## 205P CHARACTERISATION OF ASPARTATE AND GLUTAMATE INVOLVEMENT IN FOCAL ISCHAEMIA AND SPREADING DEPRESSION: AN IN VIVO MICRODIALYSIS STUDY IN FREELY-MOVING RATS

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Excitatory amino acid neurotransmitters play an important role in normal brain function and possibly in the development of tissue damage in cerebral ischaemia (Rothman & Olney, 1986). This study has measured cortical aspartate and glutamate efflux in conscious rats after focal ischaemia compared with sham surgery (a noxious insult to the cortical surface).

Microdialysis probes (Hospal membrane, 2mm) were implanted in the parietal cortex (mm from bregma; AP +1.6, L -5.5, V -6.0, jawbar set at -3.3) of male Sprague-Dawley rats (275-325g) and perfused (2µl/min) with artificial CSF. Twenty-four h later rats were anaesthetised with halothane in $O_2$ and subjected to anaesthetic alone (n=7), sham operation (cauterisation of the cortex, n=7) or permanent, middle cerebral artery occlusion (MCAo; n=7) using the method of Tamura *et al.*, (1981). Rats recovered from anaesthesia 15-20 min later. Ten min fractions were collected for 4h, followed by 20 min fractions for 2h. Following paraformaldehyde fixation, brain damage was determined using coronal sections cut at 250µm intervals, stained with haematoxylin and eosin. Aspartate and glutamate in microdialysates were measured by HPLC with electrochemical detection (Smith *et al.*, 1996). Statistical analysis of data was by determination for each animal, of area under the curve (AUC) of activity over 150% of basal levels and also the number of fractions above this threshold, using Wilcoxon rank-sum test. All data are mean ± s.e. mean.

Ischaemic damage was present 6 hours after MCAo in caudate-putamen and parietal, frontal, insular, pyriform and forelimb cortical areas. After sham operation, damage was limited to the surgical site and did not extend to the probe sampling region.

Basal levels (pmol/25µl injected onto the column) were: aspartate, 0.90±0.10 anaesthetic control; 0.93±0.11 sham-operated; 1.31±0.12 MCAo; glutamate, 2.68±0.38 anaesthetic control, 2.04±0.31 sham-operated; 2.88±0.38 MCAo. MCAo rats generally showed rapid, prolonged, increases in aspartate (48.6±11.7% fractions >150%; $P<0.001$ for AUC) and glutamate (65.2±13.3% fractions >150%; $P<0.05$ for AUC) compared with sham-operated rats. Sham-operated animals showed random, sharply delineated increases of glutamate (23.8±3.6% fractions >150%; $P<0.005$ for AUC) compared with the anaesthetic-treated group. Anaesthetic-treated rats did not show alterations of these neurotransmitters.

Elevated levels of aspartate and glutamate found after MCAo were due to anoxic depolarisation triggered by persistent, high $K^+$ levels in the ischaemic core (Katayama *et al.*, 1991). Sham-operated rats showed transient elevations of glutamate after the remote insult, resembling spreading depression. However, tissue damage was not present. Spreading depression refers to waves of depolarisation which migrate slowly through the cortex in response to injury and may enhance neuronal damage in the ischaemic penumbra (Iijima *et al.*, 1992). This study has demonstrated, using *in vivo* microdialysis in conscious rats that glutamate, but not aspartate is probably involved in spreading depression propagation and has shown dramatically increased levels of both neurotransmitters in focal ischaemia.

Iijima, T., Mies, G. & Hossman, K-A. (1992) *J. Cereb. Blood Flow Metab.* 12, 727-733
Katayama,Y., Kawamata, T., Tamura, T. *et al.* (1991) *Brain Res.* 558, 136-140.
Rothman, S. & Olney, J. (1986) *Ann. Neurol.* 19, 105-111.
Smith, S.L., Martin, K.F., Heal, D.J. *et al.* (1996) *Br. J. Pharmacol.* 120, P368.
Tamura, A., Graham, D.I., McCulloch, J. *et al.* (1981) *J. Cereb. Blood Flow Metab.* 1, 53-60.

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## 206P THE EFFECTS OF THE SELECTIVE GROUP II METABOTROPIC GLUTAMATE RECEPTOR AGONIST LY354740 ON LOCAL CEREBRAL GLUCOSE METABOLISM IN THE RAT BRAIN

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The use of metabotropic glutamate receptor (mGluR) ligands as pharmacological tools for examining receptor functions *in vivo* has been limited due to their poor bioavailability in the CNS and inadequate receptor specificity. Previous studies have demonstrated that the novel mGluR agonist (1S, 2S, 5R, 6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate, LY354740, is effective systemically (Bond *et al.*, 1997) and is selective for mGluR2/3 receptors (Schoepp *et al.*, 1997). The aim of this study was to investigate the effects of LY354740 following systemic administration on function related glucose use using the $^{14}$C-2-deoxyglucose ([$^{14}$C]-2DG) autoradiographic technique (Sokoloff *et al.*, 1977).

Twenty-three male SD rats (300-400 g) were anaesthetised with halothane. The femoral vein and artery were cannulated, and externalised at the nape of the neck. Following recovery from the anaesthetic. LY354740, dissolved in saline (0.3, 3.0, and 30 mg/kg) was administered i.v. 10 minutes prior to the i.v. injection of 50 µCi [$^{14}$C]-2DG. Control animals received vehicle (0.9% saline). Following the pulse of [$^{14}$C]-2DG, arterial samples were collected at fixed time points over the next 45 minutes. Local rates of glucose utilisation were assessed in 42 brain regions quantitatively using a computer based densitometer (MCID). Arterial blood pressure, rectal temperature and blood gases were monitored during the experiment. All the data were analysed for statistical significance by ANOVA followed by Student's unpaired *t*-tests with a Bonferroni correction factor of 3.

LY354740 produced anatomically widespread and dose-dependent changes in glucose utilisation. No significant changes in glucose use were detected in animals treated with LY354740 (0.3 mg/kg). At LY354740 (3.0 mg/kg), 4 out of the 42 brain areas measured demonstrated statistically significant changes from vehicle treated controls: the superficial layer of the superior colliculus (+50 %), red nuclei (-16 %), mammillary body (-25 %) and anterior thalamus (-29 %). An additional 15 brain regions displayed statistically significant reductions in function related glucose use ($P<0.05$) in animals treated with LY354740 (30 mg/kg). The changes were observed primarily in the limbic system and areas associated with vision. The detected changes in glucose use demonstrate the important functional involvement of the limbic system as well as the participation of components of the visual system in response to the activation of mGluR 2/3 with LY354740.

Bond, A., Monn, J. A. and Lodge, D. (1997) *NeuroReport*, 8(6):1463-1466.
Schoepp, D. D. *et al.*, (1997) *Neuropharmacology*, 36(1):1-11.
Sokoloff, L. *et al.*, (1977) *Journal of Neurochemistry*, 28:897-916.

## 207P A ROLE FOR PRESYNAPTIC GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN THE CONTROL OF GLUTAMATE RELEASE IN THE RAT STRIATUM: AN *IN VIVO* MICRODIALYSIS STUDY

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The control of glutamate release from central neurones by presynaptic autoreceptors is at present poorly understood. The metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors which are classified into 3 groups based on their pharmacology, signal transduction mechanisms and sequence homologies: group I ($mGluR_1$ and $mGluR_5$), group II ($mGluR_2$ and $mGluR_3$) and group III ($mGluR_4$ and $mGluR_{6-8}$). We have recently demonstrated that activation of group I mGluRs enhances glutamate release in the rat forebrain in vitro (Croucher et al., 1997; Thomas et al., 1997). We now report the actions of the selective group I mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) on synaptic glutamate release in vivo using rapid, dual-label microdialysis of the corpus striatum in conscious, freely-moving rats.

Male Wistar rats (275-300g) were implanted bilaterally with dialysis monoprobes aimed at the corpus striatum, under Immobilon anaesthesia (0.23ml/kg,i.m.). Preloading with [$^3$H]L-glutamate and [$^{14}$C]GABA, and subsequent dialysis with ACSF in the presence or absence of drugs, was performed as previously described (Young & Bradford, 1993). 15min samples (7.5µl/min) were collected and $^3$H and $^{14}$C contents were determined by liquid scintillation spectrometry. DHPG (0.1-300µM) enhanced the release of [$^3$H]L-glutamate with a bell-shaped dose-response curve (max. enhancement 137.0±7.0% of basal at 100µM; n=8; $P<0.01$). A significant fall in response at the highest dose tested (to 119±5.0% of basal, n=9; $P<0.05$) is consistent with the known desensitization properties of group I mGluRs (Herrero et al., 1994). (RS)-1-Aminoindan-1,5-dicarboxylic acid (AIDA), a structurally novel, selective group I mGluR antagonist (Costantino & Pellicciari, 1996), 10-300µM, dose-dependently inhibited the DHPG (10µM)-mediated release of [$^3$H]L-glutamate (max. inhibition 54% at 300µM; estimated $IC_{50}$ 255µM). 7-(Hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester (CPCCOET), another novel mGluR antagonist acting selectively at the $mGluR1_\alpha$ subtype (Batchelor et al., 1997), 30-300µM, showed no significant inhibitory activity against the agonist-induced response. Neither of the antagonists showed significant effects when tested alone. The non-selective mGluR antagonist MCPG (≤ 1mM) also caused small, but statistically insignificant, reductions in DHPG-evoked [$^3$H]L-glutamate release. However, the presence of this antagonist alone caused significant increases in efflux of label from the striatum.

These data provide in vivo pharmacological evidence for a role of presynaptic mGluRs, possibly of the $mGluR_5$ subtype, in the positive modulatory control of neuronal glutamate release in the rat striatum. Qualitatively similar responses to DHPG in [$^{14}$C]GABA release also support a role for presynaptic group I mGluRs in the control of release of other central neurotransmitters. Such receptors may provide targets for novel therapeutically useful drugs.

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Batchelor, A.M., Knöpfel,T., Gasparini, F. & Garthwaite, J. (1997) *Neuropharmacology* 36(3), 401-403.
Costantino, G. & Pellicciari, R. (1996) *J. Med. Chem.* 39, 3998-4006.
Croucher, M.J. Morant, R.J., Thomas, L.S. & Jane, D.E. (1997) *The Pharmacologist* 39(1), 215.
Herrero, I., Miras-Portugal, M.T. & Sanchez-Prieto, J. (1994) *Eur. J. Neurosci.* 6, 115-120.
Thomas, L.S, Jane, D.E. & Croucher, M.J. (1997) *This meeting*.
Young, A.M.J. & Bradford, H.F. (1993) *J. Neurochem.* 63, 231-237.

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## 208P ENHANCEMENT OF NEURONAL GLUTAMATE RELEASE IN THE RAT FOREBRAIN BY (S)-3,5-DIHYDROXYPHENYLGLYCINE, A PRESYNAPTIC GROUP I METABOTROPIC GLUTAMATE RECEPTOR AGONIST

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Glutamate is the principal neurotransmitter in the mammalian CNS mediating synaptic neurotransmission via two classes of receptors, namely the ionotropic receptors, comprising AMPA, kainate and NMDA subtypes and the metabotropic receptors. The latter are divided into three groups: group I receptors consist of subtypes $mGluR_1$ & $mGluR_5$ and are G-protein-linked to phospholipase C and polyinositolphosphate metabolism, whilst group II ($mGluR_{2\&3}$) and group III ($mGluR_{4,6,7\&8}$) receptors are both negatively coupled to adenylate cyclase (Pin & Duvoisin, 1995).

Herrero and co-workers (1992) previously demonstrated that non-selective stimulation of presynaptic mGluRs by the agonist (1S,3R)-ACPD caused an enhancement of 4-aminopyridine-stimulated glutamate release from rat cerebrocortical synaptosomes. We now report the actions of the selective group I mGluR agonist (S)-3,5-dihydroxyphenylglycine ((S)-3,5-DHPG) on basal and electrically-stimulated glutamate release in rat forebrain slices using [$^3$H]D-aspartate ([$^3$H]D-asp) as a non-metabolisable marker for glutamate.

Serial forebrain slices were cut and incubated as previously described (Patel & Croucher, 1997). After loading with [$^3$H]D-asp (40nM final concentration) slices were superfused with oxygenated Krebs buffer and the influence of drugs on basal and electrically stimulated release of label was examined using a standard protocol (Patel & Croucher, 1997). Release in this preparation is $Ca^{++}$-dependent and tetrodotoxin-insensitive, consistent with its origin from presynaptic nerve terminals. Results are means of at least 5 independent observations. (S)-3,5-DHPG, 0.1-3µM, dose-dependently potentiated electrically stimulated efflux of [$^3$H]D-asp (maximum enhancement 337% at 3µM; graphically estimated $ED_{50}$ 0.9µM) whilst having no effect on basal release. At higher doses (10-100µM), smaller, non-significant responses were apparent, consistent with desensitization of group I mGluRs. The selective mGluR antagonist (S)-α-methyl-4-carboxyphenylglycine ((S)-MCPG), 1-100µM, fully inhibited the response to (S)-3,5-DHPG, 1µM ($P<0.05$). However, the proposed $mGluR_{1\alpha}$ selective antagonist (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA) (Costantino & Pellicciari, 1996) did not significantly attenuate this response at concentrations up to 500µM. Interestingly, the previously defined broad spectrum ionotropic glutamate receptor antagonist kynurenic acid (KYN), 1-100µM, also blocked the response to (S)-DHPG, 1µM, in a dose-related manner with full inhibition apparent following KYN, 100µM ($P<0.05$). None of the antagonists influenced basal or stimulated efflux of [$^3$H]D-asp when given alone. These results demonstrate the presence of group I mGluRs on presynaptic nerve terminals in the rat forebrain acting to enhance the release of neuronal glutamate. Further characterisation using receptor sub-type selective ligands should allow us to define the specific mGluR mediating this response.

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Costantino, G. & Pellicciari, R. (1996) *J. Med. Chem.* 39, 3998-4006.
Herrero, I., Miras-Portugal, M.T. & Sanchez-Prieto, J. (1990) *Nature* 360, 163-166.
Patel, D.R. & Croucher, M.J. (1997) *Eur. J. Pharmacol.* 332, 143-151.

## 209P GLUTATHIONE: HOW IMPORTANT IS IT IN L-2-CHLORPROPIONIC ACID NEUROTOXICITY TO THE CEREBELLAR GRANULE CELL-STRUCTURE ACTIVITY STUDY?

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Administration of a single large dose of 750mg/kg L-2-chloropropionic acid (L-CPA) or daily administration of 250mg/kg for 3 days to male Alderley park rats produces selective necrosis to the granule cell layer of the cerebellum without affecting the granule cells in the forebrain (Simpson *et al*, 1996). In association with the extensive loss of cerebellar granule cells, increases in oedema and $Na^+$ and loss of aspartate and glutamate were observed in the cerebellum (Widdowson *et al*, 1996). Prior treatment with 5mg/kg/ip MK-801, a non-competitive NMDA receptor antagonist, prevents all the events seen following L-CPA treatment, suggesting that L-CPA may by activating a subpopulation of NMDA receptors located on cerebellar granule cells(Widdowson *et al*, 1996).

L-CPA undergoes rapid conjugation with glutathione (GSH) in the liver via a theta class glutathione S-transferase, whilst in the cerebellum GSH depletion takes up to 24h to reach maximal depletion (85% control), but this is not due to conjugation and the extent of depletion is greater in the cerebellum than in the forebrain (Wyatt *et al*, 1996a). It has also been shown that the cysteine conjugate of L-CPA can inhibit cystine delivery to the cerebellum and this may affect GSH synthesis and hence account for the depletion of GSH in the cerebellum(Wyatt *et al*, 1996b). In the same paper it was also shown that the maintenance of GSH concentration in the cerebellum, using the isopropyl ester of GSH, will protect rats from L-CPA neurotoxicity. Thus GSH status also appears to be important in L-CPA neurotoxicity.

We have studied whether 2-fluoro, 2-bromo and 2-iodo-propionic acids (2-FPA, 2-BPA and 2-IPA), can form GSH conjugates (which will be identical conjugates to that formed with L-CPA- 2-S-glutathionyl propionic acid), have affinity for cerebellar NMDA receptors and are neurotoxic to cerebellar granule cells.

In liver cytosol preparations (Wyatt *et al*, 1996a) 2-FPA did not conjugate with GSH whilst, L-CPA, 2-BPA and 2-IPA conjugated to GSH with Km's of 145, 95 and 151μM and Vmax's of 75, 79 and 123nmol/mg/h for L-CPA, 2-BPA and 2-IPA respectively (n=3 greater than 95% correlation). In cerebellar cytosol no conjugation with GSH was observed for 2-FPA or 2-BPA like L-CPA, however with 2-IPA conjugation was seen. In order to assess whether 2-FPA, 2-BPA and 2-IPA had any interaction at the NMDA receptor, we studied the ability at $10^{-2}$-$10^{-6}$M of the compounds to displace [$^3$H]MK-801 (2.5nM) from cerebellar and forebrain synaptosomal preparation. There was no displacement in the forebrain however, the $IC_{50}$'s in the cerebellum were 2.1, 2.3, 0.3 and 1.8mM for 2-FPA, L-CPA, 2-BPA and 2-IPA respectively (n=3). Male Alderley Park rats (180-200g body weight) were dosed with 2.3mmol/kg of neutralised halopropionic acids with 5 animals per group. Four doses of 2-FPA had no effect and minimal biochemical alterations. Twenty four hours after 3 doses of L-CPA and 2 doses of 2-BPA the cerebellar granule cells were necrotic and cerebellar water and $Na^+$ content increased and amino acids concentrations reduced with no other organ showing pathological damage. However 2-IPA proved fatal after 2 doses with the kidneys, liver and cerebellum showing pathological damage.

The inability of 2-FPA to conjugate with GSH *in vitro* and *in vivo* and the fact that it was as active at the NMDA receptor as L-CPA would indicate that GSH status in the cerebellum is important in the cerebellar lesion following L-CPA and 2-BPA treatment.

Simpson, M.G. *et al.* (1996) *Neurotoxicology* **17**, 471-480.
Widdowson,P.S. *et al.*(1996)*Toxicol. Appl. Pharmacol.***136**,138 -145.
Wyatt, I. *et al.*(1996a &b) *Arch. Toxicol.* **70**, 380-389 & 724-735.

