 60-0175 IN THE RAT SOCIAL INTERACTION MODEL OF ANXIETY

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The 5-HT_{2C} receptor agonist, 1-(3-chlorophenyl) piperazine (mCPP) is anxiogenic in humans (Seibyl et al., 1991), and induces anxiogenic-like effects in a variety of rodent tests (Kennett, 1993). It is thus of interest that Ro 60-0175 (Ro), which also has 5-HT_{2C} receptor agonist properties, is only weakly anxiogenic-like when compared with mCPP in a rat zero maze model of anxiety (Easton et al., 1999). The difference in relative anxiogenic-like activity between mCPP and Ro may reflect their different pharmacological profiles. In particular, unlike Ro, mCPP lacks efficacy at 5-HT_{2B} receptors (Porter et al., 1999). We have, therefore, assessed the contribution of 5-HT_{2B} receptor activation to the profile of Ro in the rat social interaction (SI) model of anxiety. The effect of a subthreshold dose of Ro (Easton et al., 1999) has been assessed in the presence of SB 215505 (SB), a selective 5-HT_{2B} receptor antagonist (Kennett et al., 1998).

Male, Sprague Dawley rats (Charles River; approx. 300 g; n=8-10/gp) were housed singly for 4 days pretest. On days 3 and 4, the rats were placed singly into a black test arena under red light for 10 min. On day 5, the rats were dosed p.o. either with SB (2 mg kg^{-1}) or 1% methyl cellulose (1h pretest), and then s.c. with either Ro (0.1 mg kg^{-1}), mCPP (0.7 mg kg^{-1}) or saline (40 min pretest). Rats were placed in the test arena with an unfamiliar, like-treated pair mate. Locomotor activity (line crossings) and SI (time spent sniffing, grooming, mounting, biting or crawling over) were scored blind with respect to treatment for 15 min.

The results are presented in Table 1. Ro and SB had no effect given alone. However, when combined with SB, Ro produced a significant anxiogenic-like reduction in social interaction. mCPP significantly reduced social interaction and locomotor activity, and these effects were not influenced by SB.

These results indicate that in the presence of 5-HT_{2B} receptor blockade Ro has anxiogenic-like properties in the SI test. This is in keeping with data demonstrating anxiolytic-like activity for the 5-HT_{2B} receptor agonist, BW723C86 (Kennett et al., 1996). Under normal circumstances 5-HT_{2B} receptor activation by Ro may mask 5-HT_{2C} receptor-mediated anxiogenesis, and explain its relatively mild anxiogenic-like activity in rodent tests.

Table 1

Drug dose mg kg^{-1} p.o.	Drug dose mg kg^{-1} s.c.	SI (s)	line crossings
Veh	Sal	101.7 ± 10.5	517.2 ± 32.2
SB 2	Sal	106.4 ± 11.4	502.4 ± 24.8
Veh	Ro 0.1	96.8 ± 9.2	436.7 ± 18.8
SB 2	Ro 0.1	63.1 ± 9.1*	482.9 ± 46.5
Veh	mCPP 0.7	27.1 ± 5.5**	391.2 ± 32.9**
SB 2	mCPP 0.7	35.6 ± 6.2**	316.0 ± 32.1**

Data given as mean ± s.e. mean; *, ** $p<0.05$, 0.01 vs. corresponding saline control (2 way ANOVA / Tukey's test).

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154P A SIMPLIFIED METHOD FOR THE MEASUREMENT OF ENDOGENOUS 5-HT IN RAT WHOLE BLOOD USING *IN VITRO* MICRODIALYSIS

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Earlier *in vitro* assays of 5HT release from platelet-rich plasma (PRP) have used cells preloaded with [^{3}H]5HT (*e.g.* Buczko *et al.*, 1975). Here, an *in vitro* microdialysis method has been developed to measure the more physiologically relevant endogenous 5HT in rat whole blood, thus avoiding the need for preparation of PRP.

Microdialysis loops comprised Hospal dialysis membrane and 23G x 22 mm stainless steel tubing supported by tungsten wire. Loops were soaked in (for 30 min) and then perfused (1.5 µl min^{-1}) with phosphate buffer (120 mM KH_2PO_4 and 8.5 mM NaH_2PO_4; pH 4.8) before sampling for 10 min periods. Dialysate (10 µl) was then injected onto an HPLC-ECD system to measure 5HT (and its metabolite, 5HIAA) recovered from standard solutions. If the recovery was ≥10%, loops were considered viable and were washed with phosphate buffer for at least 60 min before use. 5HT and 5HIAA were stable in phosphate buffer, provided that collected samples were kept at 4°C and analysed within 24 h.

Blood was removed from CO_2-asphyxiated male CD rats (200-400 g) by cardiac puncture and from each rat sample, 750 µl were dispensed into eight, 1000 µl Skatron tubes. A phosphate buffer-perfused loop was lowered into each tube for 30 min equilibration. Throughout, tubes were agitated using an orbital shaker (200 rpm). A 10-min basal 5HT sample was collected immediately prior to addition of test drug (in 7.5 µl). Drugs were equilibrated for 20 min before 10-min samples were taken 30, 40 and 50 min post-drug addition for 5HT assay. Vehicle (phosphate buffer) and a positive control (the 5HT-releasing agent, *d*-fenfluramine) were included in each test.

The use of whole blood or PRP as the sampling medium was investigated at room temperature (21°C) with either 110 mM sodium citrate or heparin (15 IU ml^{-1}) present as an anticoagulant. Basal 5HT values in PRP were inconsistent and frequently below detectable limits (5 pg ml^{-1}). Here, *d*-fenfluramine (50 µM), reported to cause 5HT release from PRP (Buczko *et al.*, 1975), was ineffective in heparinised PRP. In heparinised whole blood, however, both basal levels of 5HT and *d*-fenfluramine-evoked (50 µM) 5HT release were reproducibly quantified. Optimal sampling time post-drug was 40 min. Temperature effects were studied in both heparinised and citrated whole blood or PRP. Whilst 5HT release was temperature-dependent in all 4 media such that basal 5HT levels increased gradually, the most consistent basal 5HT release was in heparinised whole blood. Thus, in this medium, the effects of temperature on basal 5HT (mean pg.ml^{-1} ± s.e.mean; n=4) were: 5 ± 1 at 2°C; 19 ± 8 at 21°C; 79 ± 23 at 37°C). *d*-Fenfluramine (50 µM)-evoked 5HT release from heparinised whole blood also followed changes in temperature (mean % of 4 basal values ± s.e.mean: 454 ± 54 at 2°C; 864 ± 103 at 21°C; 1199 ± 143 at 37°C). Whilst each increase in temperature changed 5HT levels significantly from its predecessor ($p\leq0.02$, mixed linear model and Newman-Keuls' test), we preserved functionality of the system by performing subsequent experiments in heparinised whole blood at 21°C perfused with phosphate buffer (pH 4.8). At 21°C, the interpolated EC_{200} value (concentration doubling basal 5HT release) for *d*-fenfluramine in whole blood was 27.5 µM (see Lane *et al.*, 2000). This concentration consistently increased 5HT release as shown in Table 1.

Table 1. Effect of *d*-fenfluramine (27.5 µM) on basal 5HT release

Expt.	1	2	3	4	5	Mean ± s.e. mean
% basal	275	235	190	187	172	212 ± 19

In summary, a simple, yet sensitive *in vitro* microdialysis assay for measuring 5HT in rat whole blood has been developed and validated using the 5HT-releasing agent, *d*-fenfluramine.

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155P *d*-FENFLURAMINE RELEASES 5-HT FROM WHOLE BLOOD OF RATS AS MEASURED BY *IN VITRO* MICRODIALYSIS

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Primary pulmonary hypertension and cardiac valvulopathy associated with use of *d*-fenfluramine (*d*F) and the *dl*-fenfluramine/phentermine combination have been attributed, at least in part, to raised plasma 5HT levels (Fishman *et al.*, 1999). In both conditions, the compartmentalisation of 5HT between plasma and platelets is altered, such that excess levels are recorded in the plasma with reduced 5HT storage in platelets (Kolanowski *et al.*, 1999). Therefore, this study evaluated the effects of *d*F, its main metabolite, *d*-norfenfluramine (*d*NF) and phentermine on the release of 5HT from whole blood. The effect of combining *d*F with a monoamine uptake inhibitor, paroxetine (5HT-selective; SSRI) or venlafaxine (NA and 5HT; SNRI) was also investigated.

Whole blood was taken from male Sprague-Dawley rats (200-350 g) killed with CO_2. Basal 5HT levels were measured in heparinised whole blood at 21°C using the method of *in vitro* microdialysis (see Lane *et al.*, 2000). Data represent the mean ± s.e.mean or 95% confidence intervals (C.I.). Data were statistically analysed by 2-way ANCOVA (treatment and experiment as factors and baseline as covariate) and Williams' test.

Upon addition of *d*F, concentration-dependent increases in 5HT levels were seen and the EC_{200} (to evoke a doubling of vehicle-recorded levels) was calculated to be 27.5 μM (95% C.I. 8.58 μM, 80.7 μM). *d*NF also caused concentration-dependent 5HT release with an EC_{200} of 14.3 μM (95% C.I. 4.90 μM, 47.5 μM). Phentermine applied alone, from 3-300 μM, did not increase 5HT release. In combination with the EC_{200} of *d*F, phentermine (10-300 μM) did not alter the *d*F-induced 5HT release. Alone, neither paroxetine nor venlafaxine (0.01-10 μM) modified release of 5HT. However, when added prior to *d*F, each drug produced a concentration-dependent inhibition of the 5HT-releasing effect of *d*F such that in the presence of at least 0.1 μM paroxetine or venlafaxine, *d*F treatment no longer differed from vehicle treatment (see Fig. 1).

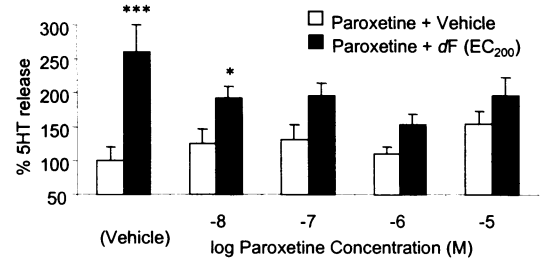

Figure 1. Effect of paroxetine on *d*F-induced 5HT release. Values are mean ± s.e.mean of 7-10 experiments. *p<0.05, ***p<0.001, significant change from vehicle-evoked release.

The results suggest that *d*F could be prevented from occupation of its binding site by the SSRI or SNRI. Alternatively, binding of the SSRI or the SNRI to the transporter may alter the shape of the *d*F binding site, thereby reducing both *d*F binding and its transport into the platelet.

These data acquired using whole blood reinforce the notion obtained using brain slices that *d*F and *d*NF act as 5HT-releasing agents, whilst uptake inhibitors do not release 5HT (Heal *et al.*, 1998). The lack of releasing action of phentermine in this *in vitro* study is consistent with *in vitro* findings using brain slices (Lancashire *et al.*, 1998).

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Lane, E.L. *et al.* (2000) This meeting.

156P THE EFFECT OF *d*-FENFLURAMINE ON FOOD INTAKE AND SOME 5-HT NEUROCHEMICAL PARAMETERS IN THE RAT

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Repeated *d*-fenfluramine (*d*F) dosing depletes 5HT and its transporter (SERT) from rat brain (Clineschmidt *et al.*, 1978). Many studies have investigated *d*F's effect on food intake but few have simultaneously analysed 5HT neurochemistry. The present study monitored (i) food intake during *d*F dosing and on withdrawal and (ii) regional brain 5HT levels together with $5HT_{2A}$ receptor and SERT densities in these rats at the end of the study.

Male Sprague-Dawley rats (300-350g) were individually-housed on reversed-phase lighting with free access to powdered diet and water. After 4 day baseline recordings of daily food intake and body-weights, vehicle (saline) or *d*F (10mg kg^{-1} p.o. *bid*) was given (day 0) for 4 days. Fourteen days after the final dose, [^{3}H]ketanserin and [^{3}H]citalopram autoradiography was used to assess brain $5HT_{2A}$ receptor and SERT densities, respectively (n=6; Pazos *et al.*, 1985; Viggers *et al.*, 2000). For the remaining brains (n=8), 5HT was measured in the frontal cortex, dorsal raphe, striatum and hypothalamus by HPLC-ECD.

Food intake ($g.kg^{-1}$) and body weights (g) are mean ± s.e.mean except for their baseline values which are means as determined by 2-way ANCOVA using day 0 baseline as a covariate. *d*F treatment reduced food intake on each day of dosing, maximally on day 1 (from a pre-drug baseline value of 69.4 to 6.3 ± 0.9; p<0.001) with slow, daily returns to 41.4 ± 4.5 on day 4, which was still lower than control (72.2 ± 1.3; p<0.001). Post-drug, food intake rose slowly but remained lower (–4 to –17%, p<0.05) than in controls for 2 more days after which, in *d*F-treated rats it exceeded that of controls daily up to the study end (saline 66.3 ± 0.9; *d*F 74.1 ± 1.2, p<0.001). The greatest loss of body-weight (g) was on day 4 of *d*F dosing (from 425 on day 0 to 388 ± 1 on day 4, p<0.001) but body-weights remained significantly lower than controls on each day post-drug (study-end values: saline 470 ± 3; *d*F 455 ± 2, p<0.001). Table 1 shows that 5HT levels were significantly decreased in all regions, $5HT_{2A}$ receptors were unaltered in all brain areas, with the exception of the frontal cortex and SERT levels were depleted in all regions apart from the dorsal raphe.

