289P THE EFFECTS OF THE ISOENZYME-SELECTIVE PHOSPHODIESTERASE INHIBITORS, ROLIPRAM AND ZAPRINAST, ON CYCLIC NUCLEOTIDE LEVELS IN THE GUINEA-PIG CEREBELLUM

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The termination of cyclic nucleotide-mediated cellular signalling is brought about through the action of phosphodiesterases (PDEs). At least five families of PDEs are currently recognised (Beavo & Reifsnyder, 1990). Of these, PDE IV is "cyclic AMP-specific" and may be selectively inhibited by rolipram and Ro 20-1724, while PDE V is "cyclic GMP-specific" and may be selectively inhibited by zaprinast (Beavo & Reifsnyder, 1990). We have investigated the effects of these agents on cyclic nucleotide levels, which we have previously suggested to be inter-related, in the guinea-pig cerebellum (Hernández *et al.*, 1994).

The accumulation of [^{3}H]-cyclic AMP or [^{3}H]-cyclic GMP was carried out at 37°C with guinea-pig (Dunkin-Hartley, either sex, 200 - 600g) cerebellar slices pre-incubated with [^{3}H]-adenine or [^{3}H]-guanine, respectively, as previously described (Hernández *et al.*, 1994). Data were expressed as a percentage conversion from the total [^{3}H]-adenine or [^{3}H]-guanine nucleotides, from experiments carried out on at least 3 separate occasions.

Accumulation of [^{3}H]-cyclic GMP in guinea-pig cerebellar slices was 0.37 ± 0.06 % and 4.02 ± 0.55 % (means ± SEM) conversion in the absence and presence of 1 mM sodium nitroprusside (SNP), respectively. Zaprinast was without effect on basal levels of cGMP, but evoked an enhancement of the SNP-evoked cGMP response at 10 (123 ± 3% SNP response) and 100 µM (188 ± 9 %). Rolipram was also without effect on basal levels of cGMP at concentrations up to 100 µM. However, in the presence of 1 mM SNP, rolipram evoked a concentration-dependent enhancement of the cGMP accumulation, with a maximal response of 212 ± 32 % of the SNP response and a pD_2 value of 8.04 ± 0.16 (slope 0.59 ± 0.12).

Zaprinast failed to alter cAMP accumulation at concentrations up to 100 µM. Rolipram and Ro 20-1724 increased the basal accumulation of cAMP without reaching an asymptote. The -log concentrations of these agents which evoked an increased cAMP accumulation of 0.1 % conversion over basal were 5.74 ± 0.19 and 4.64 ± 0.07, respectively.

We previously suggested that the elevations of cGMP levels in the guinea-pig cerebellum evoked by IBMX were mediated through direct inhibition of PDE activity. Forskolin evoked a similar enhancement of cGMP levels which we concluded derived from elevation of cAMP levels, which prevented the catabolism of cGMP, possibly through simple competition at a common PDE (Hernández *et al.*, 1994). In the same series of experiments we were unable to show any effect of SNP on forskolin-stimulated cAMP levels. This we interpreted as a consequence of the relatively low concentrations of cGMP in the cerebellum lacking an influence on cAMP catabolism. The results with the PDE isoenzyme-selective inhibitors supports these findings, since inhibition of cAMP catabolism (rolipram) enhances cGMP levels, while inhibition of cGMP catabolism (zaprinast) has no effect on cAMP levels.

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290P 5-HT NEURONAL LESION DRAMATICALLY REDUCES THE INCIDENCE OF BURST-FIRING DORSAL RAPHE NEURONES IN THE RAT

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Recently, we described a population of neurones in the rat dorsal raphe nucleus (DRN) with electrophysiological characteristics typical of 5-HT neurones except that the neurones fire bursts of spikes and not just solitary spikes (Hajós et al., 1995). As with single spiking 5-HT neurones, these burst-firing neurones are inhibited by 5-HT_{1A} agonists or 5-HT reuptake inhibitors and can be antidromically activated from the forebrain (Hajós et al., 1995; Hajós and Sharp, 1995). Here, in search of further evidence that burst-firing neurones in the DRN contain 5-HT, we have investigated whether these neurones are present in rats treated with the selective 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT).

Male Sprague-Dawley rats (265-285 g), were anaesthetised with halothane and received desipramine (25 mg/kg i.p.) followed by i.c.v. injections of 5,7-DHT (200 µg in 10 µl 1 % ascorbate) or vehicle alone. Two weeks later, rats were anaesthetised with chloral hydrate (400 mg/kg i.p.) and extracellular action potentials were recorded with stereotaxically implanted, glass microelectrodes using conventional electrophysiological methods (Hajós et al 1995). In each animal, 5-10 min baseline recordings were made from all spontaneously active neurones encountered during five electrode descents (each separated by 200 µm) into the DRN. The experimenter was blind to the treatment. At the end of each experiment, brains were removed to histologically verify the final position of the electrode and measure (by HPLC-EC) 5-HT and 5-HIAA levels in the neocortex, hypothalamus and the striatum.

In the DRN of control (n=4) and 5,7-DHT treated (n=7) rats, a total of 73 and 63 neurones were recorded, respectively. Neurones were separated into the following categories, i) neurones which fired single, broad action potentials in a slow (0.5-3 Hz) and regular pattern which is considered typical of 5-HT neurones (Aghajanian et al., 1978), ii) neurones with the latter characteristics but which fired brief bursts (Hajós et al., 1995) and iii) both fast firing (>10 Hz) regular neurones or slower firing (0.1-10 Hz) irregular neurones which are commonly seen to be non-5-HT neurones (Aghajanian et al., 1978). Figure 1 illustrates that the number of single-spiking and burst-firing neurones encountered per electrode descent was reduced by 80 % and 91 %, respectively, in rats treated with 5,7-DHT compared to controls. In comparison, the number of presumed non-5-HT neurones encountered in the two groups was not different. The electrophysiological features of the few single-spiking and burst-firing neurones remaining in 5,7-DHT-treated rats were not different from controls. Brain tissue levels of 5-HT and 5-HIAA in rats treated with 5, 7-DHT were reduced 77 - 91 %.

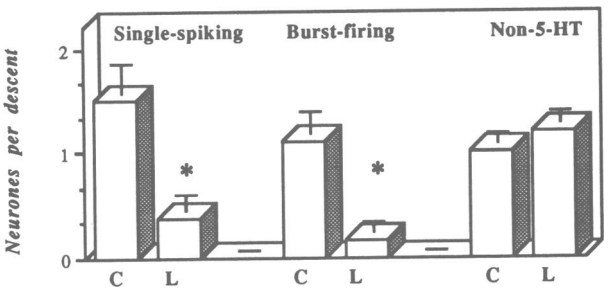

Fig. 1. Number of DRN neurones per electrode descent in control (C) and 5,7-DHT-treated (L) rats. *p < 0.001 vs C (Student's t-test).

In summary, the present data indicate that the occurrence of both single-spiking 5-HT neurones and burst-firing neurones in the DRN is much reduced in rats with 5-HT lesions. This finding is further evidence that burst-firing DRN neurones contain 5-HT.

This work is supported by the Medical Research Council.

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291P INVESTIGATION OF THE TURNOVER RATE OF 5-HT$_{2A}$ RECEPTORS IN C6 RAT GLIOMA CELLS USING PHENOXYBENZAMINE

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The application of antisense oligonucleotides to inhibit neurotransmitter receptor synthesis needs a knowledge of receptor turnover rate to optimise the exposure time of the tissue to the oligonucleotide. In the case of certain 5-HT receptor subtypes, including the 5HT$_{2A}$ receptor, the *in vivo* turnover rate is of the order of 3-4 days as determined using irreversible antagonists (eg. Gozlan et al., 1994). However, there is evidence that the *in vivo* turnover rate may not predict the turnover rate in cultured cells (Neve & Molinoff, 1986). Here we have determined the turnover rate of the 5HT$_{2A}$ receptor in C6 rat glioma cells, using the irreversible antagonist phenoxybenzamine (PBZ) (Kendall & Nahorski, 1986).

C6 cells were cultured in Duibecco's modification of Eagles medium (DMEM) plus 10% foetal calf serum (FCS) until confluent, and then DMEM plus 1% dialysed FCS, for 1 day. PBZ (10 nM-1 μM) or vehicle (acetic acid 0.1%) was then added and cells were incubated for 30 min at 37 °C. Next, cells were either washed and harvested, or washed and replenished with DMEM plus 1% dialysed FCS and incubated for a further 1.5, 3, 6 or 24 h before harvesting. In some experiments cells were incubated with cyclohexamide (0.5 μg/ml) after PBZ. Saturation binding assays were carried out on C6 membranes using [^{3}H]ketanserin and non-specific binding was defined using 10 μM mianserin (Elliott et al., 1995).

[^{3}H]Ketanserin showed specific and saturable binding to C6 cell membranes. After 30 min incubation, PBZ induced a dose-related inhibition of [^{3}H]ketanserin binding (IC_{50}=60 nM) with maximal decrease at 1 μM (6 % of control, n=3). Following incubation with PBZ (1 μM, 30 min), the B_{max} for [^{3}H]ketanserin binding showed a time-dependent (0-24 h) recovery, whereas the affinity did not change (Table 1). The half-life of recovery of [^{3}H]ketanserin binding was about 4 h. After exposure to PBZ, recovery of binding (t=6 h) was abolished by cyclohexamide (n=4), indicating that recovery required new protein synthesis.

Table 1 Recovery of [^{3}H]ketanserin binding to C6 cell membranes in controls and after 30 min exposure to 1 μM PBZ.

	Control	Recovery time (h)				
		0	1.5	3	6	24
Bmax	271±34	7±5	93±35	116±9	196±15	273±50
	(100 %)	(3 %)	(34 %)	(43 %)	(72 %)	(101 %)
pKd	9.0±0.1	8.8±0.3	8.7±0.2	9.1±0.1	9.1±0.1	9.2±0.1
n	8	8	4	4	3	3

(Bmax=fmol/mg protein ; pKd= M ; values are mean ± sem)

From these data we estimate the half-life of the rat 5HT$_{2A}$ receptor in C6 cells to be 4h. β_2-adrenoreceptors in C6 cells have a similar half-life (Neve & Molinoff, 1986). This result is relevant to experiments aimed to knock out the 5HT$_{2A}$ receptor in C6 cells using antisense probes. Furthermore, it emphasises that measures of receptor half-life *in vivo* may not reflect half-life in cultured cells.

Robert Meller is a Wellcome Prize Student.

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292P COMPARISON OF THE BINDING OF [^{3}H]PN200-110 TO RAT BRAIN, SH-SY5Y AND NK108-15 MEMBRANES

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In this study we have determined the number of L-type voltage sensitive Ca^{2+} channels (VSCC) in SH-SY5Y and NG108-15 neuroblastoma cells by measurement of [^{3}H]PN200-110 binding and compared this to rat cerebrocortical and cerebellar membranes (see Spedding & Paoletti, 1992). In addition we have made fluorimetric determination of $[Ca^{2+}]_i$ using the specific L-channel opener S(-)BayK8644 in SH-SY5Y and NG108-15 cells.

The binding of [^{3}H]PN200-110 was measured in crude membrane preparations in 1ml volumes of 50mM Tris.HCl pH7.4 at room temperature for 90mins. Non-specific binding was determined in the presence of 10μM nifedipine and bound and free [^{3}H]PN200-110 were separated by vacuum filtration. The binding reaction contained approximately 0.2mg, 0.5mg and 1.0mg of protein for cerebrocortical, cerebellar and cell membranes respectively For determination of B_{max} and K_d 0.02-2.0nM [^{3}H]PN200-110 was used and for estimation of the K_i for nifedipine 0.2nM was used. $[Ca^{2+}]_i$ was also measured in whole cell suspensions loaded with 3μM Fura2/AM for 30 mins at 37°C in Krebs/HEPES buffer, pH 7.4. Loaded cells were postincubated for a further 20mins at room temperature. $[Ca^{2+}]_i$ was measured fluorimetrically according to Grynkiewicz *et al* (1985). All data are Mean±s.e.mean (n≥5) and statistically analysed by ANOVA followed Scheffe's F test and paired t-test as appropriate

The binding of [^{3}H]PN200-110 was dose-dependent and saturable in all tissues studied. The B_{max} varied considerably but the K_d remained relatively constant (Table 1). Nifedipine produced a dose-dependent displacement of [^{3}H]PN200-110 binding with a K_i of around 4nM. In cortical membranes diltiazem *increased* the binding of [^{3}H]PN200-110 by increasing the K_d (from 88±12 to 191±16pM, $p<0.05$) without affecting the B_{max} S(-)BayK8644 produced a small but statistically significant increase in $[Ca^{2+}]_i$ in fura 2 loaded SH-SY5Y and NG108-15 cells (Table 1)

These data show that undifferentiated SH-SY5Y cells express a low density of functionally coupled L-type VSCCs. Previous studies have experienced difficulties in detecting L-currents in SH-SY5Y cells (Morton et al., 1992; Reeve et al., 1994). This study also demonstrates the presence of functionally coupled L-channels on undifferentiated NG108-15 cells.

Table 1. B_{max} and K_d for [^{3}H]PN200-110 binding and the effects of S(-) BayK8644 (n=5)

	B_{max} (fmol/mg protein)	K_d (pM)	$\Delta[Ca^{2+}]_i$(nM) with 10^{-6}M BayK8644
Cortex	137±11*	88±12	ND
Cerebellum	34± 7	78±16	ND
SH-SY5Y	9± 1	82±15	25±2#
NG108-15	4± 1	104±12	17±4#

*$p<0.05$ larger than cerebellum, SH-SY5Y and NG108-15. # $p<0.05$ increased compared with basal. ND, Not determined.

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293P MORPHINE INHIBITS GLUTAMATE RELEASE FROM RAT CEREBROCORTICAL SLICES

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The effects of opiates on the release of glutamate (and their use as neuroprotectants) are poorly understood. Enadoline, a k-agonist has been shown to inhibit 4-aminopyridine stimulated glutamate release from rodent and primate striatum and a role in neuroprotection has been suggested (Hill & Brotchie, 1995) In this study we have examined the effects of morphine on K^+ evoked glutamate release from rat cerebrocortical slices.

Rat cerebrocortical slices (350μm) were prepared from female Wistar rats (200-250g) and suspended in oxygenated (95%O_2/5%CO_2) Krebs buffer, pH 7.4. Approximately 1ml of gravity packed slices (protein was not determined) was pipetted into a perfusion chamber constructed from a 2ml syringe barrel. Slices were perfused at 1ml/min for 60mins prior to collection of 2min fractions for the estimation of glutamate concentrations. Following 6mins of perfusion with basal Krebs, 46mM K^+ was applied for 2mins (S_1). Slices were perfused for a further 30mins in basal Krebs prior to the second application of K^+ (S_2). Perfusate glutamate concentrations were determined using a fluorimetric assay (Nicholls et al., 1987). EGTA (0.1mM in Ca^{2+} free Krebs) and morphine (0.01-10μM) were applied for 30mins between S_1 and S_2. S_2/S_1 ratios were calculated from the area under the curves for control and drug treated samples. Statistical analysis was by Students paired t-test and ANOVA as appropriate and considered significant when $p<0.05$.

K^+ depolarisation produced a monophasic release of glutamate for both S_1 and S_2 stimuli. The mean S_2/S_1 control ratio was 0.98±0.13 (n=35). Glutamate release was Ca^{2+} dependent in that EGTA treatment reduced the S_2/S_1 ratio from 1.23±0.53 to 0.22±0.12 (82%) (n=5). Morphine produced a dose dependent inhibition of glutamate release with an estimated IC_{50} of 18.8nM (Table 1)

Table 1. Morphine inhibits glutamate release form rat cerebrocortical slices.

[Morphine](M)	S_2/S_1 ratio		%Inhibition
	Control	+morphine	
10^{-5}	1.04±0.26	0.45±0.08*	57
10^{-6}	1.12±0.31	0.38±0.20*	64
10^{-7}	0.89±0.13	0.46±0.10*	48
10^{-8}	0.82±0.15	0.63±0.16	23

Data are Mean±s.e.mean for n=5-10. *$p<005$ compared with paired control. In the 2min fraction immediately prior to K^+ stimulation, basal glutamate release amounted to 822±71nmoles (n=35). At the S_1 peak a value of 1552±164nmoles (n=35) was obtained.

These data show that morphine inhibits the release of glutamate from rat cerebrocortical slices and implicate a role for opioids in the prevention of glutamate induced neurotoxicity. Further studies to examine the role of δ and k opioid agonists are in progress.

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294P ELEVATION OF *IN VIVO* STRIATAL DOPAMINE RELEASE IN THE RAT VIA ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS

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Intra-striatal injection of a selective metabotropic glutamate receptor agonist (1S, 3R)-1-Aminocyclopentane-1, 3-dicarboxylic acid ((1S, 3R)-ACPD) induced contra-lateral turning behaviour in the rat (Sacaan *et al.*, 1991;1992), which was antagonised by the dopamine (DA) receptor antagonist haloperidol. This data indicates that metabotropic glutamate receptors may interact with dopaminergic neurones in the rat striatum. The present study investigates the ability of the selective metabotropic receptor agonist (1S, 3R)-ACPD to modulate basal DA release in the rat striatum *in vivo* estimated using the microdialysis technique.

For microdialysis studies, female Wistar rats (150-250 g) were anaesthetised with ketamine and medetomidine before chronic indwelling guide cannulae were stereotaxically inserted. At least 7 days after surgery, a microdialysis probe was inserted (4 mm dialysis tip; A+0.2, V-7.5, L-2.5, relative to Bregma; Paxinos and Watson, 1986) and perfused with artificial cerebro-spinal fluid (aCSF; mM: NaCl 126.6, KCl 2.4, KH_2PO_4 0.49, $MgCl_2$ 1.28 $CaCl_2$ 1.1, $NaHCO_3$ 27.4, Na_2HPO_4 0.48, glucose 7.1, pH 7.4) at 2 μl/min. Dialysate samples collected for at least the first 100 min were discarded and subsequent samples were collected every 20 min into perchloric acid (final concentration 0.1M). A stable base line was established before drugs were administered via the perfusing aCSF. Dialysate DA levels were analysed immediately using HPLC-ECD (ANTEC working electrode +700 mV versus Ag/AgCl reference electrode).

The basal dialysate DA levels were 14.58±0.77 fmol/20 min, mean±S.E.M., n=11). Administration of (1S, 3R)-ACPD (1-3 mM, administered via the microdialysis probe) enhanced the dialysate levels of DA (maximal increase 396±79%, mean±S.E.M., n=5) in a concentration-dependent manner. The selective metabotropic glutamate receptor antagonist, (+)-α-Methyl-4-carboxyphenylglycine (MCPG, 10mM, administered via the microdialysis probe) failed to modulate dialysate DA levels, but compeletely prevented the (1S, 3R)-ACPD (3 mM, administered via the microdialysis probe)-induced elevation of dialysate DA levels in the rat striatum (Figure 1).The present study indicates that activation of metabotropic glutamate receptors in the rat striatum enhances DA release.

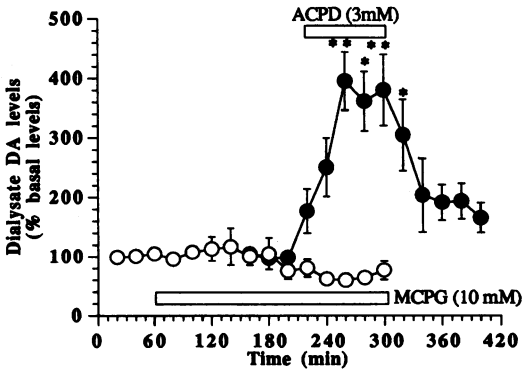

Figure 1. Ability of; (1S,3R)-ACPD (3 mM; ●) and (+)-MCPG (10 mM; ○) to modulate dialysate levels of DA in the rat striatum. Data represents mean±S.E.M., n=4-5, $P<0.01$, ANOVA, *$P<0.05$, **$P<0.01$, Dunnett's t test.

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295P AUTORADIOGRAPHIC LOCALIZATION OF TACHYKININ NK_2 RECEPTORS IN ADULT RAT BRAIN USING [^{3}H]SR48968

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In recent studies, using the tritiated peptidic tachykinin NK_2 receptor antagonist, [^{3}H]-GR100679, specific binding was observed in discrete areas of neonatal rat brain, particularly the thalamus and hippocampus (Hagan *et al.*, 1993). However, in the adult rat, results were difficult to interpret because of very high non-specific binding. We have now used the tritiated non-peptidic tachykinin NK_2 receptor antagonist, [^{3}H]-SR48968 (Emonds-Alt *et al.*, 1993) in a further attempt to localise tachykinin NK_2 receptors in the CNS of adult rat.

The binding of [^{3}H]-SR48968 to saggital and coronal sections (20μm) of adult rat brains (male Lister Hooded rats, 300-350g, n=4) was determined using in vitro receptor autoradiography. Sections were incubated with [^{3}H]-SR48968 (1.5nM) for 60min at 23°C (Beresford *et al.*, 1992). Non-specific binding (NSB) was defined as that remaining in the presence of GR159897 (1μM; Beresford *et al.*, 1995), a structurally dissimilar tachykinin NK_2 receptor antagonist. Washed and dried sections were apposed to photographic film for 4 months and the resulting autoradiographs were analysed (determination of optical densities) using an image analyser.

High levels of total [^{3}H]-SR48968 binding were observed in a number of areas including the thalamic nuclei, amygdala, striatum, habenula, hippocampus, superior colliculus and interpeduncular nucleus. Although the levels of NSB were fairly high throughout the brain, it was possible to identify a number of areas of interest with over 25% specific [^{3}H]-SR48968 binding (see Table 1). Highest specific binding was in the posteromedial cortical (PMC) amygdaloid nucleus. Within the striatum, the specific binding appeared to be in discrete granules. In contrast, other areas (e.g. globus pallidus) demonstrated minimal levels of specific binding.

Table 1 Total and non-specific optical densities (mean±s.e.mean) and specific [^{3}H]-SR48968 binding (expressed as % of total) for several regions of rat brain. n=15-31 slices from 3-4 brains.

Brain Region	Optical Density		% Specific Binding
	Total	Non-specific	
PMC amygdaloid nucleus	0.19±0.01	0.13±0.01	30±2
Interpeduncular nucleus	0.19±0.01	0.13±0.01	29±4
Habenula	0.19±0.01	0.14±0.01	27±2
Striatum (granules)	0.24±0.01	0.18±0.01	27±1
Thalamic nuclei	0.19±0.01	0.15±0.01	22±2
Central grey	0.14±0.01	0.12±0.01	14±3
Globus pallidus	0.16±0.01	0.15±0.01	4±2

This study identified a number of brain regions which appeared to express low levels of tachykinin NK_2 receptors. These results provide further evidence for the presence of tachykinin NK_2 receptors in the rat central nervous system.

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296P GR159897, A NON-PEPTIDE TACHYKININ NK_2 RECEPTOR ANTAGONIST, DECREASES 5-HYDROXYTRYPTAMINERGIC CELL FIRING IN THE DORSAL RAPHE NUCLEUS OF THE RAT

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Following either peripheral administration in rodents and primates, or discrete infusion into the dorsal raphe nucleus (DRN) of the rat, GR159897, a selective non-peptidic tachykinin NK_2 receptor antagonist (Beresford *et al.*, 1995), has anxiolytic-like activity in behavioural models of anxiety (Stratton *et al.*, 1994; Walsh *et al.*, 1994). Activation of 5-HT_{1A} autoreceptors in the DRN produces anxiolytic-like effects in rodents (Higgins *et al.*, 1988), which appear to be due to a decrease in serotonergic neuronal firing. We now report on an *in vivo* electrophysiological study to investigate, as a possible mechanism for the anxiolytic effects, an interaction between tachykinins and the serotonergic system in the DRN of the rat.