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## 210P EFFECTS OF RX821002 ON CHANGES IN EXTRACELLULAR NORADRENALINE CONCENTRATION IN RAT FRONTAL CORTEX INDUCED BY SIBUTRAMINE OR *d*-AMPHETAMINE

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Sibutramine (SIB) and *d*-amphetamine (*d*-AMP) inhibit noradrenaline (NA) reuptake (Buckett *et al.*,1988; Thornburg and Moore, 1973) but *d*-AMP, also increases NA release (Aspley *et al.*, 1996). Previous microdialysis experiments showed that increased extracellular NA induced by *d*-AMP in rat frontal cortex is more rapid than that caused by SIB (Wortley *et al.*, 1997). Also, extracellular NA after *d*-AMP, increases with dose, whereas the effect of SIB has a bell-shaped dose-response curve; maximum ~1 mg $kg^{-1}$. These findings show that, unlike *d*-AMP, NA release is diminished after high doses of SIB. The study investigated whether presynaptic $\alpha_2$-adrenoceptors are involved.

Microdialysis probes were implanted in the frontal cortex (mm:AP +3.5; L +1.5; V -5.0 from bregma) of male SD rats (250-350 g) under halothane anaesthesia which was maintained throughout the experiment. After perfusion (1μl $min^{-1}$) with aCSF for 2 h, samples were collected at 20 min intervals and analysed for NA using HPLC-ECD. After 4 basal samples, the 1st group of rats were injected with the $\alpha_2$-adrenoceptor antagonist, 2-(2,3-Dihydro-2-methoxy-1, 4-benzodioxin-2-yl)-4, 5-dihydro-1H-imidazole hydrochloride (RX, 1 mg $kg^{-1}$i.p.) and samples collected for 2 h. Following this, a further 3 mg $kg^{-1}$ RX was injected and samples collected for another 2 h. A 2nd and 3rd group of rats were pre-treated with RX (3 mg $kg^{-1}$) and, 1h later, injected with SIB (10 mg $kg^{-1}$ i.p.) or *d*-AMP (10 mg $kg^{-1}$ i.p.); samples were collected for a further 3 h. Raw data were analysed using 2-way split-plot ANOVA.

RX did not affect extracellular NA at either dose. Compared with basal samples (30 ± 2 fmol 20 $min^{-1}$), NA increased when SIB (173%, $F_{1,16}$=5.24; P=0.036) or *d*-AMP (549%, $F_{1,4}$=247.34; P<0.001) was administered. RX pretreatment significantly potentiated the effect of both SIB (1032%, $F_{1,30}$=12.85; P=0.001) and *d*-AMP (1363% $F_{1,24}$=15.85; P=0.001) on extracellular NA. However, even after RX, *d*-AMP caused a greater ($F_{1,29}$=9.58; P=0.004) and more rapid increase in NA than SIB. Finally, RX reduced the latency to reach peak extracellular NA concentration after SIB but not after *d*-AMP.

These results show that $\alpha_2$-adrenoceptor blockade with RX does not alter basal extracellular NA indicating there is little tonic activation of these receptors under drug-free conditions. Since RX pretreatment potentiated the effects of SIB and *d*-AMP on extracellular NA, it appears that the increase in NA caused by both drugs is sufficient to activate presynaptic $\alpha_2$-adrenoceptors. However, when these receptors were blocked with RX, the time to peak extracellular NA concentrations after SIB, but not *d*-AMP, was reduced suggesting that increased NA caused by SIB is by inhibition of NA reuptake, whereas the increase caused by *d*-AMP is predominantly due to NA release. *d*-AMP-induced NA release would also explain why, even in the presence of RX, the increase in extracellular NA is much greater with *d*-AMP than SIB.

KEW is a Knoll Pharmaceuticals scholar. ZAH is an MRC scholar.

Aspley, S., Broughton, D.M., Prow, M.R. *et al.*, (1996) *Br. J. Pharmacol.* 120. 353P
Buckett, W.R., Thomas, P.C., Luscombe, G.P. (1988) *Neuro-Psychopharmacol. Biol. Psychiatr.* 12. 575-484
Thornburg, J. & Moore, K. (1973) *Res. Comm. Chem. Path. Pharmacol.* 5. 81-89
Wortley, K.E., Hughes, Z.A., Mason, K., *et al.*, (1997) *Br. J. Pharmacol.* 122: 100P

## 211P EFFECTS OF ANTIDEPRESSANT TREATMENTS ON BDNF GENE EXPRESSION IN RAT BRAIN

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We have recently shown that certain voltage-dependent potassium channel genes whose corresponding proteins are exclusively located on the dendrites of the granule cells are upregulated following chronic ECS but not after a single ECS (Pei et al, 1997). This suggests that the formation of new dendritic elements is taking place after chronic but not acute ECS treatment. Brain-derived neurotrophic factor (BDNF) could be a possible mediator of such an effect, since previous studies have shown that ECS enhances gene expression of BDNF and its receptor trk*b* in rat brain (Lindefors et al, 1995). The aim of the present study was to compare the magnitude and duration of action by both chronic and single ECS on rat brain BDNF gene expression. Further, in order to confirm previous results that stimulation of BDNF gene expression might be a common effect of antidepressant treatments (Nibuya et al, 1995), we have also investigated the effect of long-term treatment with tranylcypromine (TCP) and fluoxetine (FLX) on BDNF gene expression in rat brain.

Male Sprague-Dawley rats (250-270g) were used in all experiments. In the ECS study rats were anaesthetised with halothane and received either chronic ECS (150V, 50Hz, 1s, via an ear clip electrode, 5 shocks over 10 days) or a single shock. Control rats were anaesthetised with halothane and had electrodes placed but no current delivered. Animals were sacrificed at 6h, 24h, 48h or 3 weeks after the last ECS. Rats in the drug treated groups were injected twice daily with either TCP (2.5 mg/kg, i.p.) or FLX (10 mg/kg, i.p.) for 14 days and sacrificed 24h after the last injection. Hippocampal sections (12 μm) were mounted on slides and pre-treated for ISHH using a standard procedure (Pei et al, 1997). After pre-treatment, an oligonucleotide complementary to bases 642-686 of the rat BDNF cDNA was 3'-tail labelled with [$^{35}$S]-dATP and added to the brain sections. The relative abundance of BDNF mRNA was determined by densitometric quantification of autoradiograms in dentate gyrus (DG), CA1, CA3 and piriform cortex. Values (mean±s.e.mean) are expressed as a percentage of mean of optical densities from the matched controls. Statistical analyses of mRNA changes were performed using one way ANOVA and Dunnetts t-test.

Following acute ECS, the relative abundance of BDNF mRNA in the DG and piriform cortex was increased 6h after the shock by 166±35% and 105±10.5%, (n=5 rats/group, $p<0.001$) respectively. Values were back to control levels 24h after the shock. Chronic ECS also increased BDNF mRNA abundance in the DG by 105±10.7% and in piriform cortex by 107±8.9% at 6h, but this effect remained significant at both 24h and 48h after the last shock, and returned to control levels at three weeks. Chronic administration of both TCP and FLX also elevated BDNF gene expression in DG by 76±6.2% and 43±4.2% respectively ($p<0.001$). None of the treatments induced significant changes in BDNF gene expression in any other regions measured

In summary we have shown that both acute and chronic ECS increases BDNF gene expression but that the effect of chronic ECS is much longer lasting. Long term treatment with both TCP and FLX also increased BDNF gene expression confirming previous findings by Nibuya et al, (1995) and indicating that this might be a common action of antidepressant treatments.

Lindefors, N., *et al* (1995) *Neuroscience*, **65**, 661-670
Nibuya, M., *et al* (1995) *J. Neuroscience*, **16**, 2365-2372.
Pei, Q., *et al*, (1997) *Neuroscience*, **78**, 343-350.

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## 212P VOLTAGE- AND USE-DEPENDENT INHIBITION OF VOLTAGE-GATED $Na^+$ CHANNELS IN RAT SENSORY NEURONES BY THE NOVEL ANTIHYPERSENSITIVITY AGENT 4030W92

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Neuronal voltage-gated $Na^+$ channels are essential for action potential propagation and nerve conduction and represent important molecular targets for anticonvulsant and anaesthetic drugs (Catterall, 1992). Interestingly, low concentrations of certain local anaesthetic drugs also produce analgesia (see Nagy & Woolf, 1995 for refs.). Thus, neuronal $Na^+$ channels may provide a promising avenue for research into novel analgesic compounds. Here we describe the actions of a novel chemical entity, 2,4-diamino-5-(2,3-dichlorophenyl)-6-fluoromethyl pyrimidine (4030W92) on voltage-gated $Na^+$ currents in rat sensory neurones.

Dorsal root ganglia from 3-4 week old rats (AHA) of either sex were enzymatically dissociated (0.125% collagenase, 60min followed by 0.025% trypsin, 10min). Neurones were plated on poly-DL-ornithine and laminin treated glass coverslips for whole cell patch clamp recording using an Axopatch 200B amplifier. All experiments were performed on neurones of 10-25μm somatic diameter.

Under voltage clamp and conditions designed to isolate $Na^+$ currents in these cells (Elliott & Elliott, 1993), depolarising steps from a holding potential ($V_h$) of -90mV evoked graded inward currents at potentials positive to -40mV. Both slow, tetrodotoxin-resistant (100nM; $TTX_R$) and fast, tetrodotoxin-sensitive ($TTX_S$) $Na^+$ currents were observed, either in isolation or together (Elliott & Elliott, 1993). 4030W92 produced only a weak inhibition of the peak $TTX_R$ current evoked by a step to 0mV from a $V_h$ of -90mV (geometric mean $IC_{50}$ value 103μM [95% confidence limits 58-166μM]). However, when cells were held at a $V_h$ of -60mV, 4030W92 was significantly more potent (22μM [13-38μM), indicating a voltage-dependent action ($P<0.05$; data from 5-11 cells). Use-dependent block of $TTX_R$ by 4030W92 (30μM) was also evident: the ratio of the 20th to the 1st pulse in a train (10Hz, 20ms duration) was 0.65±0.09 (mean±s.e.m) in the absence of drug compared to 0.48±0.10 in the presence ($P<0.05$, n=4). 4030W92 (30μM) produced a significant hyperpolarising shift in the steady state-inactivation curve of $TTX_R$ from a $V_{1/2}$ value of -40±2mV to -50±1mV (n=5; $P<0.05$). In 4 neurones in which $TTX_S$ alone was observed, 4030W92 also exhibited marked voltage- and use-dependent inhibitory effects. The mean $IC_{50}$ values for currents evoked from either a $V_h$ of -90 or -70mV were 37μM [22-61μM] and 5μM [4-9μM], respectively.

High threshold sustained $Ca^{2+}$ currents, obtained by depolarising steps to +20mV ($V_h$ -60mV), were only weakly inhibited by 4030W92 (20±3% inhibition at 100μM, n=8). Low-threshold, transient T-type currents, observed in 6/8 neurones ($V_{test}$ -30mV, $V_h$ -60mV) were reduced by 17±6% (n=3) and 43±8% (n=6) at concentrations of 30 and 100μM, respectively. 4030W92 did not modify the shape of the current voltage-relationship or the kinetics of activation or inactivation.

Thus, 4030W92 is a potent, voltage- and use-dependent inhibitor of $Na^+$ channels in small diameter, presumably nociceptive, sensory neurones of the rat. The enhanced effects of 4030W92 under depolarised conditions and upon repeated channel activation may underlie the preferential inhibition of sensitised *vs* non-sensitised (i.e. baseline) pain-related behavioural parameters *in vivo* (Evans *et al.*, this meeting). Indeed, recent evidence linking the hyperalgesic effects of prostaglandin $E_2$ with a positive regulation of $TTX_R$ (i.e. enhanced amplitude and decrease in activation threshold; Gold *et al.*, 1996) may provide a molecular basis for the antihypersensitivity profile of 4030W92. Further studies are in progress to test these hypothesis.

Catterall, W.A. (1992). *Physiol. Rev.*, **72**, S15-S48.
Evans K.S. *et al.* (1997). This meeting.
Elliott, A.A. & Elliott, J.R. (1993). *J. Physiol.*, **463**, 39-56.
Gold, M.S., *et al.* (1996b). *Proc. Natl. Acad. Sci.*, **93**, 1108-1112.
Nagy, I. & Woolf, C.J. (1995). *Pain*, **64**, 59-70.

## 213P THE NOVEL SODIUM CHANNEL BLOCKER 4030W92 INHIBITS CARRAGEENAN-INDUCED CUTANEOUS HYPERSENSITIVITY IN THE ANAESTHETISED RAT

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Following intraplantar carrageenan, second order dorsal horn neurone (DHN) responses to transcutaneous electrical stimulation (via A and c fibres) are sensitised. (Evans *et al.*, 1997). We have investigated the effect of the sodium channel blocker 4030W92 [R(-) 2,4-diamino-5-(2,3 dichlorophenyl)-6-fluoromethylpyrimidine; Trezise *et al.*, this meeting], on these electrically evoked DHN responses, before and after carrageenan. Male Wistar rats (250-350g) were anaesthetised (sodium pentobarbitone; 4mgkg$^{-1}$h$^{-1}$ i.v.) and prepared for extracellular single unit recording from DHN (L4-5). DHN exhibiting wind-up to trains of current pulses (0.5Hz, 3x c fibre threshold) were stimulated electrically (via A & c fibres, 0.1Hz) before and after 2% carrageenan (i.pl.). 4030W92 or vehicle (saline) was administered (i.v.) 15 minutes before carrageenan. Results (sum of action potentials per train) were expressed (% pre-drug controls, mean±s.e.mean). Student's paired and unpaired t-tests were used to determine statistical significance, $p<0.05$. Following carrageenan, DHN responses to transcutaneous electrical stimulation of A and c fibres were significantly enhanced at 2h and 4h compared with time matched controls (Table 1). 4030W92 (3mgkg$^{-1}$i.v.) significantly inhibited the sensitised A fibre evoked DHN responses at 2h and 4h and the sensitised c fibre evoked responses at 4h only (Table 1). 4030W92 had no significant effect on baseline A and c fibre evoked DHN responses (88%±11.7 & 101.6%±18.1 of pre-drug controls, respectively). In conclusion, 4030W92 significantly inhibited carrageenan-induced sensitisation of both A and c fibre evoked DHN responses but with differential effects over time. 4030W92 is therefore an effective anti-hypersensitivity agent and may provide a novel approach in the treatment of inflammatory pain conditions.

Evans, K.S., Scott, C.M. & Bountra, C. (1997). *Br. J. Pharmacol.* 122, 26P

Trezise, D., John, V.H., Nobbs, M. *et al.* (1997) This meeting.

Table 1. Effect of 4030W92 (3mgkg$^{-1}$i.v.) on the carrageenan-induced sensitisation of electrically stimulated DHN responses

| | DHN responses (% predrug controls) | | | |
|---|---|---|---|---|
| | A fibres (2h) | A fibres (4h) | c fibres (2h) | c fibres (4h) |
| time-matched controls | 68.4±9.9 | 75.4±10 | 72.5±14.1 | 93.6±25.8 |
| carrageenan+vehicle | 132.2±25.4* | 173.3±40.7* | 141.9±16.1* | 162.6±26.9* |
| carrageenan+4030W92 | 73.7±16.9# | 62.9±14.8# | 136.1±45.8# | 58.4±9.9# |

Student's unpaired t-test; * vs time-matched controls, # vs vehicle, where $p<0.05$.

## 214P THE SODIUM CHANNEL BLOCKER 4030W92 INHIBITS SPINAL C-FOS EXPRESSION IN RESPONSE TO SOMATIC AND VISCERAL NOXIOUS STIMULATION IN THE RAT

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Noxious somatic and visceral stimulation induce Fos-like immunoreactivity (Fos-LI) in discrete areas of the rat spinal cord associated with nociceptive input. We report the effect of the sodium channel blocker, 4030W92 (R (-) 2,4-diamino-5-(2,3-dichlorophenyl)-6-fluoromethylpyrimidine; Trezise et al., this meeting) on intraplantar carrageenan and repetitive colorectal distension (CRD) evoked Fos-LI in the rat spinal cord. Male Random Hooded rats (160-220g) received 4030W92 (3-10mgkg$^{-1}$p.o.) or vehicle (0.25% methyl cellulose) 30min before carrageenan injection (0.1ml; 2% i.pl.). After 3 hours, the rats were fixed (4% paraformaldehyde) and the lumbar spinal cord removed. For the CRD study, male Wistar rats (240-300g) were anaesthetised with urethane (1.5gkg$^{-1}$i.p.) and a colorectal balloon was inflated to 80mmHg for 30s, every 2min, for 120min. 4030W92 (1-3mgkg$^{-1}$i.v.) or saline were administered 10min before CRD onset. At the end of the distension period, the rats were fixed and the lumbosacral cord removed. 40µM sections were processed for visualisation of Fos-LI using standard immuncytochemical techniques. Statistical significance was assessed using one way ANOVA and Dunnett's test ($P<0.05$ significant).

Intraplantar carrageenan significantly increased ipsilateral Fos-LI nuclei to 1215±130 vs 94±35 saline injected controls (total in 6 sections, L4; $P<0.05$; n=4), with most Fos-LI nuclei in laminae I-II (492±78), lateral V-VI (229±28) and VII-X (265±23). Pretreatment with 4030W92 (10mgkg$^{-1}$ p.o.) significantly reduced total ipsilateral Fos-LI nuclei to 618±109 ($P<0.05$; n=4), with reductions of 55% in I-II, 55% in lateral V-VI and 52% in VII-X ($P<0.05$ throughout). There was no significant reduction in Fos-LI in other areas of the cord. 4030W92 (3mgkg$^{-1}$ p.o.) had no significant effect on total numbers of Fos-LI nuclei. Repetitive CRD significantly increased total bilateral Fos-LI nuclei to 1248±146 vs 49±25 in sham distended animals (total in 12 sections, L6-S1; $P<0.05$; n=5), with 345±66 in laminae I-II, 392±37 in lateral V-VI and 378±37 in medial V-X. Pretreatment with 4030W92 (3mgkg$^{-1}$ i.v.) significantly reduced total Fos-LI nuclei to 657±96 ($P<0.05$; n=4), with a 48% and 69% reduction in lateral V-VI and medial V-X respectively ($P<0.05$). There was no significant reduction in laminae I-II. A lower dose of 4030W92 (1mgkg$^{-1}$i.v.), had no significant effect of c-fos expression. In conclusion, 4030W92 inhibits somatic and visceral nociceptive neurotransmission the spinal level, in laminae associated with nociceptive processing, although there is a differential inhibition of neurones in superficial and deeper laminae following visceral stimulation. 4030W92 may therefore provide a novel approach to the treatment of inflammatory and visceral pain conditions.