Table 1. Effect of *d*F on 5HT, $5HT_{2A}$ receptors and SERT levels.

	Frontal Cortex	Dorsal Raphe	Hypothalamus	Striatum
5HT C	1214 ± 45	2124 ± 164	2604 ± 109	994 ± 60
*d*F	290 ± 55***	890 ± 178**	935 ± 88***	375 ± 56***
$5HT_{2A}$ C	72 ± 3	NT	NT	60 ± 2
*d*F	62 ± 3*	NT	NT	55 ± 3
SERT C	66 ± 3	143 ± 6	97 ± 5	26 ± 1
*d*F	12 ± 1***	143 ± 8	34 ± 1***	7 ± 1***

Values are mean ± s.e.mean $ng.g^{-1}$ tissue wet weight (5HT) or $fmol.mg^{-1}$ tissue equivalent ($5HT_{2A}$ receptors and SERT). *p<0.05, **p<0.01, ***p<0.001, by 1-way ANOVA with multiple *t*-test (5HT) or mixed linear model ($5HT_{2A}$ receptors and SERT). NT= not tested.

The profile of substantial weight loss, weight regain and rebound hyperphagia after *d*F has been reported previously (*e.g.* Roth & Rowland, 1998). Despite significant losses of 5HT and its transporter, particularly in the hypothalamus, the levels remaining are still able to support feeding behaviour. The resistance of the SERT in the dorsal raphe to depletion may indicate that the somatodendritic regions of 5HT neurones do not accumulate *d*F in the same way as terminals. Previous studies have shown marked falls in $5HT_{2A}$ binding immediately after 14 days of daily *d*F treatment (Heal *et al.*, 1998). The 14 day washout allowed $5HT_{2A}$ receptor numbers generally to return to control values, although small reductions were seen in two regions of neocortex; this could reflect either a slight delay in their recovery or a more permanent reduction.

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157P THE USE OF BEHAVIOURAL CORRELATES IN MICE TO DIFFERENTIATE PSYCHOSTIMULANTS, HALLUCINOGENS AND ENTACTOGENS

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Various psychoactive drugs that cause hallucinations in man evoke head-twitches in mice via $5HT_{2A/2C}$ receptor activation (Leonard, 1996). Also, psychostimulants which release dopamine and 5HT enhance locomotion in rodents (Calloway *et al.*, 1990; Kelly *et al.*, 1975). Therefore, this study in adult male C57/BL/6J mice (20-30g) has compared the effects of the monoamine-releasing drugs, *d*-amphetamine (psychostimulant), (±)3,4-methylene-dioxy-methamphetamine (MDMA; 'entactogen'), phentermine and *d*-fenfluramine (anti-obesity agents), the reuptake inhibitors, sibutramine (anti-obesity agent) and fluoxetine (antidepressant) and the $5HT_{2A/2C}$ agonist hallucinogen, 5-methoxy-*N*,*N*-dimethyltryptamine (5MeODMT) on these two behaviours. Head-twitches were counted 0-6, 30-36 and 60-66 min post-drug (0.3-10 mg kg^{-1}, i.p.). Locomotor activity of individual mice was monitored in test cages via infra-red beam breaks for 2 h post-drug (1-10 mg kg^{-1}, i.p.).

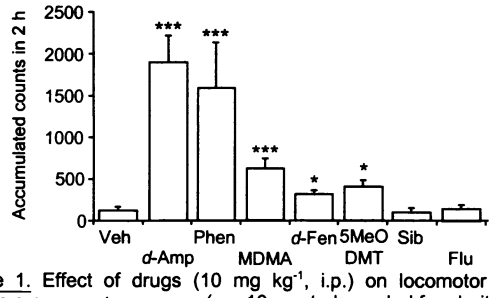

Figure 1. Effect of drugs (10 mg kg^{-1}, i.p.) on locomotor activity. Values are mean ± s.e.mean (n = 10, controls pooled for clarity).

As indicated by locomotor activity, phentermine and *d*-amphetamine are approximately equipotent as psychostimulants. The 'entactogen', MDMA, the 5HT-releasing agent, *d*-fenfluramine, and the $5HT_{2A/2C}$ agonist were considerably less potent. None of the other drugs significantly enhanced locomotor activity. In contrast, the powerful hallucinogen, 5MeODMT, was the most potent inducer of head-twitches. This response was also evoked by *d*-fenfluramine which is consistent with the observation that at high dose, this 5HT-releasing drug is hallucinogenic in man (Griffith et al., 1975). *d*-Amphetamine, phentermine and MDMA did not produce head-twitches. Rather, they suppressed this response, an action which may reflect their activation of catecholamine systems (Handley & Singh, 1986).

Table 1. Effect of drugs on head-twitch responses.

Drug (10 mg kg^{-1} i.p.)	Cumulated head-twitches	Drug (10 mg kg^{-1} i.p.)	Cumulated head-twitches
Veh	2.7 ± 0.3	*d*-Fen	11.1 ± 0.5***
d-Amp	0.1 ± 0.1†††	5MeODMT	20.5 ± 1.4***
Phen	0.4 ± 0.3†	Sib	1.3 ± 0.4
MDMA	0.2 ± 0.2†††	Flu	1.8 ± 0.5††

Values are cumulated head-twitches over 3 observation periods ± s.e.mean (n = 9-10). Vehicle-induced head twitches were pooled for clarity. Increases ***p<0.001 or decreases †p<0.05, ††p<0.01, †††p<0.001 *vs* respective vehicle one-way ANOVA and Williams' test.

Taken together, these results predict that in humans phentermine and *d*-amphetamine will have similar potencies as psychostimulants, whilst 5MeODMT and *d*-fenfluramine will be predominantly hallucinogenic. MDMA is interesting in that it appears neither markedly psychostimulant, nor hallucinogenic, which is consistent with its unique abuse classification as an 'entactogen'. The data also predict that, unlike the monoamine releasers, the reuptake inhibitors, sibutramine and fluoxetine, lack the efficacy required for psychostimulant or hallucinogenic abuse liability.

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158P BTS 72664: ITS MODE OF ACTION DEDUCED FROM RAT CORTICAL WEDGE, RAT HIPPOCAMPAL SLICE AND MOUSE CORTICAL NEURONE PATCH CLAMP STUDIES

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BTS 72664, (*R*)-7-[1-(4-chlorophenoxy)ethyl]-1,2,4-triazolo[1,5-*a*] pyrimidine, has broad spectrum anticonvulsant activity in rodent epilepsy models (Thompson et al, 2000a), but has little or no affinity for sodium, potassium, NMDA or $GABA_A$ channels or GABA synthesis or degradation enzymes (Thompson et al, 2000b). However, it reduces [^{86}Rb] efflux from depolarised synaptosomes (Thompson et al, 2000b), suggesting an effect on membrane polarisation. Consequently, the activity of BTS 72664 against spontaneous epileptiform discharges (SEDs) in rat cortical wedges, its effect on CA1 pyramidal neurone resting potential in rat hippocampal slices, and its ability to potentiate NMDA and GABA-evoked currents in voltage-clamped mouse cortical neurones, were used to reveal its mode of action.

Cortical wedges were prepared from halothane-anaesthetised 80-120g male Sprague-Dawley rats. Cortical wedge recording was as described in Phillips et al, (1996). SEDs were induced either by removal of Mg^{++} from the perfusing aCSF (Phillips et al, 1996), or by administration of 10μM (-)-bicuculline methylbromide. BTS 72664 was dissolved at 10mM in DMSO and diluted in the appropriate aCSF. Wedges were superfused with BTS 72664 at increasing concentrations for 10 min. Mean SED frequency and amplitude was calculated for the treatment interval. Hippocampal CA1 pyramidal neurone intracellular recordings were carried out using 100-150MΩ, 1M potassium acetate-filled microelectrodes, and AxoClamp 2B amplifier. Following impalement, the recording was stabilised for at least 10 min, then resting potential measured. BTS 72664, prepared as above, was perfused over the slice for 20 min (1 cell), 30 min (3 cells) or 45 min (2 cells), then resting potential measured in the presence of drug. Whole-cell-patch voltage-clamp recordings were made from primary cortical neuronal cultures of 15 day gestation Swiss mice, grown in culture for 2-3 weeks (Hertz et al, 1990). Saline contained 200nM strychnine and 200-500nM tetrodotoxin, with 1μM glycine and 10μM picrotoxin (for NMDA currents) or 1mM $MgCl_2$ (for GABA currents). Patch electrodes, of 2-3MΩ, containing 153mM CsCl, 10mM EGTA, 10mM HEPES and 4mM $MgCl_2$, and an AxoPatch amplifier, clamped cells at -60mV. NMDA (10μM) or GABA (1μM) were applied for 1-5 sec. BTS 72664 was added 20-30 sec prior to agonist.

BTS 72664 attenuated SEDs due to removal of Mg^{++} from the aCSF, with an IC_{50} (95% confidence interval in parentheses) of 189μM (106-335μM, n=3-7) for frequency, and >300μM (n=3-7) for amplitude. It also attenuated SEDs induced by bicuculline, with an IC_{50} for frequency of 94μM (56-159μM, n=7), and for amplitude of 243μM (96-614μM, n=7). BTS 72664 hyperpolarised CA1 pyramidal neurone resting potential by 9.5mV at 100μM (S.E.M. 1.89mV, n=3 cells) but had no effect at 10μM (n=3 cells). In voltage-clamp, BTS 72664 attenuated NMDA-evoked currents with an IC_{50} of 43μM (n=2-5 cells). It also potentiated GABA-evoked currents, producing increases of 194±16% at 10μM and 511±64% at 100μM. This potentiation was not blocked by 5μM flumazenil (n=4-5 cells); however, 100μM BTS 72664 alone induced an inward current that was blocked by 100μM picrotoxin (n=4 cells).

These data indicate that the broad spectrum anticonvulsant, BTS 72664, mediates its effects primarily through potentiation of a picrotoxin-sensitive $GABA_A$ current, but not by acting at the benzodiazepine binding site. In addition, the compound is a weak NMDA receptor blocker. These data are congruent with BTS 72664 binding affinities, and its activity in a broad range of electroshock and chemoconvulsant seizure models (Thompson et al, 2000a,b).

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159P 5-HT_{1B} RECEPTOR AGONIST:ANTAGONIST BINDING AFFINITY DIFFERENCES AS A MEASURE OF INTRINSIC ACTIVITY

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It is believed that G-protein coupled receptors can exist in alternative affinity states which have both high and low affinity for agonists, but which display similar affinity for antagonists. Previous studies have shown that the difference in affinity of a compound for these two states gives an indication of functional activity (Freedman *et al*, 1988). [^{3}H]GR125743, a selective 5-$HT_{1B/1D}$ receptor antagonist, is a commercially available radioligand which labels both high and low affinity states of 5-$HT_{1B/1D}$ receptors (Selkirk *et al*, 1997). We have used this in conjunction with [^{3}H]5-CT, a radiolabelled agonist. In this study, we have compared differences in compound inhibition of these two radioligands, with compound efficacy in a human (h) 5-HT_{1B} receptor expressing cell line.

Human 5-HT_{1B} receptors were stably expressed in Chinese Hamster Ovary (CHO) cells (Watson *et al*, 1996). Receptor binding studies and membrane preparations were carried out as previously described (Selkirk *et al*, 1997) using either [^{3}H]5-CT (2nM) or [^{3}H]GR125743 (1nM). [^{35}S]GTPγS binding studies and membrane preparations were carried out as previously described (Watson *et al*, 1996) Intrinsic activity was calculated as a % of basal [^{35}S]GTPγS binding, and expressed as a fraction of the maximal 5-HT response.

In saturation binding studies, [^{3}H]GR125743 labelled a higher number of h5-HT_{1B} receptors than [^{3}H]5-CT, (Kd 2.8nM and 2.0nM, B_{max} 51.3 and 11.5 pmol/mg protein respectively). Binding studies showed that agonists exhibited a higher affinity when competing with [^{3}H]5-CT than with [^{3}H]GR125743 (Table 1), whilst antagonists displayed similar affinities when competing with both radiolabels. In [^{35}S]GTPγS binding studies, both 5-HT and 5-CT were full agonists, whilst GR127935, GR125743 and SB-220272 appeared to be partial agonists. All other test compounds appeared to be inverse agonists (Table 1).

Table 1. Inhibition of [^{3}H]5-CT and [^{3}H]GR125743 binding to h5-$HT1_B$ Receptors Expressed in CHO cells

	pKi [^{3}H]5-CT	pKi [^{3}H]GR125743	pKi Difference	Intrinsic Activity
5-HT	8.3 ± 0.1	6.5 ± 0.1	1.8	1.0 ± 0.0
Sumatriptan	7.7 ± 0.0	6.0 ± 0.1	1.7	0.9 ± 0.0
GR127935	7.7 ± 0.0	7.3 ± 0.1	0.4	0.8 ± 0.0
GR125743	8.3 ± 0.1	7.8 ± 0.0	0.5	0.8 ± 0.1
SB-220272	7.4 ± 0.1	7.1 ± 0.0	0.3	0.3 ± 0.1
SB-236057	7.4 ± 0.1	7.2 ± 0.0	0.2	inv
Methiothepin	7.4 ± 0.1	7.1 ± 0.0	0.3	inv
SB-224289	7.1 ± 0.0	6.9 ± 0.0	0.2	inv
5-CT	8.5 ± 0.1	6.1 ± 0.0	2.4	1.0 ± 0.0

All values are mean +/- SEM from 3 independent experiments

These data suggest that CHO cells express h5-HT_{1B} receptors in two affinity states, although the majority of sites in the recombinant system exist in the low agonist affinity state. The antagonist radiolabel [^{3}H]GR125743 labels both the high and low agonist affinity states with similar affinity, whilst [^{3}H]5-CT mainly labels the high agonist affinity state. In systems where both affinity states exist, the agonist:antagonist affinity difference can be used to give an indication of the functional efficacy of a test compound. In this system, the intrinsic activity measured by [^{35}S]GTPγS binding and the pKi difference between [^{3}H]5-CT and [^{3}H]GR125743 show a linear relationship, with a correlation coefficient (r) of 0.78. Unfortunately, as we have described previously (Selkirk *et al*, 1997) in rat native tissue, the high agonist affinity state predominates. Therefore such studies of compound intrinsic activity from affinity differences are not feasible in brain tissue.