Male Lister Hooded rats (260-320g) were anaesthetised with urethane (1.8g.kg^{-1} i.p.) and the jugular vein was cannulated for i.v. drug administration. Glass microelectrodes, filled with 2M NaCl, were stereotaxically implanted into the DRN (midline, 1.0mm rostral of lambda, 5.0-6.5mm below brain surface). Single-unit activity was amplified, filtered, isolated and recorded in 10s sampling periods. Serotonergic cells were identified by their characteristic firing activity (0.5-2 spikes/sec) and verified by infusion of the 5-HT_{1A} receptor agonist, 8-OHDPAT (10μg.kg^{-1} i.v.), which produced a decrease in the firing rate (Table 1). Following recovery, GR159897 (0.01μg.kg^{-1} i.v.) or saline (0.15ml i.v.) were infused. The firing rate (spikes/5min) was statistically compared to pre-dose rate using a 2-tailed paired t-test.

Saline (n=3) had no effect on the firing rate of serotonergic cells in the DRN of the rat. 8-OHDPAT (n=6) significantly ($P<0.05$) inhibited neuronal firing over 20min (max. 79% inhibition at 6-10min; Table 1). GR159897 (n=4) also significantly inhibited firing over 30min (max. 67% inhibition at 20-25min; Table 1).

In conclusion, GR159897 produced a decrease in the firing rate of serotonergic cells in the rat DRN in a similar manner to 8-OHDPAT. These results suggest that tachykinin NK_2 receptors may be involved in regulating serotonergic neuronal activity in the DRN and that antagonists may produce their anxiolytic effects by reducing tachykinin-induced activation of DRN neurones.

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Table 1 Effects of saline, 8-OHDPAT and GR159897 on serotonergic neuronal firing activity in the DRN of the rat. Data are firing rate (mean±s.e.mean spikes/5min) in consecutive 5min periods. *P<0.05, **P<0.01, 2-tailed paired t-test.

Treatment	n	pre-dose	1-5min	6-10min	11-15min	16-20min	21-25min	26-30min	31-35min
saline	3	217±29	224±19	212±21	182±20	219±25	231±30	283±25	245±28
8-OHDPAT	6	274±15	85±30**	58±26**	81±36**	132±49*	155±56	164±49	199±65
GR159897	4	313±20	218±24*	167±39**	129±32**	120±48**	108±38**	162±43*	155±71

297P AFFINITY AND SELECTIVITY OF BU224 AND BU239 FOR RAT AND GUINEA-PIG BRAIN I_2 IMIDAZOLINE SITES

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It is now widely recognised that imidazoline sites exist as two principle sub-types, I_1 and I_2 and that the I_2 site can be further sub-divided pharmacologically into I_{2A} and I_{2B} (Ernsberger, 1992). Although I_2 sites are widely distributed in a variety of tissues of several species the function of these sites remains unclear. Until recently the characterisation of I_2 sites has been carried out using the α_2 adrenoceptor antagonist idazoxan (IDX) under conditions that preclude its binding to α_2 adrenoceptors (Ernsberger, 1992). Thus, it is clear that I_2 site selective ligands are required to allow the function(s) of these sites to be elucidated. We have previously shown that the derivatives of IDX, BU224 (2-(4,5-dihydroimidaz-2-yl)-quinoline) and BU239 (2-(4,5-dihydroimidaz-2-yl)-quinoxoline) have high affinity and selectivity for the I_2 site over the α_2-adrenoceptor in rabbit brain (Hudson *et al.*, 1994). We have now examined BU224 and BU239 for affinity at I_2 sites and α_2 adrenoceptors in rat and guinea pig whole brain membranes using radioligand binding.

Radioligand binding studies were performed on whole brain membranes prepared from male Wistar rats (220-250g) and male Dunkin Hartley guinea pigs (300-500g). [^{3}H]RX821002 (1nM) and [^{3}H]IDX (3nM, in the presence of 5μM rauwolscine) were used to label α_2 adrenoceptors and I_2 sites respectively. Membranes with [^{3}H]ligand and displacing drug were incubated (50mM Tris-HCl buffer, 1mM Mg^{2+}, pH 7.4) in triplicate to equilibrium (30min, final volume 1ml, 22°C). Bound and free radioactivity were separated by filtration. Specific binding of [^{3}H]RX821002 and [^{3}H]IDX was defined with 10μM rauwolscine and 10μM 6-fluoro-idazoxan (RX801023) respectively. Data were analysed by GraphPAD Inplot and the inhibition constants (K_i) are shown in Table 1

Table 1. Data are mean ± s.e.mean; n=4-6 ;** denotes 2-site fit, *** denotes 3-site fit, $p<0.05$, F test . * denotes majority I_2 sites

Compound	α_2 Ki (nM)	I_2 Ki (nM)	I_2 nH	α_2/I_2
Rat				
BU224	2231±470	6.4±0.8	0.6	347
BU224**		5.1±0.9*; 7515±6970		437
BU239	670±161	8.1±0.4	0.7	82
BU239***		0.07±0.01; 8.7±1.0*; 16854±2846		70
GuineaPig				
BU224	2984±266	1.6±0.4	0.5	1842
BU224**		0.9±0.1*:44001±39259		3316
BU239	619+106	8.8±2.6	0.4	70
BU239**		1.9±0.3*; 7743±5571		314

Both BU224 and BU239 showed high affinity and selectivity for the majority of I_2 sites* in a multiphasic complex manner, but low affinity for α_2 adrenoceptors in rat and guinea pig (Table 1). However, BU239 would appear to be less selective than BU224 for I_2 sites versus α_2 adrenoceptors in both species, as previously found in rabbit (Hudson *et al*, 1994), due to its higher affinity for α_2 adrenoceptors. The reduced α_2/I_2 selectivity of these compounds in rat and guinea pig, compared with rabbit (Hudson *et al*, 1994), may be explained by their affinity for different ratios of sub-types of I_2 sites (Table 1). We conclude that BU224 and BU239 are selective for the majority of I_2 sites over α_2 adrenoceptors in rat and guinea pig whole brain membranes and may prove useful tools in probing the function(s) of I_2 sites in the mammalian CNS.

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298P [^{3}H]RS-79948-197: A POTENT AND SELECTIVE α_2-ADRENOCEPTOR RADIOLIGAND

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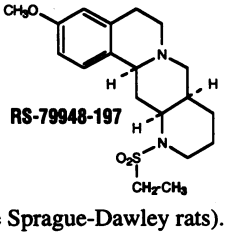
RS-79948-197 ([8a**R**, 12a**S**, 13a**S**]5, 6, 8a, 9, 10, 11, 12, 12a, 13, 13a-decahydro-12-ethanesulfonyl-3-methoxy-6**H**-isoquino[2,1-g]-[1,6]naphthyridine hydrochloride; Clark *et al.*, 1991) is an analogue of the selective, high affinity α_2-adrenoceptor antagonist, RS-15385-197 (Clark *et al.*, 1989). In the present study we have examined the binding of [^{3}H]-RS-79948-197 to a range of α_2-adrenoceptor subtypes: α_{2A} (α_2C10 cell line), α_{2B} (lung from 1 day old Sprague-Dawley rat pups), α_{2C} (OK cell line) and α_{2D} (cerebral cortex from 250-350g male Sprague-Dawley rats).



RS-79948-197

Saturation experiments using increasing concentrations of [^{3}H]-RS-79948-197 (65 Ci mmol^{-1}; 0.01-2 nM; 20-200 μg protein/tube) were incubated to equilibrium (90 min at 25°C) in a 1 ml final assay volume using 50 mM Tris HCl, 0.5 mM EDTA (pH 7.4). Non-specific binding was defined by 1 μM phentolamine (or 10 μM for the α_{2C} membranes) and represented 2-10% of total binding. Competition experiments were carried out in the presence of 0.1 nM [^{3}H]-RS-79948-197 with various drugs (1 pM-0.1 mM). Association and dissociation kinetics were determined using 0.1 nM [^{3}H]-RS-79948-197 and 1 μM unlabelled RS-79948-197 (or 10 μM for the α_{2C} membranes) to initiate dissociation.

[^{3}H]-RS-79948-197 labelled all α_2-adrenoceptor subtypes with high (and approximately equal) affinity. Saturation-derived K_d values were (mean ± s.e.mean; n = 3): α_{2A} 0.13 ± 0.04 nM, α_{2B} 0.04 ± 0.002 nM, α_{2C} 0.02 ± 0.002 nM and α_{2D} (possible species variant of the α_{2A}) 0.06 ± 0.01 nM. These values compared favourably with the kinetically derived K_d estimates: α_{2A} 0.23 ± 0.04 nM, α_{2B} 0.03 ± 0.002 nM, α_{2C} 0.02 ± 0.002 nM and α_{2D} 0.08 ± 0.01 nM.

Subtype selective compounds had affinities at the [^{3}H]-RS-79948-197 binding site in a variety of preparations which indicated that the appropriate subtype of α_2-adrenoceptor was being labelled. Displacement of [^{3}H]-RS-79948-197 by the agonists, oxymetazoline and adrenaline, exhibited low Hill slopes, indicating that [^{3}H]-RS-79948-197 labels both high and low affinity states. RS-79948-197 had low affinity for α_1, 5-HT_{1A}, 5-HT_2, β_1, β_2, D_1, D_2, I_2, M_1, M_2 and dihydropyridine binding sites ($pK_i < 6$). Finally, saturation analyses of the binding of [^{3}H]-RS-79948-197 to porcine wild type α_{2A}-adrenoceptors and to the Asp79Asn mutant variant of this receptor (both stably expressed in Rat-1 fibroblasts) indicated that [^{3}H]-RS-79948-197 had similar affinities for both receptors (K_d values in the range 0.15-0.38 nM). This finding suggests [^{3}H]-RS-79948-197 is an antagonist, as an agonist would be expected to show lower affinity for the Asp79Asn mutation, due to the uncoupling of cellular G-protein population in the mutant receptor (Ceresa & Limbird, 1994).

These results indicate that [^{3}H]-RS-79948-197 is a high affinity α_2-adrenoceptor radioligand which shows little selectivity between α_2 subtypes but very high selectivity over the non-α_2 binding sites that have so far been examined.

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299P XENOVULENE A, A NOVEL COMPOUND ACTIVE AT $GABA_A$ RECEPTORS: FUNCTIONAL STUDIES ON EXPRESSED RECOMBINANT AND MAMMALIAN NEURONAL $GABA_A$ RECEPTORS

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Mammalian $GABA_A$ receptors are characterised by the possession of allosteric binding sites for the benzodiazepines. Radioligand binding studies indicate that Xenovulene A ((2aR*,5aR*, 7E,11E,14aS*,14bS*,14cS*)-2a,5a,6,9,10,13,14,14a,14b,14c-decahydro-4-hydroxy-5a,9,9,12-tetramethyl-1,5-dioxacyclo-penta-[cd]cycloundec[f]inden-3(2H)-one), isolated from *Acremonium strictum*, can bind to the $GABA_A$ receptor (Sundaram *et al*, 1995). The functional properties of Xenovulene A, and the synthetic 11,12-epoxy derivative, XR7009, were studied using: two-electrode voltage clamp recording of recombinant $GABA_A$ receptors expressed in *Xenopus laevis* oocytes, previously injected with cDNAs encoding for $\alpha1\beta1\gamma2S$, $\alpha3\beta1\gamma2S$ and $\alpha6\beta1\gamma2S$ polypeptide subunits; and patch-clamp recording of native $GABA_A$ receptors on cultured embryonic rat (Wistar, P+2 days) cortical neurones.

Both Xenovulene A and XR7009 (0.1-1μM) were devoid of any intrinsic agonist activity on the expressed receptors. For the $\alpha1\beta1\gamma2S$ receptor, the 10μM GABA-activated response was significantly enhanced in the presence of Xenovulene A and XR7009 (1μM). The control GABA concentration-response curve (EC_{50} 10.9±0.4μM (mean±s.e.m.); n=7) was shifted to the left by Xenovulene A (EC_{50} 5.1±0.2μM; n=4) and XR7009 (EC_{50} 2.7±0.1μM; n=3), without any change in the maximum responses. This type of modulation was similar to that produced at the $GABA_A$ receptor by the benzodiazepines, e.g., flurazepam (1μM) reduced the GABA EC_{50} to 3.2±0.16μM (n=3) without changing the maximum response; however pentobarbitone (50μM) reduced the GABA EC_{50} (1.06±0.1μM; n=4) and increased the maximum response (25±4.5%). The relative potency of Xenovulene A to other positive modulators was assessed at the $\alpha1\beta1\gamma2S$ receptor by measuring the enhancement of the response to 10μM GABA. The EC_{50}s were (n=3 to 8): Xenovulene A (0.05±0.02μM); XR7009 (0.02±0.01μM); flurazepam (0.17±0.02μM); diazepam (0.03±0.001μM) and pentobarbitone (21.37±3.55μM) suggesting a potency order of: XR7009 ≥ Diazepam > Xenovulene A > Flurazepam >> Pentobarbitone.

The enhancement of GABA responses by Xenovulene A and XR7009 was antagonised by flumazenil (1-5μM).

For $\alpha3\beta1\gamma2S$ $GABA_A$ receptors, the GABA EC_{50} (104±11μM; n=6) was reduced in the presence of either 1μM Xenovulene A (45±4μM; n=3) or 1μM XR7009 (64±6μM; n=3), without affecting the maximum responses. At $\alpha6\beta1\gamma2S$ $GABA_A$ receptors, Xenovulene A and XR7009 (1μM) were ineffective, though the α6-selective agent, Ro-15-4513 (200nM), enhanced (30%) responses to 1μM GABA. Receptors formed from $\alpha1\beta1$ subunits alone, were also unaffected by Xenovulene A, XR7009, flurazepam or diazepam, but retained a sensitivity to pentobarbitone.

GABA-activated responses on cortical neurones were enhanced (52±9%) by Xenovulene A (0.5μM), and the effect was inhibited by flumazenil (1μM). In conclusion, the block of enhancement by flumazenil and dependence on the γ2 $GABA_A$ receptor subunit is entirely consistent with Xenovulene A acting at the benzodiazepine receptor, and both Xenovulene A and XR7009 appear to exhibit selectivity for the Type I (α1-containing) $GABA_A$ receptor subtype over Type II (α3-containing). These compounds, which are structurally unrelated to the benzodiazepines, may yield a new series of agents with novel therapeutic profiles with possible clinical applications in anxiety, epilepsy and muscle spasticity.

Sundaram, H., Thomas, P., Chazot, P.L. *et al* (1996) Br. J. Pharmacol. In press.

300P XENOVULENE A, A NOVEL COMPOUND ACTIVE AT $GABA_A$ RECEPTORS: CHARACTERISATION BY RADIOLIGAND BINDING

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The $GABA_A$ receptor of mammalian brain is the site of action of multiple therapeutic compounds including the often-prescribed anxiolytic benzodiazepines. Xenovulene A ((2aR*,5aR*,7E,11E,14aS*,14bS*,14cS*)-2a,5a,6,9,10,13,14,14a,14b,14c-decahydro-4-hydroxy-5a,9,9,12-tetramethyl-1,5-dioxacyclopenta[cd]cycloundec[f]inden-3(2H)-one) is a novel compound isolated from the organism, *Acremonium strictum*, which shows a specific high affinity interaction with central benzodiazepine sites associated with the $GABA_A$ receptors (Ainsworth *et al*. 1995). In competition radioligand binding assays (Duggan and Stephenson, 1988), Xenovulene A inhibited [^{3}H] flunitrazepam specific binding to adult rat forebrain membranes with an IC_{50} = 56 ± 9 nM (mean ± sem) (n = 5). Competition assays carried out in the presence of 1 mM GABA gave a positive GABA shift of 1.3. Similarly, competition assays carried out in the presence of 1 mM pentobarbital gave a 1.3-fold shift to higher affinity. Analysis of the binding curves by non-linear least squares regression using Inplot, showed that the data were fitted best to a two binding site model with K_{high} = 7 ± 2 nM and K_{low} = 192 ± 44 nM (n = 5) with the distribution of sites being 51 ± 4% and 49 ± 4% respectively (F-test results: $P<0.05$ for the more complex model). Xenovulene A inhibited [^{3}H] flunitrazepam binding to adult rat cerebellar and spinal cord membranes with K_i = 15 ± 4 nM (n = 5) and K_i = 177 ± 47 nM (n =5) respectively. In contrast to the results for forebrain, in these two regions, the binding curves fitted best to a single binding site model. Interestingly, the 11,12-epoxy analogue of Xenovulene A, XR7009, was found to have approximately 4 fold higher affinity than the parent compound. Thus competition with [^{3}H] flunitrazepam binding with XR7009 in rat forebrain membranes yielded results which best fit to a two binding site model with K_{high} = 1.7 ± 0.5 nM (comprising 42 ± 5% of the sites) and K_{low} = 42 ± 8 nM (58 ± 5% of sites, n = 7). In rat cerebellum and spinal cord, [^{3}H] flunitrazepam inhibition curves by XR7009 were best fit by a one binding site model with K_i = 6 ± 2 nM (n = 7) and K_i = 63 ± 20 nM (n = 7) respectively. Both Xenovulene A and XR7009, (100 μM) had no effect on benzodiazepine-insensitive [^{3}H] Ro 15-4513 specific binding activity. These results are similar to those observed with type I-selective ligands such as ethyl β-carboline-3-carboxylate (βCCE) or zolpidem. For example, results for competition of [^{3}H] flunitrazepam with βCCE in rat forebrain membranes were best fit to a two binding site model with K_{high} = 0.23 ± 0.05 nM, K_{low} = 4.4 ± 1.5 nM (n = 4), a single high affinity binding site in rat cerebellum with K_i = 0.36 ± 0.08 nM (n = 4) and a single low affinity binding site in rat spinal cord, K_i = 3.0 ± 0.8 nM (n = 4).

These results in conjunction with the functional studies of Thomas et al. (1995), demonstrate that Xenovulene A and XR7009 are both potent novel compounds active at the agonist benzodiazepine site. The binding profiles suggests that Xenovulene A and XR7009 are benzodiazepine type I-selective ligands. Xenovulene A thus yields a novel template for the development of $GABA_A$ receptor subtype-specific drugs.

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301P PROLONGED TREATMENT WITH SODIUM VALPROATE RESULTS IN CHANGES OF AMINO ACIDS AND MONOAMINES IN RAT HIPPOCAMPAL DIALYSATES

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Sodium valproate (NaVPA) is a widely prescribed anticonvulsant, used in the treatment of convulsive and non-convulsive epilepsies (Pinder *etal*, 1977). The mechanism of action of NaVPA remains uncertain and a variety of neurotransmitter systems may interact to mediate its anticonvulsant action (Löscher, 1993). Recently, we demonstrated that acute dosing with NaVPA resulted in modified output of central monoamines (Biggs *etal*, 1992a) and amino acids (Biggs *etal*, 1992b; Biggs *etal*, 1994) using *in vivo* microdialysis. We have now evaluated the effects of *prolonged* NaVPA treatment, at different time points during a 14-day treatment period, on release of amino acids and monoamines in the ventral hippocampus using *in vivo* microdialysis.

Male Wistar rats (250-350g) were injected i.p. twice daily (8.00h & 20.00h) with NaVPA or saline. On days 1,3,6 and 13 of treatment, groups of treated and control animals were implanted with concentric dialysis probes in the ventral hippocampus, under chloral hydrate anaesthesia (400 mg/kg). On the following days, all animals received their appropriate treatment at 8.00h. At 13.00h microdialysis was commenced. Probes were perfused with artificial CSF at 0.5µlmin^{-1} (Whitton *etal*, 1990). Following the collection of 3x60min basal samples, 100 mM K^+ was infused via the probes for 30min. Subsequently, normal artificial CSF was perfused during collection of a further 3x60min dialysates. Dialysates were analysed for amino acid and monoamine content using HPLC with fluorometric or elctrochemical detection respectively.

Both amino acids and monoamines were altered during the 14-day treatment period. Basal levels of analytes were as follows; aspartate (ASP) 2,800±445, glutamate (GLU) 34,275±6,000, γ-aminobutyric acid (GABA) 3,100±267, taurine (TAU) 60,075±9,275, 5-hydroxytryptamine (5-HT) 105±20 (of control; fmols/10µl±s.e.m; n=8) Basal dialysate ASP was significantly reduced on days 2 and 7 (47±6% and 43±7.3% of control, respectively; $p<0.05$, n=4 per treated and control group), whilst GLU levels were 33±9% of control on day 4 ($p<0.05$). A modest increase in dialysate GABA was recorded on day 2 (110±8% of control values; $p<0.05$) in treated animals, with a larger increase in 5-HT output on day 4 (175±21% of control; $p<0.05$). On days 2 and 7, high K^+-stimulation resulted in significantly lower levels of ASP in dialysates from treated animals compared to control ($p<0.05$). In contrast, high K^+ treatment tended to evoke a greater release of taurine (123±11%; $p<0.05$) and GABA (135±7;$p<0.05$) in treated versus control animals on day 4. High K^+-stimulated 5-HT overflow was significantly higher in treated animals than control on days 4 and 7; $p<0.05$).

These data suggest that prolonged treatment of rodents with NaVPA results in a variety of changes to basal and evoked neurotransmitter release *in vivo*. Such changes may represent ongoing alterations of neurotransmitter turnover, induced by the drug and raise the possibility that functionally relevent neurochemical changes occur during treatment.

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302P THE EFFECTS OF LAMOTRIGINE AND SODIUM VALPROATE (VPA) CO-ADMINISTRATION ON VERATRIDINE-EVOKED GLUTAMATE AND GABA RELEASE IN THE RAT VENTRAL HIPPOCAMPUS *IN VIVO*

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It is widely accepted that an enhanced excitability due, in part, to impaired GABAergic inhibitory feedback, could be the basis of recurrent excitatory pathways sustaining seizure activity (Meldrum, 1995). Lamotrigine (LTG) is a novel anticonvulsant, which primarily blocks Na^+-channels in a use- and voltage-dependent manner and is effective at inhibiting predominantly veratridine-evoked glutamate release *in vitro* and *in vivo*. (Leach *et al*, 1995; Ahmad *et al*, 1995). A number of reports have demonstrated an increased efficacy due to LTG and VPA co-administration over monotherapy in some seizure types (e.g. Pisani *et al*, 1994). Since, certainly part of the anticonvulsant effectiveness of VPA is achieved through a potentiation in GABA (Biggs *et al*, 1992), the possibility that the two drugs may exert complementary pharmacodynamic effects as well as interacting pharmacokinetically to prolong the half-life of LTG (Yuen *et al*, 1992) seems likely.
In the light of these findings we have investigated this possibility by *in vivo* microdialysis in freely moving rats.
Male Wistar rats (250-300g) were implanted with concentric dialysis probes in the ventral hippocampus under chloral hydrate anaesthesia (400mg/kg i.p.). The following day the probes were infused with artificial CSF (Biggs *et al*, 1992) at 0.5µl.min^{-1}. Veratridine (50µM) was then infused through the probes for 30 mins following the collection of 4 x 30 min samples. At t=180 min, vehicle (H_20), LTG, VPA or both were injected i.p. Then at t=270 min, veratridine was infused for a second 30 mins, and then a final 4 x 30 min samples were collected. Samples were analysed for glutamate (GLU) and GABA content by HPLC. Basal dialysate concentrations were: 42.8 ± 6.3 (GLU) and 2.43 ± 0.8 (GABA) pmols/ 15µl dialysate ± s.e. mean; n=5-6. Following co-administration of LTG and VPA, the magnitude of the inhibition in stimulated GABA release was significantly less than that observed following LTG treatment alone (Table 1).
These interesting *in vivo* data would imply that part of the increased efficacy observed with polytherapy may be due to the sustained potentiation in GABA probably through a pharmacodynamic effect.