Trezise, D.J., John, V.H., Nobbs, M. et al. (1997). This meeting.

## 215P THE SODIUM CHANNEL BLOCKER 4030W92 INHIBITS DEOXYCHOLIC ACID-INDUCED SENSITISATION OF DORSAL HORN NEURONE RESPONSES TO VISCERAL AND CUTANEOUS MECHANICAL STIMULATION IN THE ANAESTHETISED RAT

C.M.Kozlowski, E.J.Smith, *D.Grundy and C.Bountra (introduced by N.Clayton). Neuroscience, GlaxoWellcome Research and Development, Gunnels Wood Road, Stevenage, SG1 2NY; *Biomedical Science, University of Sheffield, Sheffield, S10 2TN.

Noxious somatic and visceral stimulation induce Fos-like immunoreactivity (Fos-LI) in discrete areas of the rat spinal cord associated with nociceptive input. We report the effect of the sodium channel blocker, 4030W92 [R (-) 2,4-diamino-5-(2,3-dichlorophenyl)-6-fluoromethylpyrimidine; Trezise & Xie, this meeting] on intraplantar carrageenan and repetitive colorectal distension (CRD) evoked Fos-LI in the rat spinal cord. Male Random Hooded rats (160-220g) received 4030W92 (3-10mgkg$^{-1}$p.o.) or vehicle (0.25% methyl cellulose) 30min before carrageenan injection (0.1ml; 2% i.pl.). After 3 hours, the rats were fixed (4% paraformaldehyde) and the lumbar spinal cord removed. For the CRD study, male Wistar rats (240-300g) were anaesthetised with urethane (1.5gkg$^{-1}$i.p.) and a colorectal balloon was inflated to 80mmHg for 30s, every 2min, for 120min. 4030W92 (1-3mgkg$^{-1}$i.v.) or saline were administered 10min before CRD onset. At the end of the distension period, the rats were fixed and the lumbosacral cord removed. 40μM sections were processed for visualisation of Fos-LI using standard immuncytochemical techniques. Statistical significance was assessed using one way ANOVA and Dunnett's test (P<0.05 significant).

Intraplantar carrageenan significantly increased ipsilateral Fos-LI nuclei to 1215±130 vs 94±35 saline injected controls (total in 6 sections, L4; P<0.05; n=4), with most Fos-LI nuclei in laminae I-II (492±78), lateral V-VI (229±28) and VII-X (265±23). Pretreatment with 4030W92 (10mgkg$^{-1}$ p.o.) significantly reduced total ipsilateral Fos-LI nuclei to 618±109 (P<0.05; n=4), with reductions of 55% in I-II, 55% in lateral V-VI and 52% in VII-X (P<0.05 throughout). There was no significant reduction in Fos-LI in other areas of the cord. 4030W92 (3mgkg$^{-1}$ p.o.) had no significant effect on total numbers of Fos-LI nuclei. Repetitive CRD significantly increased total bilateral Fos-LI nuclei to 1248±146 vs 49±25 in sham distended animals (total in 12 sections, L6-S1; P<0.05; n=5), with 345±66 in laminae I-II, 392±37 in lateral V-VI and 378±37 in medial V-X. Pretreatment with 4030W92 (3mgkg$^{-1}$ i.v.) significantly reduced total Fos-LI nuclei to 657±96 (P<0.05; n=4), with a 48% and 69% reduction in lateral V-VI and medial V-X respectively (P<0.05). There was no significant reduction in laminae I-II. A lower dose of 4030W92 (1mgkg$^{-1}$i.v.), had no significant effect of c-fos expression.

In conclusion, 4030W92 inhibits both somatic and visceral nociceptive neurotransmission at the level of the spinal cord, in laminae associated with nociceptive processing, although there is a differential inhibition of neurones in superficial and deeper laminae following visceral stimulation. 4030W92 may therefore provide a novel approach to the treatment of inflammatory and visceral pain conditions.

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## 216P INHIBITION BY CLOMETHIAZOLE OF $Ca^{2+}$ FLUX IN NEUROBLASTOMA SHSY5Y AND IMR-32 CELLS

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Clomethiazole is an effective neuroprotective agent in animal models of stroke (Green and Cross, 1994). a mechanism possibly involving increased GABA function (see Cross *et al*,1989; Green *et al.*, 1996), since certain other GABAmimetic drugs are also neuroprotective (Lyden,1997).The current investigation examined whether clomethiazole also alters $Ca^{2+}$ flux in neuronal cells by investigating its effect on $Ca^{2+}$ flux in human neuroblastoma cells.

SHSY5Y and IMR-32 cells were maintained respectively in Minimum Essential Eagles Medium (MEEM) and Hams F-12 (1:1) supplemented with 10% foetal calf serum and amino acids, and MEEM supplemented with 10% heat activated foetal calf serum plus amino acids. They were harvested by removing the medium, rinsing the monolayer with 10ml Hanks balanced salt solution and scraping the cells from the base of the flask. The cells were suspended in buffer and loaded with acetoxymethylester derivative of Fluo 3 (Fuo-3AM) for 15 min at 37°C. Following centrifugation the pellet was resuspended in buffer incubated at room temp for 20 min to allow hydrolysis of Fluo-3AM and after centrifugation cells resuspended in HEPES-Ringer buffer. 2ml aliquots were placed in quartz cuvettes and test agents (20μl) added. Following 30s incubation KCl was added and fluorescence measured (ex : 505nm; em : 530nm).

Addition of $K^+$ produced a concentration dependent increase of $[Ca^{2+}]_i$ (SHSY5Y : 75 ± 6%; IMR-32 : 119 ± 12% at $K^+$ 65 mM). Clomethiazole produced a concentration dependent inhibition of the $K^+$-induced increase in $[Ca^{2+}]_i$ in both types of cell (Fig).

These data demonstrate that, at high concentration, clomethiazole attenuates the depolarisation-induced increase in intracellular calcium in neuroblastoma cells, an effect also seen in synaptosomal preparations (unpublished observations).. However since it shares this property with other anticonvulsant compounds that are not neuroprotective, for example carbamazepine (Crowder & Bradford, 1987), it is unlikely that this effect is associated with neuroprotective activity.

Cross, A.J. *et al.*, (1989). *Br. J. Pharmacol.* **98**, 284-290

Crowder, J.M. & Bradford, H.F. (1987). *Epilepsia.* **28**, 378-382

Green, A.R. & Cross, A.J.(1994). *Progr. Neurobiol.* **44**, 463-484

Green, A.R. *et al.* (1996). *Neuropharmacology* **35**, 1243-1250

Lyden, P. (1997) *Int. Rev. Neurobiol.* **40**, 233-258.

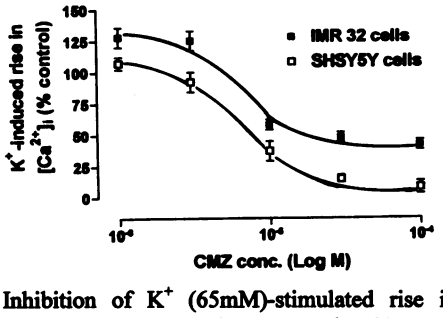


Fig. Inhibition of $K^+$ (65mM)-stimulated rise in $[Ca^{2+}]_i$ by clomethiazole (CMZ). Results expressed as % control increase in $[Ca^{2+}]_i$. Values show mean ± s.e.mean (n=4-8).

## 217P NOCICEPTIN INHIBITS GLUTAMATE RELEASE FROM RAT CEREBELLAR SLICES

B. Nicol[1], D.J. Rowbotham[1], D. Smart[2], A.T. McKnight[2] and D.G. Lambert[1]. [1]University Department of Anaesthesia, Leicester Royal Infirmary, Leicester LE1 5WW and [2]Parke-Davis Neuroscience Research Centre, Robinson Way, Cambridge CB2 2QB.

Nocieptin or Orphanin FQ is the endogenous ligand for the orphan receptor $ORL_1$ (see Henderson & McKnight, 1997) and we have previously reported that nociceptin inhibits depolarisation evoked glutamate release from rat cerebrocortical slices (Nicol et al., 1996). As nociceptin is reported to have variable effects on locomotion (see Henderson & McKnight, 1997) we have examined the effects of this peptide on 46mM $K^+$-evoked glutamate release from rat cerebellar slices.

Rat cerebellar slices (350μm x 350μm) were prepared from Wistar rats (200-250g) and suspended in oxygenated (95%$O_2$/5%$CO_2$) Krebs buffer, pH 7.4. Approximately 0.6ml of gravity packed slices (protein was not determined) was pipetted into a perfusion chamber constructed from a 2ml-syringe barrel (Nicol et al., 1996) and perfused at 1ml/min for 60mins. 2min fractions were then collected for the estimation of glutamate and 0.1% BSA added to the perfusion buffer after completion of $S_1$. Following 6mins of perfusion, 46mM $K^+$ was applied for 2mins ($S_1$). Slices were perfused for a further 30mins in Krebs prior to the second application of $K^+$ ($S_2$). Nociceptin (3-300nM in the presence of 30μM bestatin, amastatin, captopril, phosphoramidon and 0.1% BSA to prevent breakdown) was applied 10mins after $S_1$ until the end of the experiment (i.e., also present during $S_2$). Perfusate glutamate concentrations were determined using a fluorimetric based assay (Nicholls et al., 1987). $S_2/S_1$ ratios were calculated from the area under the curves for control and nociceptin treated samples. Data are mean±s.e.mean, and statistical analysis was by Wilcoxon rank sum test and considered significant when $p<0.05$.

46mM $K^+$ depolarisation produced a monophasic release of glutamate for both $S_1$ and $S_2$ stimuli. The mean $S_2/S_1$ ratio for control slices was 1.25±0.13 (n=17). Nociceptin produced a dose-dependent reduction in the $S_2/S_1$ ratio with an estimated $IC_{50}$ of 27nM. At 100nM the inhibition amounted to 32±8% (Fig 1, $p<0.05$).

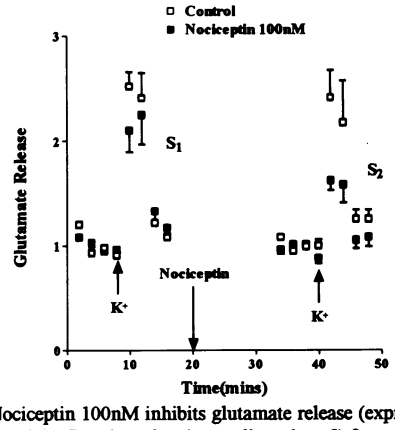


Fig 1. Nociceptin 100nM inhibits glutamate release (expressed relative to the mean of the first three fractions collected, n=5) from cerebellar slices.

These data are in agreement with our previous study in the cerebrocortex and possibly implicate reduced cerebellar glutamatergic transmission as a mechanism by which nociceptin reduces locomotor activity.

Henderson, G & McKnight, A.T. (1997) Tr. Pharmacol. Sci. 18, 293-300.
Nicol, B., Lambert, D.G., Rowbotham, D.J., Smart, D. & McKnight, A.T. (1996) Br.J. Pharmacol. 119, 1081-1083.
Nicholls, D.G., Shira, T.S. & Sanchez-Prieto, J. (1987) J. Neurochem. 49(1), 50-57.

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## 218P STUDIES ON THE COUPLING OF RECOMBINANT ORL-1 RECEPTORS TO ADENYLYL CYCLASE

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Nociceptin or Orphanin FQ is the endogenous agonist for the orphan receptor ORL-1 (see Henderson & McKnight, 1997). In this study we have compared the binding profiles of nociceptin, etorphine, naloxone-benzoylhydrazone (NalBzOH) and CAM6369 (Ac-Arg-Tyr-Tyr-Arg-Trp-Lys-$NH_2$) to recombinant ORL-1 receptors with adenylyl cyclase inhibition. These agents are reported in a number of functional/biochemical studies to have activity at ORL-1 receptors (see Henderson & McKnight, 1997).

CHO cells expressing the recombinant human ORL-1 receptor ($CHO_{ORL1}$) were maintained in DMEM:F12 (50:50) containing 5% foetal calf serum, 2mM glutamine, 200μg/ml hygromycin B and 200μg/ml G418. Maximum binding capacity ($B_{max}$) and equilibrium dissociation constant ($K_d$) for [$^{125}$I]-Tyr$^{14}$-nociceptin (Ardati et al., 1997) was determined by isotope dilution in 1ml volumes of Tris-HCl 50mM, $MgSO_4$ 5mM and BSA 0.5% buffer containing 30μM of peptidase inhibitors; captopril, amastatin, bestatin and phosphoramidon at pH 7.4. Membranes were incubated for 30 min at room temperature. In all studies ~1pM [$^{125}$I]-Tyr$^{14}$-nociceptin was used in the absence and presence of increasing concentration of unlabelled displacing drugs. $B_{max}$ and $pK_d$ were obtained from Hill analysis of specific binding curves in isotope dilution studies. Concentration of drug producing 50% displacement of specific binding was corrected for the competing mass of [$^{125}$I]-Tyr$^{14}$-nociceptin to yield $pK_i$. Inhibition of forskolin (1μM) stimulated cAMP was measured essentially as described by Hirst & Lambert (1995) in assay buffer containing 0.5% BSA and peptidase inhibitors. All curve fitting was performed using GRAPHPAD-PRIZM.

Binding of [$^{125}$I]-Tyr$^{14}$-nociceptin was dose dependent and saturable with $B_{max}$ and $pK_d$ values of 1122±65 fmol/mg protein and 9.87±0.02 respectively. [$^{125}$I]-Tyr$^{14}$-nociceptin binding was displaced dose dependently by etorphine, CAM6369 and NalBzOH with $pK_i$ values shown in Table 1. All agents tested inhibited cAMP formation with $pIC_{50}$ and $I_{max}$ values shown in Table1.

| | Binding $pK_{d/i}$ | cAMP inhibition | |
|---|---|---|---|
| | | $pIC_{50}$ | $I_{max}$ |
| Nociceptin | 9.87±0.02* | 9.95±0.08 | 95.8±1.3 |
| CAM6369 | 8.86±0.01[a] | 9.91±0.12 | 92.6±2.4 |
| Etorphine | 7.66±0.09[a,b] | 7.07±0.05[a,b] | 99.5±0.9 |
| NalBzOH | 6.70±0.16[a,b,c] | 5.80±0.20[a,b,c] | 43.7±4.5[a,b,c] |

Table 1. Displacement of [$^{125}$I]-Tyr$^{14}$-nociceptin and inhibition of cAMP formation in CHO cells expressing recombinant ORL-1 receptor. Data are mean±s.e.mean (n=4-5). *$pK_d$ from isotope dilution. [a] $p<0.05$ vs. nociceptin [b] $p<0.05$ vs CAM 6369 [c] $p<0.05$ vs.etorphine

The rank order potency in binding experiments was nociceptin>CAM6369>etorphine>NalBzOH. In cAMP inhibition studies the rank order potency was nociceptin=CAM6369>etorphine,>NalBzOH. When compared to nociceptin, CAM6369 and etorphine, NalBzOH appears to have partial agonist activity at ORL-1 receptors.

Ardati, A., Henningsen, R.A. Higelin, J. *et al.* (1997) Mol.Pharmacol. 51, 816-824.
Henderson, G & McKnight, A.T. (1997) Tr. Pharmacol. Sci. 18, 293-300.
Hirst, R.A. & Lambert, D.G. (1995) Biochem. Pharmacol. 49, 1633-1640.

Acknowledgments: we would like to thank Dr F Marshall of Glaxo-Wellcome Research and Development, Stevenage, Herts, UK. for providing $CHO_{ORL-1}$ cells.

## 219P LOCAL ANAESTHETIC AGENTS INHIBIT [$^3$H]NORADRENALINE UPTAKE INTO SH-SY5Y CELLS

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Reflex sympathetic dystrophy (RSD) is often treated using intravenous regional guanethidine (Hannington-Kiff, 1977) which is taken up via the neuronal catecholamine uptake transporter, uptake-1 and displaces noradrenaline from release vesicles (Maxwell, 1982). The end result of this treatment is a chemical sympathectomy. Local anaesthetic is co-administered to reduce the discomfort of guanethidine administration. We have previously demonstrated that lignocaine at clinically achievable concentrations non-competitively inhibits [$^3$H]-NA uptake in SH-SY5Y human neuroblastoma cells and hence would be expected to inhibit the uptake of guanethidine (Joyce et al., 1997). In this study we have examined the effects of a range of other local anaesthetic agents.

SH-SY5Y cells were maintained in minimum essential medium supplemented with 10% foetal calf serum, 100IU/ml penicillin, 100μg/ml streptomycin and 2.5μg/ml fungizone at 37°C in 5%$CO_2$/humidified air. Stock cultures were passaged weekly and refed twice weekly. SH-SY5Y cell suspensions were preincubated in 0.5ml volumes of Krebs-HEPES buffer, pH7.4 for 15mins in the absence and presence of local anaesthetic agents (3nM-10mM), [$^3$H]NA (~40nM) was added and the reaction continued for a further 5mins. Reactions were terminated by rapid vacuum filtration. Non-specific uptake was defined in the presence of 10μM imipramine. The concentration of local anaesthetic producing 50% inhibition of uptake ($pIC_{50}$) was estimated by non-linear regression using GRAPHPAD-PRIZM2. All data are mean±s.e.mean.

A range of local anaesthetic agents dose dependently inhibited [$^3$H]NA uptake into SH-SY5Y cells with $pIC_{50}$ values shown in Table 1. In this study cocaine (an ester local anaesthetic) was the most potent and prilocaine (an amide local anaesthetic) the least.