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160P MICE TRANSGENIC FOR THE HUMAN HUNTINGTON'S DISEASE MUTATION ARE NOT MORE SUSCEPTIBLE TO 3-NITROPROPIONIC ACID THAN WILD TYPE MICE

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Huntington's disease (HD) is a progressive, genetic disorder leading to degeneration of neurones in the caudate nucleus and putamen (Vonsattel and DiFiglia, 1998). The underlying pathology is not understood, and there is no known cure or treatment. A number of animal models for HD have been developed, including the use of the irreversible succinate dehydrogenase (SDH) inhibitor 3-nitropropionic acid (3-NP) (Beal, 1996). Chronic administration of 3-NP to rodents results in formation of bilateral striatal lesions comparable to the degeneration seen in HD. This has led to the suggestion that an underlying energy deficiency may lead to pathology in HD (see Beal, 1996). A transgenic mouse (R6/2) for the human HD mutation has also been developed as a model for HD. This mouse displays a progressive neurological phenotype (Miangiarini *et al.*, 1996). Thus, it is possible that if an energy deficiency underlies HD pathology, these mice would have an increased susceptibility to 3-NP.

Male mice (15 R6/2 and 15 wild type (WT) littermates, 15-20 g) were treated daily with 3-NP (s.c., in 0.9 % saline, pH 7.4) from 7 weeks of age, starting at a dose of 50 mg kg^{-1}, increasing by 10 mg kg^{-1} every 4th day for 12 days and then by 5 mg kg^{-1} every 4th day thereafter. Treatment continued until mice showed characteristic motor abnormalities indicating toxicity (Reynolds *et al.*, 1998) or died. Control mice (16 R6/2 and 14 WT) were treated in parallel with 0.9 % saline. All mice were weighed daily, and motor performance tested weekly on a rotarod at 8, 15, and 33 r.p.m. When mice became intoxicated, 3-NP injections were stopped. One week later they were killed and their brains removed and frozen. Brains of mice that died during the experiment were also collected and frozen. SDH activity was measured in 30 μm cryosections of brain. Sections were incubated with a solution containing 50 mM phosphate buffered saline, 50 mM sodium succinate, 25 mM sucrose, 2 mM EDTA and 1 mg ml^{-1} nitro blue tetrazolium for 30 min at 37^{0}C in the dark. They were washed twice with distilled water and the intensity of colour quantified spectrophotometrically (690/540 nm). Striatal lesion volume was calculated from sections stained histochemically for SDH (Reynolds *et al.*, 1998). Data were analysed by two-way analysis of variance and χ^2 test.

The mean weights of 3-NP-treated R6/2 and WT mice were significantly lower than those of controls ($p < 0.001$). 3-NP-treated R6/2 mice fell significantly earlier from the rotarod than NaCl-treated R6/2 mice (on average 25.57 ± 2.46 s earlier, at 8 - 33 r.p.m., $p < 0.01$ on last retest). 3-NP-treated WT mice also fell earlier than NaCl-treated WT mice (on average 29.82 ± 5.68 s earlier, 8 - 33 r.p.m. $p < 0.01$ on last retest), with no difference in performance of 3-NP-treated WT and R6/2 mice. Survival of 3-NP-treated mice was significantly reduced ($p < 0.01$) compared to controls. There was no difference in survival, SDH activity or lesion volume between 3-NP-treated R6/2 and 3-NP-treated WT mice. However, it was particularly interesting that although a similar number of R6/2 and WT mice became intoxicated and survived (n = 8 for both), significantly fewer surviving R6/2 mice developed lesions ($p < 0.05$).

Our data show that R6/2 mice do not have an increased susceptibility to 3-NP and in some respects appear less susceptible than wild type mice. This questions the involvement of energy deficiency in the pathology underlying early HD.

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161P EFFECT OF GASTRIN-RELEASING PEPTIDE (GRP) ON RAT HIPPOCAMPAL GABA LEVELS AND AUDIOGENIC SEIZURES IN THE DBA/2 MOUSE

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Bombesin receptors of the BB_2 subtype have been found on some GABAergic interneurones in the *stratum oriens* layer of the hippocampus (Lee *et al.*, 1999). GRP depolarised those neurones and produced a large inward current that was blocked by the selective BB_2-receptor antagonist [D-Phe^6, Leu-NHEt^{13}]bombesin(6-13) ("D-Phe"). We have now tested for a functional correlate in the whole animal by measuring changes in extracellular levels of GABA after local perfusion with GRP in the ventral hippocampus of the freely moving rat, and by testing for an action of GRP to protect against audiogenic seizures in the mouse.

Male Hooded Lister rats (250-350g) were implanted with dialysis probes (BAS, 4mm membrane, ~8000 mwt cut-off) in the ventral hippocampus 18h prior to the experiment and perfused with aCSF (mM:NaCl 140, $CaCl_2$ 1.2, KCl 4.0, glucose 11) at 1µl min^{-1}. Samples were collected every 30 min, derivatised with *o*-phthalaldehyde and *t*-butylthiol, and analysed by HPLC-ECD for their content of GABA. Peptides were infused into the hippocampus by reverse dialysis, following the collection of 3 samples that did not differ in their content of GABA by more than 10%. Male DBA/2 mice (6-10g) received intracerebroventricular (i.c.v.) free-hand injections of GRP dissolved in saline, or saline vehicle (5µl), under anaesthesia (3-4% isoflurane in 20% O_2, 80% N_2O) at lambda. Following removal from the anaesthetic chamber and 15 minutes after injection of GRP, tonic seizures were induced by ringing an electric bell (125dB, 1.4kHz) for 60s, and latency to convulse was measured. Animals failing to show a tonic seizure within this time were given a maximum latency score of 60s.

GABA levels were increased above basal (503±35.6pg, n=18) in the two samples collected while GRP (10µM in aCSF) was present in the ventral hippocampus (first sample 126.8±7.1% $p<0.01$, second sample 142.9±11.5% $p<0.01$ relative to basal levels). There was a prompt return to basal levels when GRP was removed from the aCSF (Figure 1). The effect

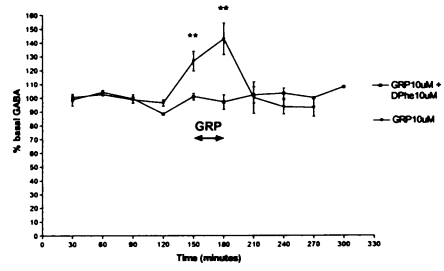

Figure 1 The effect of GRP on GABA levels in rat hippocampus (** $p<0.01$, Student's t-test versus last basal value, n=5-6)

of GRP was blocked by co-administration of the antagonist D-Phe at 10µM (Fig 1), which by itself had no effect on GABA levels (data not shown).

In the DBA/2 mouse the i.c.v. injection of GRP produced a dose-dependent increase in the latency to convulse (veh: 13.3±5.2s; 30ng GRP: 9.7±1.5s; 100ng: 37.5±10.3s; 300ng: 52.2±7.8), with a near total blockade of seizures with 300ng. The injection of GRP also produced the characteristic hind-limb scratching response at all doses tested. Co-administration of the BB_2-receptor antagonist D-Phe (100-1000ng) produced a dose-dependent antagonism of the anticonvulsant effect of 100ng GRP, with a block obtained at 1000ng (veh: 7.8±0.9s; GRP 100ng: 32.6±5.6s; GRP 100ng + DPhe^6 1000ng: 8.1±0.5s). D-Phe by itself did not induce scratching, but at 1000ng blocked this effect of the 100ng dose of GRP.

In conclusion, GRP increases the latency to convulse in the audiogenic seizure-prone DBA/2 mouse, and this effect may be due to increased GABAergic function as suggested by the ability of the BB_2 agonist to increase extracellular GABA levels in the ventral hippocampus of the rat.

Lee, K., Dixon, A.K., Gonzalez, I., Stevens, E.B., McNulty, S, Oles, R.J., Richardson, P.J., Pinnock, R.D., Singh, L. (1999) *J. Physiol*, **518**, 791-802.

162P THE LOW AFFINITY NMDA RECEPTOR ANTAGONIST ARR 15896AR REDUCED HEMINEGLECT AND INFARCT SIZE IN A PRIMATE MODEL OF STROKE

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There is good evidence for the neuroprotective efficacy of NMDA antagonists in various experimental models of stroke. The low affinity, use dependent, non-competitive NMDA receptor antagonist AR-R15896AR has been shown to be neuroprotective in transient focal cerebral ischaemia induced by occlusion of the middle cerebral artery (MCA) of rats (Cregan *et al.*, 1997). We have now examined the neuroprotective efficacy of AR-R15896AR in marmosets with a permanent MCA occlusion, using both histopathological and functional measures.

Eleven marmosets, *Callithrix jacchus*, had the M1 segment of their right MCA permanently occluded (pMCAO) by bipolar coagulation as previously described (Marshall *et al.*, 1999). Five minutes later, the monkeys received a 1ml i.v. infusion of either saline or AR-R15896AR (4.5mg kg^{-1}) and osmotic minipumps, model 2001D, implanted s.c. to provide continuous drug or saline infusion for 48 h. Drug filled pumps released AR-R15896AR at 1.1mg kg^{-1}.h. Surgery, behavioural testing and histological analysis were all done 'blind' to treatment condition. Statistical comparisons were made using ANOVA and *post hoc* Newman-Keuls *t*-tests.

The monkeys were trained pre-operatively on the Hill and Valley Staircase Tasks (Marshall *et al*, 1999) and re-tested 3 and 10 weeks after surgery. Before surgery monkeys were equally adept at reaching with each arm into either hemispace. Three weeks after surgery all the monkeys were severely impaired at reaching with their contralesional arm. However, AR-R15896AR-treated monkeys were significantly better at reaching with their ipsilesional arm into contralesional space than were saline-treated monkeys, who neglected food rewards in that hemispace, score 10.4±2.93 compared to 3.2±1.29 (t=20.1, $p<0.01$). By 10 weeks, the neglect had recovered, and there were no differences between the 2 groups. Histopathological analysis showed a reduction in the size of infarct at several stereotaxic levels (Figure), particularly in the region of the parietal cortex.

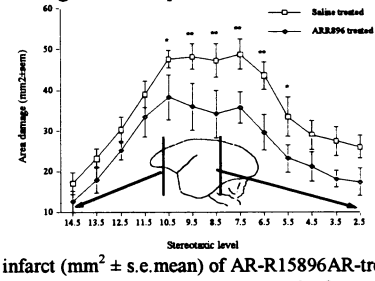

Figure. Area of infarct (mm^2 ± s.e.mean) of AR-R15896AR-treated (n=6) and saline-treated (n=5) monkeys 10 weeks after pMCAO.

This study has shown that AR-R15896AR is neuroprotective against permanent focal cerebral ischaemia and ameliorated the ensuing spatial neglect in the marmosets.

Marshall, J.W.B. *et al.* (1999) *Exp. Neurol.* **156**, 121-129.
Cregan, E.F. *et al.* (1997) *J. Pharmacol. Exp. Ther.* **283**, 1412-1424.

EJJ was supported by a Wellcome Trust Vacation Student Scholarship, Ref VS9/CAM/011/CH/TG/JG. We thank J Gabrielsson, J Lundström and S Curry for pharmacokinetic modeling of doses required.

163P POTENTIATION OF MUSCIMOL-INDUCED LONG-TERM DEPRESSION BY DIAZEPAM AND CHLORDIAZEPOXIDE

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Long-term depression (LTD) is a long lasting decreases in synaptic activity that follows some type of electrical stimulation in the hippocampus (Akhondzadeh & Stone, 1995). There is growing evidence that LTD does, in fact, occur in the nervous system and is believed to be involved in learning and memory processes (Akhondzadeh & Stone, 1996a). We have recently reported a new protocol for inducing LTD through activation of $GABA_A$ receptors in the hippocampal slices (Akhondzadeh & Stone, 1996a,b,c). This type of LTD is reversed by bicuculline and potentiated by neurosteroids. It was also shown that glutamate receptor activity or extracellular calcium are not involved in the induction of this type of LTD (Akhondzadeh & Stone, 1996b). The present study investigated the possible relation between muscimol-induced LTD and barbiturates/benzodiazepines-induced amnesia.

Hippocampal slices 450 µm thick were prepared from male Wistar rats (170-210 g) and were superfused at 30°C with ACSF (in mM : KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, Glucose 10, $MgCl_2$ 1.2 saturated with 95% O_2/ 5% CO_2). Extracellular recordings of orthodromic potentials were made in the CA1 pyramidal cell layer following stimulation of Schaffer collateral fibres in the stratum radiatum (0.01 Hz).