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SA is a BBSRC CASE student with Glaxo-Wellcome.

Table 1: The effects of LTG and VPA on veratridine-stimulated amino acid release. Figures represent maximal changes generally observed 60 mins following the second stimulus. *p<0.05- treatment vs control; Mann-Whitney U-test. N=5-6 animals/ group).

	% of basal levels (mean ± s.e. mean)		% change in GABA
STIMULUS	GLUTAMATE	GABA	
Ver. + vehicle	168 ± 21	1147 ± 227	-
Ver + LTG (13.4 mg/kg)	*49 ±19	*555 ± 187	↓48 %
Ver. + VPA (300 mg/kg)	*113 ± 29	1503 ± 796	↑31 %
Ver. + LTG (13.4 mg/kg) + VPA (300 mg/kg)	150 ± 49	*1883 ± 659	↑64 %

303P REPEATED GENERALISED SEIZURES IN THE RAT POTENTIATE CHANGES IN HIPPOCAMPAL GLUTAMATE BUT NOT GABA RELEASE

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We have reported that a single generalised tonic-clonic seizure, induced by the maximal electroshock (MES) model, altered the release of glutamate (GLU) and γ-aminobutyric acid (GABA) from rat hippocampus *in vivo* (Rowley *et al.*, 1995a and c). Here, we report the effects of repeated seizures on the MES-induced changes in GLU and GABA release.

Male, Lister hooded rats (250-350g; Harlan) received either a single MES or 5 MES spread over 10 days, via ear-clip electrodes (200V for 2s) whilst briefly anaesthetised with halothane (4% in O_2). Control animals were anaesthetised and ear-clipped only. Microdialysis experiments, performed as previously described (Rowley *et al.*, 1995c), were carried out during the single or fifth MES, using 5 and 10 min samples. Amino acid levels were determined by HPLC with ECD (Rowley *et.al.*, 1995b) and are presented as pmol/20μl dialysate sample (mean ± s.e.mean; n=6). Analysis for statistical significance was carried out by ANOVA with post hoc Dunnett's t-test.

The results show that in comparison with a single MES, the initial increase in GLU levels was significantly ($p<0.01$) increased by repeated MES (Figure 1A). In addition, the secondary elevation was also significantly ($p<0.05$ and $p<0.01$) enhanced resulting in a prolongation of this phase of the GLU response to MES (Figure 1A). By contrast, repeated MES did not significantly alter the post-ictal changes in GABA release (Figure 1B).

In conclusion, these data show that changes in GLU release are clearly amplified after 5 seizures. Since we have previously shown that the secondary, prolonged rise in GLU is due to suppression of GABA release (Rowley *et al.*, 1995c) and the present results demonstrate that GABA responses were unaltered, the mechanisms responsible for the GLU amplification remain to be determined. Elevated extracellular GLU is well known to cause neuronal damage; it is possible that after further seizures the changes reported here may result in deficits in neuronal function and viability.

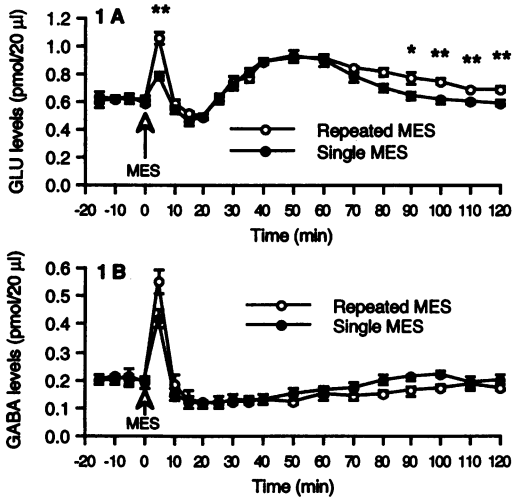

Figure 1A and 1B. Comparison of the effects of repeated seizures with a single seizure on basal hippocampal GLU (1A) and GABA (1B) levels. *p<0.05; **p<0.01 compared with single MES group.

Helen Rowley holds a BBSRC CASE Award in conjunction with Knoll Pharmaceuticals.

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304P LACK OF ALTERATION OF $GABA_B$ RECEPTOR BINDING IN THE ABSENCE EPILEPTIC WAG/Rij STRAIN OF RAT

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The WAG/Rij rat model (Wistar Albino Glaxo strain, bred in Rijswijk, Netherlands) is a genetic model of absence epilepsy. It has been suggested that the GABA system plays a role in the generation of spike wave discharges (SWD) that are a characteristic of absence seizures in the WAG/Rij model. The $GABA_A$ agonist muscimol enhanced the number of SWDs, whilst the $GABA_A$ antagonist bicuculline had the converse effect (Peeters et al 1989).
In GAERS (Genetic Absence Epilepsy Rats from Strasbourg), another rat model of absence epilepsy, GABA-mimetics including agonists for both $GABA_A$ and $GABA_B$ receptors all increase the number and duration of SWDs, whilst bicuculline did not alter the SWD. Additionally the $GABA_B$ receptor antagonist, CGP35348, has been shown to decrease the SWDs in GAERS rats (Marescaux et al 1992). Autoradiographical studies of GAERS rats and their pair bred controls showed no difference in $GABA_A$ or $GABA_B$ binding (Knight and Bowery 1992). In the present study we have investigated autoradiographic $GABA_A$ and $GABA_B$ binding in the WAG/Rij strain and the non-epileptic, control, ACI (agouti) strain.
Autoradiographic binding to $GABA_A$ and $GABA_B$ receptors (Bowery et al 1987) was determined in brains taken from male WAG/Rij rats (285-365g) and male ACI rats (255-290g). A range of [^{3}H]-GABA concentrations (10nM-400nM) were used to determine if there was any regional changes in either the number of $GABA_B$ receptors, or affinity of GABA for these receptors. 50nM [^{3}H]-GABA was used to determine if there was any regional changes in $GABA_A$ binding. There were no significant changes (Mann-Whitney) in either Bmax or Kd of $GABA_B$ binding between the WAG/Rij strain and the ACI strain (Table 1). Similarly, there was no significant difference (Mann-Whitney) in $GABA_A$ binding between the WAG/Rij strain and the ACI strain. In conclusion, the present investigation suggests that an alteration in $GABA_B$ expression is unlikely to be responsible for the occurrence of spontaneous absence epilepsy in WAG/Rij rats.

KPP is an MRC student
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Table 1: $GABA_B$ binding characteristics in WAG/Rij (epileptic) and ACI (non-epileptic) rats (Mean ± s.e.mean, n=6, * n=5).

BRAIN REGION		Bmax (fmol/mg protein)		Kd (nM)	
		WAG/Rij	ACI	WAG/Rij	ACI
Inner Frontal Cortex (layers V-VI)		239.3 ± 28.3	209.5 ± 13.8	193.0 ± 22.2	192.2 ± 22.8
Outer Frontal Cortex (layers I-IV)		323.8 ± 27.8	295.4 ± 25.9	157.8 ± 29.7	136.6 ± 8.4
Hippocampus (CA1 oriens)		150.9 ± 29.8	156.9 ± 18.1*	215.5 ± 35.0	224.3 ± 17.6*
Thalamic nuclei	Lateral dorsal	297.1 ± 29.0	351.4 ± 11.3	172.7 ± 26.3	156.6 ± 15.9
	Lateral posterior	295.4 ± 25.5	341.8 ± 11.7	182.5 ± 42.0	152.7 ± 13.8
	Ventrolateral	237.0 ± 30.8	302.3 ± 21.9	190.3 ± 25.2	237.0 ± 38.4
	Posterior	268.7 ± 39.5	275.6 ± 8.2	170.4 ± 33.7	143.9 ± 10.2
Cerebellum (molecular layer)		403.4 ± 35.4	378.1 ± 19.3	129.5 ± 15.7	128.6 ± 10.4
Cerebellum (granular layer)		94.5 ± 9.6	122.7 ± 14.1	205.4 ± 39.2	259.4 ± 35.8

305P ELECTROPHYSIOLOGICAL CHARACTERISATION OF $GABA_B$ AGONISTS AND ANTAGONISTS IN RAT DORSO-LATERAL SEPTAL NEURONES *IN VITRO*

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A number of novel $GABA_B$ receptor antagonists have been described with large differences in affinity (Waldmeier *et al.*, 1994). The object of the present investigation was to compare the actions of 5 of these antagonists on a single neuronal system in which responses to exogenous agonists and endogenous $GABA_B$-mediated inhibitory postsynaptic potentials could be measured. Intracellular recordings were made from neurones in slices of rat (male, OFA, 100-250 g) dorso-lateral septal nucleus maintained in vitro at 34°C in a modified Krebs solution (Leishman *et al.*, 1994). Membrane potential changes induced by bath application of agonists and antagonists were recorded with 3 M KCl filled microelectrodes and $GABA_B$-receptor mediated late inhibitory postsynaptic potentials (IPSPs) were elicited by focal stimulation in the septal nucleus and recorded using 4 M K-acetate filled electrodes (Stevens *et al.*, 1987).

The resting membrane potential and membrane resistance were -73 ± 8 mV and 97 ± 35 MΩ (means ± SD; n=59). In the presence of 1 μM tetrodotoxin to block all indirect neuronal effects, 2-3 min applications of the $GABA_B$ receptor agonists baclofen or SKF 97541 (3-aminopropyl[methyl]-phosphinic acid) reversibly hyperpolarised the membrane potential in a concentration-dependent manner and reduced the membrane resistance. The reversal potentials, estimated from current-voltage curves were -98 ± 4 and -103 ± 2 mV (means ± SEM; n=5-6), respectively, indicating that both compounds were increasing membrane potassium ion conductance. The EC_{50}s estimated from full concentration-response curves were 0.5 and 0.06 μM, respectively. Prolonged superfusion (≤ 10 min) with both agonists did not reveal any significant desensitisation. Responses to baclofen were antagonised in a competitive manner by the following antagonists: CGP 55845A (3-[1-(S)-(3,4-dichlorophenyl)-ethyl]amino-2-(S)-hydroxy-propyl-benzyl-phosphinic acid) HCl, CGP 52432 ([3-[[(3,4,-dichlorophenyl)-methyl]amino]propyl] (diethoxymethyl)-phosphinic acid), CGP 35348 (3-aminopropyl-diethoxy-methylphosphinic acid), CGP 36742 (3-aminopropyl-N-butyl-phosphinic acid) and 2-OH saclofen. The apparent pA_2 values calculated at the level of the EC_{50} from complete concentration-response curves (antagonist concentration in brackets) were 8.3 (0.1 μM), 6.7 (1 μM), 4.5 (100 μM), 4.0 (100 μM) and 4.2 (100 μM), respectively. Hyperpolarisations induced by SKF 97541 were antagonised by CGP 55845A with a pA_2 of 8.4 (0.1 μM), similar to the value obtained when using baclofen as agonist. Focal stimulation close to the recording area elicited a sequence of excitatory postsynaptic potential and action potential followed by a $GABA_A$-receptor mediated early IPSP and a late IPSP. The late IPSP was selectively reduced in amplitude by all five antagonists tested. The action of CGP 35348, CGP 36742 and 2-OH saclofen were readily reversed by washing for 5-10 min in drug-free Krebs solution, whereas the actions of CGP 55845A and CGP 52432 were not fully reversible. The % reduction in late IPSP amplitude compared to control was as follows: CGP 55845A (1 μM) 91 ± 5%; CGP 52432 (1 μM) 64 ± 5%; CGP 35348 (100 μM) 82 ± 5%; CGP 36742 (100 μM) 76 ± 8% and 2-OH saclofen (100 μM) 68 ± 3% (means ± SEM; n=4-6).

It is concluded that rat dorso-lateral septal neurones express conventional $GABA_B$ receptors, which are functionally involved in inhibitory synaptic transmission. Of the 5 compounds tested, CGP 55845A was the most potent antagonist in these neurones.

We thank Ciba-Geigy for supplying CGP 36742, CGP 55845A & CGP 52432

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306P THE INTERACTION OF LORECLEZOLE WITH THE $GABA_A$ RECEPTOR COMPLEX, AN *IN VIVO* AND *IN VITRO* STUDY

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Loreclezole is an anticonvulsant (Wauquier *et al.*, 1990) and anxiolytic (Dawson *et al.*, 1994) agent which has been reported to potentiate GABA via a novel allosteric site on the β-subunit of the GABA receptor (Wafford *et al.*, 1994). We have now compared the *in vivo* and *in vitro* pharmacology of loreclezole with two other GABA potentiating drugs, pentobarbitone and chlormethiazole.

Loreclezole (i.p.) produced a dose dependent rise in seizure threshold of male Wistar rats (170-220g), measured by the dose of i.v. infused pentylenetretrazole inducing a convulsion (see Green & Murray, 1989). The dose of loreclezole required to raise the threshold by 50% was comparable to that seen with barbiturates and chlormethiazole, but higher than diazepam (diazepam: 1.3, pentobarbitone: 16, chlormethiazole: 22, loreclezole: 25, phenobarbitone: 36, all in mg kg^{-1}; variance <10%). All drugs dose dependently decreased locomotion, loreclezole being the least sedative (dose required to decrease locomotion by 50%: chlormethiazole: 9, pentobarbitone: 16, loreclezole 25; all in mg kg^{-1} (variance <10%).

All three compounds were without effect on [^{3}H]-flunitrazepam binding, except at high concentration (1mM) when, confirming a previous report (Cross *et al.*, 1989), pentobarbitone enhanced binding by approximately 60%, an effect not seen with the other compounds. Both loreclezole (1mM) and chlormethiazole (1mM) partially blocked this enhancement. All three compounds failed to displace [^{3}H]-muscimol binding.

Loreclezole, pentobarbitone and chlormethiazole all fully displaced [^{35}S]-TBPS binding in a cortical membrane preparation (Cross *et al.*, 1989), loreclezole being the most potent compound (IC_{50} values in μM; loreclezole: 4.34 ± 0.68, pentobarbitone: 37.39 ± 3.24, chlormethiazole: 82.10 ± 8.52).

Addition of bicuculline (10 μM) to the medium resulted in a major rightward shift in the loreclezole and pentobarbitone displacement curves and a modest shift of the chlormethiazole curve. Bicuculline thus increased the IC_{50} values for [^{35}S]-TBPS displacement 25 times in the case of loreclezole, 6 times for pentobarbitone and 2.7 times for chlormethiazole. This may indicate a greater involvement of GABA in the interaction of loreclezole with the Cl^- channel than is the case with chlormethiazole.

These data suggest that loreclezole interacts with the GABA receptor ionophore complex in a manner very similar to a barbiturate, and further indicate that anticonvulsant activity does not appear to relate closely to [^{35}S]-TBPS displacement.

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307P INTERACTIONS BETWEEN LORECLAZOLE, CHLORMETHIAZOLE AND PENTOBARBITONE AT $GABA_A$ RECEPTORS IN RAT CUNEATE NUCLEUS *IN VITRO*

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Loreclezole ((Z)-1-[2-chloro-2-(2,4-dichlorophenyl) ethenyl]-1,2,4-triazole), a broad spectrum anticonvulsant, is proposed to mediate its effect through interaction at the $GABA_A$ receptors, although the site of action has not been identified. Recently, it was demonstrated to be highly selective for receptors containing the β_2 or β_3 subunit over those containing the β_1 subunit (Wafford *et al.*, 1994). In the present study, we investigated further the pharmacology of the action of loreclezole on $GABA_A$ receptors by comparing its effect with those of chlormethiazole and pentobarbitone, and investigating the interactions among them.

Experiments were performed on slices of cuneate nucleus prepared from male Wistar rats (100 - 150g) as previously described (Harrison & Simmonds, 1983). Each slice was placed in a two - compartment bath, with the cuneate nucleus in the first compartment and the caudal end of the dorsal funiculus in the other, and superfused with Krebs, which was continuously bubbled with 95% O_2 / 5% CO_2 to give pH 7.4. Only the first compartment was superfused with drugs. In each experiment, a muscimol control curve was first obtained, then one drug was superfused for 30 min before and during the redetermination of responses to muscimol. The same procedure was then carried out with a combination of two drugs. The effects of the drugs were measured as equi-effective muscimol dose - ratios. Chlormethiazole (Astra) (CMZ) and pentobarbitone (Sigma) (PB) were dissolved directly in the Krebs medium. Loreclezole (Janssen) (LOR) was first dissolved in a little acetone and then diluted into the Krebs medium.

CMZ dose-dependently potentiated responses to muscimol on the cuneate nucleus. CMZ, 10, 50 and 200 µM, shifted the dose-response curves of muscimol to the left by 0.156 ± 0.021, 0.339 ± 0.029 and 0.567 ± 0.037 log unit (mean ± s.e.mean, n = 4-10), respectively. LOR, however, showed a rather different profile. At 5, 10 and 20 µM, LOR gave leftward shifts of 0.085 ± 0.043, 0.121 ± 0.037 ($p<0.05$) and 0.115 ± 0.017 ($p<0.01$) log unit (mean ± s.e.mean, n = 4-6, Student's t-test), respectively. At 50 µM, the responses were very variable, ranging from a clear potentiating effect to an antagonizing effect and giving a mean shift of 0.071 ± 0.039 log unit (mean ± s.e.mean n = 22), which was not significant.

A submaximal concentration of PB (30 µM) gave a leftward shift of 0.301 ± 0.032 log unit (mean ± s.e.mean, n = 4), an effect which was unchanged at 0.336 ± 0.053 log unit (mean ± s.e.mean, n = 4) in the presence of 50 µM CMZ. LOR, however, dose-dependently decreased the potentiating effect of PB and also that of CMZ. Thus, in the presence of 10 and 50 µM LOR, CMZ (50 µM) caused significantly smaller leftward shifts of 0.283 ± 0.032 and 0.159 ± 0.028 log unit, respectively (mean ± s.e.mean, n=10, $p<0.0005$ one-way ANOVA), compared with 0.339 ± 0.029 in the absence of LOR. Similarly, in the presence of 20 and 50 µM LOR, PB (30µM) caused significantly smaller shifts to the left of 0.155 ± 0.035 and 0.091 ± 0.037 log unit, respectively (mean ± s.e.mean, n = 4, $p<0.01$ one-way ANOVA), compared with 0.301 ± 0.032 in the absence of LOR.

The attenuation of the effects of CMZ and PB by the weaker potentiator LOR could be due to competition for a common site of action, although the lack of interaction between PB and CMZ would suggest that they do not share a common site. Alternatively, LOR might depress responses to muscimol, e.g. by desensitization, as well as potentiate them, thereby limiting its own potentiating action and attenuating the potentiations by PB and CMZ.

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308P β-CARBOLINES MODULATE $GABA_A$ RECEPTORS VIA THE LORECLEZOLE SITE AS WELL AS THE BENZODIAZEPINE SITE

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The benzodiazepine site on the $GABA_A$ receptor is the principle site of action for a number of different structurally diverse compounds including the β-carbolines, many of which bind with high affinity. Several electrophysiological studies have revealed the apparent reversal of inhibition and potentiation by high concentrations of DMCM and other β-carbolines. This potentiation is not dependent on the presence of a γ2 subunit and is insensitive to the benzodiazepine antagonist Ro15-1788 (Im *et al.*, 1994).

We have studied this potentiation with β-carbolines on human $GABA_A$ receptors expressed in *Xenopus* oocytes, using an electrophysiological analysis of currents recorded by two-electrode voltage clamp. Using α6 containing receptors which have low affinity for benzodiazepines we observed a large potentiation of EC_{20} GABA responses by a 10µM concentration of DMCM (146±21% on α6β3γ2 and 112±19% on α6β2γ2). Potentiation was entirely dependent on the β-subunit variant, only present on β2 and β3 containing receptors (-19±7.9% on α6β1γ2). The similar β-subunit dependent potentiation by the anticonvulsant loreclezole is determined by a single amino acid in the putative TM2 region (Wingrove *et al.*, 1994). By using single point mutant β-subunits which discriminate the loreclezole site, we have shown that the potentiation by DMCM is dependent on the presence of the same amino acid, Asn290, in β2 or β3 (Ser in β1), suggesting that the low affinity site for β-carboline potentiation is the loreclezole site. Using α1βγ2 receptors, the potentiation was still present at 10µM, but was more pronounced on α6-containing receptors due to the low affinity of DMCM for the α6 receptor subtype benzodiazepine site.

In addition to the β-subunit dependence, the potentiation by 100µM DMCM was competitive with that of loreclezole (196±33% with both compounds, compared to 205±19% with 10µM loreclezole alone). Other β-carbolines, such as β-CCE and β-CCP behaved in a similar manner, potentiating α6β3γ2 receptors at micromolar concentrations. The finding that β-carbolines can act via the loreclezole site as well as the benzodiazepine site suggests that a wider variety of compounds may act at this site, and demonstrates that compounds can interact with more than one modulatory site on the $GABA_A$ receptor.

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309P IDENTIFICATION OF A SINGLE AMINO ACID ON THE γ SUBUNIT OF THE GABA$_A$ RECEPTOR REQUIRED FOR HIGH AFFINITY BINDING OF [^{3}H]Ro15-1788

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It is known that both an α and a γ subunit are required for the formation of a benzodiazepine binding site on the GABA-A receptor. The benzodiazepine pharmacology is dependent on the combination of the subunit isoforms (α1-α6 and γ1-γ3) which are present in the receptor complex (Whiting *et al.*, in press). GABA-A receptors containing a γ2 (or γ3) subunit, but not those containing a γ1 subunit, have high affinity binding for the benzodiazepine antagonist [^{3}H] Ro15-1788 when expressed transiently in HEK293 cells (Whiting *et al.*, in press). This was the criteria used to identify the residue in the γ2 subunit which confers high affinity binding of this ligand.

A series of six chimeric γ1/γ2 subunits were constructed of which two were able to form a high affinity binding site for [^{3}H] Ro15-1788 when co-expressed in HEK293 cells with α1 and β1. Both of these chimeras (γ1Δ2.2 and γ1Δ2.6) contain residues 72 to 198 of the γ2 subunit. Since γ2 and γ3 subunits both confer high affinity benzodiazepine binding, it was hypothesized that the critical residue would be common to these two subunits but different from that at the homologous position of γ1. Alignment of the deduced amino acid sequences of the γ subunits reveals six such positions. The γ1 subunit was mutated to incorporate each of these changes individually and each mutant was co-expressed with α1 and β1 subunits by transient transfection in HEK293 cells. Only one mutant subunit - γ1ΔIle114Phe - was able to confer binding of [^{3}H] Ro15-1788.