Table 1. Effects of a range of local anaesthetic agents on [$^3$H]NA uptake. *$p<0.05$ less potent than cocaine. E=Ester, A=Amide. [a], from Joyce et al (1997).

| Agent | Class | n | $pIC_{50}$ | $IC_{50}$ |
|---|---|---|---|---|
| Cocaine | E | 4 | 6.81 ± 0.01 | 154nM |
| Tetracaine | E | 8 | 4.91 ± 0.04* | 12.2μM |
| Procaine | E | 7 | 4.24 ± 0.04* | 57μM |
| Dibucaine | A | 8 | 4.29 ± 0.08* | 52μM |
| Bupivacaine | A | 7 | 3.73 ± 0.03* | 188μM |
| Prilocaine | A | 8 | 3.72 ± 0.07* | 189μM |
| Lignocaine[a] | A | 4 | 2.97 ± 0.01* | 1.07mM |

In this study we have demonstrated that in common with lignocaine (Joyce et al., 1997) cocaine, tetracaine, procaine, bupivacaine, and prilocaine also inhibit uptake-1. Prilocaine is a commonly used local anaesthetic agent in regional guanethidine sympathetic block and was around 5 fold more potent than lignocaine. Based on a simple bench study in volunteers of varying body size we estimate that 0.5% prilocaine (19.5mM) when injected into an arm would be diluted around 17 fold. Assuming homogenous distribution this would yield an 'in the arm' concentration of around 1mM (Joyce et al., 1997). The degree of uptake inhibition (and hence of guanethidine) would therefore be ~90% for prilocaine. These data question the co administration of guanethidine and local anaesthetic agents for the treatment of RSD.

Hannington-Kiff, J.G. (1977) Lancet. 1, 1132-1133.
Joyce PI, Atcheson R, Lambert DG. (1997) Brit. J. Anaesthesia 79, (5), 677P.
Maxwell, R.A. (1982) Brit. J. Clin. Pharmacol. 13, 35-44.

## 220P CGRP INDUCES MECHANICAL HYPERALGESIA IN RATS WHICH IS REDUCED BY IL-1ra, KININ B1 AND B2 RECEPTOR ANTAGONISTS BUT NOT A SELECTIVE NK1 RECEPTOR ANTAGONIST

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Activation of C fibres not only causes transduction of nociceptive information centrally, but also results in peripheral release of neuropeptides. Neuropeptides released into the periphery can cause inflammation, and this neurogenic inflammation has been implicated in the pathogenesis of arthritis (Levine et al., 1984).

Previously, we have showed that capsaicin-induced neurogenic hyperalgesia could be blocked by both kinin B1 and B2 receptor antagonists (Davis & Perkins 1996). We have now studied the role of CGRP in capsaicin-induced hyperalgesia as well as the hyperalgesia induced by intra-articular injection of CGRP alone.

The method used for assessment of mechanical hyperalgesia was as described previously (see Davis & Perkins 1996). Female Sprague Dawley rats (90-110 g) were injected with either capsaicin or CGRP into a single knee joint and the load tolerated by that joint was assessed at various time points after treatment.

Capsaicin (10nmol) caused a hyperalgesia which was prevented when coadministered with 0.5nmol CGRP(8-37) see table 1. CGRP (0.001-1nmol) administered to naïve joints induced a dose dependent hyperalgesia with a 41±1 % reduction in tolerated load 1 hr after administration of 1 nmol CGRP.

The hyperalgesia induced by CGRP (0.1nmol) was antagonised when given concomitantly with CGRP(8-37) (0.5nmol), desArg9Leu8BK (0.5nmol), icatibant (5pmol) and IL-1ra (0.1μg). The NK1 receptor antagonist RP 67580 (1nmol) had no effect see table 1.

Administration of CGRP(8-37) (0.5 nmol i.artic), icatibant (100pmol/kg i.v.), desArg9Leu8BK (10nmol/kg iv) or IL-1ra (0.1μg i.artic.) 3.5 h after CGRP (0.1nmol i.artic.) caused a significant reversal of hyperalgesia when assessed 30min later (56±11, 76±13, 54±10 and 66±8% reversal respectively).

Indomethacin (1mg/kg sc) pretreatment caused a small but significant reduction in the level of CGRP-induced hyperalgesia. desArg9Leu8BK (10nmol/kg iv) did not reverse the hyperalgesia present in animals pretreated with indomethacin.

In this model, the neurogenic hyperalgesia caused by capsaicin can be blocked by a CGRP receptor antagonist and CGRP alone can cause hyperalgesia. These data suggest a role for CGRP in the generation of kinins prostaglandins and IL-1 leading to a sensitisation of peripheral nocieptive afferents and hyperalgesia.

Davis and Perkins (1996). Br. J. Pharmacol., 118, 2206-2212.

Levine J.D., Clark R., et al., (1984). Science, 226, 547-549.

Table 1. Summary of effects of specific antagonists on capsaicin and CGRP-induced hyperalgesia in rat knee joints

| | alone | + CGRP(8-37) (0.5nmol) | + desArg9Leu8BK (0.5nmol) | + icatibant (5 pmol) | + IL-1ra (0.1 μg) | + RP 67580 (1 nmol) |
|---|---|---|---|---|---|---|
| Capsaicin (10 nmol) | 57±4 | 85±1* | 90±1* † | 95±3*† | 92±4*† | 94±3*† |
| CGRP (0.1 nmol) | 64±4 | 97±3* | 97±3* | 97±5* | 94±3* | 66±4 |

*P<0.05 compared to the agonist administered alone. All results are expressed as load tolerated ± s.e. mean (g) by the treated joint 4 h after intra-articular injection, n=8 animals per treatment group. † Data taken from Davis and Perkins 1996.

## 221P THE SELECTIVE NK-1 RECEPTOR ANTAGONIST SDZ NKT 343 INHIBITS BOTH INFLAMMATORY AND NEUROPATHIC HYPERALGESIA IN THE GUINEA PIG

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Recently several highly selective non-peptide NK-1 receptor antagonists have been described and developed for different indications (Kidd et al, 1996, Dray & Urban 1996). The anti-hyperalgesic activity of SDZ NKT 343, a novel, selective, non-peptide NK-1 receptor antagonist, was tested in models of inflammatory (Patel et al, 1995) and neuropathic (Panesar et al, 1996) hyperalgesia in the guinea pig and compared to other NK-1 receptor antagonists currently in development.

Male Dunkin Hartley guinea pigs (200-220g, n=6/group) were anaesthetised with enflurane in $N_2O$:$O_2$ and peripheral nerve damage induced to the left sciatic nerve using partial ligation (Seltzer et al, 1990). Inflammatory hyperalgesia was induced by intraplantar injections of 1% carrageenan. Mechanical hyperalgesia was assessed using an Ugo Basile analgesymeter. Drugs were tested orally 12-14 days post-surgery in neuropathic animals or 24h following carrageenan injection in the inflammatory model. Drug effects were expressed as the D30 values (the dose in mg/kg at which a 30% reversal of hyperalgesia is achieved) and mean ± sem percentage reversal of hyperalgesia compared by Student's t-test.

As shown below in table 1, oral SDZ NKT 343 inhibited carrageenan-induced inflammatory hyperalgesia by 68.4 ± 1.6% with a D30 of 1.1mg/kg. It was significantly more potent and efficacious than the compounds GR 205171, LY 303870 and RPR 100893 and had a duration of action of >6 hours.

When administered orally in the model of neuropathic pain SDZ NKT 343 inhibited hyperalgesia by 85.0 ± 8.8% with a D30 of 0.02mg/kg. Again, its duration of action of >6 hours and it was more potent and efficacious than the other NK-1 antagonists tested.

In models of chronic inflammatory and neuropathic pain in the guinea pig the selective NK-1 receptor antagonist, SDZ NKT 343 is a potent and long lasting orally active analgesic with a rapid onset of action.

Dray,A & Urban,L.(1996), *Annu.Rev.Pharmacol.Toxicol.* 36: 253-280.
Kidd, B.L.,Morris,V.H.,Urban,L. (1996), *Ann.Rheum.Dis.* 55:276-283.
Panesar,M., Patel,S., Gentry,C., *et al.* (1997), *Br.J.Pharmacol.* 120:231P.
Patel,S.,Gentry,C., Campbell,E.(1996),*Br.J.Pharmacol.*117: 248P.
Seltzer, Z., Dubner, R., Shir, Y. (1990), *Pain* 43: 205-218.

Table 1: Comparisions of D30 values and maximum reversal of mechanical hyperalgesia at 1,3 and 6 hrs following oral drug administration in the guinea pig inflammatory and neuropathic models.

| | Carrageenan model | | | | Neuropathic model | | | |
|---|---|---|---|---|---|---|---|---|
| | | maximum % reversal | | | | maximum % reversal | | |
| Compound | D30 @ 3hr mg/kg | 1hr | 3hr | 6hr | D30 @ 3hr mg/kg | 1hr | 3hr | 6hr |
| SDZ NKT 343 | 1.1 | 50.2±9.3 * | 68.4±1.6 * | 61.7±3.1 * | 0.02 | 71.2±4.0 * | 85.1±8.8 * | 90.4±25.2 * |
| RPR 100893 | 17 | 20.4±5.1 * | 35.7±5.8 * | 27.6±3.9 * | >100 | 19.9±4.9 * | 15.6±4.2 * | 13.8±3.5 * |
| LY 303870 | >30 | 22.0±0.2 * | 28.5±6.7 * | 37.3±5.9 * | 5.7 | 25.2±3.2 * | 45.0±6.1 * | 52.2±3.6 * |
| GR 205171 | 4.5 | 15.1±2.7 * | 57±12.8 * | 17.2±5.7 * | NT | NT | NT | NT |

* $P < 0.01$ compared to the relevant vehicle control group. n = 6 -18 animals per drug group NT = not tested

## 222P EFFECTS OF t-BUTYL HYDROPEROXIDE ON ION CHANNELS IN CULTURED RAT CEREBELLAR GRANULE NEURONES

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Formation of free radical species is a major contributing factor to neuronal damage during ischaemic episodes. In the present study, we have investigated the effects of t-butyl hydroperoxide (t-BH), a free radical generating compound, upon ion channel currents in neurones from the central nervous system.

Cerebellar granule neurones from neonatal male Wistar rats (6-8 d.o.) were grown in culture as previously described (Pearson *et al*, 1995). Whole cell patch-clamp recordings were made from cells after 7-14 days in culture (20-22°C). For recording of $K^+$ channel currents, cells were bathed in a medium containing (mM): NaCl 120, KCl 1.3, HEPES 10, glucose 5, $CaCl_2$ 1.3 and $MgCl_2$ 1.2. Patch pipettes (2-5MOhm) were filled with (mM): KCl 140, $CaCl_2$ 0.5, EGTA 5 and HEPES 10. For measurement of $Na^+$ channel currents, 30mM TEA was substituted for NaCl in the bath solution and pipettes were filled with a solution containing (mM): Cs-HEPES 100, EGTA 30, $CaCl_2$ 1, $MgCl_2$ 2.5 and $K_2ATP$ 3.3. $Ca^{2+}$ channel currents were measured using the same pipette solution as used for $Na^+$ channel currents; whilst the bath solution contained TEA-acetate 70, N-methyl-D-glucamine 70, KOH 3, Mg acetate 0.6, glucose 4, Ba acetate 10 and HEPES 10. t-BH and the antioxidant, butylated hydroxytolulene (BHT), were applied by superfusion of cells or by pre-incubation in the culture medium. All data are expressed as mean ± s.e.mean and differences between means were calculated with Student's paired or unpaired t-test as appropriate.

Superfusion of cells with 500µM t-BH resulted in a steady increase in the outward $K^+$ current at a test poential of +50mV. The peak current was increased by 13±6%, 49±19% and 70±34% after 1, 3 and 5 minutes perfusion with t-BH respectively ($p<0.05$; n=5-9). This increase was confined to the inactivating component of the current while the non-inactivating component (measured at the end of the pulse) was unaffected. The increase in peak $K^+$ current caused by t-BH was prevented by prior application of 50µM BHT. The effects of 50µM t-BH were measured on $Ca^{2+}$ channel currents following 1 hour preincubation in the culture medium. Incubation was necessary to avoid problems with run-down. Preincubation with t-BH inhibited the $Ca^{2+}$ channel current by 50-60%. For instance, the $Ca^{2+}$ current at +10mV was reduced from -210±19pA to -103±19pA by t-BH ($p<0.01$, n =25 control cells, 10 t-BH treated cells). Incubation with 50µM BHT for 1 hour prior to t-BH application had a no significant effect in reversing the inhibitory effect of t-BH (-124±21pA at +10mV, n=11). By contrast to the other channels studied, TTX-sensitive $Na^+$ channels were unaffected by t-BH (500µM) perfused for up to 3 minutes.

The results suggest that free radical species may have a selective effect on the various ion channel currents in cultured rat cerebellar granule neurones. $Na^+$ channels were unaffected by t-BH. However, $K^+$ currents were activated by a mechanism that appears to involve the oxidative properties of t-BH. Inhibition of the $Ca^{2+}$ channel current by t-BH appeared to be unrelated to oxidation as inhibition was not prevented by the antioxidant BHT.

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References

Pearson HA *et al* (1995) J. Physiol. **482**, 493-509

## 223P EFFECT OF THE $NK_1$ RECEPTOR ANTAGONIST, GR205171, ON APOMORPHINE-INDUCED FOS-EXPRESSION IN THE RAT BRAINSTEM

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The tachykinin $NK_1$ receptor antagonist GR205171 has previously been found to block apomorphine-induced conditioned taste aversions in rats (McAllister and Pratt, 1996), a phenomenon which has been hypothesised to recruit the same neural circuits as emesis (Grant, 1987). Therefore the aim of the present study was to investigate the effects of GR205171 on apomorphine-induced Fos expression in the brainstem of the rat. Experiments were carried out on male Lister Hooded rats (250-350g; n = 4-5/group). Rats were randomly assigned to 1 of 6 treatment groups and were either pre-treated with vehicle or GR205171 (0.3 and 3.0 mg $kg^{-1}$ s.c.) 30 minutes prior to treatment with apomorphine (0.5 mg $kg^{-1}$ s.c.) or vehicle. Two hours post-treatment the rats were deeply anaesthetised and perfused with phosphate buffered saline, followed by 4% formalin and the brain removed. Fos-like immunoreactivity (Fos-LI) was localised in 24 - 30 sections (40μm) from the brainstem region by the avidin-biotin-peroxidase method (Vectastain). The number of stained nuclei within each section were counted manually throughout the entire region and the average calculated per group. Differences in the levels of Fos-LI between groups were analysed by one-way ANOVA, followed by a Dunnett's t-test where appropriate.

Apomorphine (0.5 mg $kg^{-1}$ s.c.) stimulated an increase in Fos-LI in both the nucleus tractus solitarius (NTS) (stained nuclei: control = 57.6 ± 8.0; apomorphine = 144.0 ± 5.2, $p<0.05$) and the dorsal motor nucleus of the vagus (DMNV) (stained nuclei: control = 35.8 ± 6.6; apomorphine = 71.8 ± 7.6 $p<0.05$). Pre-treatment with GR205171 (3.0 mg $kg^{-1}$) significantly reduced apomorphine-induced Fos expression in the NTS by 47% to 76.3 ± 15.8 stained nuclei ($p<0.05$) but did not reduce Fos-LI evoked by apomorphine in the DMNV. Conversely GR205171 (3.0 mg $kg^{-1}$) alone did not cause a significant increase in Fos expression compared to the equivalent control group in either of brainstem region examined. The ability of GR205171 to modify changes in Fos-LI in the NTS stimulated by apomorphine accords with previous work which has shown that GR205171 significantly reduces apomorphine-induced changes in local cerebral glucose utilisation in the NTS measured using 2-deoxyglucose autoradiography (McAllister and Pratt, 1997). Therefore the NTS may reflect an important site of action regarding the ability of $NK_1$ antagonists to block the aversive effects of apomorphine in rats.

Grant, L. (1987) *Psychopharmacologia,* 93, 405 - 441
McAllister, K.H.M. & Pratt, J.A.(1996) *Soc. Neurosci. Abstr.* 188.16
McAllister, K.H.M. & Pratt, J.A. (1997). *Br. J. Pharmacol.* Proc. Suppl. In Press.

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## 224P BTS 74 398: A NOVEL NONOAMINE REUPTAKE INHIBITOR FOR THE TREATMENT OF PARKINSON'S DISEASE

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BTS 74 398 (1-[1-(3,4-dichlorophenyl)cyclobutyl]-2-(3-dimethyl-aminopropylthio)ethanone monocitrate) has undergone preclincal evaluation as a potential treatment for Parkinson's Disease. In this study, we have examined the effects of BTS 74 398 on [$^3$H]monoamine uptake and release in rat brain tissue *in vitro*, apomorphine-induced climbing (AC) in the mouse and 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxytryptophan (5-HTP) levels in rodent brain. Where appropriate nomifensine and bupropion were used as comparators.

Male Sprague-Dawley rats (100-250g) and CD1 mice (18-35g) were used. [$^3$H]5-Hydroxytryptamine (5-HT), [$^3$H]noradrenaline (NA) and [$^3$H]dopamine (DA) uptake into rat brain synaptosomes and release from rat brain slices *in vitro* were determined (Heal *et al.*, 1992; 1996). Apomorphine HCl (0.35mg/kg sc) was given to CD1 mice 30 min after BTS 74 398 (15-120mg/kg, po), nomifensine (3.75-30mg/kg, po) or bupropion (15-120mg/kg, po). At 10 and 20 min post-apomorphine, climbing was assessed (score 0=all four paws on the floor, 1=two front paws on the cage grid and 2=all four paws on the cage grid). The dose giving 60% of maximum response was estimated by logistic regression. MHPG and 5-HTP levels in mouse and rat brain, respectively, were quantitated using hplc-ECD.

BTS 74 398 is a potent dopamine, noradrenaline and 5-HT reuptake inhibitor *in vitro* (Table 1). BTS 74 398 potentiates apomorphine-induced climbing and decreases the concentrations of the noradrenaline metabolite MHPG and the 5-HT precursor 5-HTP demonstrating reuptake inhibition *in vivo* (Table 1). As a dopamine reuptake inhibitor, BTS 74 398 is, respectively, 20 and 100 times more potent than nomifensine and bupropion *in vitro*, however, *in vivo* the order of potency is nomifensine>BTS 74 398>bupropion (Table 1). BTS 74 398 does not evoke the release of dopamine, noradrenaline or 5-HT *in vitro*.