Under normal experimental conditions, when Schaffer collateral/commissural fibers were stimulated at a frequency of 0.01 Hz, the addition of $GABA_A$ agonist muscimol at concentration of 10 µM for 10 minutes induced a stable LTD of population potentials in which spike sized was reduced by 97.6%±0.1 (mean±s.e.m., P<0.001, n=4, Bonferroni post hoc test). When used at concentrations of 0.5 to 2 µM, perfused for 10 min, this inhibition was readily reversible by washing. The LTD induced by muscimol was concentration and time dependent as described previously (Akhondzadeh & Stone, 1995). There was no sign of recovery for at least 120 minutes. Pentobarbital sodium was superfused over the slices at concentrations of 100 and 200 µM. Neither concentration had any effect itself on population potentials. However, pentobarbital 200 µM proved able to potentiate the ability of muscimol to induce LTD. A normally ineffective concentration of 2 µM muscimol, applied for 10 minutes was able to induce LTD in the presence of pentobarbital 200 µM. As for muscimol alone, increasing stimulation frequency to 1 Hz for 10 seconds was sufficient to reverse the LTD and restore potentials to their control size (98% ± 2.2 of control, non-significant., n=4).

In another set of experiments, diazepam and chlordiazepoxide were superfused over the slices at concentrations of 10 and 20 µM. Neither compounds had any effect itself on population potentials. However, both agents potentiated the ability of muscimol to induce LTD. A concentration of 2 µM of muscimol, which was not able to induce LTD was applied for 10 minutes in the presence of 10 µM diazepam or 20 µM chlordiazepoxide. These combinations of $GABA_A$ agonist and benzodiazepines proved able to induce LTD. Both agents either potentiate the inhibitory effect of muscimol on population spike or the ability of muscimol to induce LTD.

It is possible that the results suggest that the potentiation of this type of LTD by benzodiazepines and barbiturates may explain one of adverse effects of these drugs, amnesia and cognitive impairment.

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Akhondzadeh, S. & Stone, T.W. (1996b). Neuroscience., 71, 581-588.

Akhondzadeh, S. & Stone, T.W. (1996c). Life Sciences., 58, 1023-1030.

164P NORADRENERGIC INVOLVEMENT IN THE EXPLORATORY BEHAVIOUR OF ISOLATION REARED RATS

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The ascending noradrenergic (NA) system from the locus coeruleus has been implicated in attention and arousal. However, there are conflicting evidence on the effects of central NA depletion on fear and anxiety reactions (Delini-Stula *et al.*, 1984). Isolation rearing from weaning induces behavioural and neurochemical changes in the adult (Fulford & Marsden, 1996). The most consistent behavioural effect of social isolation in rats is locomotor hyperactivity in a novel environment. This study investigated the effect of central NA depletion by the selective neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), on the exploration and locomotor activity (LMA) of isolated rats.

Male Lister hooded rats (24) were housed either singly or in groups (6/cage) after weaning (21 days). During week 2, the rats were initially tested in LMA photocell cages (Lapiz *et al.*, 1999) and were then injected with DSP-4 (25 mg/kg, i.p.) or 0.9% saline at 1 ml/kg (6/group). During week 4, rats were tested in a novel open field for 10 min. Following two 5-min habituation sessions in the open field, the rats were subjected to the novel exploration test in the same field (Giustino *et al.*, 1996). At trial 1 (T1), rats were exposed to two identical objects while at trial 2 (T2), they were exposed to one familiar and one novel object. Activity was tracked by a computerised system (Ethovision). Parameters measured included distance travelled, exploration, rearing, grooming and defecation.

Changes in cortical noradrenaline levels were initially analysed 72 hours after DSP-4 (i.p.) using high performance liquid chromatography with electrochemical detection in a separate group of rats. Cortical noradrenaline was also determined 28 days after DSP-4 treatment in the animals used for behaviour. All data were analysed using t-test or ANOVA with Bonferroni's test as post hoc, where appropriate.

Cortical noradrenaline was significantly ($P<0.01$) reduced 72 h after DSP-4. 28 days after DSP-4 injection, noradrenaline in the cortex was still reduced ($P<0.05$) in both group and isolation reared rats.

Isolates had significantly higher locomotor ($P<0.001$) and rearing activity ($P<0.05$) in the LMA cages compared to group controls. They travelled greater distance ($P<0.05$) in the open field, although exploration and discrimination of novel objects were comparable with the group reared controls. Group reared DSP-4 treated rats had enhanced ($P<0.05$) inner zone activity in the open field compared to saline controls although their exploration of the novel object was comparable. DSP-4 in the isolates had no effect on open field behaviour and object exploration in T1 compared to saline injected isolates. However, their exploration of the novel object in T2 was reduced (group reared DSP-4: 46±7.0 vs isolation reared DSP-4: 34±1.9, $P<0.05$) while their rearing was enhanced (group reared DSP-4: 3.2±0.6 vs isolation reared DSP-4: 8.7±1.5, $P<0.05$). Neither social isolation nor DSP-4 treatment altered grooming and defecation.

The results indicate that isolation rearing influences the behavioural effects of central NA depletion produced by DSP-4. This may be suggestive of isolation-induced changes in the central NA system in isolation reared rats.

Delini-Stula, A *et al.* (1984) *Pharmacol Biochem Behav* 20: 613-618.
Fulford, A and Marsden, CA (1996) *Psychopharmacol* 116: 183-190.
Guistino, A *et al.* (1996) *Brain Res* 728:149-156.
Lapiz, MDS, Parker, TL and Marsden, CA (1999) *Proc Aust Neurosci Soc*10: 200.
MDL holds a University of Nottingham and an ORS scholarship.

165P INHIBITORY EFFECT OF THE ADENOSINE A_1 AND κ-OPIOID SYSTEMS ON THE EXPRESSION OF THE μ-WITHDRAWAL RESPONSE IN THE GUINEA-PIG ILEUM: REVERSAL BY CHOLECYSTOKININ

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In the isolated guinea-pig ileum (GPI) a brief exposure to the selective μ-agonist, dermorphin (D), indirectly activates the κ-opioid system, which in turn inhibits the expression of the naloxone-induced withdrawal contraction (Valeri et al., 1996). Under some experimental conditions, the adenosine system may also control the GPI μ-withdrawal expression (Romanelli et al., 1997). Cholecystokinin octapeptide (CCK8) strongly enhances the expression of the μ-withdrawal response; this effect can be partially ascribed to the absence of κ-opioid system indirect activation in presence of CCK8 (Romanelli et al., 1999). Aims of the present work were to investigate: i) whether, in addition to the κ-opioid system, the GPI adenosine system is also activated following exposure to D; ii) the relative importance of the two systems in controlling the expression of the μ-withdrawal response; iii) to verify if CCK8 can also inhibit the activation of the adenosine system. GPI segments from males (300 –400 g) were set up under 1 g tension in an organ bath containing Tyrode's solution. The contractile responses were recorded by an isotonic force transducer and expressed as per cent of the maximal ACh response. Tissue segments were exposed for 5 min to D (1.2 or 12 nM) and then challenged with naloxone (NL, $5{,}4\times10^{-7}$ M); in the subsequent D/NL tests, the selective κ-opioid antagonist, nor-binaltorphimine (BNI, 3.4×10^{-8} M) and/or the selective adenosine A1 antagonist, 8-cyclopentyl-1,3 dimethylxanthine (CPT, 4×10^{-7} M), were added 30 sec before NL. Tissues were then tested with D/CCK8/NL (CCK8 2.2 nM was added 2 min after D, NL 3 min after CCK8); this test was then repeated but CPT (4×10^{-7} M) was added 30 sec before NL. Both CPT and BNI increased the response to NL (Table 1). In presence of both antagonists, the response to NL further increased (Table 1), reaching an intensity similar to that observed in the D/CCK8/NL tests (68 ± 5.6 with D 1.2 nM, 74 ± 5.9 with D 12 nM). CPT, added 30 sec before NL, did not alter the response to NL in the D/CCK8/NL tests (not shown).

Table 1. Responses to NL after a 5 min exposure to D. Values are means; s.e.mean is shown in brackets; n=20.

Antagonist added before NL	-	CPT	BNI	CPT+BNI
D 1.2 nM	16 (3.9)	57 (5.4)*	49 (7.7)*	70 (2.7)*,§,#
D 12 nM	15 (3.7)	36 (9.4)*	37 (9.0)*	68 (6.6)*,§,#

*: $p<0.01$ *vs.* the response in the absence of antagonists; §: $p<0.01$ *vs.* CPT; #: $p<0.01$ *vs.* BNI (ANOVA followed by Student-Newman-Keuls test).

These results suggest that in the GPI a brief exposure to D activates both the κ-opioid and adenosine systems; both systems, in turn, inhibit the expression of the μ-withdrawal response to a similar extent. In presence of CCK8, the adenosine system may not be activated.

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Romanelli L. *et al.* (1997) *Pharmacol. Res.* **35**(Suppl.), 54.
Romanelli L. et al. (1999) *Br. J. Pharmacol.*, **127**, 909-918

166P SENSITIVITY OF INTESTINAL Na^+-K^+ ATPase TO INHIBITION BY DOPAMINE IN SPONTANEOUS HYPERTENSIVE AND WISTAR-KYOTO RATS

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The present study aimed to evaluate, in male spontaneous hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats, the activity of jejunal Na^+-K^+-ATPase and its sensitivity to inhibition by dopamine and selective dopamine agonists during low salt (LS), normal salt (NS) and HS intake. Isolation of jejunal epithelial cells and Na^+-K^+-ATPase assay was performed as previously described (Vieira-Coelho et al., 1998). Results are given as arithmetic means ± s.e. mean, n=5. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. A P-value less than 0.05 was assumed to denote a significant difference. Basal jejunal Na^+-K^+-ATPase activity (in nmol Pi mg protein^{-1} min^{-1}) in SHR on LS intake (141±5) was higher ($P<0.05$) than in WKY rats (101±7). Jejunal Na^+-K^+-ATPase activity in WKY rats, but not in SHR, on LS intake was significantly ($P<0.05$) reduced (20% decrease) by dopamine (1 μM). The effect of changing from LS to NS or HS intake in WKY rats was a significant increase in basal jejunal Na^+-K^+-ATPase activity (from 101±7 to 145± and 160±12) and attenuation of the inhibitory effect of dopamine. In SHR, changing from LS to NS or HS intake increased basal jejunal Na^+-K^+-ATPase activity (from 141±5 to 162±5 and 157±4). As shown in figure 1, in WKY rats on LS intake the effect of both dopamine (1 μM) and SKF 38393 (10nM) (Jose et al., 1992), but not quinerolane (10 nM), was a significant reduction in jejunal Na^+-K^+-ATPase activity, this being antagonised by the D_1 receptor antagonist SKF 83566 (Jose et al., 1992), but not the D_2 receptor antagonist S-sulpiride. It is concluded that inhibition of jejunal Na^+-K^+-ATPase activity by D_1 dopamine receptor activation is dependent on salt intake in WKY rats, and SHR animals fail to respond to dopamine, irrespective of their salt intake.

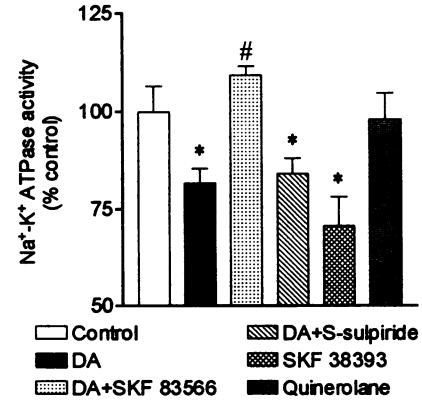

Figure 1. Effect of dopamine (DA, 1 μM) alone and in the presence of SKF 83566 (1 μM) or S-sulpiride (1 μM), SKF 38393 (10 nM) and quinerolane (10 nM) on jejunal Na^+-K^+-ATPase activity from WKY rats on LS intake. Columns represent means of four determinations per group and vertical lines show SEM. Significantly different from corresponding control values (* $P<0.05$) or values for dopamine alone (# $P<0.05$).

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Jose, P.A., et al. (1992). *J. Am. Soc. Nephrol.*, **2**, 1265-1278
Supported by grant SAU 14010/98.