Saturation curves for the binding of [^{3}H] Ro15-1788 were constructed for recombinant receptors containing α1 and β1 and either γ2 or γ1ΔIle114Phe and the K_d values calculated by Scatchard analysis to be 0.91 ± 0.22 nM and 3.13 ± 0.20 nM respectively (mean ± s.e.mean). The Ile at position 114 of the γ1 subunit was also mutated to an Asp, Glu, His, Tyr or Trp residue to assess the nature of the interaction between the receptor and ligand. Only the Tyr mutant - γ1ΔIle114Tyr - had a high affinity binding site for [^{3}H] Ro15-1788 (K_d=5.56 ± 2.20 nM).

Thus Phe116 is necessary and sufficient for the high affinity binding of [^{3}H] Ro15-1788 to the GABA-A receptor. Tyr can also substitute for Phe, suggesting that the benzene ring of these residues is the site of interaction with the benzodiazepine. It is of interest to note that Phe116 is homologous to the Phe at position 92 of the α1 subunit which has been implicated as a critical residue at the GABA binding site (Sigel *et al.*,1992), suggesting some structural homology between binding sites for different ligands.

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310P CLONING OF cDNA ENCODING THE α4 SUBUNIT OF THE HUMAN γ-AMINOBUTYRIC ACID TYPE A RECEPTOR AND CHARACTERISATION OF THE PHARMACOLOGY OF α4-CONTAINING RECEPTORS

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We have cloned and sequenced a cDNA encoding the α4 subunit of the human γ-aminobutyric acid type A ($GABA_A$) receptor. Recombinant human α4β1γ2s $GABA_A$ receptors have been expressed in *Xenopus* oocytes and the pharmacology of these receptors investigated using the two-electrode voltage clamp method.

Concentration-response curves to GABA were constructed and revealed an EC_{50} of 33.1 ± 3.1 μM (n=5) with a Hill coefficient of 1.29 ± 0.05. This EC_{50} is similar to that obtained for α1β1γ2s (25 ± 4 μM) while lower than that obtained for α6β1γ2s (14.4 ± 1.4 μM). EC_{20} responses to GABA on human α4β1γ2s receptors were sensitive to the competitive antagonists, bicuculline (100 μM) and SR95531 (1 μM), and the non-competitive antagonist picrotoxin (100 μM).

A number of different compounds, which act via the benzodiazepine site, were examined at a concentration of 1 μM. The effects of these compounds on α4β1γ2s when compared to α1β2γ2s and α6β2γ2s revealed a unique profile (Table 1). GABA responses (EC_{20}) were potentiated by the steroid, 5α-pregnan-3α-ol-20-one, (367 ± 26%). This potentiation was not significantly different to that obtained on α1β2γ2s (298 ± 60%) or α6β2γ2 (312 ± 79%). Marked potentiation of the GABA response (706 ± 82%) was observed with pentobarbital (100 μM). This compared to 288 ± 32% on α1β1γ2s and 719 ± 52% on α6β1γ2s. The direct activation by pentobarbital on α4β1γ2s receptors was extremely small (6% of maximum GABA at 1 mM) compared to 31% and 147% on α1β1γ2s and α6β1γ2s respectively.
These experiments reveal a unique pharmacology for α4 containing receptors with similarities to both α6 and α1.

Table 1. Percent modulation of control GABA EC_{20} currents by benzodiazepine ligands.

	α4β1γ2s	α1β2γ2s	α6β2γ2s
Flunitrazepam	-1 ± 1.2	117 ± 7.3	5.5 ± 2.7
CL 218872	0.8 ± 1.9	64 ± 8.6	6 ± 1.2
Zolpidem	-4.8 ± 1.1	159 ± 30	1 ± 1.7
Ro15-4513	52.8 ± 3.5	-8.5 ± 1.1	45 ± 5
Ro15-1788	37.8 ± 2.3	7 ± 9	33.3 ± 7
Bretazanil	78.2 ± 6.7	29.3 ± 3.6	50.5 ± 7.9
FG 8205	51.3 ± 5.5	54 ± 9	88 ± 13
DMCM	-25.8 ± 4.1	-39 ± 3.9	34 ± 8.6
Abecarnil	7.8 ± 2.1	74 ± 20	86 ± 10

Values shown are the mean ± s.e.mean, n=3 or more oocytes.

311P EFFECT OF STRIATAL FLUORO-GOLD INJECTION IN THE RAT NEONATE ON DOPAMINE RELEASE AND AUTORECEPTOR FUNCTION

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Fluoro-Gold (FG) is a fluorescent retrograde axonal tracer, which lables dopamine (DA) islands that form striosomes in the adult rat, when injected into the striatum of the neonatal rat (Snyder-Keller, 1988). When injected at high concentrations (5-10% w/v) FG causes tissue necrosis, possibly making it unsuitable for functional studies (Schmeud & Fallon, 1986). *In vitro* use of FG has been shown to inhibit vesicular DA release (Bowyer *et al.*, 1993). The aim of this study was to establish whether FG is suitable as a marker for striosomes in functional studies, by investigating its effects on DA neurotransmission in the rat caudate putamen (CPu).

FG (1μl, 2% w/v in 0.9% w/v NaCl) was injected into the right dorsal CPu of Wistar pups at post natal day 4. The non-injected contalateral side served as an internal control. Coronal brain slices (350μm thick) containing both the FG and control striatal hemispheres were prepared after week 6. Electrically stimulated DA efflux was measured by fast cyclic voltammetry as described by Patel *et al.*, 1992. DA efflux was evoked by single pulse (1P) stimulation (20V,0.1ms), or trains of 20 pulses applied at 50Hz (20P/50Hz), at 6 sites in the FG injected CPu and at symmetrical sites in the control CPu. Students t-tests were used for statistical comparisons.

In the FG injected CPu there were 10 out of 36 occasions when it was not possible to detect evoked DA efflux but only 1 such occasion out of 39 in the control CPu. On occasions when DA efflux could be detected, there were no significant differences ($P > 0.05$) in the amount of DA released from each recording site between the FG and control CPu following either 1P or 20P/50Hz stimulation. In addition, no significant differences ($P > 0.05$) were found in the mean amount of DA released (pooled from all sites) following 1P stimulation (control: 39.08 $\pm$ 4.36nM, n = 38; FG: 34.75 $\pm$ 8.51nM, n = 26) or 20P/50Hz (control: 69.13 $\pm$ 11.24nM, n = 38; FG: 72.42 $\pm$ 17.11nM, n = 26).

Quinpirole (1nM - 1μM) was significantly less potent at inhibiting 1P stimulated DA efflux in FG injected CPu compared to control (EC_{50} values with 95% confidence limits were FG: 40.16nM (21.01 - 75.40), n = 5; control: 16.22nM (7.53 - 34.92), n = 5; $P < 0.05$. There was no significant reduction ($P > 0.05$) in the maximum inhibition achieved (93.18 $\pm$ 3.20% and 92.90 $\pm$ 3.74% for control and FG CPu respectively, n = 5). (-)Sulpiride (10 - 300nM) was less effective at displacing concentration-response curves to quinpirole in the FG injected CPu (pA_2: 8.83 and 7.42 for control and FG respectively).

These findings indicate that FG may interfere with DA autoreceptor function but the mechanism is unclear. Therefore, FG may not be a suitable inert fluorescent marker of striosomal compartments in functional studies.

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312P FLUOROGOLD AND DOPAMINERGIC FUNCTION IN THE RAT CAUDATE PUTAMEN

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Fluorogold (FG), is used extensively as a retrograde tracer in anatomical studies of the central nervous system and in particular the developement of striosomes in the striatum (Snyder-Keller, 1988). A recent study has shown that *in vitro,* superfusion of brain slices with FG inhibited the release of dopamine (DA) evoked by K^+ or methamphetamine (Bowyer *et al.*1993). In this study we investigated whether a single injection of FG into the caudate putamen (CPu) of a neonate rat altered dopaminergic neurotransmission in the CPu with respect to neuronal uptake function.

Male Wistar rats (4 days old) were injected with 1μl of a 2% solution of FG into the right CPu. The contralateral side was used as the control. Animals were killed 6 -12 weeks later and brain slices containing both the FG injected and non-injected CPu were prepared. The effect of cocaine on DA overflow evoked by a single pulse (1p: 0.1ms, 20V) and 20 pulses at 20Hz (20p/20Hz) was measured using fast cyclic voltammetry in the ventro - lateral quadrant of the CPu (Wieczorek & Kruk, 1994).

The extracellular concentration of DA evoked by 1p or 20p/20Hz was not significantly different between control (39.2$\pm$7, n=9; 69$\pm$18 nM, n=4, respectively) and FG treated slices (49$\pm$12 nM, n=9; 62.3 $\pm$ 14 nM, n=4; respectively).

Superfusion of the brain slice with increasing concentrations of cocaine (30nM - 3μM) produced a concentration - dependent potentiation of DA overflow evoked by either 1p or 20p/20Hz. In control slices the maximum potentiation by cocaine(1μM), of DA overflow evoked by 1p (256 $\pm$ 21%, n=4) or 20p/20Hz (320 $\pm$63%, n=4) was significantly lower than that produced by cocaine (3μM) in FG treated slices (1p: 478 $\pm$ 98%; 20p/20Hz: 682 $\pm$ 116%, P<0.05, n=4).

The EC_{50} values for cocaine (the concentration required to potentiate DA overflow by 50% of maximum response) were significantly lower in control slices (1p: 1.02 $\pm$ 0.3 μM; 20p/20Hz : 1.12 $\pm$ 0.4 μM, n=4) than in FG slices (1p: 2.9 $\pm$ 0.9μM; 20p/20Hz:: 3.6 $\pm$ 1.1 μM, n=4; P<0.02).

The results from this study appear to indicate that a single injection of FG in neonate rats can alter the functioning of the dopaminergic neuronal uptake system. Whether this is due to desensitization of or the reduction in the number of transporter sites is not known.

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313P MODULATION OF 5-HT$_3$ RECEPTOR-EVOKED CURRENTS BY L-TYPE Ca^{2+} CHANNEL ANTAGONISTS

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We have previously shown that Ca^{2+} signals evoked by activation of 5-HT$_3$ receptors in HEK 293 cells transfected with the 5-HT$_3$R-As subunit (HEK/5-HT$_3$R-As cells) (Hargreaves *et al.*, 1994) were inhibited by the L-type Ca^{2+} channel antagonists, (+)verapamil, (-)verapamil, diltiazem and nimodipine. This inhibition was independent of blockade of L-type Ca^{2+} channels (Hargreaves *et al.*, 1995). (+)Verapamil, (-)verapamil and diltiazem, but not nimodipine, also inhibited binding of the 5-HT$_3$ receptor agonist, [^{3}H]m-chlorophenyl biguanide, to membranes prepared from Sf9 cells overexpressing the 5-HT$_3$R-As receptor (Hargreaves *et al.*, 1995).

We have used whole-cell voltage-clamp to determine the effects of L-type Ca^{2+} channel antagonists on the currents evoked by activation of 5-HT$_3$ receptors. Application of 50 μM 5-HT to HEK/5-HT$_3$R-As cells, held at -60 mV, evoked an inward current that decayed monoexponentially during continued application of 5-HT. Mean peak current (I_p) evoked by 50 μM 5-HT was 651 ± 101 pA and the mean current decay time constant (τ) was 8.03 ± 0.88 s (n = 15). Co-application of 5-HT and (+)verapamil (1-10 μM) caused concentration-dependent increases in the rate of current decay with little effect on I_p. At 10 μM (+)verapamil, τ was reduced to 17 ± 2 % (n = 3) of that obtained in the absence of (+)verapamil. Similar results were obtained with (-)verapamil and nimodipine. The effect of (+)verapamil on 5-HT$_3$ receptor currents was similar when cells were held at +50 mV, indicating that the blockade was not voltage-dependent.

Application of 5-hydroxyindole (1 mM) (Kooyman *et al.*, 1994) to cells held at -60 mV significantly reduced the rate of decay of currents evoked by 50 μM 5-HT (τ = 13.0 ± 2.4 s and 36.3 ± 3.0 s in the absence and presence of 5-hydroxyindole, respectively; n = 3). However, application of 10 μM (+)verapamil reduced the τ of 5-HT-evoked currents to a similar degree irrespective of the presence of 5-hydroxyindole (τ = 1.86 ± 0.17 s and 2.42 ± 0.27 s in the absence and presence of 5-hydroxyindole, respectively; n = 3).

We conclude that some L-type Ca^{2+} channel antagonists can directly inhibit 5-HT$_3$ receptor function by increasing the rate at which the 5-HT$_3$ receptor evoked current decays. These results and our earlier data, demonstrating inhibition of radioligand binding to 5-HT$_3$ receptors by verapamil, suggest that L-type Ca^{2+} channel antagonists may act at a novel modulatory site on the 5-HT$_3$ receptor.

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314P NALOXONE AND DESAMINOYFLFQPQRAMIDE BLOCK POTENTIATION BY FLFQPQRFAMIDE (NPFF) OF THE RAT ISOLATED SPINAL CORD MONOSYNAPTIC REFLEX (MSR)

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RFamides, including NPFF have been isolated from bovine brain extracts (Yang *et al*, 1985) and NPFF binding sites are located postsynaptically in the rat spinal cord (Lombard *et al*, 1995). In contrast μ opioid receptors are preferentially located presynaptically on primary afferent fibres. In a previous study, NPFF was shown to potentiate the amplitude of the MSR of the isolated rat spinal cord preparation (Huang *et al*, 1996). In the present study the rat isolated spinal cord preparation was used to examine the effect of pretreatment with naloxone and desaminoYFLFQPQRamide on NPFF-induced potentiation of the MSR. DesaminoYFLFQPQR-amide attenuates morphine tolerance in the rat and may be a useful tool in studying the role of NPFF in nociception (Lake *et al*, 1992).

The dissection and recording methods have previously been described (Bagust *et al*, 1985). However in the current experiments a suction electrode was used to record MSR activity in response to single stimuli (0.5msec, 5 times threshold, 0.033Hz) and the saline was magnesium-free to prevent blockade of NMDA receptors. Pretreatment with 1μM naloxone decreased the MSR amplitude to 87.96%±3.76% (mean±s.e.mean, n=5) compared with control. Following naloxone pretreatment, NPFF was perfused in sequence from 10nM to 10μM to potentiate the MSR. However this potentiation was significantly (student's t-test) reduced at NPFF concentrations of 100nM and 1μM, such that control values of 116.89%±5.14% (n=7) and 123.99%±5.65% (n=7) respectively were reduced to 92.08%±4.54% (n=5) and 96.02%±5.55% (n=5) respectively, $p<0.01$. Desamino-YFLFQPQRamide was applied in the same manner and slightly reduced the MSR to 93.72%±2.64% (n=5) at 1μM. This peptide also reduced NPFF-induced potentiation. At 100nM and 1μM NPFF, the increase in MSR amplitude was reduced, such that control values of 116.89%±5.14% (n=7) and 123.99%±5.65% (n=7) respectively were reduced to 85.94%±11.21% (n=5) and 88.58%±12.52% (n=5) respectively, $p<0.05$.

The blockade of the NPFF response by naloxone may suggest an indirect interaction between opioid and NPFF receptors since they are unlikely to share a common receptor (Allard *et al*, 1989). The blockade by desaminoYFLFQPQRamide suggests a possible interaction between it and NPFF at NPFF receptors though its ability to bind to NPFF receptors has yet to be demonstrated. Since desaminoYFLFQPQRamide has a slight direct effect on the MSR, it could be competing with endogenous NPFF in the cord. DesaminoYFLFQPQRamide may be of value in investigating the role of NPFF in nociception.

Note: F, Phenylalanine; L, Leucine; P, Proline; Q, Glutamine; R, Arginine; Y, Tyrosine.

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315P INHIBITION OF WHOLE-CELL CALCIUM CHANNEL CURRENTS IN RAT CEREBELLAR GRANULE NEURONES BY NICARDIPINE AND THE CONUS PEPTIDE ω-CONOTOXIN-MVIIC

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Cerebellar granule neurones are the most abundant cell type in the mammalian central nervous system. They receive inputs from mossy fibres and give rise to parallel fibres which synapse onto the dendrites of Purkinje cells having a summating influence on excitability of the Purkinje cell. With synaptic transmission being intimately related to calcium entry, the wide range of calcium channel types present in the granule neurone provide us with a rich area of investigation. Several groups (De Ward *et al.*, 1991;Randall and Tsien, 1995.) have worked to characterise the different types of calcium channel present in these neurones but a certain degree of overlap exists in their biophysical and pharmacological properties leading to difficulties in the interpretation of the findings.(Pearson et al., 1995). The contribution of calcium channel types to the whole-cell calcium current in cerebellar granule neurones is still an area requiring investigation. In addition to the dihydropyridine-sensitive L-type, the ω-conotoxin GVIA-sensitive N-type, and the ω-Agatoxin IVA-sensitive P-type channels a fourth component termed Q-type has been characterised on the basis of its sensitivity to the *Conus magus* toxin ω-conotoxin-MVIIC (Randall and Tsien, 1995). Here we investigate the effects of ω-CTx-MVIIC and the dihydropyridine nicardipine on calcium currents.

Whole-cell calcium currents were recorded from granule cells cultured for 8-12 days from 6 day-old post-natal rats similar to the methods of Huston *et al.*, 1993. In recording solutions designed to minimise sodium, potassium and chloride conductances, and using 10mM barium as the charge carrier an inward current activated at -30mV, reached a peak at +10mV and reversed at +55mV.

ω-CTx MVIIC (5μM) inhibited calcium channel currents at a test potential of +10mV by 18.8±3.0% (mean±sem, n=9, $p<0.005$ paired *t* test). Over a range of concentrations the EC_{50} was calculated to be 0.62μM. Nicardipine applied at 1μM caused an inhibition of 16.1± 6.7% (n=6, $p<0.05$). When nicardipine was applied following inhibition by ω-CTx-MVIIC a further inhibition of 20.2±4.5% (n=9, $p<0.01$) was observed. This figure expressed as a percentage of the total calcium current before block by ω-CTx-MVIIC was found to be 16.5±3.6%, thus very similar to inhibition by nicardipine alone, and therefore it could be concluded that the proportion of the whole-cell calcium current blocked by nicardipine was independent of the prior application of ω-CTx-MVIIC. However, higher concentrations of nicardipine produced a greater degree of inhibition. The EC_{50} was 4.4μM, and 100μM nicardipine produced >60% inhibition of the calcium current (n=4). Further experiments are in progress to examine whether the additional block produced by concentrations of nicardipine greater than 1μM remains selective for L-type calcium channels.

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316P EVIDENCE THAT CANNABINOIDS HAVE NO EFFECT ON ACETYLCHOLINE RELEASE IN THE RAT CEREBRAL CORTEX

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Cannabinoids have a wide range of central actions, but their action at a cellular level remains unclear. Cannabinoid receptors may be negatively linked to adenylyl cyclase and an influence over calcium channels has also been demonstrated (Howlett, 1995). In comparison to other agents which show this profile, it might be predicted that cannabinoids should have an influence on neurotransmitter release. Therefore in the present study the effects of two cannabinoid agents on acetylcholine (ACh) release were investigated using two methods. The first involved indirect measurement of ACh release by monitoring phosphoinositide (PI) hydrolysis following stimulation with KCl in the presence of physostigmine (Kendall & Nahorski, 1987). The second method involved direct measurement of ACh overflow following KCl stimulation of [^{3}H]choline pre-labelled cortical slices using a suprafusion apparatus. Agonists and antagonists of other receptors coupled negatively to adenylyl cyclase were also tested.

Depolarization-evoked ACh release was examined by monitoring PI hydrolysis in rat cross-chopped cortical slices (male, Wistar, 160-400g) using a [^{3}H]myo-inositol pre-labelling assay of total [^{3}H]inositol phosphates accumulation in the presence of lithium (Kendall & Nahorski, 1987). The release of ACh was stimulated by KCl (18mM) in the presence of physostigmine (50μM). Using a superfusion apparatus [^{3}H]choline pre-labelled rat cortical slices underwent two periods of KCl stimulation (18mM) in the presence and absence of atropine (1μM) and the results are expressed as a ratio of the two stimulations (S2/S1).

High KCl (18mM) in the presence of physostigmine (50μM) stimulated [^{3}H]inositol phosphates accumulation compared to basal conditions (KCl 5.5mM) (basal dpm 1359±104, high KCl dpm 8433±696; p< 0.01 Students t-test; n=4). The cannabinoid receptor agonist, CP 55,940 (1μM) (Howlett, 1995), and the cannabinoid receptor antagonist, SR 141716 (1μM) (Howlett, 1995), had no effect on the inositol phosphates accumulation stimulated by high KCl in combination with physostigmine (109 ±12, 121±30 % control, respectively). The A1 adenosine receptor agonist, N6-cyclopentyladenosine (0.1μM) and the antagonist, 8-cyclopentyl-1,3-dipropylxanthine (50nM), the α_2-adrenoceptor agonist, 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14304) (10μM) and antagonist, idazoxan (1μM) and the opioid agonist, morphine (10μM) and antagonist, naloxone (10μM) had no effect on depolarization-evoked inositol phosphate accumulation (101±9, 102 ±12, 97±8, 99±5, 106 ±10, 114±20 % control, respectively ;p> 0.05 Students t-test; n=3).

To determine whether depolarization-elicited ACh overflow produced a saturation of muscarinic autoreceptors the effects of atropine on the release of [^{3}H] from [^{3}H]choline-pre-labelled slices was examined. There was no effect of the antagonist (S2/S1 control 58±2, atropine 66±4, n=3).

These data indicate that endogenous ACh release in the rat cerebral cortex is not modulated by cannabinoid, opioid, A1 adenosine or α_2- adrenoceptors.

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317P NICOTINE-INDUCED LONG-LASTING POTENTIATION IN THE RAT DENTATE GYRUS

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Acute systemic injections of nicotine have been shown to increase hippocampal noradrenaline release and the expression of tyrosine hydroxylase in the locus coeruleus (Mitchell, 1993). A challenge injection of nicotine given 4 weeks later, after sufficient time has elapsed for the increased enzyme activity to reach the hippocampus and increase hippocampal noradrenaline synthesis, resulted in an enhanced release of hippocampal noradrenaline (Mitchell et al., 1993). Within the hippocampus, iontophoretic application of noradrenaline has been shown to produce a prolonged increase in the excitatory post-synaptic potential (EPSP) evoked by medial perforant path (MPP) stimulation which could be prevented by β-adrenoceptor blockade (Dahl and Sarvey, 1989). This long-lasting potentiation (LLP) resembles the long-term potentiation (LTP) evoked by brief, high-frequency electrical stimulation and may be a mechanism underlying the cognitive enhancing effects of nicotine (Warburton, 1992). The aim of this *in vivo* study was to determine whether a challenge injection of nicotine would produce LLP in nicotine-primed rats, via a β-adrenoceptor-sensitive mechanism.