Thus, BTS 74 398 is a monoamine reuptake inhibitor *in vitro* and *in vivo*. As such, it has the potential not only to treat the motor deficits associated with Parkinson's Disease (Smith *et al.*, 1997) but also to reverse the depression often associated with this disorder.

Heal, D. *et al.* (1992) *Drug Dev. Res.* 27:121-135.
Heal, D.J. *et al.* (1996) *Br. J. Pharmacol.* 117:325P.
Smith, L.A. *et al.* (1997) This meeting.

Table 1. Effect of BTS 74 398 on monoamine uptake and release.

| | Uptake ($K_i$ nM) | | | Release (% increase $10^{-6}$M) | | | AC | MHPG | | 5-HTP | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | DA | NA | 5-HT | DA | NA | 5-HT | $ED_{60}$ | C | DT | C | DT |
| BTS 74 398 | 4.2±0.4 | 6.9±0.8 | 19±2 | 16±18 | 2±6 | 20±10 | 29(14-44) | 94±6 | 75±4** | 136±6 | 111±3* |
| Nomifensine | 88±8 | 8.0±0.9 | 2660±132 | - | - | - | 12(6-26) | - | - | - | - |
| Bupropion | 409±43 | 2590±12 | 18312±753 | - | - | - | 45(29-72) | - | - | - | - |

Values are mean ± s.e. mean except $ED_{60}$. $K_i$ (n = 3-4); % increase at $10^{-6}$M over basal fractional efflux (n = 6-7); potentiation of apomorphine-induced climbing (AC) $ED_{60}$ (95% limits) mg/kg po. MHPG and 5-HTP levels 1hr after dosing (C = vehicle, DT = drug-treated, 10 mg/kg po MHPG, 30 mg/kg po 5-HTP), ng/g tissue wet weight (n = 6-8) *P<0.05, **P<0.01 Dunnett's test. - = Not tested.

## 225P LOCALISATION OF CYP2D ENZYMES IN NAÏVE RAT BASAL GANGLIA

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Mutations in the *CYP2D6* gene (the only functional gene of this subfamily in man) result in a 2-6 fold increased risk of Parkinson's disease (PD). CYP2D6 expression has been detected in human substantia nigra and is lost in PD. In the rat, 6 functional *CYP2D* genes have been identified. Of these, CYP2D1 is not expressed in the substantia nigra pars compacta (SNpc) (Riedl *et al.*, 1996). The expression of other members of the CYP2D subfamily in naïve rat basal ganglia is unknown. For this reason, we have investigated the expression and distribution of individual members of the CYP2D subfamily using highly specific anti-peptide antisera (Edwards *et al.*, 1995). Furthermore, we have examined the effect of 6-hydroxydopamine (6-OHDA) lesioning of the nigro-striatal tract on CYP2D expression.

Naïve male Wistar rats (180-200g) were anaesthetised and transcardially perfused with 0.1M phosphate buffered saline (PBS), followed by PBS containing 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde. Contiguous cryosections (30µm) were taken from the striatum to the interpeduncular nucleus and incubated overnight with either pre-immune serum or anti-peptide antiserum to CYP2D2, CYP2D3, CYP2D4/18 or CYP2D5 (n=3). Immunoreactive products were visualised by peroxidase immunohistochemistry. In further experiments, 6-OHDA (8µg free base, n=5), or vehicle (n=5) was injected stereotaxically into the left medial forebrain bundle of male Wistar rats under pentobarbital anaesthesia. Six weeks post surgery, midbrain tissue was prepared as described above and incubated overnight with either rabbit anti-rat CYP2D5 (1:1000) or rabbit anti-rat tyrosine hydroxylase (TH) (0.2µg/ml). TH and CYP2D5 positive neurones were counted only in the SNpc (-5.6mm and -4.8mm from Bregma, respectively). Four sections were counted per brain (n=5). Cell counts (expressed as mean ± SD) were analysed by Student's t-test with Bonferroni correction for multiple comparisons.

Weak immunoreactivity to CYP2D2 was observed in neurones in subthalamic nucleus, SNpc and interpeduncular nucleus and outside the basal ganglia. No immunoreactivity to CYP2D3 or CYP2D4/18 was observed in the basal ganglia, although very weak immunoreactivity to these enzymes was observed outside the basal ganglia. In contrast, strong CYP2D5 immunoreactivity was evident in cell bodies within the subthalamic nucleus, SNpc and interpeduncular nucleus, but was absent in the globus pallidus. In addition, extensive CYP2D5 immunoreactivity was present outside the basal ganglia. Following 6-OHDA lesioning of the nigro-striatal tract, there was complete loss of TH-positive and 50% loss of CYP2D5 positive cells in the SNpc, compared to the sham-lesioned group (Table 1).

Table 1. TH & CYP2D5 cell counts in defined regions of the SNpc following 6-OHDA lesioning of the nigro-striatal tract.

| | TH | CYP2D5 |
|---|---|---|
| Sham | 70±30 | 176±56 |
| 6-OHDA | 1±1** | 89±49* |

* P<0.05 compared to sham; ** P<0.01 compared to sham

These results indicate that CYP2D5 is the most prominent member of the CYP2D subfamily expressed in rat basal ganglia. Additionally, CYP2D5 neurones in the SNpc are destroyed by 6-OHDA lesioning, implying that this enzyme is expressed in dopaminergic neurones.

Edwards, R.J. *et al.* (1995) *Biochem. Pharmacol.* 49, 39-47.
Riedl, A.G. *et al.* (1996) *Brain Res.* 743, 324-328.

## 226P REPEATED ADMINISTRATION OF FLUOXETINE AND DESIPRAMINE INCREASES $D_2$ BUT NOT $D_1$ RECEPTOR EXPRESSION IN THE NUCLEUS ACCUMBENS OF THE RAT BRAIN

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We have recently shown in behavioural studies that repeated administration of antidepressant drugs enhances the function of dopamine $D_2$-like receptors (Ainsworth *et al.*, 1997). Specifically, fluoxetine (Flu), desipramine (DMI) and tranylcypromine (TCP) potentiated, albeit to varying degrees (Flu>DMI>>TCP), the behavioural response to $D_2$-like but not $D_1$-like receptor agonists. In the present study we have examined the effect of these antidepressants on the expression of $D_2$ and $D_1$ receptors in the nucleus accumbens and striatum.

Groups of male Sprague-Dawley rats (200-250 g) were injected i.p. twice daily for 14 days with saline vehicle (1 ml/kg), Flu (10 mg/kg), DMI (10 mg/kg) or TCP (2.5 mg/kg). Animals were sacrificed and their brains dissected out 24 h after the final injection. The abundance of mRNA encoding $D_1$ and $D_2$ receptors was determined by in situ hybridization histochemistry (Smith *et al.*, 1995). $D_2$ binding site density was determined by receptor autoradiography using [$^3$H]YM-09151-2 (Yokoyama *et al.*, 1994). Image analysis of the resulting autoradiograms was performed on four distinct brain regions; the nucleus accumbens shell (Shell), the nucleus accumbens core (Core), the ventromedial striatum (VMS) and the dorsolateral striatum (DLS). Data were analysed for statistical significance using one-way ANOVA and *post-hoc* Dunnett's test.

A summary of results is presented in Table 1. In brief, rats treated with Flu, DMI and TCP showed enhanced $D_2$ mRNA levels in the Shell. In contrast, $D_1$ mRNA levels were not altered in any of the regions examined following antidepressant administration. In the case of Flu and DMI, the increase in $D_2$ mRNA in the Shell was coincident with an increase in [$^3$H]YM-09151-2 binding. However, TCP did not increase $D_2$-like receptor binding site density in the nucleus accumbens and significantly decreased binding in the VMS and DLS.

In summary, the present results indicate that repeated administration of Flu, DMI and TCP enhances $D_2$ but not $D_1$ mRNA levels in the nucleus accumbens. In the case of Flu and DMI, the increase in $D_2$ mRNA is associated with an increase in $D_2$-like binding site density. These results provide evidence that repeated antidepressant drug administration alters dopamine receptor expression in the nucleus accumbens. Since dopamine function in this region is known to facilitate reward and motivated behaviour (Koob, 1992), these effects may be relevant to the antidepressant actions of these drugs.

| | Vehicle | Flu | DMI | TCP |
|---|---|---|---|---|
| ***D1 mRNA*** | | | | |
| Shell | 32.3±2.5 | 33.0±2.8 | 33.8±3.5 | 28.5±3.7 |
| Core | 27.2±2.3 | 27.2±1.8 | 30.0±1.1 | 24.2±2.7 |
| VMS | 27.6±2.1 | 28.6±1.9 | 30.0±1.8 | 26.5±2.6 |
| DLS | 29.0±2.8 | 29.2±1.0 | 30.2±1.5 | 26.0±2.4 |
| ***D2 mRNA*** | | | | |
| Shell | 101.2±6.6 | 137.7±4.9** | 124.2±6.4* | 127.6±5.9** |
| Core | 132.0±12.6 | 162.8±7.0 | 150.2±8.0 | 137.0±5.1 |
| VMS | 133.8±8.1 | 161.8±9.2 | 151.4±3.1 | 141.2±4.8 |
| DLS | 154.4±10.0 | 188.7±8.1** | 172.2±7.9 | 164.8±2.6 |
| ***D2 binding site density*** | | | | |
| Shell | 126.5±6.9 | 160.8±6.1** | 157.0±6.6** | 113.2±4.2 |
| Core | 102.3±5.6 | 132.8±8.5** | 112.8±5.0 | 106.8±4.0 |
| VMS | 148.5±4.8 | 157.3±4.1 | 153.7±5.6 | 125.2±1.6** |
| DLS | 187.2±4.4 | 189.2±2.3 | 188.3±1.2 | 171.2±2.1** |

Table 1. Effect of repeated administration of antidepressant drugs on $D_1$ and $D_2$ mRNA levels (µCi/g tissue) and $D_2$-like binding site density (fmol/mg tissue). Data expressed as mean ± s.e.mean, N=5-6/group. * P<0.05, ** P<0.01 *vs.* vehicle control.

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Ainsworth, K., *et al.* (1997) *J. Psychopharmacol.* (in press).
Koob, G.F. (1992) *Trends Pharmacol. Sci.* 13, 177-85.
Yokoyama C. *et al.* (1994) *J. Comp. Neurol.* 344, 121-36.
Smith, S.E., *et al.* (1995) *Psychopharmacology* 120, 333-40

## 227P L-745,870 AND U101958 AS DOPAMINERGIC $D_4$ RECEPTOR AGONISTS

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The dopamine $D_4$ receptor subtype has stimulated much interest in schizophrenia research because the atypical neuroleptic, clozapine has some affinity and a small degree of selectivity for it over $D_2$ receptors. Recently, several dopaminergic $D_4$ receptor selective antagonists have been developed, in the hope of treating schizophrenia without eliciting side effects. In the present work, we have characterised the functional activities of two such compounds, L-745,870 (3-(4-[4-chlorophenyl]piperazin-1-yl)-methyl-1H-pyrrolo[2,3-b]pyridine; Kulagowski *et al.*, 1996) and U101958 ((1-benzyl-piperidin-4-yl)-(3-isopropoxy-pyridin-2-yl)-methyl-amine; Schlachter *et al.*, 1995) at human recombinant dopamine $D_4$ receptors.

A human retinal cDNA library constructed in the bacteriophage λgt10, was screened for the human dopamine $D_4$ receptor cDNA using appropriate radiolabelled PCR fragments as probes. Positively hybridising clones were isolated, subcloned into Bluescript SK(-) and sequenced. The sequence of one clone was confirmed as being that of the human dopamine $D_{4.4}$ receptor (Van Tol *et al.*, 1992). It was subcloned into a mammalian expression vector with the CMV promoter. This expression construct was cotransfected with the pRSVNeo plasmid (in a 9:1 ratio) into HEK293 cells by the calcium phosphate precipitation method. Selection for stable integration was performed by adding 0.8 mg/ml geneticin to the culture medium. Cells were used for cAMP accumulation studies (Schoeffter *et al.*, 1995) and conventional radioligand binding assay using [$^3$H]spiperone (Schoeffter *et al.*, 1997). Compounds were synthesized at Novartis Pharma Inc. Results are given as mean ± s.e.mean.

HEK293/$D_4$ cells expressed 330-570 fmol receptors/mg protein. Dopamine induced a concentration-dependent inhibition of forskolin-stimulated cAMP accumulation in HEK/$D_4$ cells ($pEC_{50}$ 8.6 ± 0.1, n=7). Spiperone and clozapine were without such effect but competitively antagonised the response to dopamine with $pK_B$'s of 8.8 ± 0.2 (n=4) and 7.1 ± 0.3 (n=9), respectively, consistent with their affinities for the receptor estimated in the binding assay ($pK_D$ 9.6 ± 0.1, n=3 and 7.4 ± 0.1, n=4). In contrast, L-745,870 and U101958 acted as agonists in the model, with $pEC_{50}$'s of 9.0 ± 0.2 (n=4) and 8.7 ± 0.3 (n=3) and $E_{max}$ values of 71 ± 3 % and 93 ± 4 % of that of dopamine, respectively, consistent with binding $pK_D$'s of 8.5 ± 0.1 (n=3) and 8.9 ± 0.1 (n=3). Dopamine, L-745,870 and U101958 were devoid of effects in non-transfected HEK293 cells.

These results show that the putative $D_4$ receptor antagonists, L-745,870 and U101958 are not devoid of intrinsic activity at dopamine $D_4$ receptors, contrary to spiperone and clozapine. Therefore, they question the relevance of their use to test the benefit of $D_4$ receptor antagonism in schizophrenia.

Kulagowski, J.J., Broughton, H.B., Curtis, N.R. *et al.* (1996). *J. Med. Chem.*, 39, 1941-1942.

Schlachter, S.K., Poel, T.J., Lawson, C.F. *et al.* (1995). *Soc. Neurosci.*, 21, abstract 252.7

Schoeffter, P., Bobirnac, I., Boddeke, E. & Hoyer, D. (1997). *Neuropharmacology*, 36, 429-437.

Schoeffter, P., Pfeilschifter, J. & Bobirnac, I. (1995). *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 351, 35-39.

Van Tol, H.H.M., Wu, C.M., Guan, H.-C. *et al.* (1992). *Nature*, 358, 149-152.

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## 228P [$^3$H]NEMONAPRIDE BINDING DEFINES DOPAMINE D4 RECEPTORS IN RAT RETINAL MEMBRANES

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The interest in dopamine D4 receptors as a site of action for antipsychotic drugs emerged from the observation that clozapine had a relatively high affinity for these receptors, and from the reports, now discredited, of an increase in D4 receptors in the striatum in schizophrenia (reviewed by Reynolds, 1996). The levels of mRNA for this receptor in brain tissue are, however, very low, and most pharmacological studies have relied on the production of cloned receptors in cultured cells. Of the neural tissues expressing D4 mRNA, it is the retina that has the highest levels (Matsumoto et al, 1995). We have undertaken a study of [3H]nemonapride binding to the D2-like receptors in this tissue of the rat, employing selective D4 antagonists to determine the D4 component of this binding. [3H]Nemonapride was used at concentrations of approx. 0.5nM to define D2-like receptors in tissue homogenates following previously published protocols (Reynolds and Mason, 1995). Crude tissue homogenates were prepared as before, except that rat eyes were initially homogenised in 10vol. buffered 0.32M sucrose, followed by three centrifugation/wash procedures before being resuspended and incubated at 2.5mg/ml with radioligand and, where appropriate, displacing drug.

Displacements of radioligand binding were undertaken with 3-[4-(4-chlorophenyl)piperazin-1-yl]methyl-1H-pyrrolo[2,3-b]pyridine (SB-223326; L-745870), and (2-methoxy-3-naphthyl)-1-benzyl-4-piperidinyl acetate (SB-235753) (Boyfield et al, 1996). We find these to have high affinity for the cloned human D4 receptor, with pKi values of 8.4 and 8.2, and selectivity vs. D2 sites of 340 and 1200 respectively. While in human putamen these drugs showed no displacement of [3H]nemonapride binding at concentrations below 1μM, indicative of low affinity for the D2 site, their effect in the retinal preparation was at substantially lower concentrations. These results are shown in the table, along with the effects of three antipsychotic drugs.

Table1. pKi values for displacement of [3H]nemonapride binding to rat retinal membrane preparation

| | |
|---|---|
| Haloperidol (n=3) | 8.07 ± 0.06 |
| Sulpiride (n=3) | 5.78 ± 0.21 |
| Clozapine (n=3) | 7.54 ± 0.12 |
| SB-223326 (n=9) | 8.28 ± 0.47 |
| SB-235753 (n=6) | 8.00 ± 0.62 |

Values are mean ± SD calculated from Kd of 0.166nM (n=4).

The addition of 1μM (+)WAY-100135 in the assay had no effect on the results, indicating that 5-HT1A receptors make no contribution to the effects observed here. In addition to the results with the D4-specific compounds, the affinities for clozapine and (-)sulpiride, which have respectively a slight D4 and a substantial D2 selectivity, are consistent with the presence of dopamine D4 receptors in the rat retina.

Boyfield I. et al (1996) *J. Med. Chem.* 39, 1946-1948.
Matsumoto M. et al. (1995) *Mol. Brain Res.* 29, 157-162.
Reynolds G.P. (1996) *Drugs* 51, 7-11.
Reynolds G.P. & Mason S.L. (1995) *Eur. J. Pharmacol.* **281**, R5-R6.

## 229P CELLULAR DISTRIBUTION OF 5-HT$_7$ RECEPTOR mRNA IN RAT BRAIN

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The distribution of the 5-HT$_7$ receptor has been assessed using both northern blot analysis and *in situ* hybridisation histochemistry. *In situ* hybridisation studies have demonstrated that 5-HT$_7$ receptor mRNA is discretely localised in rat and guinea pig brain with strong hybridisation signals detected in hippocampus, thalamus, hypothalamus and cortical brain regions (Gustafson et al., 1996; Ruat et al., 1993; To et al., 1995). Furthermore, autoradiographic studies report a similar distribution of radiolabelled 5-HT$_7$ receptors (Gustafson et al., 1996; To et al., 1995). However the detailed cellular distribution of the 5-HT$_7$ receptor mRNA and the phenotypes of neurones expressing these transcripts have yet to be reported. This study performed *in situ* hybridisation in rat brain in attempt to detect 5-HT$_7$ receptor mRNA at the cellular level.