167P MOUSE NEUROBLASTOMA NEURO 2A CELLS TAKE UP L-DOPA THROUGH THE L-TYPE AMINO ACID TRANSPORTER

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The treatment of Parkinson's disease with L-DOPA, the immediate precursor of dopamine, has been in the past decades the ultimate strategy to activate the dopaminergic system in brain. The aim of present study was to define the kinetics of L-DOPA uptake in Neuro 2A cells. This cell line has its origin in mouse neuroblastoma cells, and has been used as an *in vitro* neuronal model (Calderon *et al.*, 1999). In an additional set of experiments, 2-aminobicyclo (2,2,1)-heptane-2-carboxylic acid (BHC) and N-(methylamino)-isobutyric acid (MeAIB) were used to define the type of amino acid transporter involved in the apical inward transfer of L-DOPA and its sodium dependence. Neuro 2A cells (ATCC CCL-131; passages 170-181) were grown at 37° C in a humidified atmosphere (5% CO_2) on 2 cm^2 plastic culture clusters in Minimum Essential Medium adjusted to contain 1.5 g/L sodium bicarbonate and 1.0 mM sodium pyruvate supplemented with 10% fetal bovine serum and 100 U ml^{-1} penicillin G, 0.25 µg ml^{-1} amphotericin B and 100 µg ml^{-1} streptomycin. After 6 days, the cells formed a monolayer and each 2 cm^2 culture well contained about 100 µg of cell protein; 24 h before the experiments the cell culture medium was changed to a serum free medium. In uptake studies, cells were preincubated (30 min) with Hanks' medium with added tolcapone (1 µM) and benserazide (10 µM). L-DOPA was assayed by h.p.l.c. with electrochemical detection. L-DOPA was applied from the apical cell side at non-saturating (2.5 µM) and saturating (up to 1000 µM) concentrations for 6 min. Results are arithmetic means with s.e.mean, n=4-6. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. A P value less than 0.05 was assumed to denote a significant difference. Non-linear analysis of the saturation curve for L-DOPA revealed a K_m value (in µM) of 50±3 and a V_{max} value (in nmol mg protein^{-1} 6 min^{-1}) of 27±0.4. L-DOPA uptake was markedly decreased by 2,4-dinitrophenol (88% reduction) and incubation of cells at 4°C (91% reduction). Increasing extracellular sodium (from 0 mM to 140 mM) significantly ($P<0.05$) reduced the accumulation of L-DOPA (2.5 µM). However, manoeuvres which affect the cellular flux of sodium, such as the addition of amphotericin B (2.5 µg ml^{-1}), amiloride (100 µM) or ouabain (500 µM) failed to affect the accumulation of L-DOPA. N-(methylamino)-isobutyric acid (MeAIB; 1 mM) failed to affect the uptake of L-DOPA, whereas 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BHC) produced a concentration-dependent inhibition of L-DOPA uptake (IC_{50}=82±4 µM). The inhibitory effect of 80 µM BHC on the accumulation of L-DOPA was of the competitive type, as evidenced by the increase in K_m (75±6) but not V_{max} (25±0.6) values for L-DOPA uptake. It is concluded that Neuro 2A cells are endowed with the L-type amino acid transporter through which L-DOPA can be taken up.

Calderon, F.H., *et al.* (1999). *J. Neurosci. Res.*, **56**, 620-631

Supported by grant SAU 123/96.

168P Ca^{2+}/CALMODULIN-MEDIATED PATHWAYS REGULATE THE UPTAKE OF L-DOPA IN MOUSE NEUROBLASTOMA NEURO 2A CELLS

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The dopamine percursor, L-3,4-dihydroxyphenylalanine (L-DOPA), has been used in the therapy in Parkinson's disease to activate the dopaminergic system in brain. The present study examined the result of manoeuvres that affect molecular mechanisms, namely those concerning protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG), protein tyrosine kinase (PTK) and Ca^{2+}/calmodulin mediated pathways, on the uptake of L-DOPA in Neuro 2A cells, an *in vitro* model of neuronal cells (Calderon *et al.*, 1999). Neuro 2A cells (ATCC CCL-131; passages 170-181) were grown at 37° C in a humidified atmosphere (5% CO_2) on 2 cm^2 plastic culture clusters in Minimum Essential Medium adjusted to contain 1.5 g/L sodium bicarbonate and 1.0 mM sodium pyruvate supplemented with 10% fetal bovine serum and 100 U ml^{-1} penicillin G, 0.25 µg ml^{-1} amphotericin B and 100 µg ml^{-1} streptomycin. After 6 days, the cells formed a monolayer and each 2 cm^2 culture well contained about 100 µg of cell protein; 24 h before the experiments the cell culture medium was changed to a serum free medium. In uptake studies, cells were preincubated (30 min) with Hanks' medium with added tolcapone (1 µM) and benserazide (10 µM). L-DOPA was assayed by h.p.l.c. with electrochemical detection. Results are arithmetic means with s.e.mean, n=4-6. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Student's t test. A P value less than 0.05 was assumed to denote a significant difference. Non-linear analysis of the saturation curves for L-DOPA revealed K_m values (in µM) of 27±0.4 and a V_{max} value (in nmol mg protein^{-1} 6 min^{-1}) of 50±3; uptake of saturating concentrations of L-DOPA at 4°C was ~ 15% of that occurring at 37°C. Cyclic AMP (100 µM), forskolin (100 µM), isobutylmethylxanthine (100 µM) and cholera toxin (10 µg ml^{-1}) failed to affect the accumulation of a non-saturating (2.5 µM) concentration of L-DOPA. Similarly, cyclic GMP (1 mM), zaprinast (10 µM), LY 83583 (10 µM) and sodium nitroprusside (300 µM) failed to affect the accumulation of L-DOPA (2.5 µM). The PKC activator phorbol 12,13-dibutirate (PDBu, 1 µM), the inactive phorbol ester 4α-phorbol 12,13-didecanoate (PDDC, 1 µM) and the PKC inhibitors chelerythrine (30 µM) bisindolylmaleimide (100 µM) also failed to affect the accumulation of L-DOPA (2.5 µM). However, another PKC inhibitor, staurosporine (0.3 to 3 µM) reduced L-DOPA uptake by 92±2%. The PTK inhibitors genistein (300 µM) and tyrphostin 25 (300 µM) failed to change the accumulation of L-DOPA (2.5 µM). The Ca^{2+}/calmodulin inhibitors calmidazolium and trifluoperazine produced concentration-dependent inhibition of L-DOPA (2.5 µM) uptake with IC_{50} 's of 49±1 and 70±1 µM, respectively. The inhibitory effect of calmidazolium (50 µM) and trifluoperazine (100 µM) on the accumulation of L-DOPA was of the non-competitive type, as evidenced by the decrease in V_{max} (7.3±0.4 and 11±0.3 nmol mg protein^{-1} 6 min^{-1}, respectively), but not K_m (41±9 and 33±4 µM) values for L-DOPA uptake. It is concluded that L-DOPA uptake in Neuro 2A cells is a carrier-mediated system that is temperature dependent and appears to be under the control of Ca^{2+}/calmodulin mediated pathways.

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169P EFFECTS OF DOPAMINE UPON INTRACELLULAR pH IN OPOSSUM KIDNEY (OK) CELLS

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Recovery of cell pH after acid and alkaline loads has been shown in a variety of cells to depend on the activation of H^+ and HCO_3^- transport mechanisms. OK cells have been shown to express several transport systems characteristic of proximal tubular cells, namely the Na^+/H^+ exchanger that is involved in extruding H^+ from the cell after an acid load, and a Na-independent HCO_3^- transport system (Pastoriza et al., 1992). Dopamine, a renal natriuretic hormone, has an inhibitory effect on Na^+/H^+ exchanger and Na^+,K^+-ATPase activity (Jose et al., 1992). In the present study we used OK cells (ATCC 1840-CRL) as an *in vitro* model of renal tubular cells, and evaluated the effects of dopamine on steady-state intracellular pH (pH_i). The subtype of dopamine receptors involved in this effect was also investigated. Furthermore, we attempted to identify the mechanisms by which dopamine exerts its action, namely via the Na^+/H^+ exchanger and the HCO_3^- transporter. Intracellular pH was measured in cells grown on glass coverslips loaded for 40 min with 5 μM BCECF-AM (Pastoriza et al., 1992), at 37°C. Fluorescence of BCECF was monitored at the excitation wavelengths of 490 and 440 nm and at the emission wavelength of 525 nm. To correlate excitation ratio 490/440 with pH_i, a calibration curve was constructed using the high K^+-nigericin technique, with pH values between 6.6-7.4. Results are arithmetic means with s.e.mean, n=4-5. Statistical differences between experimental groups were determined by ANOVA followed by the Newman-Keuls test. At steady-state, intracellular pH in OK cells perfused with Krebs buffer at pH 7.4 was 7.37±0.04 (n=31). Addition of dopamine (10, 30, 100, 300 and 1000 nM) to the perfusion fluid, induced a concentration dependent acidification (ΔpH_i : 0.010; -0.019; -0.039, -0.053 and -0.084 pH units, respectively), with an IC_{50} value of 70±2 nM. The acidifying effect of 300 nM dopamine was partially prevented by SKF 83566 (~50% reduction) (Jose et al., 1992), a D_1 antagonist, and not by (S)-sulpiride, a D_2 antagonist. Dopamine (300 nM) was also found to markedly decrease (~20% reduction) the recovery of pH_i after exposure to sodium-free medium for 600 s; which is essentially mediated via the Na^+/H^+ exchanger. HCO_3^- transport system was studied as described (Pastoriza et al., 1992). In a HCO_3^- containing medium, removal of CO_2/HCO_3^- caused an initial cell alkalinization (from 7.23±0.07 to 7.40±0.07), as a result of CO_2 loss from the cell with subsequent return of pH_i to basal values. To assess whether HCO_3^- transport in OK cells is linked to Na^+ and Cl^-, via a Cl/HCO_3 exchanger and a Na/HCO_3 cotransporter, respectively, Cl^- and Na^+ in the perfusion medium were replaced by gluconate and choline. Under these conditions, recovery of pH_i after removal of CO_2/HCO_3^- was not affected. DIDS (200 μM) (Pastoriza et al., 1992) significantly slowed the cell acidification rate after CO_2/HCO_3^- removal. Dopamine (1 μM) failed to affect the recovery of pH_i after removal of CO_2/HCO_3^- in the absence of sodium. It is concluded that in OK cells dopamine induces a concentration-dependent acidification, which involves D_1 receptors. These results suggest that dopamine exerts an intracellular acidification via inhibition of the Na^+/H^+ exchanger, being devoid of effect on the Na-independent HCO_3^- transport system.

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170P TRANSEPITHELIAL FLUX OF SODIUM AND HANDLING OF L-DOPA IN RENAL EPITHELIAL CELLS

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There is evidence suggesting that uptake of L-DOPA, the precursor of natriuretic hormone dopamine, in renal epithelial cells is dependent on apical-to-basal flux of Na^+ and the Na^+/H^+ exchanger. On the other hand, the addition of L-DOPA from the apical cell border resulted in a decrease in intracellular pH suggesting that changes in intracellular pH may have to do with inhibition of Na^+/H^+ exchange resulting from increases in L-DOPA-Na^+ co-transport (Gomes & Soares-da-Silva, 1999). The present study describes the kinetics of L-DOPA uptake through the apical cell border in two clones of OK cells (ATCC 1840-CRL) endowed with marked differences in their ability to translocate sodium from the apical to basal cell side, the high capacity (OK_{HC}) and the low capacity (OK_{LC}) cell lines. 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% foetal 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. 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. The ability to translocate sodium from the apical to the basal cell side was evaluated by measuring the activities of Na^+,K^+ ATPase and the Na^+/H^+ exchanger, as previously described (Vieira-Coelho et al., 1998; Thwaites et al., 1993). Na^+,K^+ ATPase activity (nmol Pi mg $protein^{-1}$ min^{-1}) in OK_{LC} (30.0±0.12) was markedly lower than that in OK_{HC} (57.6±5.70) cells. The activity of the Na^+/H^+ exchanger was evaluated by measuring the initial slope for intracellular pH recovery (dpH_i/dt [pH units min^{-1}]) after an acid load imposed by NH_4Cl pulse, this being greater ($P<0.05$) in OK_{HC} (0.254±0.016) than in OK_{LC} (0.094±0.011) cells. Basal intracellular pH in OK_{HC} (7.37±0.04) was lower ($P<0.05$) than in OK_{LC} (7.65±0.05) cells. Non-linear analysis of the saturation curve for L-DOPA uptake revealed a K_m value (in μM) of 162 (99, 225) and 160 (105, 214) and a V_{max} value (in pmol mg $protein^{-1}$ 6 min^{-1}) of 31703±1264 and 19123±758 in OK_{HC} and OK_{LC} cells, respectively. Ouabain (100 μM) was found not to affect L-DOPA accumulation in OK_{LC} cells, whereas in OK_{HC} cells a significant decrease (13.2±0.9 % reduction) was observed. The inhibitory effect amphotericin B (2.5 μg ml^{-1}) upon L-DOPA accumulation in OK_{HC} (33.7±1. 9 % reduction) was greater than that in OK_{LC} cells (12.9±2.5 % reduction). It is concluded that enhanced ability to translocate sodium from the apical to the basal cell side correlates positively with their ability to accumulate L-DOPA, which is in agreement with the role of sodium in taking up the precursor of renal dopamine.