Male Sprague-Dawley rats (250-350g) were given (-)-nicotine bitartrate (0.8 mg free-base kg^{-1} s.c., pH 7.4) or saline (1ml kg^{-1} of a 0.9% w/v NaCl solution) daily for 7 consecutive days commencing 4 weeks before experiment. On the day of the experiment, the rats were anaesthetized with urethane (1.5 g kg^{-1}) placed in a stereotaxic frame and a bipolar stimulating electrode (pair of twisted 100 μm stainless steel wires insulated except for the tip) placed in the MPP (AP - 6.5 mm from bregma; L ± 4.5 mm from the midline; H 3.0 mm from the surface of the skull; angled to obtain a response exhibiting paired-pulse depression at inter-pulse intervals of 200 and 400 ms) and a similar electrode placed in the dentate gyrus (AP - 3.8 mm from bregma, L ± 2.0 mm from the midline, H 3.7 mm below the skull surface, Paxinos and Watson, 1986) to record field potentials; current intensity was adjusted to evoke half-maximal responses. When responses had stabilized (at least 30 min), a challenge injection of either nicotine (0.4 mg kg^{-1}, s.c.) or saline was given (0.5 ml kg^{-1}, s.c.) and the change in EPSP slope recorded as a change from baseline. In some nicotine-primed rats, propranolol hydrochloride (15 mg kg^{-1}, i.m.) was given either 30 min before acute nicotine challenge, or 90 min after acute challenge.

In nicotine-primed rats (n = 6), a challenge injection of nicotine gradually increased the slope of MPP-evoked EPSPs in the dentate gyrus to approximately 50 % above baseline 90 min after injection. This potentiation persisted for at least 140 min (ANOVA, $P < 0.001$, compared to saline-primed rats given saline, n = 5) and was not seen in saline-primed rats given nicotine. The β-adrenoceptor antagonist, propranolol, given 30 min before nicotine to nicotine-primed rats (n = 5), did not affect pre-injection baseline EPSPs but prevented induction of LLP ($P < 0.0001$ compared to nicotine-primed rats given nicotine) confirming involvement of β-adrenoceptors in nicotine-induced LLP in the rat dentate gyrus. However, propranolol did not attenuate nicotine-induced LLP once it had been established (n=5), indicating that its maintenance did not involve β-adrenoceptors.

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318P MODULATION BY OESTROGEN AND PROGESTERONE OF THE RESPONSE TO STIMULATION OF DOPAMINE RECEPTORS IN THE ENTORHINAL CORTEX ON DOPAMINE EFFLUX IN THE NUCLEUS ACCUMBENS OF OVX RATS

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Dopaminergic dysfunction arising from an abrupt fall in oestrogen and/or progesterone, has been implicated in post-partum affective disorders, onset of which may be predicted by an enhanced growth hormone response to apomorphine (Wieck et al., 1991). In a previous study (Takada et al., 1994), focal injections of apomorphine into the entorhinal cortex of conscious ovariectomised (OVX) rats were shown to reduce dopamine release in the nucleus accumbens of ovariectomized (OVX) rats. The peak reduction, 15 %, occurred 40 min after injection with recovery at 2 h and the effect was doubled by oestrogen and progesterone priming. Both these sites express oestrogen and dopamine receptors (Loy et al., 1988; Mansour et al., 1990) and are constituents of a cortico-striatal loop in which dysfunctions may lead to affective and cognitive symptoms (Alexander et al., 1986). This study describes the individual roles played by the two hormones in modulating the response to apomorphine and the effects of the hormone combination on responses to selective D1 and D2 receptor agonists.

Female Sprague-Dawley rats (250 g) were anaesthetized, ovariectomized and guide cannulae positioned into the entorhinal cortex and nucleus accumbens of the right hemisphere. Dialysis experiments were performed using a cross-over design, in hormone-primed (17-β-oestradiol, 10 μg s.c. in sesame oil 72 h and 48 h before experiment and/or progesterone, 500 μg s.c. 5 h before experiment) and control (sesame oil) rats at an interval of 1 week. Prior to experiment, a dialysis probe was positioned in the nucleus accumbens and connected to an HPLC system (Eicom, Kyoto) with an Eicompak CA-5ODS column for dopamine separation.

Oestrogen priming alone did not significantly affect the response to apomorphine (10 μg in 0.5 μl) injected into the entorhinal cortex via the guide cannula (n = 8) whereas in rats primed with progesterone only, the reduction in dopamine efflux during the 3 h after apomorphine injection was significantly greater than in rats treated with sesame oil ($P < 0.003$) but was less than that obtained in rats primed with both hormones (Takada et al., 1994). Intra-entorhinal injections of the D2 receptor agonist, quinpirole (10 μg in 0.5 μl), did not alter nucleus accumbens dopamine efflux either in control rats or in rats primed with both hormones. In contrast, injections of the D1 receptor agonist, SKF38393 (10 μg in 0.5 μl), into the same site of control rats increased the amount of dopamine in the dialysates of control OVX rats by approximately 23 % at 40 min, an effect which was completely abolished in rats primed with both hormones ($P<0.0001$, n=12).

Priming OVX rats with oestrogen and progesterone potentiated the reduction in dopamine efflux in the nucleus accumbens caused by injection of apomorphine into the entorhinal cortex (Takada et al., 1994). This priming effect required the presence of both hormones; oestrogen alone was ineffective and progesterone exerted only a small effect. Selective D1 receptor stimulation increased dopamine release in the accumbens and D2 receptor stimulation lacked effect suggesting a permissive role of D1 receptor stimulation. Thus dopaminergic modulation of a cortico-accumbens pathway, dysfunction of which could give rise to post-partum affective disorders is itself modulated by oestrogen and progesterone.

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319P NOVEL PHARMACOLOGICAL CHARACTERISTICS OF P_2 PURINOCEPTORS MEDIATING INCREASES IN FIRING RATE OF RAT LOCUS COERULEUS NEURONES *IN VITRO*

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The purine nucleotide, αβ-methylene ADP (αβmeADP), potently inhibits 5'-nucleotidase, the enzyme responsible for breakdown of AMP to adenosine (see Michel *et al.*, 1995). In addition, αβmeADP exhibits high affinity for [^{3}H]-αβ-methylene ATP (αβmeATP) binding sites in the rat brain, sites which may represent binding to P_{2X} purinoceptors (Michel *et al.*, 1994). However, in view of the known weak activity of αβmeADP at peripheral P_{2X} purinoceptors this high affinity for sites in the brain raises the possibility that [^{3}H]-αβmeATP instead labels a brain ectonucleotidase. To help address this issue, we have looked for evidence for functional responses to αβmeADP in the rat locus coeruleus (LC), a brain area in which excitatory effects of purines mediated via P_2 purinoceptors have been described previously (Tschöpl *et al.*, 1992).

Extracellular recording of neuronal activity in the rat LC was performed using standard techniques. Pontine slices containing the LC were prepared from male AHA rats (150-200g) and placed in a perspex chamber and superfused with artificial CSF at 32°C. Unit activity was recorded using carbon-fibre electrodes, and spikes were discriminated using Spike 2 software (Cambridge Electronic Design). Typically, LC neurones exhibited regular biphasic action potentials of 3-4ms duration (mean basal rates 1.06±0.04Hz (n=25)), and firing could be reversibly inhibited by noradrenaline (30μM). Addition of single concentrations of either αβmeADP (1-300μM) or αβmeATP (1-300μM) for periods of 4-5min at 10min intervals evoked sustained, concentration-related increases in firing rate. For each nucleotide, the threshold concentration for this action was 1-3μM, but no clear maxima for the concentration-effect (c-e) curve could be obtained even at concentrations up to 300μM. Peak mean increases in firing rate amounted to 1.66±0.13Hz (n=14) and 1.68 ±0.20Hz (n=11) for αβmeADP and αβmeATP, respectively. Consecutive c-e curves to both agonists were highly reproducible (respective 1st and 2nd c-e curve peak increases were 1.57±0.33 and 1.39±0.19Hz for αβmeADP, and 1.44±0.35 and 1.50±0.11Hz for αβmeATP (n=4)). Responses to αβmeADP and αβmeATP were both attenuated by the P_2 purinoceptor antagonist suramin (100μM for 30min; αβmeADP control 2.09±0.27 vs treated 0.43±0.13Hz (n=3), αβmeATP control 2.22± 0.29 vs treated 0.33±0.08Hz (n=4); each $P<0.05$, Student's paired *t*-test). Pyridoxal-5-phosphate (P-5-P, 100μM) produced only modest inhibition of these responses (αβmeADP control 1.72±0.11 vs treated 1.42±0.13Hz (n=3), αβmeATP control 1.25±0.12 vs treated 0.81±0.14Hz (n=3)). The adenosine receptor antagonist DCPCX (0.1μM) did not modify responses to αβmeADP (control 1.39±0.17 vs treated 1.21±0.24Hz (n=4)). Of the antagonists, only suramin significantly reduced basal firing rate (control 1.09±0.05Hz vs treated 0.82±0.07Hz; n=7).

These preliminary studies show that αβmeADP, like αβmeATP, increases firing rate in rat LC neurones. The resistance of these responses to antagonism by DCPCX suggests that the effects of αβmeADP are not mediated via inhibition of 5'-nucleotidase, thereby reducing endogenous adenosine levels. Rather, it appears that αβmeADP activates P_2 purinoceptors that are sensitive to suramin, but only weakly antagonised by P-5-P. These unusual pharmacological characteristics suggest that the purinoceptors on rat LC neurones are different from P_2 purinoceptors found elsewhere (but see Bailey & Hourani, 1992). The sites labelled by [^{3}H]-αβmeATP in the rat brain could represent binding to this putative novel receptor, although further studies are required to confirm this.

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320P AUTORADIOGRAPHICAL STUDIES WITH THE SOMATOSTATIN sst_2-RECEPTOR-SELECTIVE NOVEL RADIOLIGAND [^{125}I]-BIM-23027 IN RAT BRAIN

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Somatostatin (SRIF) is thought to act as a neurotransmitter within the central nervous system (Epelbaum *et al.*, 1994). There are five known recombinant somatostatin receptor types (sst_1 - sst_5), however, more detailed study of the pharmacological properties and functional significance of these has been hampered until recently by the lack of selective ligands. BIM-23027 has been shown to be a potent and selective agonist for the recombinant sst_2 receptor type (Raynor *et al.*, 1993; Castro *et al.*, 1994). The purpose of this study was to examine the distribution of the SRIF-sensitive BIM-23027 binding sites in the rat brain using the novel radioligand [^{125}I]-BIM-23027 (Holloway *et al.*, 1995) which was custom synthesised at Amersham International U.K.

Coronal sections (20μm) of frozen rat brain (male Sprague-Dawley, 170 - 220g; n=5) were thaw-mounted onto gelatin-subbed slides and pre-incubated in 50mM TRIS HCl buffer containing 5mM $MgCl_2$, pH 7.4 at 21°C for 60min, then incubated in buffer containing leupeptin (10μg/ml), soyabean trypsin inhibitor (1μg/ml) and bacitracin (0.2mg/ml) for 60min at 21°C with ~ 0.03nM [^{125}I]-BIM-23027 (specific activity 2000Ci/mmol). Non-specific binding was estimated from adjacent sections incubated in the presence of 1μM SRIF. The sections were washed for 3 x 2min in ice-cold buffer and for 10s in ice-cold distilled water. The dried sections were exposed to ^{3}H- Hyperfilm for 2 weeks and quantified using iodine standards.

The highest levels of specific binding (expressed as specific fmol bound/mg tissue equivalent ± SEM; n=5) were found in the medial habenula (0.123 ± 0.021), dentate gyrus (0.134 ± 0.053), medial amygdaloid nuclei (0.094 ± 0.034) and the basolateral amygdaloid nucleus (0.093 ± 0.017). Moderate levels of binding were seen in the cerebral cortex, notably the cingulate (0.074 ± 0.009), piriform (0.074 ± 0.014) and parietal (0.078 ± 0.015) regions, dorsal lateral septal nucleus (0.085 ± 0.014) and the claustrum (0.086 ± 0.022). Specific binding was also observed in the lateral septum (0.067 ± 0.015), subiculum (0.041 ± 0.009), superficial grey layers of the superior colliculus (0.037 ± 0.006), locus coeruleus (0.027 ± 0.006), interpeduncular nucleus (0.037 ± 0.007) and the CA1 region of the hippocampus (0.028 ± 0.009). No significant binding was detected in the cerebellum.

The distribution of [^{125}I]-BIM-23027 binding sites shows general agreement with studies using [^{125}I]-MK-678 (Martin *et al.*, 1991) and, importantly, agrees closely with the distribution of the sst_2 receptor protein in rat brain identified by the use of immunohistochemistry (Schindler, M. *et al.*, this meeting). The distribution of binding sites in rat brain is consistent with the hypothesis that somatostatin may be important in the modulation of complex functions such as cognition and polymodal sensory processing.

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321P POSSIBLE INVOLVEMENT OF A G-PROTEIN RECEPTOR KINASE IN THE RAPID DESENSITIZATION OF SOMATOSTATIN RECEPTORS IN NG108-15 CELLS

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The inhibition of calcium channel currents (I_{Ca}) by somatostatin and δ-opioid agonists can exhibit rapid desensitization (Shapiro & Hille, 1993; McFadzean & Docherty, 1989). We have previously shown that a component of this rapid desensitization is mediated by protein kinase A (Hepworth & Henderson, 1994). In the present study we have examined the possible role of G-protein receptor kinases (GRKs) in rapid desensitization.

NG108-15 cells were differentiated by treatment with IBMX (50μM) and prostaglandin E1 (10μM) for three days and then superfused (5-7ml.min^{-1}) at room temperature with a solution containing (in mM): TEACl 121; CsCl 5; $BaCl_2$ 10; $MgCl_2$ 1; HEPES 10; glucose 10 and sucrose 50. Whole cell patch clamp recordings were made with electrodes containing (in mM): CsCl 100; BAPTA 10; MgATP 5, NaGTP 0.5 and HEPES 20. All solutions were adjusted to pH 7.3 with CsOH. Cells were held at -90mV and currents were activated by 100ms depolarizing voltage commands to 0mV. Data are presented as mean ± s.e.mean.

Neither somatostatin (300nM) nor the δ-opioid agonist [D-pen^2, D-pen^5]-enkephalin (DPDPE; 300nM) inhibited the L- or T-type I_{Ca}, but these agents did inhibit the N-type I_{Ca} by 46±2% (n=38) and 50±5% (n=7) respectively. After 3 min of continuous agonist application the response to somatostatin desensitized by 52±3% (n=38). In contrast, after 3 min of continuous DPDPE application the response had desensitized by only 23±4% ($p<0.05$ relative to somatostatin; n=7, Student's unpaired t-test). The rate of recovery from the desensitization to somatostatin was determined by comparing the degree of inhibition caused by two 3 min applications of somatostatin (300nM) separated by 1-10 min. The $t_{1/2}$ for recovery from desensitization to somatostatin was approximately 2 min.

Zinc ions have been shown to inhibit GRK activity in a number of cell lines (Benovic et al, 1987). Intracellular dialysis for at least 10 min with Zn^{2+} (free concentration 30μM) failed to produce any significant change in the rate or degree of desensitization to DPDPE (30±6%; n=4). However, a 15 min dialysis with Zn^{2+} (free concentration 30μM) significantly reduced the degree of desensitization to somatostatin to 25±2% ($p<0.05$; n=4). The effect of Zn^{2+} was not dependent on Zn^{2+} being dialysed into the cell. Superfusion of the cells for 5 min with $ZnCl_2$ (10μM) in the extracellular solution also reduced the desensitization to somatostatin to 36±7% ($p<0.05$; n=6).

The phosphatase inhibitor microcystin-LR was used to examine the role of dephosphorylation in the recovery from desensitization to somatostatin. Intracellular dialysis with microcystin-LR (1μM for 15 min; n=6) had no effect on the degree of desensitization or rate of recovery from desensitization to somatostatin.

The findings of this study suggest that a GRK is involved in the desensitization to somatostatin, but not to the δ-opioid agonist.

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322P OPERATIONAL CHARACTERISTICS OF SOMATOSTATIN RECEPTORS MEDIATING INHIBITION OF SPONTANEOUS FIRING OF RAT LOCUS COERULEUS NEURONES

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We have previously described inhibitory actions of somatostatin (SRIF) and related analogues on the spontaneous firing of rat locus coeruleus (LC) neurones, measured using extracellular recording techniques (Black *et al.*, 1995). In the present study, we have extended this work to attempt to characterise the mechanism of action and receptor transduction events involved in the mediation of these responses.

Brain slices (350μm) containing the LC were prepared from male AHA Sprague-Dawley rats (150-200g). Slices were placed in a perspex recording bath and superfused with oxygenated artificial CSF (ACSF) at 32°C (flow rate = 2ml min^{-1}). Simultaneous single unit recording and fast-cyclic voltammetry (FCV) was performed using a single carbon-fibre electrode (Stamford *et al.*, 1993). LC neurones were identified by their regular firing (0.5-3 Hz, spike duration 1-2 msec) and sensitivity to noradrenaline (NA).

Both SRIF and NA concentration-dependently and reversibly inhibited the spontaneous firing of LC neurones (EC_{50} values of 11.8 [8.4-16.6] nM [geometric means with 95% confidence intervals], n=30 and 2.9 [0.5-16.4] μM, n=4, respectively). However, the low sensitivity of FCV for NA detection (threshold of approximately 3-10 μM) precluded using this technique to determine unequivocally whether the effects of SRIF were mediated by NA release. Addition of the monoamine uptake inhibitor, desipramine (100nM), caused a significant (approx. 25 fold) leftward shift of the NA concentration-effect (c-e) curve (EC_{50}=126 [34-477] nM, n=4), but did not significantly affect responses to SRIF (EC_{50}=8.0 [2.0-32.4] nM).

Somatostatin receptors have been reported to inhibit cAMP formation (Patel *et al.*, 1994). To determine whether this action was responsible for inhibition of LC neurone firing, 8-bromoadenosine-cyclic monophosphate (8-Br-cAMP; 500 μM) was superfused over the slice for 30min prior to construction of a second SRIF c-e curve. 8-Br-cAMP significantly increased the basal firing rate (223±24 % over basal, n=4, $P<0.05$, Student's paired *t*-test), but did not affect inhibitory responses to SRIF, which completely inhibited firing both in the absence and presence of 8-Br-cAMP (EC_{50} values, 7.6 [4.1-13.9] and 10.2 [2.7-38.2] nM, n=4, respectively).

Incubation of slices in a modified buffer containing 500ng ml^{-1} pertussis toxin (PTX) for 18h prior to extracellular recording significantly attenuated inhibitory responses caused by concentrations of SRIF (3-100 nM) which were effective in slices incubated for 18h in the absence of PTX (e.g. 86.8±7.4% and 19.5±15.9% of basal firing rate with 30nM SRIF, presence and absence of PTX, respectively, $P<0.05$, Student's *t*-test, n=3). In contrast, in the same experiments, inhibitory responses to muscimol were not affected (EC_{50} values, 1.2 [0.7-1.9] and 2.2 [0.7-6.8] μM, presence and absence of PTX, n=3).

We conclude that the inhibitory effects of SRIF on LC neurones are mediated by a direct effect which does not involve release of NA. This receptor, resembling the recombinant sst_2 receptor (Black *et al.*, 1995), couples via a pertussis toxin- sensitive G-protein, and mediates its response by a mechanism apparently independent of inhibition of cAMP formation.

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323P COMPARISON OF THE EFFECTS OF SIBUTRAMINE AND OTHER MONOAMINE REUPTAKE INHIBITORS ON FOOD INTAKE IN THE RAT

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Sibutramine is a 5-hydroxytryptamine (5-HT) and noradrenaline (NA) reuptake inhibitor (Buckett *et al.*, 1988) which decreases food intake in rats (Fantino *et al.*, 1994). This study explores the relative contribution of 5-HT and NA reuptake inhibition to this response by comparing sibutramine's effects on food intake with those of the monoamine reuptake inhibitors, fluoxetine, nisoxetine and venlafaxine, and the 5-HT releaser and 5-HT reuptake inhibitor, d-fenfluramine.

Individually-housed male Sprague-Dawley rats (350-500g; n=6-8) were maintained on reversed phase lighting (lights off 09.00-17.00h) with free access to powdered diet and water. Feeding jars were weighed at p.o. drug administration (09.00h) and after 2 and 8h.

Sibutramine, venlafaxine and d-fenfluramine significantly decreased food intake in a dose-related manner (Table 1). Fluoxetine and nisoxetine had no effect. A combination of these drugs (30 mg/kg p.o. of each) markedly inhibited food intake (2h: vehicle 11.2±2, fluoxetine+nisoxetine 2.2±1.1**; 8h: vehicle 44.1±3.6, fluoxetine+nisoxetine 17.1±4.7**; units as in Table 1).

These results demonstrate that selective uptake inhibition of 5-HT (fluoxetine) or NA (nisoxetine) does not reduce food intake. However, when these actions are combined (nisoxetine + fluoxetine; high doses of venlafaxine, which has 5-fold selectivity for 5-HT, over NA, reuptake (Bolden-Watson & Richelson, 1993)) food intake is markedly decreased. The data are, therefore, consistent with the hypothesis that sibutramine attenuates food intake by inhibiting both 5-HT and NA reuptake. In addition, they argue that the hypophagic effects of d-fenfluramine are unlikely to be due to 5-HT uptake inhibition, confirming the different mechanisms underlying the actions of sibutramine and d-fenfluramine.

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Table 1. Effect of p.o. administration of sibutramine, d-fenfluramine, fluoxetine, nisoxetine and venlafaxine on food intake in the rat

Time	Dose	Sibutramine	d-Fenfluramine	Dose	Fluoxetine	Nisoxetine	Dose	Venlafaxine
2h	Vehicle	14.1±2.7	14.0±1.3	Vehicle	13.6±1.3	12.4±1.3	Vehicle	14.1±1.0
	1 mg/kg	14.7±1.2	5.2±1.6**	3 mg/kg	12.2±2.9	15.5±1.0	30 mg/kg	8.8±1.8
	3 mg/kg	6.0±1.8*	3.1±0.9**	10 mg/kg	13.3±2.1	15.9±3.1	100 mg/kg	5.6±1.4**
	10 mg/kg	2.7±1.2**	2.4±1.0**	30 mg/kg	12.2±0.9	7.6±2.7	300 mg/kg	7.9±2.1*
8h	Vehicle	46.7±3.8	33.4±2.1	Vehicle	40.5±2.1	41.0±2.9	Vehicle	38.7±1.9
	1 mg/kg	41.8±4.3	24.5±2.6*	3 mg/kg	37.7±3.7	39.7±2.5	30 mg/kg	29.9±3.3
	3 mg/kg	28.5±3.8**	10.2±1.2**	10 mg/kg	34.6±5.9	38.3±3.6	100 mg/kg	19.4±3.6**
	10 mg/kg	18.4±3.8**	6.0±2.3**	30 mg/kg	27.0±1.9	34.3±2.0	300 mg/kg	17.2±3.8**

Results are expressed as mean intakes (g/kg rat weight)± s.e.mean; n=6-8; *P<0.05, **P<0.01 vs vehicle (ANOVA and Dunnett's test).

324P EFFECT OF SIBUTRAMINE ON TISSUE GLUCOSE UTILISATION IN THE RAT

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Selective β_3-adrenoceptor agonists, eg BRL 35135 (R*,R*-(±)-methyl-4-{2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl}phenoxyacetate hydrobromide), stimulate thermogenesis in brown adipose tissue (BAT), but also increase glucose utilisation (GU) in BAT and skeletal muscle (Liu & Stock, 1995). Sibutramine is a potent in vivo inhibitor of noradrenaline and 5-hydroxytryptamine (5-HT) reuptake (Buckett *et al.*, 1988) and we have demonstrated it activates thermogenesis possibly via indirect activation of an atypical or β_3-adrenoceptor (Connoley *et al.*, 1995). We have now determined the effects of sibutramine on GU to compare its actions with those of BRL 35135.