Whole rat brain from male Wistar rats (250-300g) were frozen in isopentane at -40°C for 5 mins and stored at -80°C until sectioning. Cryostat sections (12 µm) were cut at -15 to -20°C and mounted onto charged microscope slides. Sections were fixed with 4% paraformaldehyde in ice-cold phosphate buffered saline (PBS) pH 7.2 for 5 min, washed in fresh PBS, dehydrated through a series of alcohols and stored in 95% ethanol at 4°C until hybridisation.

An antisense oligonucleotide probe to nucleotides 1341-1385 in the carboxy tail region of the rat 5-HT$_7$ receptor (Lovenberg et al., 1993) was labelled with [$^{35}$S] on the day of hybridisation to a specific activity of $10^9$ d.p.m. µg$^{-1}$ and diluted in hybridisation buffer to a concentration of $1.5 \times 10^4$ d.p.m. µl$^{-1}$. The hybridisation buffer was composed of 50% formamide, 1x Denhardt's solution (0.2% polyvinylpyrrolidine, 0.2% Ficoll, 0.2% bovine serum albumin) 4 x sodium citrate buffer (1 x SSC = 0.15M NaCl and 0.015M sodium citrate), 0.5 mg ml$^{-1}$ salmon sperm DNA, 0.5 mg ml$^{-1}$ yeast tRNA, 50mM dithiothreitol and 10% dextran sulphate. Sections were removed from alcohol, allowed to dry and hybridised overnight at 42°C. Sections were then washed in 2 x SSC for 5 min at room temperature followed by two changes of 1 x SSC for 1 hour at 55°C, rinsed in 1 x SSC and 0.1 x SSC at room temperature, dehydrated using a series of alcohols and air-dried. Slides were apposed to Kodak XAR-5 film for 3 weeks at 4°C and then dipped in Ilford K5 emulsion. After 6 - 8 weeks at 4°C, the slides were developed in Kodak D-19 developer, fixed and counterstained with methylene blue. Non-specific hybridisation was assessed in the presence of 100-fold excess of unlabelled oligonucleotide.

5-HT$_7$ mRNA was discretely localised in rat brain. From film, the strongest hybridisation signals were observed in the cortex, hippocampus, amygdala and thalamus (n=3). Other regions displaying hybridisation signals included the hypothalamus, dentate gyrus, habenula and subiculum. Analysis of the emulsion dipped slides revealed silver grains over large neurone cell bodies in all of the above mentioned areas with particularly dense grains over cells in the cingulate cortex, retrosplenic granular cortex, piriform cortex, CA3 region of the hippocampus, thalamus, hypothalamus and the amygdaloid complex particularly cortical, medial, and central nuclei. Areas with weak hybridisation signals included layers 1-3 of the cortex, CA1 and CA2 of the hippocampus, striatum, and cerebellum. Non-specific hybridisation was uniformly distributed, just above background. Furthermore, in the majority of the regions investigated, hybridisation signal was associated with medium-sized (10 - 15 µm) neurones whereas no signal was associated with the magnocellular neurones (> 20 µm). The phenotype(s) of the neurones expressing 5-HT$_7$ receptor mRNA is currently being determined.

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Gustafson, E. L. et al (1996) Brit. J. Pharmacol., 117, 657-666.
Lovenberg, T. W. et al (1993) Neuron, 11, 449-458.
Ruat, M. et al (1993) Proc. Natl. Acad. Sci. USA. 90, 8547-8551.
To, Z. P. et al (1995) Brit. J. Pharmacol., 115, 107-116.

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## 230P FURTHER CHARACTERISATION OF [$^3$H]5-CT BINDING IN RAT HYPOTHALAMUS

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The 5-HT$_7$ receptor has been cloned from rat, mouse, guinea pig and human tissue. 5-HT$_7$ receptor mRNA is localised in cortical and limbic brain regions which together with a high affinity for many antipsychotic agents suggests a role for the receptor in control of affective behaviour (e.g. Ruat et al; 1993). Competition assays using [$^3$H]5-CT ([$^3$H]5-carboxamidotryptamine) to label the 5-HT$_7$ receptor in rat brain have demonstrated shallow curves when methiothepin competes for the labelled receptor (Boyland et al; 1996). We have previously reported that in a rat hypothalamus preparation, [$^3$H]5-CT may label a homogenous population of sites with a pharmacological profile of the 5-HT$_7$ receptor (Stowe and Barnes 1996). This study used [$^3$H]5-CT to further characterise this 5-HT$_7$ receptor binding in rat hypothalamus.

Hypothalamus from female Wistar rats (180-220g) was frozen at -80°C prior to preparation of binding homogenate. To prepare the radioligand binding homogenate, brain tissue was defrosted and homogenised in ice-cold Tris buffer (50 mM; pH 7.4 using a Polytron blender (full power; 10 s). The homogenate was washed three times by centrifugation/resuspension in Tris buffer (50 mM; pH7.4) incubated at 37°C for 30 mins and finally resuspended in Tris buffer (50 mM; pH 7.4 containing 0.1% ascorbic acid, 10 µM pargyline and 4 mM $CaCl_2$) at a concentration of 75mg wet weight /ml. For competition radioligand binding experiments, [$^3$H]5-CT (0.5 nM) was incubated with brain homogenate for 2 hours at 37°C in the absence (total binding) and presence of the competing drug. Non-specific binding was determined in the presence of 10 µM 5-HT. Binding was terminated by rapid filtration under vacuum through 0.3% PEI- pre-treated Whatman GF/B glass fibre filters followed by immediate washing with ice-cold Tris buffer. Radioactivity remaining on the filters was assayed by liquid scintillation spectroscopy.

In homogenates of rat hypothalamus, a range of structurally diverse compounds competed for (±)-pindolol (10µM)-insensitive [$^3$H]5-CT binding in a monophasic manner with Hill coefficients around unity (see Table 1) and at concentrations consistent with their affinities for the recombinant 5-HT$_7$ receptor (Table 1).

However, in this hypothalamus preparation, the antipsychotic drug, pimozide failed to consistently compete for [$^3$H]5-CT binding with unity Hill slopes.

Table 1. Affinities and Hill coefficients for a range of compounds competing for (±)-pindolol (10µM)-insensitive [$^3$H]5-CT binding to homogenates of rat hypothalamus.

| Compound | pK$_i$ | Hill coefficient | 'n' |
|---|---|---|---|
| 5-CT | 8.99 ± 0.02 | 0.92 ± 0.02 | 3 |
| Methiothepin | 8.19 ± 0.01 | 1.19 ± 0.13 | 7 |
| 5-HT | 8.05 ± 0.06 | 0.95 ± 0.06 | 3 |
| Pimozide | 8.02 ± 0.12 | 0.84 ± 0.04 | 5 |
| Dihydroergotamine | 7.76 ± 0.05 | 1.09 ± 0.06 | 3 |
| Ritanserin | 6.87± 0.15 | 1.00 ± 0.15 | 5 |
| 8-OHDPAT | 6.83 ± 0.02 | 0.96 ± 0.04 | 3 |
| Sumatriptan | 5.88 ± 0.10 | 0.96 ± 0.04 | 3 |
| Clozapine | 6.81 ± 0.11 | 1.05 ± 0.03 | 4 |
| Ketanserin | 6.27 ± 0.14 | 0.99 ± 0.11 | 5 |
| Ondansetron | << 5 | | 3 |
| GR 113808 | << 5 | | 3 |
| (-)Sulpiride | << 5 | | 3 |
| DOI | << 5 | | 3 |

These results indicate that whilst the overall pharmacological profile of [$^3$H]5-CT binding in rat hypothalamus homogenate is very similar to the 5-HT$_7$ receptor, under the present binding conditions pimozide appears to recognise more than one population of [$^3$H]5-CT binding site, suggesting that [$^3$H]5-CT binding is not homogenous.

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

Boyland, P.S., Eastwood, S., Ellis, C. et al. (1996) Br. J. Pharmacol. 117, 132P.
Ruat, M., Traiffort, E., Leurs, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90, 8547-8551.
Stowe , R.L. and Barnes, N. M. (1996) Br. J. Pharmacol. 119, 366P.

## 231P MANIPULATION OF BRAIN 5-HT LEVELS AFFECTS GENE EXPRESSION FOR BDNF IN RAT BRAIN

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The gene expression for brain derived neurotophic factor (BDNF) has been shown to be regulated by a number of processes amongst which are neurotransmitter actions and second messenger cascades (Duman et al, 1997). The possible existence of an interaction between the central 5-HT and BDNF systems has recently attracted wide interest. It has for example, been shown that the chronic administration of 5-HT selective re-uptake inhibitors leads to an up-regulation of BDNF mRNA expression in the hippocampus, suggesting that 5-HT enhances BDNF gene expression (Nibuya et al, 1995). In contrast, a more recent study has shown that acute 5-$HT_2$ receptor stimulation results in decreased BDNF mRNA abundance in the the rat dentate gyrus (DG), while there was an increase in neocortical areas (Vaidya et al, 1997). The aim of the present study was to investigate whether changes in brain 5-HT levels affect BDNF gene expression in the rat brain. Brain 5-HT levels were elevated by co-administration of the monoamine oxidase inhibitor tranylcypromine (TCP) and the 5-HT precursor L-tryptophan (L-TP). 5-HT levels were depleted using the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine (*p*CPA) or *p*-chloroamphetamine (PCA), which destroys the majority of 5-HT axon projectons in the hippocampus ( Koe et al, 1966, Mamounas et al, 1991).

Rats were divided into three different treatment groups, A: TCP (20 mg/kg, i.p.), 30 min prior to L-TP (100 mg/kg, i.p.), rats were sacrificed at 2 h after the L-TP injection. B: *p*CPA, (300 mg/kg, i.p.) for two days, followed by 100 mg every other day for seven days and killed on day 11. C: PCA (10 mg/kg, i.p) for two days and killed 14 days after the last injection. After completion of the various treatments, rats were anaesthetised with sodium pentobarbitone, transcardially perfused and brains were removed and processed for ISHH (Zetterström et al, this meeting). The extent of 5-HT depletion following *p*CPA or PCA was verified using 5-HT immunochemistry (Mamounas et al, 1991). Values (mean±s.e.mean, n=5 rats/group) are expressed as a percentage of mean of optical densities from the matched control groups. Statistical analyses were performed using one way ANOVA and Dunnetts t-test.

Co-administration of TCP and L-TP decreased BDNF gene expression in the DG by 42±7.8%, ($p<0.002$, compared to controls) while it elevated BDNF gene expression in frontal cortex by 50±5.5%, ($p<0.002$, compared to controls). *p*CPA or PCA injections resulted in marked reductions of 5-HT immunoreactive axons, *p*CPA being most effective. Both drugs increased BDNF mRNA abundances in DG: *p*CPA by 44±6.2%, ($p<0.005$), compared to controls: PCA by 23± 4.2%, ($p<0.005$ compared to controls).

In summary we have shown that the acute co-administration of TCP and L-TP inhibits BDNF gene expression in DG and increases it in the frontal cortex. In contrast, treatments with *p*CPA or PCA increased BDNF gene expression in the DG. Future studies are in progress to investigate the underlying mechanisms following acute and chronic 5-HT stimulation in causing opposite changes in BDNF gene expression in the DG (Zetterström et al, this meeting).

Koe, B.K. *et al* (1966) *J. Pharmac. exp. Ther*, **154**, 499-516.
Mamounas, L.A. *et al* (1991) *J. Comp. Neurol*, **314**, 558-586.
Duman, R.S., *et al* (1997) *Arch Gen Psychiatry*, **54**, 597-606.
Nibuya, M., *et al* (1995) *Neuroscience*, **16**, 2365-2372.
Vaidya, V.A. *et al* (1997) *J. Neurosci*, **17**, 2785-2795.

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## 232P EFFECT OF PAROXETINE IN COMBINATION WITH THE β-BLOCKERS/5-$HT_{1A}$ ANTAGONISTS, (±)-PINDOLOL, (-)-TERATOLOL AND (-)-PENBUTOLOL, ON EXTRACELLULAR 5-HT IN THE RAT CORTEX

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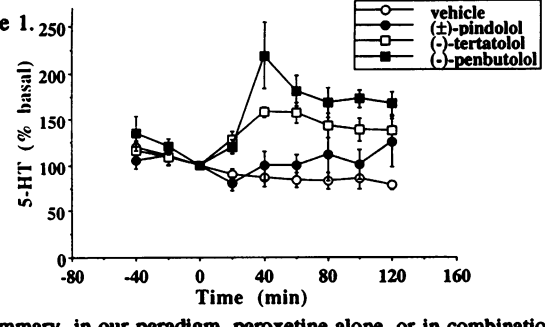
SSRIs block 5-HT uptake but only weakly increase extracellular 5-HT in the rat frontal cortex (FCx). However, SSRIs markedly elevate FCx extracellular 5-HT in animals pretreated with a 5-$HT_{1A}$ antagonist (Gartside *et al.*, 1995). Since increasing forebrain 5-HT function may be crucial for antidepressant action, 5-$HT_{1A}$ antagonists might enhance the antidepressant efficacy of SSRIs. Indeed, the β–blocker/5-$HT_{1A}$ antagonist, (±)-pindolol, augmented the antidepressant effect of SSRIs in open trials (Artigas *et al.*, 1996). However, the results of more recent double-blind clinical trials with (±)-pindolol are contractictory (Berman *et al.*, 1997), a fact which may be explained by recent evidence that (±)-pindolol is a partial 5-$HT_{1A}$ agonist (Clifford *et al.*, in preparation). Here, we have examined the effect of the combination of paroxetine and (±)-pindolol, on extracellular 5-HT in rat FCx. We also examined the interaction between paroxetine and two further β–blockers ((-)-tertatolol and (-)-penbutolol), reported to be silent 5-$HT_{1A}$ receptor antagonists (Sanchez *et al.*, 1996).

Male Sprague-Dawley rats (260-320 g) anaesthetised with chloral hydrate (500 mg/kg i.p.), were mounted in a stereotaxic frame. A concentric microdialysis probe (4 mm window) was implanted in the FCx, and perfused (2 µl/min) with artificial CSF. Dialysates (20 min samples) were analysed for 5-HT by HPLC-ED. Drugs were given i.v.. Animals received (±)-pindolol (4 mg/kg), (-)-tertatolol (2.4 mg/kg), (-)-penbutolol (2.4 mg/kg), or vehicle, and 10 min later (at t=0 min), all groups received paroxetine (0.8 mg/kg). Results (mean ± s.e.mean (n)) are expressed as % of 5-HT in the t=0 sample, or area under the curve (AUC) relative to no change (100 %). AUCs were compared by ANOVA and Dunnett's test.

Figure 1 shows the time course of the effect of paroxetine (0.8 mg/kg) in the 4 treatment groups. In vehicle-pretreated animals, paroxetine did not increase 5-HT in FCx dialysates. In the (±)-pindolol-pretreated group, although 5-HT levels after paroxetine were slightly higher than in vehicle pretreated animals, the AUC was not significantly different (AUC: 102 ± 14.1 % (6) *vs* 87 ± 6.4 % (9)). However, in (-)-tertatolol-pretreated animals, and in (-)-penbutolol-pretreated animals, paroxetine induced a distinct increase in 5-HT in FCx dialysates (Figure 1.). In both cases, the AUC was significantly greater than that in the vehicle pretreated group (137 ± 8.5 % (5), and 161 ± 6.3 % (5) respectively: $P<0.01$ *vs* vehicle).

Figure 1.




In summary, in our paradigm, paroxetine alone, or in combination with (±)-pindolol, did not increase extracellular 5-HT in the FCx. In contrast, paroxetine markedly increased 5-HT when combined with either (-)-tertatolol, or (-)-penbutolol. As an alternative to (±)-pindolol, we suggest that (-)-tertatolol or (-)-penbutolol might be useful in augmenting the antidepressant effects of SSRIs.

Artigas, F., Romero, L., de Montigny, C., & Blier, P. (1996) *Trends Pharmacol. Sci.* 15, 220-226.
Berman, R.M., Darnell, A.M., Miller, H.L., et al. (1997) *Am. J. Psychiatry* 154, 37-43.
Gartside, S.E., Umbers, V., Hajós, M., & Sharp, T. (1995) *Br. J. Pharmacol.* 115, 1064-1070.
Sanchez, C., Arnt, J. & Moltzen, E. (1996) *Eur. J. Pharmacol.* 315, 245-254.

## 233P BRL 54443, A POTENT AGONIST WITH SELECTIVITY FOR HUMAN CLONED 5-$ht_{1E}$ AND 5-$ht_{1F}$ RECEPTORS

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The 5-$ht_{1E}$ receptor was originally proposed to define a [$^3$H]-5-HT binding site in human brain remaining when 5-$HT_{1A}$, 5-$HT_{1D}$ and 5-$HT_{2C}$ receptors are masked (Leonhardt et al., 1989). Receptors having binding properties similar to this site and designated 5-$ht_{1E}$ and 5-$ht_{1F}$ were subsequently cloned (McAllister et al., 1992; Adham et al., 1993). The physiological roles of these receptors in brain is unknown. In order to identify potent selective ligands for these receptors we have examined the ability of a range of indoles to compete with binding of [$^3$H]-5-HT to membranes from CHO cells expressing cloned human 5-$ht_{1E}$ or 5-$ht_{1F}$ receptors. Selectivity was assessed from the radioligand binding profile of the ligands at a range of other monoamine (mostly human recombinant) receptors. Functional activity of compounds at 5-$HT_1$ receptors was assessed by their ability to stimulate binding of [$^{35}$S]-GTPγS to CHO cell membranes expressing these receptors, as described previously (Thomas et al., 1993).

BRL-54443 (3-(1-methylpiperidin-4-yl)-1H-indol-5-ol) was identified as a potent ligand for 5-$ht_{1E}$ and 5-$ht_{1F}$ receptors with more than 30-fold selectivity against human cloned 5-$HT_{1A}$ and 5-$HT_{1D}$ and more than 50-fold selectivity against a range of other 5-HT and dopamine receptors (Table 1). In studies using rat brain cortical membranes, BRL-54443 caused biphasic inhibition of total [$^3$H]-5-HT binding. When binding to 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1D}$ and 5-$HT_{2C}$ receptors was masked with 5-carboxamidotryptamine (100 nM) and mesulergine (100 nM), inhibition of [$^3$H]-5-HT binding became monophasic with an apparent pKi of 8.7 ± 0.1. In functional studies, BRL-54443 was a full agonist at 5-$ht_{1E}$ and 5-$ht_{1F}$ receptors ($pEC_{50}$s: 8.5 ± 0.1 and 8.6 ± 0.1 respectively) and was also a full agonist at 5-$HT_{1A}$, 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors.