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171P INHIBITION OF Na^+-K^+ ATPase IN OK CELLS BY D_1 AND D_2 DOPAMINE RECEPTOR STIMULATION

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In renal proximal tubular cells, where both D_1 and D_2 receptors are co-expressed, D_2 agonists have been reported either to have no effect or to act in concert with D_1 agonists to inhibit Na^+-K^+ ATPase activity (Bertorello & Aperia, 1990). In the present study, we evaluated the effects of dopamine and selective agonists upon Na^+-K^+ ATPase activity, using OK cells (ATCC 1840-CRL) as an *in vitro* model of proximal tubular function. Furthermore, we attempted to identify the subtype of dopaminergic receptors present in this cell line, by means of radioligand binding studies, and characterise the intracellular signaling pathways. Results are arithmetic means with s.e.mean, n=4-5. Statistical differences between experimental groups were determined by ANOVA followed by the Newman-Keuls test. OK cells mounted in Ussing chambers connected to an automatic voltage current clamp were continuously monitored for changes in short circuit current (I_{sc}, $\mu A\ cm^2$) after the addition of amphotericin B, to increase the sodium delivered to Na^+-K^+ ATPase to the saturating level. Under these conditions, a decrease in I_{sc} is assumed to represent the sodium transport mediated by Na^+-K^+ ATPase. Amphotericin B (0.3 - 3.0 $\mu g\ ml^{-1}$), added from the apical cell border of the monolayer, induced a concentration dependent decrease in I_{sc}, the maximum effect being attained at 1.0 $\mu g\ ml^{-1}$. The addition of ouabain (100 μM) to the fluid bathing the basolateral side markedly reduced the effect of amphotericin B (from -36.4±2.4 to -6.0±2.2 $\mu A\ cm^2$). Similarly, activation of PKA and PKC with cAMP (0.5 mM) and PDBu (1 μM), respectively, markedly ($P<0.05$) reduced the response to 1.0 $\mu g\ ml^{-1}$ amphotericin B (from -36.4±2.4 to -11.6±2.3 and -6.5±1.6 $\mu A\ cm^2$). Dopamine (1 μM) applied from the basal side failed to affect the amphotericin B-induced I_{sc} decrease. By contrast, dopamine applied from the apical side reduced by 28±2% ($P<0.05$) the amphotericin B-induced I_{sc} decrease. This effect was completely abolished by the D_1 selective antagonist SKF 83566 (1 μM) (Jose et al., 1992) and the D_2 antagonist (S)-sulpiride (1 μM). SKF 38393 (Jose et al., 1992), a D_1 selective agonist, and quinerolane, a D_2 selective agonist, applied from the apical cell side mimicked the effect of dopamine in a concentration dependent manner. Specific binding of [^{3}H]Sch 23390, a D_1 antagonist, and [^{3}H]YM-09151-2, a D_2 antagonist, to OK cell membranes (Nash et al., 1993), revealed the presence of both D_1 (B_{max} =185.8±15.6 fmol/mg protein; K_d =0.34±0.09 nM) and D_2 (B_{max} =326.9±73.2 fmol/mg protein; K_d =1.85±1.20 nM) receptors in this cell line. The effect of dopamine receptor agonists and antagonists on adenosine 3',5'-cyclic monophosphate (cAMP) formation in OK cells was also studied. The stimulation of cAMP production by dopamine (in the presence of 100 μM IBMX) was concentration dependent (EC_{50} = 220±2 nM). SKF 38393 (0.3 μM) but not quinerolane (1 μM) stimulated cAMP production (from 6.4±1.0 to 13.9±1.5 pmol mg $protein^{-1}$). The specific D_1-receptor antagonist, SKF 3566 (1 μM), abolished the stimulatory effects of dopamine and SKF 38393. Unexpectedly, the D_2-receptor antagonist, (S)-sulpiride, had the same inhibitory effect on dopamine-stimulated cAMP production. This may have to do with lack of selectivity of this antagonist at D_2 receptors. It is concluded that in OK cells dopamine inhibits Na^+-K^+ ATPase activity through stimulation of both D_1 and D_2 receptor, by cAMP-dependent and cAMP-independent pathways, respectively.

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172P FUNCTIONAL STUDIES WITH THE SOMATOSTATIN (SRIF) sst_2 RECEPTOR ANTAGONIST, L-TYR^8-CYANAMID-154806 IN RAT COLONIC MUCOSA

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Somatostatin (SRIF) is a potent antisecretory neuropeptide in mammalian gastrointestinal mucosae *in vitro* (Ferrar *et al.*, 1990; McKeen *et al.*, 1995). Functional characterisation of the somatostatin receptor (sst) type(s) mediating these inhibitory effects has been limited by the lack of specific SRIF antagonists. Here we describe the competitive inhibition of SRIF responses in the rat decending colon mucosa by the first selective sst_2 receptor antagonist, L-Tyr^8-Cyanamid-154806 (L-Tyr^8-CYN 154806, Bass *et al.*, 1996; Feniuk *et al.*, 1998).

Mucosal preparations of rat (male Sprague-Dawley, 250-300g) descending colon were prepared as described previously (Ferrar *et al.*, 1990) placed in Ussing chambers (exposed area, 0.6 cm^2) and voltage-clamped at 0 mV, recording the resultant short-circuit current (I_{sc}) continuously. Tissues were allowed to stabilise in the presence of tetrodotoxin (TTX, 100 nM, basolateral) before addition of peptides to the basolateral reservoir alone. Reductions in I_{sc} in response to cumulative addition of SRIF were pooled and the means ± 1 s.e.m. for each agonist concentration were analysed using GraphPad Prism (version 2.0). EC_{50} values are given with 95% confidence limits and degrees of freedom (d.f.).

TTX reduced basal I_{sc} values (in $\mu A.cm^{-2}$) from 26.7 ± 1.5 to 20.1 ± 1.7 (n = 40). Following this SRIF (0.1 - 300 nM) caused prolonged reductions in I_{sc} with an EC_{50} of 14.6 nM (14.1 - 15.1 nM, 7 d.f. n=4) as observed previously (Ferrar *et al.*, 1990). In the presence of increasing L-Tyr^8-CYN 154806 concentrations (10, 100 nM and 1 μM) SRIF-response curves were displaced to the right in a parallel fashion with no decrease in maxima. A pA_2 value of 8.2 was obtained with a slope of 0.9.

The relative effects of L-Tyr^8-CYN 154806 upon two other SRIF analogues, namely BIM23190 (a sst_2-preferring agonist) and BIM23268 (a sst_5-preferring agonist) were also investigated. Both analogues reduced I_{sc} but the maximal responses to BIM23268 (440 nM) were reduced compared with those of SRIF (440 nM, $P < 0.01$). 100 nM L-Tyr^8-CYN 154806 displaced the BIM23190 response curve to the right (control EC_{50} 2.5 nM (1.9 - 3.3 nM, 7 d.f. n=5) and plus antagonist; 63.0 nM (47.6 - 83.5 nM, 8 d.f. n=5)). A pK_B of 8.4 was obtained. In contrast the response-curve for BIM23268 (0.1nM - 5 μM) was unaltered by L-Tyr^8-CYN 154806 (100 nM, control EC_{50} 32.0 nM (11.9 - 86.7 nM, 7 d.f. n=3-5) and plus antagonist, 61.4 nM (43.1 - 87.6 nM, 8 d.f. n=3-5)).

In conclusion, we have shown that L-Tyr^8-CYN 154806 selectively inhibits antisecretory responses to SRIF and BIM23190 but not to BIM23268 in preparations of rat descending colon mucosa *in vitro*. This data corroborates that from Warhurst *et al.* (1996) who recently found mRNA for sst_2 (with sst_1 and sst_5) in crypt epithelia from rat colon. The pA_2 and pK_B values observed for L-Tyr^8-CYN 154806 in colonic mucosa are similar to those described in rat isolated vas deferens and also in guinea-pig ileum (Feniuk *et al.*, 1998).

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173P FUNCTIONAL SOMATOSTATIN (SRIF) RESPONSES IN HUMAN ADENOCARCINOMA CELLS TRANSFECTED WITH HUMAN OR RAT sst_2 RECEPTORS

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SRIF inhibits both basal and stimulated electrogenic anion transport in rat colonic mucosa, a response which may be mediated at least in part by epithelial sst_2 receptors (McKeen *et al.*, 1995). We have therefore stably transfected the HCA-7 Colony 1 adenocarcinoma cell line (which lacks SRIF responses; Holliday *et al.*, 1997) with cDNAs encoding either the human sst_2 receptor or the rat sst_{2a} long splice variant (also containing an amino terminal epitope tag). Here we report that the resultant clones (hS2-16 and rS2A-1 respectively) show SRIF responses in short-circuit current (I_{SC}) studies, which are inhibited by the cyclic peptide sst_2 antagonist AcNH-4-NO_2-Phe-c[D-Cys-Tyr-D-Trp-Lys-Thr-Cys]-Tyr-NH_2 (L-Tyr^8-CYN 154806; Bass *et al.*, 1996).

Clones hS2-16 and rS2A-1 were isolated after introduction of human sst_2 or rat sst_{2a} receptor cDNAs into Colony 1 cells by calcium phosphate co-precipitation and glycerol shock, and the subsequent identification and functional screening of G418 resistant colonies. Epithelial layers (area 0.2 cm^2) were voltage-clamped at 0 mV (Holliday *et al.*, 1997) and peak changes in the resulting I_{SC} were measured in response to basolateral peptide additions (given as $\mu A\ cm^{-2}$). SRIF concentration-response curves in the absence or presence of L-Tyr^8-CYN154806 (added 10 min earlier) were constructed non-cumulatively. EC_{50} and IC_{50} values are quoted with 95 % confidence limits; the pK_B estimate was calculated using the Gaddum equation.

hS2-16 and rS2A-1 clones exhibited stable basal I_{SC} levels and responded to a maximal concentration of vasoactive intestinal peptide (VIP, 30 nM) with sustained elevations in I_{SC} ($+15.2 \pm 0.5$, $n = 53$ in hS2-16 and $+69.5 \pm 1.4$, $n = 193$ in rS2A-1). In contrast to its lack of effect in wild type Colony 1 cells, SRIF attenuated VIP-elevated I_{SC} in both hS2-16 and rS2A-1 cells with similar potency ($n = 3$ - 6; Table 1). In each clone the I_{SC} reduction to 100 nM SRIF reached a maximum at 1.5 - 2 min after agonist addition (representing a 23 - 30 % reduction in total I_{SC}) and rapidly recovered (the residual I_{SC} decrease at 5 min being only -0.3 ± 0.2 in hS2-16 and -1.0 ± 0.4 in rS2A-1, both $n = 6$). 10 min pretreatment with L-Tyr^8-CYN154806 fully inhibited 100 nM SRIF responses (with IC_{50} values given in Table 1; $n = 3$ - 6). In the presence of 30 nM L-Tyr^8-CYN154806, SRIF inhibited VIP-stimulated I_{SC} with an EC_{50} value of 91.1 (51.9 - 160) nM; $n = 4$ - 5) in rS2A-1 epithelial layers, yielding an antagonist pK_B estimate of 7.8 (as observed in rat vas deferens; Feniuk *et al.*, 1998). Thus human sst_2 and rat sst_{2a} receptors exhibit very similar functional characteristics when expressed in Colony 1 epithelial cells, suggesting that both may be coupled and desensitised in an identical manner.

Table 1. SRIF functional data in hS2-16 and rS2A-1 cells.

Clone	*100 nM SRIF* ($\mu A\ cm^{-2}$)	*SRIF* EC_{50} *(nM)*	*L-Tyr^8-CYN154806* IC_{50} *(nM)*
hS2-16	-8.3 ± 1.0	21.2	74.4
	($n = 6$)	(12.8 - 35.1)	(14.1 - 391)
rS2A-1	-22.4 ± 2.2	21.5	32.9
	($n = 6$)	(14.2 - 32.8)	(21.9 - 49.5)

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174P EFFECTS OF NITRIC OXIDE RELEASING NON-STEROIDAL ANTI-INFLAMMATORY DRUG DERIVATIVES ON SECRETORY ACTIVITY IN RAT GASTRIC EPITHELIAL CELLS

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In an attempt to overcome the ulcerogenic properties of non-steroidal-anti-inflammatory drugs (NSAIDs), a new class of NSAIDs has been developed that incorporates a nitric oxide (NO) donating group called NO-NSAIDs (Wallace, 1997). Previous studies have demonstrated a stimulatory role of NO in mediating mucus secretion from suspensions of rat gastric epithelial cells (Brown *et al.*, 1993). The aim of this study was to examine the effect of NO-NSAIDs on gastric mucus secretion from suspensions of rat gastric epithelial cells.

Rat gastric epithelial cells were isolated by pronase digestion and intermittent calcium chelation according to a previously described method (Brown *et al.*, 1993). To examine the effects of NO-NSAIDs (10-1000 μM) on mucus secretion, cells were incubated with either NO-Aspirin (NCX 4016) or NO-Flurbiprofen (HCT 1026) for 30 min at 37°C. An ELISA method was used to provide an estimate of mucus secretion. Trypan blue dye exclusion and acid phosphatase activity in the culture medium were measured to assess the effect of the NO-NSAIDs on cell viability. The rate of NO release from the NO-NSAIDs was assessed using the NO-sensitive fluorescent dye 4,5-diaminofluorescein diacetate.

The results of our experiments are shown in table 1. Both NCX 4016 and HCT 1026 (10-1000μM) caused a concentration-dependent increase in mucus release from gastric epithelial cells. This response could be inhibited by co-incubation with the NO scavenger oxyhaemoglobin (10 μM) which reduced the response to 500 μM NCX 4016 to 8.6 ± 1.3 % above basal ($P<0.01$ for difference from 500μM NCX 4016 alone). In addition, the parent compounds of

Table 1. Effects of NO-NSAIDs on mucus secretion from gastric epithelial cells (mean ± s.e.mean. (n)), where **P<0.01 for difference from basal by ANOVAR and a Dunnett's post-hoc test.

Concentration	Mucus Secretion (% increase above basal)	
	NCX 4016	HCT 1026
Basal (μg mucin/10^7 cells)	10.1±1.4 (6)	14.5 ± 3.6 (3)
10μM	9.0 ± 1.4 (6)	18.8 ± 1.9 (3)**
100μM	38.4 ± 3.7 (6)**	37.3 ± 0.8 (3)**
500μM	101.3 ± 5.4 (6)**	57.5 ± 7.3 (3)**
1000μM	118.1 ±13.6 (6)**	92.4 ±10.6 (3)**

NCX 4016 and HCT 1026, aspirin and flurbiprofen respectively were without effect. However, when gastric epithelial cells were co-incubated with aspirin (500 μM) and the NO-donating compound *S*-nitroso-*N*-acetylpenicillamine (500 μM), a similar response was observed to that in the presence to NCX 4016 (500 μM) with a 78.9 ± 6.7 % increase in mucus secretion ($P<0.01$). Neither NCX 4016 nor HCT 1026 had an effect upon either the ability of the cells to exclude Trypan blue or acid phosphatase activity ($P>0.05$). Both NCX 4016 and HCT 1026 produced a concentration-dependent increase in fluorescence intensity in cells loaded with the NO-sensitive dye DAF-2 DA although the response to NCX4016 was more rapid than that with HCT 1026.