Sixteen adult (200g) female Wistar rats were anaesthetised (1.2g urethane/kg ip) and colonic temperature was measured continuously. Tissue GU was determined by the accumulation of 2-deoxy-[^{3}H]-glucose-6-phosphate 60 min after intravenous injection of 2-deoxy-[^{3}H]-glucose (2DG) (Liu & Stock, 1995). Rats received sibutramine (10mg/kg ip) or saline 60 min before 2DG iv. GU was determined in leg muscles (gastrocnemius, soleus, tibialis anterior, extensor digitorum longus [EDL], adductor longus [ADL]), diaphragm, heart, cerebral cortex, parametrial white adipose tissue [WAT] and interscapular BAT.

The thermogenic effect of sibutramine was evident from the difference in colonic temperature at the end of the experiment, (sibutramine, 38.01±0.19°C, n=7, Control 35.86±0.51°C, n=8; P<0.01 unpaired t-test). There were no significant differences in GU in any of the tissues studied, apart from BAT (Table 1). The 18-fold increase of GU utilisation in BAT compares with the much smaller 12-fold increase observed with a maximal thermogenic dose of the β_3-adrenoceptor agonist, BRL 35135 (Liu & Stock, 1995).

Sibutramine is a potent inhibitor of noradrenaline reuptake in vivo (Buckett *et al.*, 1988), but has no affinity (Ki>1000 nM) for β_3-adrenoceptors (unpublished observation). The results presented demonstrate that activation of thermogenesis by sibutramine is selective for BAT and may, therefore, involve the discrete central activation of sympathetic nerves innervating BAT. The inhibition of noradrenaline (and 5-HT) reuptake by sibutramine does not, however, appear to affect responses in other tissues innervated by sympathetic nerves.

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Table 1. The effect of sibutramine on tissue glucose utilisation

	Control	Sibutramine		Control	Sibutramine		Control	Sibutramine
Gastrocnemius	1.98±0.15	2.56±0.14	ADL	5.07±0.37	4.61±0.30	BAT	7.00±0.85	128.99±15.93†
Soleus	4.28±0.45	4.42±0.43	Diaphragm	10.95±1.09	19.45±2.61	Brain	16.09±0.70	16.80±0.88
Tibialis	2.91±0.17	2.75±0.24	Heart	87.80±12.63	73.95±10.91	Blood		
EDL	3.10±0.25	2.88±0.24	WAT	2.61±0.37	3.89±0.69	glucose (mM)	8.30±0.60	7.22±0.36

Results (ng/min/mg) are mean±s.e.mean (n=8 for control; n=7 for sibutramine). †P<0.001 (unpaired t-test) vs Control.

325P EFFLUX OF [^{3}H]-DOPAMINE FROM SUPERFUSED RAT STRIATAL SLICES: PREDICTIVE VALUE FOR DETECTING STIMULANT DRUGS OF ABUSE

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It is postulated that enhanced limbic dopamine (DA) function mediates reinforcement and reward and, consequently, underpins the actions of abusable drugs, particularly psychostimulants (Di Chiara *et al.*, 1993). DA release is a primary mechanism for enhancing central DA function. We have now compared the actions of drugs from various classes on [^{3}H]DA release, viz stimulants, weight-modifiers and monoamine reuptake inhibitors, including sibutramine and its major pharmacologically active metabolites BTS 54 354 (N-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-N-methylamine) and BTS 54 505 (1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine). Sibutramine is a noradrenaline/5-hydroxytryptamine reuptake inhibitor which is in development as an antiobesity drug.

Striata were removed from the brains of male Sprague-Dawley CD rats (180-300g). The methodology for determining the effects of drugs (10^{-7}-10^{-5}M; n≥4) on the unstimulated release of [^{3}H]DA from superfused striatal slices is described by Heal *et al.* (1992).

Unstimulated (basal) release of [^{3}H]DA expressed as a fraction of the total tritium content was in the range 0.084 - 0.319 (n=≥4). The most potent releasers with increases at ≥10^{-7}M were d-amphetamine (10^{-7}M = 56 ± 9%, P<0.01; 10^{-6}M = 122 ± 6%, P<0.001) and methamphetamine (10^{-7}M = 37 ± 10%, P<0.05; 10^{-6}M = 82 ± 9%, P<0.001), followed by amfonelic acid and mazindol which were active ≥10^{-6}M (amfonelic acid, 10^{-6}M = 82 ± 11%, P<0.001; mazindol, 10^{-6} = 38 ± 21%, P<0.05). Cocaine, fencamfamine, methylphenidate and nomifensine were active only at 10^{-5}M. Bupropion, venlafaxine, sibutramine, BTS 54 354 and BTS 54 505 were inactive.

The data indicate DA release is a major effect of d-amphetamine and methamphetamine and it also probably contributes to the pharmacological actions of the other stimulants investigated. In terms of predictive value, [^{3}H]DA release is a sensitive test because it is able to detect compounds for which abuse potential is marked (d-amphetamine, methamphetamine, cocaine, methylphenidate), moderate/low (mazindol, fencamfamine, amfonelic acid) or even absent (nomifensine). Finally, the data also argue that sibutramine and its major active metabolites are unlikely to have stimulant drug abuse potential.

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Table 1. Comparison of the effects of stimulants, weight-modifiers and reuptake inhibitors on [^{3}H]DA efflux from rat striatal slices

Drug	% Increase	Drug	% Increase	Drug	% Increase
d-Amphetamine	138±15***	Fencamfamine	44±6***	Sibutramine	2±8
Methamphetamine	140±10***	Methylphenidate	29±7***	BTS 54 354	13±12
Mazindol	91±21***	Bupropion	7±13	BTS 54 505	4±11
Amfonelic acid	137±35***	Nomifensine	20±6**		
Cocaine	54±19*	Venlafaxine	-12±7		

% Increase over basal fractional efflux (mean±s.e.mean; n≥4) produced by 10^{-5}M drug. *P<0.05, **P<0.01, ***P<0.001 (Williams' test).

326P LACK OF EFFECT OF SYSTEMICALLY ADMINISTERED 5-HT$_4$ AGONISTS ON DOPAMINE LEVELS MEASURED FROM THE NUCLEUS ACCUMBENS AND STRIATUM: AN *IN VIVO* MICRODIALYSIS STUDY IN FREELY-MOVING RATS

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The role of 5-HT$_4$ receptors in the CNS is unclear although high concentrations are found in the nigrostriatal pathway of various species (Jakeman et al., 1994). 5-HT and 5-HT$_4$ receptor agonists when given locally into the striatum of anaesthetised rats have been shown to induce an increase in dopamine release and these effects were reduced by 5-HT$_4$ receptor antagonists (Bonhomme et al., 1995). In this study, we have investigated systemic injection of BIMU-8 (Bonhomme et al., 1995) and RS 67333 (Eglen et al., 1995), selective 5-HT4 receptor agonists, on dopamine release from the rat nucleus accumbens and striatum.

Male Sprague Dawley rats (250-350g) were anaesthetised with fentanyl (0.45 mgkg^{-1}, ip) and medetomidine (0.4 mgkg^{-1}, sc) and placed in a stereotaxic frame. Concentric dialysis probes were implanted into either the striatum (A 0.0mm; L 2.8mm from bregma; V 6.6mm from dura, according to Paxinos and Watson, 1982) or the nucleus accumbens (A 2.7mm; L 1.6mm from bregma; V 7.4mm from dura). Anaesthesia was reversed with atipamezole HCl (1 mgkg^{-1}, ip) and nalbuphine HCl (2 mgkg^{-1}). After overnight recovery, the probes were perfused with physiological salt solution (NaCl, 125mM, KCl, 2.5mM, $MgCl_2$, 1.18mM, $CaCl_2$, 1.26mM) at a flow rate of 2µlmin^{-1}. Samples were collected at 20 min intervals for three hours post-dosing and analysed for dopamine by HPLC with electrochemical detection (essentially as in Bristow et al., 1994). Data was analysed for statistical significance by analysis of variance with post hoc Dunnett's test where appropriate.

Basal dopamine levels were 42.2 ± 6.6 and 57.3 ± 12.7 fmoles/40 µl for the nucleus accumbens and striatum respectively. BIMU-8 (30 mgkg^{-1} ip, n=4) had no significant effect compared to vehicle controls on dopamine levels measured from the nucleus accumbens (maximal dopamine levels (%basal): vehicle, 127 ± 8%; BIMU-8, 125 ± 10%,). RS 67333 (100 µgkg^{-1}, ip, n=4) had no significant effect relative to vehicle controls on dopamine levels measured from the nucleus accumbens (vehicle, 127 ± 8%; RS 67333, 148 ± 36%) or striatum (vehicle, 131 ± 21%; RS 67333; 101 ± 18%).

These results suggest that at the dose tested, these 5-HT$_4$ receptor agonists do not influence dopamine release on systemic administration, in contrast to the results when they are given directly into the CNS. Whether this is due to their partial agonist activity and/or ability to penetrate the CNS is unknown.

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327P AROMATIC HYDROXYLATION OF PHENYLALANINE TO MEASURE OXIDATIVE STRESS IN RAT BRAIN *IN VIVO*

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Free radicals and reactive oxygen intermediates (collectively termed reactive oxygen species-ROS) are implicated in the pathogenesis of disorders affecting the central nervous system. It has proven particularly difficult to monitor ROS production *in vivo* due to their transitory nature but some studies have been reported based upon assays measuring the end products of reactions eg. malonaldehyde (from lipid peroxidation), spin trapping or aromatic hydroxylation. Fewer studies have attempted to measure oxidative stress *in situ*. We have developed a method for the measurement of malonaldehyde in rat brain *in situ* using microdialysis coupled to HPLC with UV detection (Waterfall *et al.*, 1995). Here we describe preliminary data from other development work attempting to measure ROS production *in vivo* by combining microdialysis sampling with an assay for tyrosines- the aromatic hydroxylation products of phenylalanine.

The detection of oxidative stress is based upon the use of phenylalanine (phe) as a trapping agent. The aromatic hydroxylation of phe (Kaur and Halliwell 1994) considers the reaction of hydroxyl radicals at the ortho, meta and para positions on the aromatic ring of phe to form the corresponding isomers of tyrosine (tyr). Of these products p-tyr is already present in tissue due to the action of the enzyme phenylalanine hydroxylase on L-phenylalanine, hence in previous studies (eg. Sun et al 1993) this assay has concentrated upon the m-tyr and o-tyr products only, measured by electrochemical detection. However, D-phenylalanine (D-phe) is not thought to be metabolised by the enzyme and infusion of this isomer should provide useful additional data.

Male Lister Hooded rats 250-350 g were anaesthetised and maintained with chloral hydrate (600 mg/kg i.p.). A single microdialysis probe was implanted uni-laterally through the cortex and striatum and perfused either with artificial CSF (pH 7.3) alone (to measure endogenous tyrosine) or ACSF supplemented with D-phe (5mM). Loop design microdialysis probes and perfusion lines were constructed entirely of PTFE tubing with a cellulose acetate microfibre. The loop was maintained with an in-dwelling stainless steel wire rather than tungsten (Waterfall et al 1995) or nylon, as these materials generate contaminants. After collection, the dialysate was immediately measured by reverse phase HPLC using a C18 column with citrate/acetate buffer in methanol/water (Kaur and Halliwell 1994) coupled to fluorescence detection (excitation 275 nm, emission 303 nm).

Co-elution of p-tyr was evident from the *in vivo* dialysate samples at a concentration of 1.75 µM ± 0.25 (n=6), m- and o- tyr were not present. D-phe (Aldrich) in solution (5mM) revealed the presence of p-, m-, and o- tyr contaminants at less than 0.25 µM. *In vivo*, perfusion of D-phe led to an immediate 4-fold increase in p-tyr to 7 µM ± 1 (n=6) which was significant ($p<0.002$, unpaired t-test), on the other hand m- and o-tyr were unaffected.

Microdialysis sampling coupled to HPLC with fluorescence detection appears to be a viable method for the measurement of oxidative stress. A clear, unambiguous and readily quantifiable signal for p-tyr is produced by the perfusion of D-phe (as the hydroxyl radical trapping agent), over and above the endogenous signal. Hence, our preliminary work suggests that D-phe might be preferentially substituted in this paradigm for L-phe, which is metabolised enzymatically. Furthermore the preliminary data provides evidence for the continuous presence of hydroxyl radicals in the extracellular fluid of rat brain in this acutely anaesthetised preparation.

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328P L-701,324, A SELECTIVE ANTAGONIST AT THE GLYCINE/NMDA SITE, ATTENUATES STRESS-INDUCED ACTIVATION OF MESOCORTICAL DOPAMINE NEURONES

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Previous studies have demonstrated that R-(+)-HA966, a low efficacy partial agonist at the glycine/NMDA receptor, is anxiolytic (Dunn *et al.* 1992) and blocks stress-induced activation of dopamine (DA) metabolism in the medial prefrontal cortex (Morrow *et al.*, 1993). In the present study we have compared the effects of L-701,324, a highly selective, potent (IC_{50} = 2 nM verses [^{3}H]L-689,560 binding in rat brain membranes), full antagonist at the glycine/NMDA receptor (Kulagowski *et al.*, 1994) with zolpidem a $GABA_A$ receptor agonist on stress-induced activation of prefrontal cortex DA metabolism.

Male Sprague Dawley rats (weight range 250-300 g, B & K Ltd, U.K.) were pre-treated with either zolpidem (5 mg/kg, i.p.), R-(+)-HA-966 (20 mg/kg i.p.), L-701,324 (7-chloro-4-hydroxy-3(3-phenoxy)phenylquinolin-2-(H)-one) (1 or 5 mg/kg, i.p.), L-701,357 (7-chloro-4-hydroxy-3(3-phenoxy)phenylquinolin-2-(1H)-one) (10 mg/kg, i.p), the inactive regio-isomer of L-701,324 (27.9% inhibition @ 10 µM verses [^{3}H]L-689,560 binding in rat brain membranes) or vehicle (0.5% carboxymethylcellulose in 0.9% NaCl, 1ml/kg, i.p.). Thirty min later rats were either left in the home cage or immobilised for 30 min and immediately killed by stunning and decapitation. Brains were removed, the medial prefrontal cortex dissected and frozen on solid CO_2 , before being stored at -80°C until required for analysis of dopamine and the acidic metabolite DOPAC by HPLC with electrochemical detection (Hutson *et. al.* 1991). Data were subjected to analysis of variance followed where significant, by Tukey's Test. A value of $P<0.05$ was considered significant.

Dopamine metabolism, as indicated by the concentration of DOPAC, in medial prefrontal cortex was significantly increased following 30 min immobilisation stress. Pre-treatment of rats with either zolpidem (5 mg/kg, i.p.), R-(+)-HA-966 (20 mg/kg, i.p.) or L-701,324 (5 but not 1 mg/kg, i.p.) significantly attenuated stress induced increase of medial prefrontal cortex DOPAC concentration without affecting dopamine metabolism per. se. In contrast, L-701,357 (10 mg/kg, i.p.), the inactive regioisomer of L-701,324 did not significantly affect either basal or stress induced increase of dopamine metabolism in the medial prefrontal cortex (Table 1). The concentration of dopamine in the medial prefrontal cortex was unaffected by stress or drug treatment (data not shown).

Results in the present study demonstrate that activation of mesocortical dopamine neurones by acute stress is comparably blocked by the $GABA_A$ receptor agonist zolpidem and by L-701,324 and R-(+)-HA-966, antagonists at the NMDA/glycine site.

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

	DOPAC (ng/g)				
Treatment	R(+)HA966 (20 mg/kg)	L-701,357 (10 mg/kg)	L-701,324 (1 mg/kg)	L-701,324 (5 mg/kg)	Zolpidem (5 mg/kg)
Vehicle/Non-Stress	11.70 ± 3.08	36.89 ± 1.10	15.02 ± 1.79	15.52 ± 1.68	19.38 ± 3.16
Vehicle/Stress	27.60 ± 3.43*	58.55 ± 2.92*	60.72 ± 0.66*	38.54 ± 2.87*	43.05 ± 3.43*
Drug/Non-Stress	8.30 ± 1.97	36.16 ± 6.22	27.22 ± 2.52	17.82 ± 1.99	22.04 ± 3.46
Drug/Stress	14.20 ± 1.86+	60.77 ± 5.05**	66.87 ± 10.98**	18.34 ± 2.13+	21.67 ± 4.16+

Values are mean ± s.e.mean, n = 6. * $P<0.05$ compared with vehicle treated non-stressed animals, + $P<0.05$ compared with vehicle treated stressed animals and ** $P<0.05$ compared to drug treated non-stressed animals using Tukeys' studentised range method following significant ANOVA.

329P EVIDENCE THAT DIZOCILPINE (MK-801) DOES NOT DOWN-REGULATE EITHER β_1-ADRENOCEPTORS OR 5-HT_{2A} RECEPTORS IN RAT BRAIN

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The most widely described receptor changes following repeated administration of antidepressant drugs to rodents are down-regulation of cortical β_1-adrenoceptors and 5-HT_{2A} receptors (Sugrue, 1983). Dizocilpine (MK 801) is a non-competitive NMDA receptor antagonist which has been reported to produce antidepressant-like effects in the Porsolt swim and tail suspension tests (Trullas & Skolnick, 1990; Maj et al., 1992; Love et al., 1995). In addition, dizocilpine has been reported to down-regulate cortical β-adrenoceptors (Paul et al., 1992). In this study, we have determined the effects of acute and chronic administration of dizocilpine and desipramine on the number and affinity of β_1-adrenoceptors and 5-HT_{2A} receptors in rat frontal cortex.

Male CD rats (100-160 g) were given desipramine, dizocilpine or saline ip for 1 or 14 days, or via subcutaneously implanted minipumps for 28 days (Table 1). Frontal cortices were removed 24h after the final dose (ip) or 28 days after implantation. β_1-Adrenoceptors were quantitated using [^{3}H]CGP 12177 (0.025-1.2 nM), non-specific binding was defined by 500 nM CGP 20712A (Ogilvie et al., 1995). 5-HT_{2A} receptors were measured using [^{3}H]ketanserin (0.05-4.8 nM), non-specific binding was defined by 5 μM methysergide. Binding parameters (K_d and B_{max}) were determined using LIGAND.

The data presented in Table 1 confirm that chronic administration of desipramine decreases the number of β_1-adrenoceptors and 5-HT_{2A} receptors. Repeated administration of dizocilpine had no significant effect on the number or affinity (data not shown) of β_1-adrenoceptors or 5-HT_{2A} receptors (Table 1). These data contrast with those of Paul et al. (1992), who measured the total population of β-adrenoceptors in mouse whole cortex. Therefore, these discrepant findings may relate to methodological differences.

As shown in this study and by others (reviewed by Sugrue, 1983) antidepressants which exert their effects by enhancing noradrenergic function markedly down-regulate β_1-adrenoceptors and 5-HT_{2A} receptors. Thus, the failure of dizocilpine to produce such effects suggests that the antidepressant-like actions of this compound at 0.1-1 mg/kg in animal models (Trullas & Skolnick, 1990; Love et al., 1995) are unlikely to be mediated via the noradrenergic axis.

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Table 1. Effect of repeated administration of dizocilpine on the number of β_1-adrenoceptors and 5-HT_{2A} receptors

14-Day	β_1	5-HT_{2A}	28-Day	β_1	5-HT_{2A}
Vehicle (saline 2 ml/kg)	89 ± 3	473 ± 13	Vehicle (saline)	100 ± 6	578 ± 23
Desipramine (10 mg/kg)	60 ± 5**	385 ± 18**	Desipramine (10 mg/kg/24h)	59 ± 4**	464 ± 11**
Dizocilpine (1 mg/kg)	88 ± 4	457 ± 23	Dizocilpine (0.3 mg/kg/24h)	90 ± 2	561 ± 24
Dizocilpine (1 mg/kg, bd)	82 ± 3	426 ± 14	Dizocilpine (1 mg/kg/24h)	91 ± 2	525 ± 15

B_{max} values (fmol/mg protein) are mean ± s.e. mean (n = 7 - 20); bd = bidaily. **P<0.01 Dunnetts multiple comparison test.

330P METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION CONTRIBUTES TO THE NOCICEPTIVE REFLEX RESPONSE IN THE NEONATAL RAT SPINAL CORD *IN VITRO*

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The contribution of metabotropic glutamate receptor (mGluR) activation to the nociceptive spinal segmental reflex response has been examined in an *in vitro* preparation of neonatal (11-14 day) rat spinal cord (Thompson et al., 1992). Segmental nociceptive reflex responses were recorded as a ventral root potential evoked by electrical activation of high threshold nociceptive afferent fibres or by agonist application to the spinal cord. Superfusion of the selective mGluR agonist, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD), to the spinal cord produced a concentration-dependent, reversible ventral root depolarization (EC_{50}=58.1±7.2μM, n=4), which was blocked in a concentration-dependent manner by the selective mGluR antagonist, (+)-α-methyl-4-carboxyphenylglycine (MCPG) (IC_{50}=243±61μM, n=4). MCPG, over the same concentration range (100μM-5mM) did not affect N-methyl-D-aspartic acid (NMDA) induced ventral root depolarizations. The specific NMDA receptor antagonist D(-)-2-amino-5-phosphonopentanoic acid (D-AP5) at a concentration specific for NMDA receptors (40μM) (Thompson et al. 1992) did not affect responses evoked by (1S,3R)-ACPD (100μM).

High intensity single electrical shock to the dorsal root stimulated C-fibres and evoked a prolonged ventral root potential. MCPG, at concentrations specific for (1S,3R)-ACPD-induced responses (100μM-5mM), reduced the prolonged phase of the single shock C-fibre-evoked response. Low frequency high intensity stimulation (50V, 200μs, 1-10Hz) of the dorsal root evoked a windup response, the amplitude of which was reduced by both D-AP5 and MCPG (maximum reduction of the control response was 35.7±1.9% (n=27) and 26.0±3.1% (n=9) respectively) in a concentration-dependent manner. The ventral root depolarisation evoked by capsaicin application was blocked by both MCPG (IC_{50}=808.6±35.3μM, n=4) and D-AP5 (IC_{50}=142.6±42.5μM, n=4). These data suggest that both D-AP5 and MCPG are effective in blocking C-fibre induced ventral root responses. In addition to NMDA receptors, mGlu receptor activation appears to be involved in the generation of the segmental spinal nociceptive reflex in the neonatal rat spinal cord *in vitro* under normal conditions.

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331P DEPOLARISING EFFECTS OF CERTAIN ANALOGUES OF (*S*)-WILLARDIINE UPON NEONATAL RAT DORSAL ROOTS *IN VITRO*

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Dorsal roots isolated from neonatal rats are preferentially depolarised by kainate (KA) and certain analogues of (*S*)-willardiine while *N*-methyl-D-aspartate (NMDA), (*S*)-2-amino-3-[3-hydroxy-5-methylisoxazol-4-yl]propanoic acid (AMPA) and (*S*)-willardiine itself are virtually inactive (Agrawal & Evans, 1986). Speculation that this preparation is a source of "pure" KA receptors make it useful for the examination of KA receptor agonists. Here, we report upon the actions of various agonists, including novel analogues of (*S*)-willardiine extending our previous studies (Blake *et al.*, 1991).