Table 1. Binding affinities (pKi) for BRL-54443 at a range of monoamine receptors (human recombinant, unless indicated)

| Receptor | pKi | Receptor | pKi |
|---|---|---|---|
| 5-$HT_{1A}$ | 7.2 ± 0.1 | 5-$HT_{2C}$ | 6.5 ± 0.1 |
| 5-$HT_{1B}$ | 6.9 ± 0.1 | 5-$HT_4$ (piglet) | <6 |
| 5-$HT_{1D}$ | 7.2 ± 0.1 | 5-$HT_7$ (g.pig) | <6 |
| 5-$ht_{1E}$ | 8.7 ± 0.1 | $hD_2$ | 6.3 ± 0.1 |
| 5-$ht_{1F}$ | 8.9 ± 0.1 | $hD_3$ | 6.2 ± 0.1 |
| 5-$HT_{2A}$ | 5.9 ± 0.1 | $\alpha_{1B}$ | 5.9 ± 0.1 |
| 5-$HT_{2B}$ | 7.0 ± 0.1 | | |

Data are mean ± sem for n ≥ 3 determinations

BRL-54443 is therefore a potent selective agonist for the 5-$ht_{1E}$ and 5-$ht_{1F}$ receptors and may be a useful tool for analysing the physiological roles of these receptors.

Adham, N., Kao, H-T, Schecter, L.E. et al., (1993). *Proc. Natl. Acad. Sci.* **90**, 408-412.

Leonhardt, S., Herrick-Davis, K. and Titeler, M. (1989). *J. Neurochem.* **53**, 465-471.

McAllister, G., Charlesworth, A., Snodin, C. et al., (1992). *Proc. Natl. Acad. Sci.* **89**, 5517-5521.

Thomas, D.R., Faruq, S.A., Balcarek, J.M. and Brown, A.M. (1995). *J. Recept.Signal. Transduction. Res.* **15**, 199-211.

## 234P USE OF FLIPR TECHNOLOGY TO INVESTIGATE AN ENDOGENOUSLY EXPRESSED 5-HT RECEPTOR IN SKNBE(2) CELLS

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G-protein coupled receptors that modulate phospholipase C isoforms can be studied by monitoring the production of phosphoinositides inside the cell. However, an alternative approach is to measure $Ca^{++}$ mobilisation within the cell using fluorescence techniques. In this study we have investigated a 5-HT receptor endogenously expressed in a neuroblastoma cell line (SKNBE(2)) using a Fluorescence Imaging Plate Reader (FLIPR, Molecular Devices).

Cultured SKNBE(2) cells were plated onto polytronic 96 well plates (20,000 cells/well) and incubated in the presence of the fluoresence dye indicator, FLUO-3AM (0.5μg), at 37$^0$C for 90 min. The cells were then washed 4x with Tyrode buffer, placed into the FLIPR and individual wells subsequently exposed to increasing concentrations of test compound. For antagonist studies, washed cells were incubated for a further 30 min in the presence of antagonist before exposure to agonist. Mobilisation of cytoplasmic $Ca^{++}$ was measured as an increase in fluorescence intensity (F.I.) detected at excitation and emission wavelengths of 488nM and 540nM respectively. Peak increase in fluorescence was recorded and expressed as a percentage of the maximum 5-HT response.

Increasing concentrations of 5-HT caused a dose-dependent increase in F.I. with $pEC_{50}$ = 6.0. Sumatriptan, (+)-8-Hydroxy-dipropyl aminotetralin HBr (8-OH-DPAT), SKF99101, m-CPP ( 1-(-3-Chlorophenyl)piperazine dihydrochloride) and 2-methyl-5-HT had no effect on F.I. up to 10μM.

Table 1.

| Compound | % Inhibition of max. 5-HT response | apparent $pK_h$ |
|---|---|---|
| ritanserin (1nM) | 41 ± 5 | |
| ritanserin (3nM) | 68 ± 5 | |
| ritanserin(10nM) | 96 ± 2 | |
| ketanserin (1nM) | 62 ± 3 | 8.9 ± 0.1 |
| ketanserin (3nM) | 91 ± 4 | 8.8 ± 0.1 |
| ketanserin (10nM) | 100 | |
| DOI (300nM) | 34 ± 6 | 6.7 ± 0.1 |

GR127935 (30nM), WAY100635 (30nM) and metoclopramide (1μM) had no effect on the 5-HT concentration-effect curve suggesting it is not due to a 5-HT1B/1D, 1A or 3 receptor subtype respectively. The 5-$HT_2$ selective antagonists ritanserin and ketanserin did not effect F.I. up to 1μM, but attenuated the 5-HT response in a dose-dependent, apparently, non-competitive manner (Table1). The 5-$HT_2$ selective ligand DOI (R(-)-2,5-Dimethoxy-4iodo-amphetaminehydrobromide) had no effect on its own but at 300nM also caused a reduction in the maximum 5-HT response and produced a small rightward shift of the 5-HT concentration-effect curve.

Our studies have shown the existence of a 5-HT receptor endogenously expressed in SKNBE(2) cells. Of several selective 5-HT compounds tested DOI, ketanserin and ritanserin inhibited this response suggesting the presence of a 5-$HT_2$ receptor subtype. The lack of agonist effect of DOI alone may be explained by the fact that DOI has previously been reported as a partial agonist (Pierce & Peroutka, 1988) and the level of receptor expression is too low to display significant intrinsic activity. Further studies are required to fully characterise this endogenous receptor.

Pierce, P.A. & Peroutka, S.J. (1988) *J. Pharmacol. Exp.Ther.*, 247, 918-925.

## 235P MODULATION OF SECRETONEURIN-INDUCED 5-HT RELEASE FROM THE HIPPOCAMPUS OF BEHAVING RATS

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Secretoneurin (SN) is a novel peptide derived by proteolysis of secretogranin II (chromogranin C). In a recent study our group has shown that SN induces a concentration-dependent release of dopamine from the striatum of awake rats. It is proposed that SN may act as a natural neurotransmitter (Agneter *et al.*, 1995). The present study expands our investigations on SN-induced serotonin (5-HT) release from the hippocampus of behaving rats (male Sprague-Dawley rats weighing 280-320 g) utilising in vivo microdialysis.

A guide cannula was inserted into the right hippocampus (interaural line as reference; tip of probe; rostrocaudal +4.2mm, lateral -4.6mm, dorsoventral +1.0 mm). After a recovery period of two days a microdialysis probe was inserted through the guide cannula of the awake animal and perfused with artificial cerebrospinal fluid (aCSF) at a flow of 1.5 µl/min. The dialysate was collected in 15 min fractions and the content of 5-HT quantified by HPLC with electrochemical detection. SN and test drugs were added to the aCSF (number of experiments: 5 - 12).

SN at concentrations of 3, 10 and 30 µM induced a concentration-dependent and reversible increase of the 5-HT concentrations in the dialysate. The effect of SN was quantified by the area under the 5-HT-concentration vs. time-curve (AUC in arbitrary units) and amounted to 183 ± 79, 387 ± 129, 929 ± 175 units for 3, 10, and 30 µM, respectively.

To test a possible indirect effect of SN on 5-HT efflux via excitatory aminoacid release NMDA- and non-NMDA-antagonists were added to the aCSF before and during the SN-stimulation. The non-NMDA-antagonist GAMS (D-γ-Glutamyl-Aminomethanesulfonic-Acid) concentration-dependently (10, 30, 100 µM) reduced the SN-effect (10 µM) on 5-HT efflux, and 300 µM caused an inhibition of 5-HT efflux below baseline. The AUC-values amounted to 456 ± 80, 346 ± 98, 172 ± 70, and -134 ± 109 units for 10, 30, 100, and 300 µM respectively.

The non-NMDA-antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) also concentration-dependently (300, 1000 µM) decreased SN-induced 5-HT efflux but to a lesser extent than GAMS. A lower concentration of CNQX (100 µM) increased the effect of 10 µM SN (AUC: 820 ± 129 units). By contrast the NMDA-antagonist D-AP5 (D(-)-2-Amino-5-Phosphopentanic-acid) did not inhibit the SN-effect (100µM) but displayed a potentiating effect at the higher concentrations of 300 and 1000 µM (AUC-values : 410 ± 78, 783 ± 168, and 1888 ± 782 units for 100, 300, and 1000 µM, respectively). The effects of the antagonists alone have not been investigated.

The results point to a possible involvement of excitatory aminoacid neurotransmitters in the effect of SN on 5-HT efflux.

Agneter E., Sitte HH., Stöckel-Hiesleitner S., et al., (1995) *J Neurochem* 65, 622-625.

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## 236P TRANSIENT UP-REGULATION OF CORTICOSTEROID RECEPTORS FOLLOWING LONG-TERM ADMINISTRATION OF DESMETHYLIMIPRAMINE

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Long-term administration of some antidepressant drugs increases the density of corticosteroid receptors (CR) in hippocampus of adrenalectomised rats. (Przegalinsky & Budziszweska, 1993; Reul *et al*, 1993, 1994). The aim of this study was to measure CR following desmethylimipramine (DMI), in cortex from rats with intact adrenals.

Male Sprague-Dawley rats (~200g at the start of the study) were given DMI (10mg $kg^{-1}$, p.o. once daily) or vehicle for up to 28 days. Rats were killed 24 hours after 1, 14 and 28 days of drug administration. Dissected tissues were frozen on dry ice and stored at –70°C until assay. CR binding experiments were carried out by incubating 100µL aliquots of the cytosolic fraction prepared from cortical tissue with $^3$H-dexamethasone (concentrations ranging from 0.625-20nM) for 20-24 hours at 0-4°C. Non-specific binding was defined using 5µM hydrocortisone. Bound and free $^3$H-dexamethasone were separated by filtration under vacuum (Rosser *et al*, 1995). Binding parameters (Bmax and Kd) were determined by non-linear regression analysis to a one-site binding model. Statistical analysis was carried out using two-way ANOVA, followed by Student's t-test. Administration of DMI for 14 but not 28 days caused a significant increase (36%) in the density of CR as compared to control values (Table 1). Acute administration did not alter the density of these receptors relative to control values but caused an increase in their affinity for $^3$H-dexamethasone. Differences in Bmax and Kd were also observed within the control group at different time points. Although our results show a transient up-regulation of CR by DMI, interpretation of this should consider the observations within the control group. These indicate that factors (e.g. stress produced by handling) which may cause fluctuations in plasma corticosterone could influence binding of $^3$H-dexamethasone to CR and these effects may be influenced by antidepressants.

Przegalinsky E. & Budziszweska B. (1993) *Neurosci.Lett.*161,215-218
Reul J.M.H.M *et al* (1993) *Endocrinology* 133, (No.1) 312-320
Reul J.M.H.M *et al* (1994) *Neuroendocrinology* 60, 509-519
Rosser D.J. *et al* (1995) *Br.J. Pharmacol.*116, Proc.Suppl.(Oct); 101P

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**Table 1. Effect of repeated administration of DMI on 3H-dexamethasone binding in rat cortex.**

| Duration of drug administration (days) | 1 | | 14 | | 28 | |
|---|---|---|---|---|---|---|
| | Control | DMI | Control | DMI | Control | DMI |
| Bmax | 114 ± 12 | 103 ± 10 | 92 ± 6 | 125 ± 11* | 121 ± 10 | 108 ± 17 |
| Kd | 3.64±0.45 | 2.13 ± 0.36* | 1.59±0.22# | 1.61± 0.2 | 3.29±0.39 | 2.62±0.35 |

Bmax (fmol/mg protein) and Kd (nM) values are mean ± s.e.m.(n = 6-8). * p<0.05 compared to respective controls. # p<0.05 compared to control at days 1&28.

## 237P EFFECT OF BRL 54443 (3-(-1-METHYLPIPERIDIN-4-YL)-1H-INDOL-5-OL), A 5-$HT_{1E/1F}$ RECEPTOR AGONIST, ON GENERAL BEHAVIOUR AND MAXIMAL ELECTROSHOCK SEIZURE THRESHOLD IN THE RAT

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In vitro radioligand binding studies reveal that BRL 54443, 3-(-1-methylpiperidin-4-yl)-1H-indol-5-ol, has selective affinity for the human cloned 5-$HT_{1E}$ and 5-$HT_{1F}$ receptors (pKi 8.8) vs. other 5-HT (including the 5-$HT_{2C}$ receptor - $pK_I$ 6.5) and dopamine receptors (Brown et al., this meeting), and is a full agonist at each. We have studied the effects of BRL 54443 on general behaviour and maximal electroshock seizure threshold in the rat.

a) Rats (Sprague Dawley; approx. 300g; 6-8/group) were administered BRL 54443 (1-5 mg/kg i.p.) 20 min before assessment in an automated locomotor activity (LMA) monitoring apparatus (57x17x25cm) for 20 min under red light. Since 5-$HT_{2C}$ receptor activation is associated with a change in LMA, in a subsequent experiment rats were pre-dosed with the selective 5-$HT_{2C}$ receptor antagonist SB-221284 (3 mg/kg p.o. 1hr pretest) (Bromidge et al., 1997) or 1% methyl cellulose soln. before BRL 54443 (3 mg/kg i.p.) and LMA assessment.

b) Rats (Sprague Dawley; approx. 300g; 6-14/group) were administered BRL 54443 ($10^{-4}$-$10^{-1}$ mg/kg i.p.) or saline 20 min before being placed in a circular cage (34cm diameter) under white light. Time spent sniffing any point for longer than 1 s during 10 min was recorded by an observer blind with respect to treatment.

c) Rats (Sprague Dawley; approx. 100g; 27-29/group) were administered BRL 54443 ($10^{-4}$-$10^{-1}$ mg/kg i.p. 30 min pretest) or saline, and assessed for hindlimb tonic extensor seizure following a single shock using the "Up and Down" method described by Upton et al. (1997). CC50 (current producing convulsions in 50% of rats) for each treatment group were determined.

Results are given as mean ± s.e.m.

a) LMA was significantly (ANOVA and Dunnett's) reduced by BRL 54443 at doses of 3 and 5 mg/kg, from a control of 16.6±2.0 apparatus transits to 7.4±1.6 and 9.0±1.9, respectively, with no effect at 1 mg/kg. A reduction in LMA produced by 3 mg/kg BRL 54443 (12.2±2.0 vs. 20.0±1.5 transits) was significantly (2 way-ANOVA and Newman Keuls) reversed by SB-221284 to 19.2±2.1 transits.

b) BRL 54443 significantly (ANOVA and Dunnett's test) increased time spent sniffing at doses of $10^{-3}$, $10^{-2}$ and $10^{-1}$ mg/kg from a control of 46.7±5.4 to 89.2±7.4, 77.4±9.5 and 82.0±4.9 s, respectively. No significant effect was seen at lower doses.

c) At doses of $10^{-2}$ and $10^{-1}$ mg/kg, BRL 54443 significantly (Litchfield and Wilcoxan) increased the seizure threshold from a control CC50 of 20.4±1.4 to 27.9±2.0 and 28.7±2.4 mA, respectively. No effect on CC50 was produced by the lower doses tested.

BRL 54443, a selective 5-$HT_{1E/1F}$ receptor agonist, stimulates sniffing behaviour and is weakly anticonvulsant in rats at doses below which it activates 5-$HT_{2C}$ receptors in vivo. Firm pharmacological characterisation of these responses awaits an appropriate antagonist tool.

Bromidge, S. M., Dabbs, S., Davies, D. T. et al. (1997) J. Med. Chem., In Press.
Upton, N., Blackburn, T. P, Campbell, C. A. et al. (1997) Br. J. Pharmacol. 121, 1679-1686.

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## 238P EFFECTS OF SELECTIVE 5-$HT_{1B}$ RECEPTOR LIGANDS ON INDICES OF APPETITE CONTROL IN THE RAT

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It has previously been proposed that the 5-$HT_{1B}$ receptor is involved in the hypophagic properties of the 5- $HT_{1B/1A}$ receptor agonist, RU 24969 (RU) (Kennett et al., 1987). In the present study, the effects of the selective 5-$HT_{1B}$ receptor antagonist, SB-224289 (Roberts et al., 1997) and agonist, SKF-99101 (SKF) (Hagan et al., 1995), the 5-HT $_{1B/1D}$ receptor antagonist, GR 127935 (GR) (Skingle et al., 1995), the selective 5-$HT_{2C}$ receptor antagonist, SB-242084 (Kennett et al., 1997) and the selective 5-$HT_{1A}$ receptor antagonist, WAY 100635 (WAY) (Fletcher et al., 1996) on feeding behaviour have been studied.

Male Sprague-Dawley rats (200-250 g) were singly housed under a 12 h light cycle (lights on 0700 h) with free access to food and water. Some rats were then food deprived for 23 h overnight and the following day given antagonist and agonist treatment prior to restoring food and monitoring intake over 2 h. Alternatively, freely-feeding rats were orally dosed and food-deprived 1 h before restoring food and monitoring over 24 h. All data was analysed by ANOVA and Newman-Keuls test and are cited as means ± sem.

RU (1-5 mg/kg i.p. 30 min pre-test), reduced the food intake of food-deprived rats. SB-224289 (5 or 10 mg/kg p.o. 1 h pre-test) had no effect on RU (5 mg/kg i.p.)-induced hypophagia, unlike WAY (0.01 or 0.1 mg/kg s.c. 30 min pre-test) which reversed the action of RU (food intake (g); vehicle + saline 5.2 ± 0.5, vehicle + RU 0.03 ± 0.05, WAY 0.01 + RU 2.37 ± 0.99, $p<0.01$, WAY 0.1 + RU 4.93 ± 1.4, $p<0.01$). Another 5-$HT_{1B}$ receptor agonist, SKF (20 mg/kg i.p. 30 min pre-test) also decreased the food intake of food-deprived rats from 5.6 ± 0.5 to 4.1 ± 0.4 g, $p<0.01$.