These data suggest that NO-NSAIDs may act as NO-donating compounds and may have reduced ulcerogenic properties due to their ability to stimulate mucus secretion.

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175P RELAXATION TO β-ADRENOCEPTOR AGONISTS IN ILEUM FROM β_3-ADRENOCEPTOR KNOCK-OUT MICE IS MEDIATED BY β_1-ADRENOCEPTORS

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β_3-Adrenoceptors (ARs) are the predominant β-AR mediating relaxation of ileal smooth muscle in several species including rat, mouse and guinea-pig. However, all three β-AR mRNA's are expressed in rat ileum and β_1-ARs are also capable of mediating smooth muscle relaxation (Roberts *et al.*, 1999).

This study examines the role of β-ARs in mediating relaxation to β-AR agonists in ileum from β_3-AR knockout (KO) (-/-) and FVB (WT) (+/+) mice. KO (Susulic et al., 1995) and WT mice were genotyped by tail clipping, DNA extraction and polymerase chain reaction (PCR) prior to use. Isolated ileal segments (~ 2 cm long) were set up in organ baths gassed with 95%O_2/5%CO_2 in Krebs-Henseleit buffer containing EDTA (0.04μM) and ascorbic acid (0.1M). Isotonic responses were recorded using UgoBasile transducers (model 7006, resting force 0.4mN) connected to a MacLab system. Mouse ileum was pre-contracted with carbachol (1μM) and cumulative concentration-response curves constructed to isoprenaline (non-selective β-AR agonist), RO363 (β_1-AR agonist) and CL316243 (β_3-AR agonist) expressed as a percentage of the relaxation to papaverine (10μM). Antagonists (CGP20712A, β_1-AR; ICI118551, β_2-AR; SR59230A, β_3-AR; 100nM) were equilibrated for 30min before carbachol contraction.

Responses to isoprenaline or RO363 were not significantly different in KO or WT mice, whereas responses to CL316243 were abolished in KO mice. pK_B values for CGP20712A were significantly greater (9.60 ± 0.40; n=6) in KO mice compared with WT (7.81 ± 0.38; n=3) mice (P<0.05) and pK_B values for SR59230A were also greater (8.14 ± 0.14; n=4) in WT mice compared with KO (7.56 ± 0.20; n=5) mice (P=0.06). Responses to isoprenaline were weakly antagonized by ICI118551 in both KO (pK_B 7.11 ± 0.45; n=3) and FVB (7.46 ± 0.24; n=3) mice (NS). Curves were fitted and pEC_{50} values calculated using Graphpad Prism® (table 1).

	pEC_{50}	
	β_3-AR KO	FVB
CL 316243	NA	8.14 ± 0.10 (6)
RO363	8.39 ± 0.26 (12)	8.50 ± 0.16 (6)
Isoprenaline	7.92 ± 0.11 (9)	7.80 ± 0.25 (6)
+ ICI118551	7.60 ± 0.16 (6)	7.31 ± 0.09 (3)
+ CGP20712A	5.66 ± 0.46 (6) ***	6.73 ± 0.23 (4) *
+ SR59230A	7.03 ± 0.20 (5) *	6.57 ± 0.09 (4) **

Values are means ± s.e.mean: * P<0.05, **P<0.01 and ***P<0.005 by Student's unpaired t-test.

These results suggest that (1) loss of the β_3-AR in KO mice is functionally compensated for by β_1-AR (2) CL316243 is a highly selective β_3-AR agonist (3) SR59230A has limited selectivity for β_3-AR in mouse ileum.

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176P ROLE OF N- AND L-TYPE CALCIUM CHANNELS IN THE RELEASE OF NORADRENALINE FROM SYMPATHETIC NEURONS IN RABBIT CAROTID ARTERY

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Transmitter release from nerve terminals is dependent on the entry of extracellular Ca^{2+} through prejunctional voltage-dependent calcium channels. In sympathetic neurons both N- and L-type calcium channels are present (Lipscombe *et al.*, 1988). Potassium channel blockade increased Ca^{2+} entry into sympathetic neurons, mostly through L-type calcium channels (Doležal *et al.*, 1996). The aim of this study was to examine the putative role of L-type calcium channels in the stimulation-evoked release of noradrenaline from vascular postganglionic sympathetic neurons.

Albino rabbits (weight 1.8-2.7 kg) of either sex were killed by stunning and exsanguinated. Rings of carotid artery were preincubated with (-)-^{3}H-noradrenaline and the fractional ^{3}H-overflow evoked by electrical-field stimulation (200 mA; 600 pulses; 0.5 ms; 2 Hz) was determined as described in detail (Jensen & Nedergaard 1999).

The selective N-type calcium channel blocking agent ω-conotoxin GVIA (single concentrations: $3x10^{-10}$-$3x10^{-9}$M) caused a slowly developing reduction of the stimulation-evoked ^{3}H-overflow. At $3x10^{-8}$M, ω-conotoxin GVIA caused a rapid (15 min) equilibrium block. The inhibition was irreversible. After 2 h exposure to ω-conotoxin GVIA (single concentrations: $3x10^{-10}$-$3x10^{-8}$M) the inhibition was steady (pA50 (-log M): 9.43; Emax: 91%; n = 4-6). The selective L-type calcium blocking agent nifedipine (cumulative addition: 10^{-7}-10^{-5}M) had no effect on the stimulation-evoked ^{3}H-overflow (P>0.05; n=5). This was also the case when the artery was exposed for 2 h to nifedipine (10^{-5}M). The potassium channel blocking agent 4-aminopyridine (cumulative addition: 10^{-5}-10^{-3}M) enhanced the stimulation-evoked ^{3}H-overflow up to 466% (P<0.001; n=5). In the presence of 4-aminopyridine (10^{-4}M), nifedipine (10^{-5}M) enhanced the stimulation-evoked ^{3}H-overflow by 21% (P<0.05; n=5). 4-Aminopyridine (10^{-4}M) attenuated the inhibitory effect of ω-conotoxin GVIA ($3x10^{-9}$M) by up to 15% (P<0.05; n=4-5). ω-Conotoxin GVIA, nifedipine and 4-aminopyridine in the employed concentrations had no effect on the passive ^{3}H-outflow.

We conclude that the stimulation-evoked release of noradrenaline from sympathetic neurons in rabbit carotid artery is mainly mediated by N-type calcium channels and that L-type channels are not involved even when potassium channels are blocked by 4-aminopyridine.

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177P CHARACTERISATION OF THE ATPase RELEASED DURING SYMPATHETIC NERVE STIMULATION OF THE GUINEA-PIG ISOLATED VAS DEFERENS

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Electrical field stimulation (EFS) of the sympathetic nerves of the guinea-pig vas deferens results in the release of adenosine 5'-triphosphate (ATP) as a cotransmitter. We have shown that EFS also releases nucleotidases that dephosphorylate ATP to adenosine (Todorov *et al.*, 1997). The aims of this study were a) to investigate if similar activity is released from the vas deferens of other species and from other guinea-pig tissues in which ATP is a neurotransmitter and b) to further characterise the biochemical properties of the releasable ATPase.

Segments of guinea-pig, rat and mouse vas deferens and guinea-pig urinary bladder and taenia coli (~ 150 mg) were superfused at 2 ml min^{-1} with Krebs solution at 37°C. EFS was applied to the tissues at 8 Hz, 0.1 ms pulse width, supramax. voltage for 25 s. The superfusate was collected and divided into 80 µl aliquots. 10 µl of a stock ATP solution was added to each aliquot, along with 10 µl of H_2O or enzyme inhibitor and incubated at 20°C for 0-100 min. The ATP content of the samples at the end of the incubation was measured using the luciferin-luciferase method.

Superfusate collected in the absence of EFS did not break down ATP. but that collected during EFS of sympathetic nerves of rat, guinea-pig and mouse isolated vas deferens and parasympathetic nerves of guinea-pig isolated urinary bladder, contained ATPase activity (n=3-8). ATP breakdown was fastest in superfusate collected from the guinea-pig isolated vas deferens. In contrast, EFS of the enteric nerves of the guinea-pig isolated taenia coli did not release any detectable ATPase (n=4).

The biochemical properties of the ATPase released from the guinea-pig isolated vas deferens were further characterised. ATP was metabolised at similar rates at incubation temperatures of 37°C and 20°C (n=4). Analysis of the initial rates of ATP hydrolysis using the Lineweaver-Burke equation gave a K_M of 39 µM and a V_{max} of 1039 pmol ATP metabolised min^{-1} ml^{-1} superfusate (n=5-6). 6-N,N-diethyl-D-β,γ-dibromomethyleneATP (ARL 67156), pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) and pyridoxal-5'-phosphate (P-5-P) all inhibited the ATPase activity in a concentration-dependent manner with a potency order of ARL 67156 = PPADS > P-5-P (n=4-7). ARL 67156 and PPADS both inhibited enzyme activity by 50% at ~30 µM.

In conclusion, EFS of several tissues in which ATP is a neurotransmitter results in the release of an ATPase and activity is greatest in the guinea-pig vas deferens. The molecular identity of the ATPase is not known, but it has similar characteristics to ectonucleoside triphosphate diphosphohydrolases.

Todorov, L.D., Mihaylova-Todorova, S., Westfall, T.D *et al.*, (1997) *Nature*, **387**, 76-79.

178P RELEASE OF ATPase FROM THE RABBIT VAS DEFERENS DURING SYMPATHETIC NERVE STIMULATION

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We have shown that electrical field stimulation (EFS) of the sympathetic nerves of the guinea-pig vas deferens releases not only adenosine 5'-triphosphate (ATP) and noradrenaline as cotransmitters, but also nucleotidases that dephosphorylate ATP to adenosine (Todorov *et al.*, 1997). Similar release is seen in the rat and mouse vas deferens and the guinea-pig urinary bladder (Westfall *et al.*, 1999). Here we report the results of similar experiments using the rabbit isolated vas deferens.

Segments of rabbit or guinea-pig vas deferens were superfused at 2 ml min^{-1} with Krebs solution at 37°C. Unless stated otherwise, EFS was applied to the tissues at 8 Hz, 0.1 ms pulse width, supramax. voltage for 25 s. The superfusate was collected and divided into 80 µl aliquots. 10 µl of a stock ATP solution was added to each aliquot, along with 10 µl of H_2O or 6-N,N-diethyl-D-β,γ-dibromomethylene ATP (ARL 67156) and incubated at 20°C for 0-20 min. The ATP content of the samples at the end of the incubation was measured using the luciferin-luciferase method.

The ATPase released from the rabbit isolated vas deferens, metabolised ATP at a substantially greater rate than that released from the guinea-pig isolated vas deferens. ATPase activity was released into the superfusate in a frequency-dependent manner with significantly less activity seen at 2 Hz than at 4 Hz and 8 Hz ($P<0.01$, n=8). When EFS was applied three times at 30 min intervals, ATP metabolism was slightly, but significantly less at each time point in superfusate collected during the 2nd and 3rd stimulation periods compared with the first period ($P<0.05$, n=6).

There was no difference in the time-course and extent of ATP breakdown by superfusate when it was stored for 1 h or 24 h at 20°C (n=4). Increasing dilution of the superfusate progressively decreased the rate of ATP breakdown, but significant ATPase activity was still present after 95% dilution (n=5). ARL 67156 (10-300 µM) inhibited ATPase activity in a concentration-dependent manner and at 300 µM almost abolished the breakdown of ATP (n=4).

In conclusion, EFS of the rabbit isolated vas deferens releases substantially greater ATPase activity than any other tissue studied. It is not clear if this reflects differences in the density of innervation of these tissues or if other factors are involved.

Todorov, L.D., Mihaylova-Todorova, S., Westfall, T.D *et al.*, (1997) *Nature*, **387**, 76-79.
Westfall, T.D., Sarkar, S., Ramphir, N. *et al.*, (1999), this meeting.

179P EVIDENCE TO DEMONSTRATE THAT LACTATE PRODUCTION CAN BE USED AS A METABOLIC RATE MARKER IN IMMORTALISED C_2C_{12} SKELETAL MUSCLE MYOCYTES *IN VITRO*

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The measurement of oxygen consumption in cell lines is an insensitive measure of metabolic rate. C_2C_{12} myocytes were used in combination with the uncoupling agent, dinitrophenol (DNP), to evaluate lactate production as a metabolic rate marker in vitro.

Lactate production and acidification were determined in myocytes seeded at $0.25x10^6$ cells.ml^{-1} in 2ml Dulbeccos' Modified Eagles Medium (DMEM [10% fetal bovine serum, 1% antibiotics (5000 units penicillin, 5000µg streptomycin.ml^{-1}]). Myocytes were incubated for 48h in culture-treated 24-well plates with or without DNP (1µM-1mM) at 37 °C in 5% CO_2, 95% air. Lactate was measured using a commercial kit (Sigma 735) and results expressed against drug-free controls. pH was measured using a standard electrode. Oxygen consumption was measured in trypsinised myocytes suspended in DMEM at $3\text{-}4x10^6$ cells.ml^{-1} using a Clark-type oxygen electrode with 3ml cell suspension at 37°C. An initial oxygen consumption rate was measured, DNP (in DMSO) was added cumulatively (1µM-10mM) and results expressed against the drug-free control. All data are expressed as the mean ± s.e.mean (3 experiments). EC_{50} values were calculated using non-linear regression (Prism 2.01) and expressed with 95% confidence intervals in parentheses.