Isolated dorsal roots from neonatal rats (3-6 days old) were placed across a grease-gap with the peripheral end on a wick recording electrode and the central end exposed to superfusion of artificial cerebrospinal fluid (aCSF). aCSF contained (in mM) NaCl 118, KCl 3, $CaCl_2$ 2.5, $NaHCO_3$ 28, D-glucose 11 and was gassed with 95%/5% O_2/CO_2. Roots were maintained at 25°C and were superfused at 1 ml aCSF min^{-1}. Drug effects were measured as D.C. potential shifts. To reduce the degree of desensitisation induced by agonist applications, concanavalin A (ConA) was employed (Pook *et al.*, 1993). Prior to each experiment dorsal roots were superfused with ConA (1mg ml^{-1}, in glucose-free aCSF) for 20 min.

The potencies of (*S*)-5-iodo- (5-IW), (*S*)-5-iodo-6-aza- (5-I-6AW), (*S*)-5-bromo- (5-BrW), (*S*)-5-bromo-6-aza- (5-Br-6AW), (*S*)-5-chloro-6-aza- (5-Cl-6AW), (*S*)-6-aza- (6-AW), (*S*)-5-trifluoromethyl- (5-CF_3W), (*S*)-5-methyl-6-aza- (5-Me-6AW) and (*S*)-willardiine itself were compared with KA, L-glutamate (L-Glu) and domoate in ConA treated preparations. Applications of each agonist for 1 min gave potencies relative to KA of (mean ± s.e.mean, with the number of preparations given in parentheses): 5-IW (most potent), 59.0 ± 9.1 (3); domoate, 57.7 ± 9.8 (4); 5-Br-6AW 16.3 ± 2.84 (4); 5-CF_3-W, 15.7 ± 2.5 (3); 5-Br-W, 13.1 ± 0.89 (4); 5-Cl-6AW, 10.6 ± 1.7 (4); 5-I-6AW, 4.49 ± 0.26 (4); KA, 1.0; L-Glu, 0.097 ± 0.011 (4); 6AW, 0.053 ± 0.0047 (3); 5-Me-6AW, 0.047 ± 0.0104 (4); (*S*)-willardiine, <0.02 (3). Interestingly, the analogues with the 6-aza substitution in the uracil ring exclusively exhibited shallower concentration-response slopes than those of kainate and domoate and in addition, a maximum response comparable with kainate could not be achieved. Experiments confirmed that ConA was able to attenuate the fade associated with L-Glu-induced responses, however, it is possible that the lower efficacy observed with the 6-aza analogues is the result of a ConA resistant desensitisation phenomenon. Experiments currently underway indicate that they are reversibly antagonised by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX).

To conclude, while none of the 6-aza analogues displayed exceptional potency their apparently ConA resistant desensitisation characteristics may prove important in the study of the mechanism of KA receptor desensitisation.

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332P THE EFFECT OF A 6-AZA SUBSTITUTION ON THE AFFINITY OF WILLARDIINE ANALOGUES FOR THE AMPA RECEPTOR

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Electrophysiological and ligand binding studies, using structural analogues of the amino acid willardiine, have highlighted the importance of the ionization of the uracil group in conferring agonist activity at the AMPA receptor. Consequently the most potent willardiine analogues have an electron-withdrawing substituent at the 5-position of the uracil ring (Patneau *et al.*, 1992; Wong *et al.*, 1994; Hawkins *et al.*, 1995). It is known that 6-aza uracils have lower pKa's than their corresponding uracils (Jonas and Gut, 1961). In order to further reduce pKa's of the willardiines analogues synthesized so far, a series of azawillardiines, with a nitrogen atom substitution at the 6-position of the uracil ring have been synthesized (for general structures see Figure 1). In this study we have investigated the relative affinities of these 6-azawillardiine compounds for the AMPA receptor using radioligand binding.

Radioligand binding studies were performed on rat whole brain (minus cerebellum) synaptic membranes prepared from male Wistar rats (250-300g) as described by Hawkins *et al.*, (1995). Binding was performed in 50mM Tris-HCl /100mM KCl buffer (pH 7.4 at 4°C) in the presence of 10nM (S)-[^{3}H]AMPA for 40min at 4°C. Non-specific binding was defined in the presence of 1mM L-glutamate. The reaction was terminated by centrifugation (13,000g for 7 min). The data were analysed by a one site fit and the IC_{50} values determined using GraphPAD Prism.

The IC_{50} values of a series of 6-azawillardiine analogues to displace (S)-[^{3}H]AMPA binding are summarized in Table 1. The 6-azawillardiines displayed the same stereoselectivity observed with previous willardiine derivatives, with the potent activity residing in the (S)-enantiomer. The halogenated 6-azawillardiines, halogenated at the 5-position of the 6-aza uracil ring, were more potent displacers of (S)-[^{3}H]AMPA binding than the 5-halowillardiine analogues (Hawkins *et al.*, 1995). Furthermore, the 6-aza substitution converted the relatively inactive compounds (S)-5-methylwillardiine and (S)-5-isowillardiine into more potent displacers of (S)-[^{3}H]AMPA binding.

In conclusion, aza-substitution at the 6-position of the uracil ring increased the affinity of willardiine analogues for the AMPA receptor. This provides further evidence that ionisation of the uracil ring is important in determining affinity for the AMPA receptor.

Figure 1. The structures of willardiine and 6-azawillardiine.

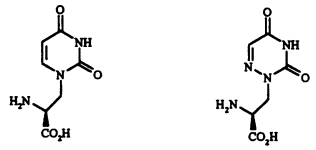

Table 1. A summary of the IC_{50} values (± S.E.M) for the azawillardiines at displacing (S)-[^{3}H]AMPA (10nM) binding.

Compound	IC_{50}
(S)-6-azawillardiine	1.6 ± 0.5 μM
(S)-5-methyl-6-azawillardiine	2.5 ± 0.4 μM
(S)-5-iodo-6-azawillardiine	0.5 ± 0.3 μM
(S)-5-chloro-6-azawillardiine	53.0 ± 25.0 nM
(S)-5-bromo-6-azawillardine	19.0 ± 9.0 nM
(R,S)-5-iso-6-azawillardiine	5.4 ± 1.8 μM
(R)-6-azawillardiine	>1mM

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333P POTENTIATION OF 4-AMINOPYRIDINE-STIMULATED [^{3}H]GLUTAMATE RELEASE FROM RAT CEREBROCORTI-CAL SYNAPTOSOMES BY (RS)-3,5-DIHYDROXYPHENYLGLYCINE IN THE ABSENCE OF ARACHIDONIC ACID

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To date eight metabotropic glutamate receptors (mGluRs) have been cloned and classified into three groups. Group 1 (1 and 5) mGluRs are found to be coupled to phosphoinositide (PI) hydrolysis. mGluR5 is prominently expressed in the cerebral cortex and has recently been found to be present both pre- and post synaptically, suggesting that this receptor may have a role as an autoreceptor modulating glutamate transmission (Romano *et al.*, 1995). Previously it has been shown that the non-specific mGluR agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) can potentiate 4-aminopyridine (4AP)-stimulated release of endogenous glutamate from rat cerebrocortical synaptosomes in the presence of low concentrations of arachidonic acid (AA). AA is thought to act as a retrograde messenger which sensitises protein kinase C (PKC) to diacylglyerol (Herrero *et al.*, 1992). Here we provide evidence for the potentiation of 4AP-stimulated [^{3}H]glutamate release from preloaded rat cerebrocortical synaptosomes in the absence of AA by the selective group 1 mGluR agonist (RS)3,5-dihydroxyphenylglycine ((RS)DHPG).

Cerebrocortical synaptosomes were prepared from Wistar rats (250-300g) by the method of Dodd *et al.*, (1987). Synaptosomes were resuspended in oxygenated Krebs-bicarbonate medium, incubated (37°C,10 min) in the presence of 7.5μCi L-[^{3}H]-glutamate and centrifuged (500g, 10min, room temperature) onto Whatman GF/C filters. Loaded filters were transferred to a superfusion apparatus and superfused for 15min at 2ml/min prior to the collection of ten 1min fractions. Agonists were present in the medium 1min prior to and during a 1min stimulation with 200μM 4AP (approximate EC_{50} in this assay). (S)-4-carboxyphenylglycine ((S)4CPG) was present 1min before and for the duration of the presence of the agonist and the stimulation with 4AP. Release of [^{3}H]-glutamate was determined by liquid scintillation spectometry. All experiments were performed at least three times in quadruplicate.

In the absence of added AA, the non-specific mGluR agonist (1S,3R)ACPD inhibited 4AP-stimulated [^{3}H]glutamate release (EC_{50}=32±3μM). This may suggest activity at group II and III mGluRs, which are known to be present presynaptically and act to downregulate glutamate transmission. When 2μM AA was included in the buffer, (1S,3R)ACPD (between 1 and 10μM) potentiated 4AP-stimulated [^{3}H]glutamate release; with greater than 10μM release was inhibited. Higher concentrations of AA (up to 100μM) failed to potentiate release further. The presence of AA may enable activity at group I mGluRs to be detected as AA is thought to sensitize PKC to diacylglyerol, whereas at higher concentrations of (1S,3R)ACPD activity at other mGluRs may predominate.

In contrast to this, the selective group I mGluR agonist (RS)DHPG potentiated 4AP-stimulated [^{3}H]glutamate release in the absence of added AA (EC_{50}=1.6±0.25μM), this could not be further potentiated by the addition of AA (0.1-100μM) to the buffer. This effect is unlikely to be due to the presence of endogenous AA as inclusion of BSA (known to bind free fatty acids (Rhoads *et al.*, 1983)) in the medium (1mg/ml) had no effect on the potentiation. The effect was found to be PKC-dependent as preincubation with the PKC inhibitor Ro-318220 (10μM) abolished the potentiation. The potentiation was also significantly reduced (by 68±13%) by the group I mGluR antagonist (S)4CPG (100μM).

The data presented here may provide evidence for a positive feedback mechanism for presynaptic glutamate release without the requirement of AA as a retrograde messenger.

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334P α-METHYL-3-PHOSPHONOPHENYLALANINE AND α-CYCLOPROPYL-4-PHOSPHONOPHENYLGLYCINE ARE POTENT ANTAGONISTS AT mGluRs NEGATIVELY COUPLED TO ADENYLYL CYCLASE

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(2S,1'S,2'S)-2-(2-carboxycyclopropyl)glycine (L-CCG-1) and L-2-amino-4-phosphonobutyric acid (L-AP4) potently and relatively selectively stimulate group 2 and group 3 metabotropic glutamate receptors (mGluRs) in adult rat cortical slices (Bedingfield *et al.*, 1995a). These mGluRs are negatively coupled to adenylyl cyclase via G-proteins.

The antagonist effects of a large series of phenylglycine derivatives against the agonists L-AP4 and L-CCG-1 acting at these mGluRs have previously been reported Bedingfield *et al.*, 1995a, b). In this study we report the antagonist effects of (S)-α-methyl-3-phosphonophenylalanine (MPPA) and (RS)-α-cyclopropyl-4-phosphonophenylglycine (CPPG). The latter compound effectively antagonises the depression of monosynaptic excitation elicited by L-AP4 and (1S,3S)-1-aminocyclopentane-1,3-dicarboxylate in neonatal rat motoneurones (Thomas *et al.*, this meeting).

Adult rat cortical slices (0.2x0.2mm) were prepared and adenylyl cyclase stimulated with 30μM forskolin in the presence of 10μM L-AP4 or 0.3μM L-CCG-1 (previously determined EC_{50} values for cyclic AMP inhibition in this system), adenosine deaminase (0.2 units), the phosphodiesterase inhibitor Ro 20-1724 (100μM) and antagonists.

Accumulated cyclic AMP was assayed by displacement of [^{3}H] cyclic AMP from a cyclic AMP binding protein obtained from bovine adrenal medulla. Results for the inhibition of the agonist effects of L-AP4 and L-CCG-1 by the novel antagonists are presented as IC_{50} values in table 1.

MPPA is a very potent antagonist of cortical mGluRs stimulated by both L-AP4 and L-CCG-1. It is approximately three times as potent against L-AP4 than L-CCG-1. CPPG is the most potent antagonist yet reported against both agonists. It is approximately twenty times more potent against L-AP4 than L-CCG-1.

CPPG thus demonstrates a substantial enhancement in both potency and selectivity over previously described mGluR antagonists and represents an important new pharmacological research tool for investigating mGluRs.

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Table 1. IC_{50} ± SEM values for test compounds *versus* 10μM L-AP4 or 0.3μM L-CCG-1 n=6

		L-AP4	L-CCG-1
(S)-α-methyl-3-phosphonophenylalanine	MPPA	18.8 ± 7.7nM	52.8 ± 13.7nM
(RS)-α-cyclopropyl-4-phosphonophenylglycine	CPPG	2.2 ± 0.6nM	46.2 ± 18.2nM

335P STATE-DEPENDENT BLOCK OF NEURONAL SODIUM CHANNELS BY (+)- AND (–)-MK-801

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Although classified as an *N*-methyl-D-aspartate channel blocker, the experimental anticonvulsant and neuroprotective agent, (±)-MK-801 (Wong *et al.*, 1986), also has blocking actions (albeit at higher concentrations) at nicotinic receptor ion channels and voltage-sensitive Na^+ and K^+ channels (Rothman, 1988; Halliwell *et al.*, 1989). In the present study we have used (+)- and (-)-MK-801 to examine whether inhibition of Na^+ currents by this compound is stereoselective. The neuroblastoma cell line, N1E-115, was used as the test system. Voltage-dependent Na^+ currents were recorded using the whole-cell voltage clamp technique (for methods see McGivern *et al.*, 1995).

In the first series of experiments (+)- or (-)-MK-801 was applied to N1E-115 cells held at a membrane potential of -80 mV and the effect of the isomers on the peak Na^+ current developed in the *I/V* relation was investigated. When applied at a concentration of 30 µM, (+)- and (-)-MK-801 reduced the peak current by 20.6 ± 6.0% (5 cells) and 27.0 ± 1.9% (7 cells), respectively. Similarly, when applied at 100 µM, (+)- and (-)-MK-801 reduced the peak current by 40.8 ± 3.8% (5 cells) and 56.3 ± 7.5% (6 cells), respectively. The inhibitory potencies of the isomers (compared at each concentration of MK-801) were not significantly different ($P > 0.05$; unpaired, 2-tailed, Student's *t* test).

To investigate the nature of the Na^+ current inhibition in more detail, the effects of (+)- and (-)-MK-801 on Na^+ channel steady-state inactivation were investigated. Steady-state inactivation curves were constructed by clamping the membrane for 15 s to a series of prepulse potentials ranging from -120 to -30 mV, after which a test current was evoked by stepping to 0 mV for 10 ms. When applied at a concentration of 100 µM, (+)- and (-)-MK-801 reduced the amplitude of the Na^+ current generated from the -120 mV prepulse potential by 27.2 ± 1.7% (5 cells) and 30.4 ± 4.2 % (5 cells), respectively (corresponding to apparent affinities of the isomers for the resting state of the Na^+ channel of 273 ± 22 µM and 264 ± 66 µM; see Bean *et al.*, 1983). Furthermore, (+)- and (-)-MK-801 (100 µM) induced shifts in the midpoint of the steady-state inactivation relation of -6.1 ± 0.6 mV and -8.4 ± 1.9 mV, respectively (corresponding to apparent affinities for the inactivated state of 43 ± 5 µM and 32 ± 11 µM). The affinities of the isomers for the each state of the channel were not significantly different ($P > 0.05$; unpaired, 2-tailed, Student's *t* test).

These results indicate that (+)- and (-)-MK-801 inhibit voltage-gated Na^+ currents by a non-stereoselective interaction predominantly with the inactivated state of the channel. However, it is also possible that the present test system is not sufficiently sensitive to detect small differences in the potencies of the isomers.

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336P REGIONALLY DIFFERENT N-METHYL-D-ASPARTATE REECEPTOR DISTINGUISHED BY *IN VITRO* BINDING AND QUANTITATIVE AUTORADIOGRAPHY OF [^{3}H]-CGP 39653 IN RAT BRAIN

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CGP 39653 (D,L-(E)-2- amino -4-[^{3}H]- propyl -5- phosphono -3-pentenoic acid) is a high affinity, selective antagonist at the glutamate site of the NMDA receptor (Sills *et al.*, 1991). In rat cerebral cortex homogenates, [^{3}H]-CGP 39653 binding is inhibited by glycine through an allosteric mechanism, involving the strychnine-insensitive glycine site of the NMDA receptor channel complex (Mugnaini *et al.*, 1993). In the present study we have explored the characteristics of [^{3}H]-CGP 39653 binding in rat brain tissue in vitro as well as its negative modulation by glycine.

Striatal and cerebellar membranes were obtained from adult (250g) male Sprague-Dawley rats as described previously (Mugnaini *et al.*, 1993). The assays were performed by incubation (25 min, 25 °C) of the membrane suspension in buffer solution (50 mM Tris HCl, 2.5 mM $CaCl_2$) containing 2 nM [^{3}H]-CGP 39653 (displacement experiments), or at radioligand concentrations ranging from 1 to 60 nM (saturation experiments). For autoradiography, animals were killed by intracardiac perfusion with ice cold saline, under pentobarbital sodium anaesthesia. Brains were removed, frozen in isopentane in dry ice and horizontal sections (14 µm) cut at -20°C and thaw-mounted on to glass slides. On the day of the experiment, sections were rinsed in buffer (30 min, 37 °C), incubated (25 min, 25 °C) with 20 nM [^{3}H]-CGP 39653, washed (5 min, 4 °C) in buffer, rapidly dipped in purified water, air dried and apposed to tritium sensitive films for 28 days.

As shown in Table 1, the affinity of [^{3}H]-CGP 39653 for binding sites in brain membranes was the same in striatum, cerebellum and cerebral cortex. In addition, glutamic acid and CPP (3-(±)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) completely displaced specific binding, with Ki values not significantly different between areas. Glycine inhibited the binding of [^{3}H]-CGP 39653 in all three regions but in this case the displacement curves could be resolved into high (Ki_H) and low (Ki_L) affinity components. The apparent Ki_H values differed between the regions with the striatum (33 nM) being significantly lower than cortex (104 nM). Regional binding of [^{3}H]-CGP 39653 to sections of rat brain revealed that in the areas examined (cortex, caudate putamen, thalamus, geniculate nuclei, hippocampus) 10 µM glycine reduced the binding to 80% of control (e.g., binding in caudate putamen, thalamus and hippocampus passed from 65, 145 and 283 to 53, 115 and 199 fmol/mg brain tissue, respectively). 7-CKA (7-chlorokynurenic acid), a competitive antagonist of the glycine site of the NMDA receptor channel complex, was able to reverse the inhibitory action of glycine. Reversal by 100 µM 7-CKA was not the same in all regions ($p<0.05$, ANOVA). The effect was greater ($p<0.05$, Dunnett's test) in caudate putamen (131 ± 6 %; 85 fmol/mg) with respect to thalamus (112 ± 5 %; 162 fmol/mg) and hippocampus (104 ± 2 %; 294 fmol/mg). (mean ± s.e.mean, n = 4 experiments)

It is concluded that [^{3}H]-CGP 39653 binding is inhibited by glycine in the major rat brain regions containing NMDA receptors. Moreover, the existence of regionally distinct NMDA receptor subtypes , with a different allosteric mechanism of [^{3}H]-CGP 39653 binding modulation through the associated glycine site, is suggested.

Table 1.: [^{3}H]- CGP 39653 binding profile in striatal, cerebellar and cortical membranes (mean ± s.e. mean, n = 3-7 experiments).

Compound		Striatum	Cerebellum	Cerebral Cortex
[^{3}H]-CGP 39653	pKd	7.81 ± 0.05	8.00 ± 0.08	7.97 ± 0.07
Glutamic acid	pKi	6.66 ± 0.06	6.72 ± 0.26	6.53 ± 0.09
CPP	pKi	6.97 ± 0.01	7.17 ± 0.12	6.97 ± 0.12
Glycine	pKi_H	* 7.48 ± 0.05	7.26 ± 0.13	6.98 ± 0.02
	pKi_L	3.89 ± 0.06	3.65 ± 0.37	3.49 ± 0.11

* significantly different from cortex ($p<0.005$, ANOVA; $p<0.05$, Dunnett's test)

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337P NMDA RECEPTOR SUBTYPES EXPRESSED BY RAT CULTURED CORTICAL NEURONES CHANGE WITH TIME *IN VITRO*

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The NMDA receptor is a multimeric assembly of subunits comprised of a common NR1 together with one or more distinct NR2 subunits. The subunit composition and stoichiometry of natively expressed NMDA receptors is currently unknown. However, predictions of subunit composition of native NMDA receptors expressed by cultured neurones have been possible using the subunit-selective receptor antagonist, ifenprodil. This noncompetitive antagonist has >300-fold higher affinity for human recombinant receptors comprising NR1a/NR2B compared to NR1a/NR2A subunits (Priestley *et al.*, 1995) and has been shown to discriminate subpopulations of NMDA receptors expressed by individual rat cortical and cerebellar granule cells (Priestley *et al.*, 1994). In the present experiments we have used ifenprodil to study time-dependent changes in NMDA receptor subtype expression in rat cultured cortical neurones. All experiments were performed on whole-cell, voltage-clamped (holding potential = -60mV) cells, prepared as described previously (Priestley *et al.*, 1989) and used between 13 and 65 days *in vitro* (DIV).

Concentration-inhibition curves for the antagonism of inward current responses evoked by combined applications of NMDA (100μM) and glycine (10μM) usually comprised two components representing high and low affinity antagonism ($pIC_{50}(h)$ and $pIC_{50}(l)$, respectively). In all experiments there was a significant difference ($P<0.05$, range = 95-130-fold) between the high and low affinity components. In the case of a single 65DIV neurone, however, the ifenprodil inhibition curve appeared monophasic and lacked a high affinity component.

The pIC_{50} values for each component of the inhibition curves showed some variation across treatment groups. In two instances these minor (<2.5-fold) differences in affinity were statistically significant (Table 1). However, the most striking time-dependent change was in the ratio of high to low affinity antagonism. Thus, there appeared to be a time-dependent decrease in the relative contribution of the high affinity component (% high) of ifenprodil antagonism (Table 1).

Table 1. Curve-fit parameters

DIV	pIC_{50} (*h*)	pIC_{50} (*l*)	% high	*n*
13	5.99 ± 0.06	3.87 ± 0.08	69.1 ± 5.5	8
28	5.59 ± 0.09	3.51 ± 0.04*	37.6 ± 3.1*	4
65	5.48 ± 0.11*	3.60 ± 0.004*	20.7 ± 4.5*	6

* = $P < 0.05$, *t* test, all represent within column comparisons to 13DIV.

These experiments confirm that rat cortical neurones express a mixed population of NMDA receptors with high and low affinity for ifenprodil. By analogy with experiments performed on recombinant NMDA receptors, the initial component of the ifenprodil inhibition curve has an affinity which resembles that of a NR1/NR2B subunit assembly. The second component of the ifenprodil inhibition curve has a lower affinity which resembles that of a recombinant NR1/NR2A receptor. Thus, it appears as though cortical neurones express a high complement of NR1/NR2B-like receptors shortly after plating but that this expression declines with time such that the most dominant receptor after prolonged periods in culture resembles a NR1/NR2A subunit assembly.

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338P PHENYTOIN INHIBITS NON-NMDA GLUTAMATE RECEPTOR AGONIST-INDUCED DEPOLARISATION IN RAT CORTICAL WEDGES

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The primary action of the anticonvulsant, phenytoin, is to modify voltage-gated sodium channels, reducing neuronal excitability and neurotransmitter release (McLean and Macdonald, 1983; Willow et al., 1985). However, reports of phenytoin's effects on glutamate receptors are conflicting: some authors report it blocks NMDA receptors on mouse central neurons (Wamil & McLean, 1993), while others report no effect on NMDA receptors, but find competitive blockade of non-NMDA receptors (Lampe & Bigalke, 1990; Kawano et al., 1994). This study addresses these issues by determining phenytoin's effect on the extracellularly-recorded population response of rat cortical neurones to both NMDA and non-NMDA glutamate agonists, using the *in vitro* cortical wedge preparation (Harrison and Simmonds, 1985).

Cortical wedges, cut from 500μm coronal sections of hemibrains of halothane-anaesthetised male CD rats (80-120g; Charles River), were placed in a two compartment chamber with the pial side of the cortex isolated from the callosal side. Both sides were perfused with Mg^{++}-free aCSF at 21°C. Glutamate receptor agonists (NMDA, AMPA, kainate, quisqualate) were bath applied for 1min to the pial side. Agonist-evoked depolarisation amplitudes were used to generate concentration response curves. EC_{50} values were: NMDA, 23μM (n=3); AMPA, 9μM (n=5-7); kainate, 10μM (n=7); quisqualate, 6μM (n=8). Phenytoin (3-300μM) was bath applied to the pial side for 10min before glutamate receptor agonists were added (NMDA, 30μM; AMPA, 10μM; kainate, 10μM; quisqualate, 10μM). Reduction of agonist responses, and changes in frequency and amplitude of spontaneous epileptiform discharges (SEDs) were determined.

Phenytoin dose-dependently decreased the response to the non-NMDA agonists, kainate, quisqualate and AMPA. IC_{50} values were: kainate, 61μM (30-124μM), [10*, 30*, 100** and 300μM**]; AMPA, 163μM (63-421μM), [100** and 300μM**]; and quisqualate 248μM (125-491μM), [30*, 100** and 300μM**] (95% confidence limits in parentheses; concentrations giving significant differences from controls in square parentheses; * P<0.05; ** P<0.01, William's test; n=4 for each sample). Phenytoin at 100* and 300μM** reduced the response to NMDA, but the IC_{50} was >300μM. Phenytoin also dose-dependently reduced both the frequency and amplitude of SEDs, but with IC_{50} values >300μM in both cases.

These results show phenytoin blocks responses to non-NMDA glutamate receptor agonists in rat cortex and it weakly blocks NMDA responses, agreeing with previously reported studies using glutamate receptors expressed in *Xenopus* oocytes (Kawano et al., 1994). Blockade of non-NMDA receptors will prevent the voltage-dependent activation of NMDA receptors; this may partially underlie phenytoin's ability to prevent epileptic seizure generation and spread. Effects of phenytoin on SEDs were also seen, but at concentrations >300μM. Since SEDs are initiated by the removal of Mg^{++} from the aCSF, which ameliorates the voltage-dependency of the NMDA receptor, the reduction in SED frequency and amplitude caused by phenytoin is unlikely to be due to its effects on non-NMDA receptors, but may be the result of weak NMDA receptor blockade.

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339P BINDING OF [^{3}H]SCH 58261, A NEW A_{2A} RECEPTOR ANTAGONIST, IN RAT STRIATUM

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The present study describes the binding to rat striatal A_{2A} adenosine receptors of the tritium-labeled form of the new potent and selective antagonist 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo [1,5-*c*] pyrimidine, SCH 58261 (Baraldi et al., 1994; Zocchi et al., *in press*).
When incubated for 30 min at 25 °C, pH 7.4, 0.2 nM [^{3}H]-SCH 58261 bound to rat striatal membranes with specific binding of 92 (90-94)%. Specific binding was saturable, reversible and dependent upon protein concentration. The presence of 10 mM $MgCl_2$ in the assay mixture did not modify specific binding (89%). Kinetic studies (n=4) showed that [^{3}H]-SCH 58261 binding reached equilibrium after approximately 5 min and was stable for at least 4 h. [^{3}H]-SCH 58261 binding was rapidly reversed by the addition of 50 μM NECA. Association and dissociation kinetics were monophasic with the following rate constants (geometric mean, with 95% confidence limits in parentheses): K_{obs} = 0.85 (0.70-1.04)/min and K_{-1} = 0.62 (0.55-0.69)/min from a $T_{1/2}$ = 1.12 (1.00-1.26). These values gave a kinetic dissociation constant (Kd) of 0.54 nM.
Saturation experiments (n=4) showed that [^{3}H]-SCH 58261 bound to a single class of receptors in rat striatal membranes, with a Kd value of 0.70 (0.64-0.76) nM and an apparent Bmax value of 971 (859-1099) fmol/mg of protein. The presence of 100 μM GTP in the incubation mixture did not modify [^{3}H]-SCH 58261 binding parameters.
The ability of several adenosine receptor agonists, (5'-N-ethylcarboxamidoadenosine, NECA, 2-hexynyl-NECA, HE-NECA, 2-[4-(2-carboxyethyl)-phenetylamino]-5'-N-ethyl-carboxamidoadenosine,CGS21680, 2-phenylaminoadenosine, CV1808,R-N^6-phenylisopropyladenosine,R-PIA, N^6-cyclohexyl-adenosine, CHA, S-N^6-phenylisopropyladenosine, S-PIA, and antagonists 5-amino-9-chloro-2-(2-furyl)-[1,2,4]-triazolo[1,5-*c*] quinazoline, CGS 15943, 5-amino-8-(4-fluorobenzyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo [1,5-*c*] pyrimidine, 8FB-PTP, SCH 58261, xanthine amine congener, XAC, (*E*,18%-Z,82%)7-methyl-8-(3,4-dimethoxystyryl)-1,3-dipropylxanthine, KF 17837S, 8-cyclo pentyl-1,3-dipropylxanthine, DPCPX, 8-phenyltheophylline, 8-PT) in inhibiting 0.2 nM [^{3}H]-SCH 58261 binding was examined in competition experiments (n=4-8). Binding affinities are reported in the following Table:

AGONIST	Ki (nM)	ANTAGONIST	Ki (nM)
HE-NECA	3.1 (2.4-4.0)	CGS 15943	0.4 (0.3-0.5)
NECA	61 (48-77)	8FB-PTP	0.8 (0.6-1.2)
CGS 21680	111 (83-148)	SCH 58261	1.1 (0.8-1.3)
CV 1808	332 (233-473)	XAC	9.0 (6.6-12)
R-PIA	992 (834-1183)	KF 17837S	9.4 (7.5-12)
CHA	2840 (2367-3408)	DPCPX	234 (124-445)
S-PIA	8504 (8178-8843)	8-PT	383 (263-557)

The order of potency of both agonists and antagonists was consistent with a selective interaction occuring at A_{2A} receptors. The Ki values for adenosine antagonists were similar to those labeled with the A_{2A} agonist [^{3}H]-CGS 21680 whereas affinities of agonists were generally lower (Jarvis et al., 1989). Except for 8-PT, both adenosine agonists and antagonists inhibited [^{3}H]-SCH 58261 binding with Hill coefficients not significantly different from unity.
The present results indicate that [^{3}H]-SCH 58261 is the first non-xanthine adenosine antagonist radioligand which directly labels A_{2A} striatal receptors. Thus, it appears to be an excellent probe for studying A_{2A} adenosine receptors.

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340P BOTH ISOMERS OF MEDETOMIDINE INHIBIT SYNAPTOSOMAL NORADRENALINE UPTAKE *IN VITRO* BUT NOT AT THERAPEUTICALLY RELEVANT CONCENTRATIONS *IN VIVO*

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It was recently reported that the α_2-adrenoceptor agonist, medetomidine (MED), inhibited rat noradrenaline (NA) uptake both in vitro and in vivo after infusion via a microdialysis probe in frontal cortex (Dalley & Stanford, 1995). The aims of this study were to investigate i) whether uptake inhibition was due to either or both of the isomers of MED i.e. d-MED, the isomer responsible for α_2-receptor activation or l-MED, which has only weak biological activity; ii) if d-MED can cause uptake blocking effects in vivo at therapeutically relevant doses.

^{3}H-NA uptake was measured in vitro using the method of Komulainen & Tuomisto (1981) in synaptosomes from frontal cortex of Wistar rats. In vivo NA uptake was assessed by i) the ability of MED isomers to block mouse frontal cortex NA-depletion by DSP-4 (N-2-chloroethyl-N-ethyl-2-bromobenzylamine 50 mg/kg i.p. 45 min after d- or l-MED 100 μg/kg, s.c., sacrifice 7 days later and ii) the ability of d- or l-MED (0.1 - 100 μg/kg i.v.) to modify cardiovascular responses to tyramine (TYR, 100 μg/kg, i.v.) in pentobarbitone-anaesthetized, hexamethonium-treated (10 mg/kg i.v) ganglion blockaded rats (≈ 400 g).

Both isomers of MED were active in vitro at inhibiting NA uptake; IC50 values (± s.e. mean of 5 cortices) were; d-MED 13.2 ± 3.8 μM; l-MED 14.9 ± 2.4 μM, slightly higher values than the 8 μM reported by Dalley & Stanford (1995) for racemic MED. However, neither d-MED 100 μg/kg, which induced loss of the righting reflex, nor l-MED (100 μg/kg) were able to prevent DSP-4 induced depletion in frontal cortex NA levels (table 1). DSP-4 is a drug which enters the nerves via NA uptake processes. None of the tested doses of d- or l-MED were able to prevent TYR-induced tachycardia, nor did l-MED block TYR-induced increase in blood pressure. D-Med (doses > 0.1 μg/kg) itself caused a marked increase in blood pressure by activation of post-synaptic α_2-receptors.

Table 1: Mouse frontal cortex NA concentrations 7 days after DSP-4 (50 mg/kg) with pretreatment with d- or l-MED (100 μg/kg, s.c. 45 min prior to DSP-4)

Treatment (no. of mice)	NA (nmol/g) ± s.e. mean
No DSP-4 (5)	2.28 ± 0.11
DSP-4 (4)	0.58 ± 0.11*
d-MED + DSP-4 (4)	0.53 ± 0.09*
l-MED + DSP-4 (4)	0.41 ± 0.10*

(* $P < 0.001$ vs No DSP-4 mice, ANOVA + Scheffe test)

In conclusion, both isomers of MED possess the ability to block NA uptake into synaptosomes in vitro. Thus, this property is not related to α_2-receptor activation (only found with d-MED). However, at anaesthetic concentrations in mice and at substantially greater levels in rats, neither d-MED nor l-MED were able to prevent the actions of compounds which exert their effects only after they have entered NA nerves via the uptake system. This indicates that functional uptake blockade does not occur at concentrations which can be achieved in vivo.

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341P AN INVESTIGATION OF THE EFFECT OF IMIDAZOLINE$_2$-SITE SELECTIVE COMPOUNDS 2-BFI AND BU224 ON [^{3}H]NORADRENALINE UPTAKE IN RAT BRAIN SLICES

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Imidazoline$_2$ (I_2) sites appear to have a functional interaction with monoamine oxidase (MAO). A number of I_2-site ligands inhibit MAO activity in rat adipose and liver tissue (Carpéné *et al* 1995) and chronic administration of the MAO_A inhibitor clorgyline downregulates I_2-sites in rat brain (Olmos *et al.*, 1993).

2-(-2-Benzofuranyl)-2-imidazoline (2-BFI) is an I_2 site selective ligand which elevates extracellular levels of noradrenaline in the rat hippocampus and frontal cortex *in vivo* (see Lalies & Nutt, 1995). Whilst MAO inhibition could account for this observation, a similar effect could result from inhibition of noradrenaline uptake. We have now examined the effects of 2-BFI and another selective I_2-site ligand, 2-(4,5-dihydroimidaz-2-yl)-quinoline (BU224), on [^{3}H]-noradrenaline ([^{3}H]NA) uptake into rat hippocampal and cortical slices. Their effects were compared with those of the selective NA uptake inhibitor, desipramine (DMI).

Cross-chopped (200 μm intervals) hippocampal and cortical slices were prepared from male Wistar rats (225-250g) and pre-incubated (5 min at 37°C in Tris-Krebs buffer, pH 7.4) with the test ligands (within the range 1nM-1mM) or buffer (to determine total uptake). [^{3}H]NA (50nM) was added for a further 10 min incubation and the assay rapidly terminated by vacuum filtration. Non-specific uptake was similarly determined at 4°C. The [^{3}H]NA content of the slices was then assayed by scintillation counting. The data were analysed and IC_{50}s determined from the inhibition curves using GraphPad Inplot software.

DMI potently inhibited NA uptake into hippocampal slices at nM concentrations. In cortical slices DMI also blocked uptake with nM efficacy. However, a second low affinity (μM) component, not apparent in the hippocampus, was also recognised (Table 1). This finding agrees with that of Michel et al (1984) in cortical synaptosomes. The selective I_2-site ligands, 2-BFI and BU224 only inhibited NA uptake at μM concentrations in both hippocampal and cortical slices (Table 1).

Table 1. IC_{50} values (mean ± s.e.mean, n = 3 - 4 experiments) for the inhibition of [^{3}H]NA uptake into rat hippocampal and cortical cross chopped slices.

Compound	Hippocampus	Cortex
DMI	41.5±18.9 nM	7.7±3.6 nM (high) 14.2±3.6 μM (low)
2-BFI	26.9±5.7 μM	33.6±4.4 μM
BU224	16.3±8.1 μM	27.8±3.2 μM

These results demonstrate that the selective I_2-site ligands, 2-BFI and BU224, are weak inhibitors of noradrenaline uptake, exhibiting a 1000 fold less potency than DMI. It is concluded that inhibition of noradrenaline uptake is unlikely to predominantly account for the ability of these ligands to increase extracellular levels of noradrenaline *in vivo*.

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342P DIFFERENCES IN THE NATURE OF NICOTINE-STIMULATED DOPAMINE (DA) RELEASE FROM THE TERMINAL REGIONS OF THREE ASCENDING DOPAMINERGIC PATHWAYS

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The ability of nicotine to enhance DA release in the mesolimbic dopaminergic pathway is thought to underlie nicotine's reinforcing properties (Di Chiara *et al.*, 1994). Systemically administered nicotine results in greater DA release from accumbens than from striatum or frontal cortex (Imperato *et al.*, 1986; Nisell *et al.*, 1994). Why the mesolimbic system is more sensitive to nicotine and the precise site of action remain unclear. Previously (Marshall *et al.*, 1995) we examined, using *in vivo* microdialysis, the mechanism by which nicotine, given locally via the dialysis probe, enhanced DA release in the striatum of male Sprague-Dawley rats (250-350g). At a low nicotine concentration ($3x10^{-3}$M) DA release was blocked by prior local administration of either mecamylamine or tetrodotoxin. Using the same technique we have here compared DA release from the striatum with that from the nucleus accumbens and the frontal cortex. All data analysed using two way ANOVA for repeated measurements.

In all 3 brain areas nicotine (10^{-3}-$3x10^{-1}$M) produced an immediate and dose-dependent increase in DA release (n=6-8). In the presence of 10^{-4}M mecamylamine, nicotine 10^{-3}-10^{-2}M failed to stimulate DA release in all 3 brain regions, whereas nicotine $3x10^{-2}$-$3x10^{-1}$M increased release to levels comparable with that in the abscence of mecamylamine (n=4). EC_{50} values for receptor-mediated mecamylamine-sensitive release were $3.5x10^{-3}$M in the accumbens and the cortex and $4x10^{-3}$M in the striatum. Release from the accumbens was of a similar magnitude to that of the striatum whereas release from the cortex was significantly ($p<0.05$) lower (e.g. peak DA release at $3x10^{-3}$M nicotine was 329% of basal in the striatum, 319% in the accumbens and 109% in the cortex). In the presence of 10^{-5}M tetrodotoxin, basal DA release was reduced in accumbens and cortex. Subsequent challange with a low nicotine concentration failed to stimulate DA release in accumbens (n=6) but did increase release in cortex (n=5) by an amount similar to that in the absence of tetrodotoxin.

These data suggest that similar receptor subtypes are involved in the nicotine-stimulated DA release in all 3 brain regions studied but that release from the cortex proceeds predominantly via a different mechanism, possibly by direct stimulation of presynaptic nicotinic receptors on dopaminergic terminals (Wonnacott *et al.*, 1990: Whiteaker *et al.*, 1995).

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343P CHARACTERISATION OF CYSTINE ACCUMULATION INTO RAT CEREBELLAR SLICES AND INHIBITORY CHARACTERISTICS of 2-S-L-CYSTEINYL PROPIONIC ACID

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Oral administration of L-2-chloropropionic acid (L-CPA; 750 mg/kg) to Alderley Park (AP) rats produces selective cerebellar granular cell necrosis within 48 hours following administration (Widdowson et al., 1995). This treatment also depletes cerebellar glutathione (GSH) in a time dependent manner with maximal depletion occurring 24 h after dosing (Wyatt et al., 1995), prior to granule cell necrosis. The depletion of GSH was not due to conjugation with L-CPA or as a result of oxidative stress (Wyatt et al., 1995). Preliminary studies had indicated that reduced L-cystine transport into the cerebellum, might be involved in the GSH depletion produced by L-CPA, since 100 µM L-2-cysteinyl propionic acid (2-CP) the cysteine conjugate of L-CPA, inhibited 10 µM [^{14}C]cystine accumulation into cerebellar slices by 50%. L-cysteine is one of the amino acids required for GSH synthesis (however, in-vitro, cysteine is rapidly converted to cystine which is transported into the brain as cystine and then rapidly reduced to L-cysteine once inside cells). We examined the kinetics of transport of cystine into the cerebellum and the effect of 2-CP on this process to see whether this may account for the decrease in cerebellar GSH seen after L-CPA treatment. Male AP rats (200-220 g) were killed by halothane overdose. Cerebellar slices (0.5 mm thick) were cut using a McIlwain tissue chopper and incubated at 37 °C under O_2 in Krebs buffer (pH 7.4) containing 5, 10, 20, 50, 100 and 200 µM [^{14}C]cystine. The accumulation of radiolabelled [^{14}C]cystine was measured at 0, 30, 60, and 120 min with three separate analyses performed using cerebellar slices from different animals. The rate of accumulation of [^{14}C]cystine was expressed as nmol/g wet weight/h and determined for each concentration using a linear regression fit. The accumulation of cystine into cerebellar slices was found to obey saturation kinetics with an apparent Km of 77 µM and a Vmax of 450 nmol/g wet weight/h using a linear regression fit to the data (correlation coefficient = 0.99). To characterise the inhibition of cystine accumulation further by 2-CP, cerebellar slices were incubated in 5, 10, 20, 50 and 100 µM [^{14}C]cystine with and without 100 µM 2-CP, for 2 h at 37 °C under O_2 with 3 separate determinations per concentration. The rate of accumulation was determined by subtracting the zero time point intercept, obtained from the studies used to derive the saturation kinetic constants, from the concentration of cystine found at 2h. Using a Hane plot and linear regression analysis, the inhibition observed was non-competitive with correlation coefficients of 0.93 and 0.97 for control and 100 µM respectively giving a Ki for 2-CP of approximately 60 µM. The addition of 1 mM L-glutamate to cerebellar slices gave only diffusion rates for [^{14}C]cystine uptake. The lack of active transport of [^{14}C]cystine into the slices was due to L-glutamate-induced cytotoxicity. Cytotoxicity was monitored by incubating cerebellar slices in [1-^{14}C]glucose and measuring the evolution of [^{14}C]CO_2 over 2 h. 1 mM L-glutamate reduced [^{14}C]CO_2 production by 18% ($P>0.01$) for 4 observations (Student's t-test) demonstrating a decreased cellular metabolism. The non-competitive inhibition of [^{14}C]cystine uptake into cerebellar slices by the metabolite of L-CPA, 2-CP suggests that the L-CPA-induced reductions in GSH concentrations in the cerebellum *in vivo* could be due to reduced GSH synthesis as a result of a reduction in substrate supply, notably cysteine.

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344P A COMPUTER SIMULATION TO DEMONSTRATE THE EFFECTS OF PHARMACOLOGICAL AGENTS OR PROCEDURES ON BLOOD PRESSURE AND HEART RATE OF THE ANAESTHETIZED RAT *IN VIVO*

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In recent years a large number of computer programs which simulate undergraduate pharmacological experiments or preparations have been demonstrated to the Society. These have a number of potential uses. For example, they may be used: to better prepare students who will the perform the experiment(s) at a later date; to debrief students after they have performed the experiment(s); as a fallback to provide data for students whose experiments were unsuccessful; as an alternative to real experiments if equipment and/or expertise is not available in a particular department. This program simulates a range of experiments which may be performed on the anaesthetized rat (*in vivo*) to investigate the action of a number of pharmacological agents or procedures on blood pressure and heart rate. It is suitable for undergraduates from science, medical and a range of biomedical courses which include pharmacology modules.

The program was developed using Multimedia Toolbook® (Asymetrix) to run on IBM PC compatibles (minimum delivery platform: 386 SX, 20 MHz PC running Windows™ version 3.1 (Microsoft), a sixteen colour VGA monitor and a mouse). The main menu allows students to access sections covering different aspects of the laboratory class. **Introduction** covers Home Office Licence requirements; **Preparation** covers anaesthesia and anaesthetization, cannulation of trachea, jugular vein and carotid artery; **Apparatus** covers the equipment used to maintain body temperature, record blood pressure and heart rate; **Measurements** describes how to take measurements from the simulated chart recorder display and how to calculate mean BP and pulse pressure. Each of these sections combines text, high quality colour graphics, and animation with interactive questions designed to reinforce learning. **Experiments** is a large section providing typical data for 16 different experiments.These include actions of: catecholamines (noradrenaline (NA), adrenaline, isoprenaline); pressor agents (adrenaline, vasopressin, phenylephrine); acetylcholine (acetylcholine (high and low doses), atropine); ganglion stimulants (DMPP in normal and atropinised animal); uptake$_1$-blockers (cocaine on NA, tyramine, phenylephrine); alpha-blockers (prazosin on NA, vasopressin and phenylephrine); beta-blockers (propranolol on isoprenaline, adrenaline); adrenaline reversal (NA, NA+prazosin, NA+prazosin+ propranolol); guanethidine (on NA, vasopressin, sympathetic nerve stimulation (SNS)); SNS (effects of hexamethonium, prazosin, propranolol and guanethidine on responses to NA and SNS); depressor drugs (histamine, acetylcholine, isoprenaline, hexamethonium) ; ganglion blockade (hexamethonium on responses to NA, vasopressin); quantitative effects of alpha-blockade (several doses of adrenaline with/without prazosin); quantitative effects of beta-blockade (several doses of adrenaline with/without propranolol); reserpine (NA, tyramine, and SNS in normal and reserpine-treated rats); pithing (NA, tyramine and SNS in normal and pithed rats.

Students are expected to record and tabulate data from the screen display and to then complete student assignments. Two different assignments are offered for each experiment: (i) a series of MCQ questions, with feedback, to assess accuracy of data collection and data interpretation; (ii) a student task (typical of a traditional lab-class report) to be completed in their own time. Tutors may direct students to complete either or both of these tasks or design their own assessments. In addition there is a section containing a selection of MCQ's with feedback covering cardiovascular pharmacology which students can use for revision.