In drug-free preliminary experiments, lactate concentration increased to 2.46 ± 0.08 mM after 24h (initially 1.20 mM) and subsequently increased by 288 and 1770% (relative to 24h) after 48 and 120h respectively. After 48h DNP increased lactate production above control in a concentration dependent manner (Figure 1). The mean control lactate production was 4.4 ± 0.3 mM and was maximally increased to 202.8 ± 8.3% of control at 300µM DNP. The EC_{50} value was determined to be 29µM (12µM, 67µM).

The measurement of pH provided an almost equivalent measure to lactate production. Mean control pH was 7.15 ± 0.04 and was maximally decreased to 6.99 ± 0.05 at 300µM DNP. The EC_{50} value was determined to be 29µM (8µM, 110µM). Myocytes exposed acutely to DNP produced concentration dependent increases in oxygen consumption. The mean control rate of oxygen consumption was 1.4 ± 0.1nmoles/min/10^6 cells and was increased maximally to 345.9 ± 35.4% of control at 1mM DNP. The EC_{50} value was determined to be 24µM (8µM, 110µM).

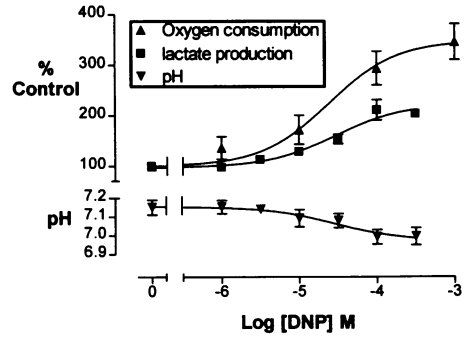

Figure 1. Effect of dinitrophenol on metabolic parameters measured in C_2C_{12} myocytes.

In conclusion increased lactate production above control at 48 hours mirrors the increase in extracellular acidification and increased oxygen consumption and therefore represents a quick and simple measure of metabolic rate in C_2C_{12} myocytes.

180P NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE DEGRADATION IN MAMMALIAN TISSUE

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent endogenous Ca^{2+} mobilising agent described to date. In sea urchin eggs and mammalian cells (Cancela *et al.*, 1999; Bak *et al.*, 1999), it activates a Ca^{2+} release mechanism distinct from IP_3 or cADPR (Genazanni *et al.*, 1996). An intracellular messenger role for NAADP demands that mechanisms are present for its intracellular synthesis and degradation. The aims of the present investigation were to assess the degradation of NAADP in a number of mammalian tissues and to characterise the enzyme and products involved.

Rat heart, pancreas, and spleen homogenates were prepared and subsequently incubated (200 µl, 37°C) with NAADP. Samples were removed at various time points and assayed for Ca^{2+} release in sea urchin egg homogenate. NAADP standards were then used to convert assayed samples to NAADP concentration. Statistical significance was determined by analysis of variance and a post hoc Fisher's least significance difference.

The initial rate of NAADP (5 µM) degradation was assessed in both heart, pancreas and spleen homogenate. The fastest rate was seen in the heart homogenate, 1.37 ± 0.09 nmol/mg of protein/minute. The degradation was significantly slower in both the pancreas and the spleen the relative rates being 0.49 ± 0.13 (mean ± s.e.mean, n = 5, $P < 0.001$) and, 0.27 ± 0.12 (n = 3, $P < 0.001$) nmol/mg of protein/minute, respectively.

Anion exchange HPLC was used to identify the endogenous metabolite. Injection of an homogenate sample with NAADP (5 µM) at time zero produced a peak consistent with NAADP standards. Ten minutes incubation with NAADP resulted in a second peak. This second peak was subsequently shown to co-elute with NAAD, suggesting the action of a phosphatase enzyme.

Vanadate and $[AlF_4]^-$, both of which are known to inhibit phosphatases, were studied. Vanadate (1 mM) and $[AlF_4]^-$ (1 mM) were pre incubated for 5 minutes with the heart homogenate prior to the addition of NAADP (5 µM). Comparison of the initial rate of degradation showed a significant reduction in rate from 1.37 ± 0.09 to 0.30 ± 0.11 nmol/mg protein/minute (n = 3, $P < 0.001$) in the presence of vanadate and 0.41 ± 0.21 nmol/mg protein/minute (n = 3, $P < 0.002$) in the presence of $[AlF_4]^-$. Furthermore, when vanadate was incubated with NAADP 10 minutes prior to HPLC injection the metabolite peak NAAD, was no longer present.

In conclusion, these results demonstrate that NAADP is metabolised in mammalian tissues. Furthermore, this is the first report showing the endogenous metabolite of NAADP is NAAD, suggesting that the degradation observed is due to the actions of phosphatases.

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181P MECHANISM OF PEROXYNITRITE TOXICITY TO HUMAN CULTURED SYNOVIAL FIBROBLASTS: A COMPARISON WITH HYPOCHLOROUS ACID

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Peroxynitrite (Oxoperoxonitrate 1-; $ONOO^-$) is a cytotoxic reactive nitrogen species formed from the rapid reaction between nitrogen monoxide ($^{\bullet}NO$) and superoxide ($O_2^{\bullet-}$). Its formation *in vivo* is implicated in numerous diseases including rheumatoid arthritis (RA). Hypochlorous acid (HOCl) is a reactive chlorine species formed by activated phagocytes *via* myeloperoxidase, hydrogen peroxide and chloride ions. Part of the toxicity of $ONOO^-$ and HOCl involves the oxidation, nitration and chlorination of biological substrates such as DNA, protein, lipids and carbohydrates.

Here we compare the toxicity of these two potent biological oxidants in cultured human synovial fibroblasts. Cells were cultured in DMEM with 1% (v/v) penicillin, 10% (w/v) foetal calf serum, 5% CO_2: 95% O_2 with ~95% humidity to 90% confluency before use.

After incubation in PBS for 5 min, cells were exposed to HOCl (0-200μM) or $ONOO^-$ (0-1mM), washed, lysed with 0.2ml trichloroacetic acid (TCA; 6% w/v) and incubated on ice for 10 min. The TCA extract was then used to determine cellular levels of ATP (Strehler, 1968), reduced glutathione (GSH) and oxidised glutathione (GSSG; Hissin & Hilfin, 1976) and NAD^+ (Hinz *et al.*, 1973). Mitochondrial respiration and cell leakage were assessed by MTT reduction and LDH release, DNA strand breakage by the alkaline unwinding method (Birnboim & Jevcak, 1992) and oxidative DNA base damage by GC-MS (Spencer *et al.*, 1996).

HOCl and $ONOO^-$ caused time and concentration-dependent loss of GSH, NAD^+, ATP and caused substantial DNA strand breakage but only HOCl caused LDH leakage. Table 1 shows the concentrations of HOCl and $ONOO^-$ used to achieve 50% change in these markers. With the exception of GSH loss, $ONOO^-$ concentrations required to achieve 50% loss were higher than those required for HOCl. HOCl (100μM) and $ONOO^-$ (250μM) also oxidatively damaged cellular DNA. Most notably, $ONOO^-$ caused 3 fold increases in hypoxanthine and xanthine (products of adenine and guanine deamination respectively) whereas HOCl exposure generated 2 fold increase in 5-hydroxyhydantoin and 5-chlorocytosine and four fold increases in thymine glycol. 8-Hydroxyguanine levels did not increase.

	Concentration Change (μM)	Causing 50%
Marker Measured	**+ $ONOO^-$**	**+ HOCl**
ATP loss	487.2 ± 10.2	207.6 ± 15.3
GSH loss	204.3 ± 21.2	248.0 ± 5.9
NAD^+ loss	351 ± 26.5	68.2 ± 6.3
MTT reduction	244.5 ± 3.8	54.3 ± 2.8
GSSG accumulation	306.6 ± 19.5	250.1 ± 15.1
LDH leakage	> 1mM	102.6 ± 5.3
DNA strand breakage	250.0 ± 22.1	62.3 ± 4.3

Table 1. Toxicity of $ONOO^-$ and HOCl to cultured human synovial fibroblasts. Cells were exposed to either $ONOO^-$ or HOCl for 10 min in PBS. LDH leakage was assessed 24hr after initial exposure. Data are mean ± sem of 6 separate experiments.

Elucidating the mechanisms by which HOCl and $ONOO^-$ cause cell toxicity is of interest in the development of potential therapeutic agents for conditions where these oxidants are formed. The data presented here show HOCl and $ONOO^-$ oxidise the intracellular antioxidant GSH to GSSG and perturb respiration. Both oxidants attacked cellular DNA to cause strand breakage and oxidation of individual DNA bases. Damage to DNA also perturbs respiratory function. Interestingly, there was no detectable increase in the formation of the most widely used bio-marker of oxidative DNA base damage, 8-hydroxyguanine. This was probably due to the destruction of 8-hydroxyguanine by HOCl and $ONOO^-$ (Whiteman *et al.*, 1997).

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182P BTS 67 582, AN ANTI-DIABETIC AGENT, IS MORE EFFECTIVE IN LOWERING PLASMA GLUCOSE THAN THE SECOND-GENERATION SULPHONYLUREA, GLIBENCLAMIDE, IN *ob/ob* MICE

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BTS 67 582 (1,1-dimethyl-2-(2-morpholinophenyl)guanidine fumarate is not structurally related to the sulphonylurea class of K_{ATP} channel blockers, eg glibenclamide, widely used for the treatment of NIDDM. Previously, we have shown that BTS 67 582 lowers blood glucose in normal rats and this is associated with increases in plasma insulin (Jones *et al.*, 1997). In this study, the effect of BTS 67 582 and glibenclamide were compared in fasted and obese (*ob/ob*) mice and homozygous lean (+/+) controls.

Male and female ob/ob mice (20-25 weeks, n=14-19) were obtained from Aston University. Mice were starved 18 hours prior to study. Blood, obtained by tail clipping, was collected in lithium heparin tubes and plasma assayed for glucose, using a GM7 meter and insulin, by RIA (Amersham, RPA 547 Kit). Obese mice were administered BTS 67 582 (3-300 mg kg^{-1} po) or glibenclamide (0.1-100 mg kg^{-1} po) and samples taken at 0, 2 and 4 h. In (+/+) lean mice, BTS 67 582 and glibenclamide were evaluated at 100 and 10 mg kg^{-1} po, respectively. Statistical analysis was by a linear model with baseline as covariate, treatment as a fixed effect and experiment as a random effect. Treatments were compared to control using Williams t-test.

In *ob/ob* mice, BTS 67 582 caused dose-dependent reductions in plasma glucose at 30, 100 and 300 mg kg^{-1}. ED_{50} values for the reduction of plasma glucose were 73.3 mg kg^{-1} (s.e.mean = 15.6) at 2h and 38.9 mg kg^{-1} (s.e.mean = 8.9) at 4h. This was associated with dose-dependent increases in plasma insulin at 100 and 300 mg kg^{-1}. In contrast, no dose-related effects were observed with glibenclamide. Glibenclamide at 1, 10 and 100 mg kg^{-1} caused small falls in plasma glucose. No significant falls were observed after 4h. Glibenclamide dosing caused no significant changes in plasma insulin (Table 1). In lean mice, BTS 67 582 and glibenclamide caused similar reductions plasma glucose (mM), 7.59 (0h) to 4.64 (4h) and 7.70 (0h) to 4.49 (4h), respectively.

The observed reduction in plasma glucose clearly indicates the effectiveness of BTS 67 582 in the *ob/ob* mouse. The associated increase in plasma insulin suggests that stimulation of insulin secretion is the mechanism by which BTS 67 582 exerts it action. Glibenclamide was only weakly active in the *ob/ob* mouse. Interestingly, glibenclamide and BTS 67 582 were equally effective in the lean mouse. The lack of effect with glibenclamide may be specific to the *ob/ob* strain of mouse. Exactly where and how BTS 67 582 exerts it's effects in the *ob/ob* mouse awaits further investigation, but the data clearly differentiate BTS 67 582 from the sulphonylurea class of anti-diabetic agents.

Jones R.B. *et al.* (1997) *Br. J. Pharmacol.* 120, 1135-1143.

Table 1 Comparison of the effects of BTS 67 582 and glibenclamide on plasma glucose and insulin levels in *ob/ob* mice

		Plasma glucose (mM)			Plasma insulin (ng/ml)		
Drug	Dose (mg kg^{-1})	0h	2h	4h	0h	2h	4h
BTS 67 582	30	13.44±1.6	7.68±1.8**	6.59±1.6**	16.83±2.4	20.29±3.0	16.05±1.9
	100	12.65±1.5	4.41±0.4**	3.79±0.5**	14.57±1.9	41.71±10.0**	22.94±4.6**
	300	12.30±1.4	3.18±0.3**	3.62±0.2**	7.04±0.8	21.46±3.5**	11.96±1.8**
Glibenclamide	1	11.16±1.3	5.73±0.8*	7.10±1.3	14.69±3.0	16.96±2.7	12.48±2.8
	10	11.97±1.8	6.89±1.3*	7.46±1.4	14.56±3.9	25.14±5.3	13.68±4.2
	100	10.58±1.7	5.96±0.9*	7.81±0.9	7.81±1.5	7.03±0.8	7.16±0.9

Significant difference from control (0h) *P<0.05, **P<0.01.