Pretreatment with SB-224289 (10 mg/kg p.o 1 h pre-test) attenuated this effect, SB-224289 + saline 7.0 ± 0.4, SB-224289 + SKF (20 mg/kg i.p.) 6.2 ± 0.4 but this may have been because the drug increased food intake when given alone (vehicle + saline 5.6 ± 0.5, SB-224289 + saline 7.0 ± 0.4 g $p<0.01$). SB-224289 5 mg/kg p.o. also modestly increased the food intake of freely-feeding rats over 4 h from 2.78 ± 0.3 to 4.5 ± 0.5 g $p<0.01$ while at 10 mg/kg p.o. an increase over 24 h was observed from 24.58 ± 0.6 to 26.4 ± 0.7 g $p<0.05$. Finally, the hypophagic action of d-fenfluramine, (5 mg/kg i.p. 30 min pre-test), in food-deprived rats, was not significantly affected by prior treatment with GR (1 or 2 mg/kg s.c. 30 min pre-test), but was partially blocked by SB-242084 (2 and 6 mg/kg p.o. 1 h pre-test).

In conclusion, RU-induced hypophagia is 5-$HT_{1A}$ and not 5-$HT_{1B}$ receptor mediated in the rat and there was no clear evidence that the 5-$HT_{1B}$ receptor mediates the hypophagic responses to either SKF or d-fenfluramine. However, the hyperphagic action of SB-224289 suggests that acute blockade of the 5-$HT_{1B}$ receptor may nevertheless affect appetite.

Fletcher, A., Forster, E. A., Bill, D. J., et al., (1996) Behav. Brain Res., 73, 337-353.
Hagan, J. J., Hatcher, J. P., Slade, P. (1995). Eur. J. Pharmacol., 294, 743-751.
Kennett, G. A., Dourish, C. T., Curzon, G., (1987). Eur. J. Pharmacol., 141, 429-435.
Kennett G. A., Wood, M. D., Bright, F., et al., (1997), Neuropharmacology, 36, 609-620.
Roberts, C., Price, G. W., Gaster, L., et al., (1997). Neuropharmacology, 36, 549-557.
Skingle, M., Scopes, D. I. C., Feniuk, et al., (1993), Br. J. Pharmacol., 110, 9P.

## 239P EFFECT OF DESTRUCTION OF THE ASCENDING 5-HYDROXYTRYPTAMINERGIC PATHWAYS ON SWITCHING BETWEEN CONCURRENT REINFORCEMENT SCHEDULES

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Destruction of the ascending 5-hydroxytryptaminergic (5-HTergic) pathways alters performance in operant behaviour paradigms which entail temporal regulation of behaviour (Ho *et al.*, 1995; Al-Ruwaitea *et al.*, 1997). Al-Ruwaitea *et al.* (1997) proposed that this effect may be mediated by an increase in the rate of switching between alternative behavioural states. We report here the effect of lesions of these pathways on switching between two concurrently available reinforcement schedules.

Under halothane anaesthesia, 12 female Wistar rats (250-300 g) received injections of 5,7-dihydroxytryptamine (4 μg base in 2 μl vehicle) into the dorsal and median raphe nuclei; 15 rats received sham lesions (method: Ho *et al*, 1995). They were then trained in operant conditioning chambers using a sucrose reinforcer (0.6 M, 50μl). Daily training sessions consisted of 40 minutes exposure to a pair of concurrent variable-time reinforcement schedules (Zeiler, 1977) specifying equal interreinforcement intervals, scheduled according to the changeover procedure (Findley, 1958). The two schedules were associated with the presence or absence of a light (1.2 W); the rat could switch between the two schedules by pressing a lever (changeover response). The experiment consisted of 7 phases (30 sessions each) in which the scheduled interreinforcement interval specified by each of the two schedules was 30 s, 60 s, 120 s, 240 s, 480 s, 960 s, and 30 s (redetermination condition). Then, approximately 7 months after surgery, the rats were killed and their brains dissected for assay of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline and dopamine by high-performance liquid chromatography with electrochemical detection (method: Ho *et al.*, 1995).

Both groups showed a significant inverse relation between rate of changeover responses and the interreinforcement interval specified by the two concurrent schedules. The lesioned group's changeover rate was consistently higher than that of the sham-lesioned group under all schedules: Table 1. The levels of 5-HT and 5-HIAA in the parietal cortex, hippocampus, amygdala and nucleus accumbens of the lesioned group were <16% of those of the control group (*t*-test, $P<0.001$ in each case), but the levels of noradrenaline and dopamine did not differ significantly between the groups ($P>0.1$ in each case).

**Table 1**: Rate of changeover responses (changeovers $min^{-1}$, mean ± s.e. mean) under each condition of the experiment

| | scheduled interreinforcement interval (s) | | | | | |
|---|---|---|---|---|---|---|
| Group | 30[a] | 60 | 120 | 240 | 480 | 960 |
| Control | 13.1±0.8 | 12.5±0.6 | 12.5±0.9 | 9.6±1.1 | 4.3±0.6 | 2.6±0.4 |
| Lesioned | 15.6±1.1 | 15.3±1.2 | 15.8±1.1 | 11.1±0.7 | 6.5±0.8 | 5.1±0.6 |

[a] mean of two determinations. Statistical analysis [ANOVA]: group $F_{1,25}=7.2$, $P<0.02$; condition $F_{5,125}=105.2$, $P<0.01$; interaction, $F<1$.

The finding that changeover rate was enhanced by destruction of the 5-HTergic pathways is consistent with the suggestion that these pathways may contribute to the inhibitory regulation of switching between behavioural states (Al-Ruwaitea *et al.*, 1997).

Al-Ruwaitea, A.S.A. *et al.* (1997) *Psychopharmacology*, in press.
Findley, J.D. (1958) *J. Exp. Anal. Behav.*, 1, 123-144.
Ho, M.-Y. *et al.* (1995) *Psychopharmacology*, 120, 213-219.
Zeiler, M.D. (1977) In *Handbook of operant behavior*, ed. Honig, W.K. & Staddon, J.E.R. Prentice-Hall, Englewood Cliffs.

## 240P CARBACHOL INFUSIONS INTO THE MEDIAN RAPHE NUCLEUS ELEVATE HIPPOCAMPAL THETA FREQUENCIES IN THE RAT

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One of the most thoroughly characterized and studied electrical phenomena in the hippocampus is the rhythmical, slow, sinusoidal, high voltage wave, called theta (Bland, 1986). Considerable research has demonstrated the existence of ascending brainstem synchronizing systems that generate hippocampal theta activity (Vertes *et al.*, 1993); these are known to be activated by acetylcholine. There is less known about afferents mediating non-theta states. Electrical stimulation of the median raphe (MnR) desynchronizes theta activity, while MnR inhibition, either through lesions or pharmacologically, produces long trains of hippocampal theta (Kinney *et al.*, 1995). In the present study, we have examined if theta is affected by intra-MnR carbachol infusions.

Adult male, Lister hooded rats weighing between 350-500 g, were anaesthetized with isofluorane and implanted with jugular catheters. They were then switched to urethane (0.8 g/ml) anaesthesia and placed in a stereotaxic frame. A theta recording electrode was placed in the stratum moleculare of the hippocampus (A-P -3.3, M-L 2.5, D-V 2.7 mm). A 30 gauge cannula was positioned in the MnR (A-P -7.8; M-L 0; D-V 8.0 mm) for local drug infusions. Rats were infused with 30 μg of carbachol (CARB) (n=7) into the MnR in 0.5-1.0 μl saline. Pre-drug theta activity (frequency and amplitude) was monitored and again at 30 min following infusions. At the conclusion of each experiment a small amount of dye was infused into the MnR to localize cannula placements. Changes in frequencies (Hz) and amplitude (mV) of the theta signals were analyzed using ANOVA.

ANOVA revealed a significant effect of CARB on theta frequency $F(1,6)=10.2$, $P<.001$, but no effect on theta amplitude $F(1,6)=0.55$, ns. Similar control infusions were without effect. As shown in Figure 1, CARB elevated frequencies compared to the pre-drug condition.

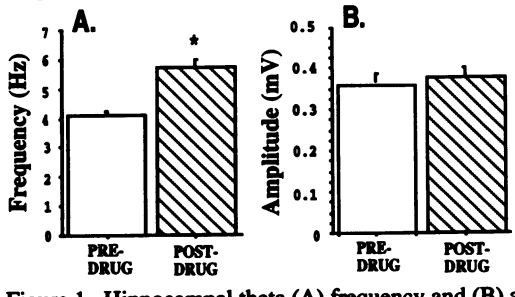


Figure 1. Hippocampal theta (A) frequency and (B) amplitude under spontaneous conditions following CARB infusions (Means ± SEM are shown).
Significantly different from pre-drug condition *(P<.05).

Local inhibition of 5-HT cells in the MnR is associated with long, uninterrupted trains of hippocampal theta. 5-HT cells in the MnR may receive an inhibitory input from local GABAergic interneurons (Vertes *et al.*, 1994). Direct infusions of carbachol may excite these GABAergic neurons and produce feed-forward inhibition of 5-HT neurons thereby blocking an ascending theta desynchronizing signal. In conclusion, these data suggest that brainstem carbachol infusions may directly activate ascending theta synchronizing mechanisms, and inhibit ascending theta desynchronizing signals.

Bland, B.H. (1986) *Prog. Neurobiol.* 26, 1-54.
Kinney, G.G.*et al.*, (1995) *Psychopharmacol.* 120, 244-248.
Vertes, R.P.*et al.*, (1993) *Exp. Brain Res.* 96, 419-429.
Vertes, R.P.*et al.*, (1994) *Neuroscience.* 60, 441-451.

## 241P THE SELECTIVE 5-HT$_{2A}$ RECEPTOR ANTAGONIST MDL 100,907 ATTENUATES THE BEHAVIOURAL AND NEUROCHEMICAL EFFECTS OF PCP IN RODENTS

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In rodents, phencyclidine (PCP) stimulates locomotor activity and increases mesolimbic dopamine metabolism/release, effects which may relate to the psychotomimetic effects seen in man. Recent studies suggest that MDL 100,907, a selective 5HT$_{2A}$ receptor antagonist, attenuates PCP-induced hyperactivity in the rat (Maurel-Remy *et al.*, 1995). In the present studies, we have extended these observations and examined the effects of MDL 100,907 on both PCP-induced hyperactivity and on the increase in dopamine metabolism and release induced by PCP in rat nucleus accumbens.

Male BKTO mice (21-34 g) were habituated to individual activity cages equipped with 2 infra-red beams positioned one at each end of the base of the cage to record cage crossings ie consecutive beam breaks. Animals (n = 7-8/group) were pretreated with either vehicle (veh; water; 10 ml/kg, s.c.) or MDL 100,907 (0.003 - 3 mg/kg, s.c.) 15 min prior to injection of either saline (sal; 10 ml/kg, s.c.) or PCP (5 mg/kg, s.c.). Photocell beam breaks were then monitored for 140 min. Dopamine (DA) efflux was measured in conscious Sprague Dawley rats (250 - 350 g; n = 5-6/group) implanted 18 h previously with a concentric dialysis probe in the nucleus accumbens (A/P +1.7 mm, L +1.5 mm from bregma; V -7.5 mm from dura (Paxinos and Watson, 1982)) under isoflurane anaesthesia. Probes (2 mm Hospal membranes) were perfused with Ringer (composition mM: NaCl (125), KCl (2.5), $MgCl_2$ (1.18), $CaCl_2$ (1.26)) at 1 µl/min and samples collected every 20 min and analysed for DA content by HPLC with electrochemical detection. Two h after beginning perfusion, rats were pretreated with either veh (1 ml/kg, s.c.) or MDL 100,907 (1 mg/kg, s.c.) 20 min prior to injection of either PCP (10 mg/kg, i.p.) or saline (1 ml/kg, i.p.) and samples collected for a further 2 h. To examine effects on DA metabolism, rats (n = 6/group) were dosed with MDL 100,907 (0.3 mg/kg, s.c.) or veh (1 ml/kg, s.c.) 30 min prior to saline or PCP (10 mg/kg, i.p.) injection. Animals (n = 6/group) were killed 60 min later, the nucleus accumbens dissected and the levels of DA and the metabolites DOPAC and HVA measured by HPLC with electrochemical detection. All data were analysed by analysis of variance followed by either Tukey's or Dunnett's t test (*P<0.05 compared to veh/PCP-treated animals).

Pretreatment with MDL 100,907 significantly reduced PCP-induced hyperlocomotion with maximal inhibition (74%) achieved at a dose of 0.03 mg/kg (mean ± s.e. mean total cage crosses: veh/sal = 29 ± 8*; veh/PCP = 925 ± 197; MDL 100,907(0.003)/PCP = 859 ± 195; MDL 100,907(0.03)/PCP = 262 ± 63*; MDL 100,907(0.3)/PCP = 331 ± 100*; MDL 100,907(3.0)/PCP = 327 ± 130*). Systemic injection of PCP also increased DA efflux (measured by dialysis) in the rat nucleus accumbens, an effect which was maximal 40 min after dosing (mean ± s.e. mean DA content (expressed as a % of basal efflux): veh/sal = 99 ± 8%*; veh/PCP = 289 ± 45%). MDL 100,907 (1 mg/kg, s.c.) had no effect on DA efflux *per se*, but abolished the increase induced by PCP (MDL 100,907/sal = 108 ± 22%; MDL 100,907/PCP = 127 ± 16%*). The increase in DA metabolism (expressed as the ratio [DOPAC + HVA]/DA) induced by PCP was also abolished in rats pretreated with 0.3 mg/kg MDL 100,907 (mean ± s.e. mean: veh/sal = 0.25 ± 0.01*; veh/PCP = 0.34 ± 0.01; MDL 100,907/sal = 0.25 ± 0.01*; MDL 100,907/PCP = 0.28 ± 0.02*).

These results clearly demonstrate that MDL 100,907 attenuates the behavioural and neurochemical effects of PCP in rodents and support the suggestion that 5HT$_{2A}$ receptor antagonists have antipsychotic potential in man.

Maurel-Remy, S., Bervoets, K. and Millan, M.J. (1995). Eur. J. Pharmacol., 280, R9-R11.

Paxinos, G. and Watson, C. (1982). The rat brain in stereotaxic coordinates. Sydney: Academic Press.

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## 242P INHIBITORY AND DISINHIBITORY PROFILES OF THE INTERACTION BETWEEN M-CHLOROPHENYLPIPERAZINE AND M-CHLOROPHENYLBIGUANIDE IN THE MOUSE LIGHT DARK TEST

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The 5-HT receptor agonists m-chlorophenylpiperazine (MCPP) and m-chlorophenylbiguanide (MCPBG) have anxiogenic profiles of action in rodent models of anxiety (Kennett *et al.*, 1989; Costall & Naylor, 1995). The present study investigates the effect of their interaction in the mouse light dark test.

Male albino BKW mice (Bradford strain) weighing 25-30g received an intraperitoneal injection of vehicle + vehicle/ diazepam/MCPP/MCPBG or MCPP plus MCPBG, with 40 min between treatments and behavioural testing 40 min after the last treatment. Mice were placed into the centre of the light compartment of the test box and the latency of first movement from the light to the dark compartment and line crossings in both the light and dark compartments was video recorded over a 5 min period (for detailed methodology see Cheng *et al.*, 1994).

MCCP and MCPBG when administered alone decreased and the reference anxiolytic agent diazepam increased the latency of first movement from the light to the dark compartment, in the absence of non-specific changes in line crossings. The inhibitory effect of MCPP and MCPBG was reversed to disinhibition by a combined treatment of MCPP plus MCPBG or MCPBG plus MCPP. The anxiolytic profile of the combined administration of MCPP and MCPBG in doses that normally induce inhibition may relate to complex agonist, partial agonist and antagonist interactions at 5-HT$_2$, 5-HT$_3$ and possibly other 5-HT receptors (Kennett *et al.*, 1989; Kilpatrick *et al.*, 1990) in the presence of varying degrees of endogenous 5-HT tone.

Costall, B. & Naylor, R.J. (1995) *Br. J. Pharmacol.*, in press
Cheng, C.H.K., Costall, B., Kelly, M.E. *et al.* (1994) *Eur. J. Pharmacol.* 255, 39-49.
Kilpatrick, G.J., Butler, A., Burridge, J. *et al.* (1990) *Eur. J. Pharmacol.* 182, 193-197.
Kennett, G.A., Whitton, P., Shah, K. *et al.* (1989) *Eur. J. Pharmacol.* 164, 445-454.

Table 1. The actions and interactions of MCPP and MCPBG to modify mouse behaviour in the light (L) dark (D) test

| Treatment | Latency L>D (s) | Treatment | Latency L>D (s) |
|---|---|---|---|
| Vehicle + vehicle | 11.0 ± 1.3 | Vehicle + vehicle | 12.2 ± 1.6 |
| Vehicle + MCPP (0.005mg kg$^{-1}$) | 12.1 ± 2.0 | Vehicle + MCPBG (0.005mg kg$^{-1}$) | 13.8 ± 2.0 |
| Vehicle + MCPP (0.01mg kg$^{-1}$) | 9.0 ± 2.1 | Vehicle ± MCPBG (0.01mg kg$^{-1}$) | 6.3 ± 2.5 |
| Vehicle + MCPP (0.05mg kg$^{-1}$) | 2.1 ± 0.3* | Vehicle + MCPBG (0.05mg kg-1) | 2.2 ± 0.4* |
| MCPP (0.05mg kg$^{-1}$) + MCPBG (0.005mg kg$^{-1}$) | 1.8 ± 0.4* | MCPBG (0.05mg kg$^{-1}$) + MCPP (0.005mg kg$^{-1}$) | 2.6 ± 0.7* |
| MCPP (0.05mg kg$^{-1}$) + MCPBG (0.01mg kg$^{-1}$) | 24.2 ± 3.1*+ | MCPBG (0.05mg kg$^{-1}$) + MCPP (0.01mg kg$^{-1}$) | 11.7 ± 2.1+ |
| MCPP (0.05mg kg$^{-1}$) + MCPBG (0.05mg kg$^{-1}$) | 26.4 ± 2.5*+ | MCPBG (0.05mg kg$^{-1}$) + MCPP (0.05mg kg$^{-1}$ | 24.8 ± 2.9*+ |
| Vehicle + Diazepam (0.5mg kg$^{-1}$) | 23.6 ± 2.1*+ | MCPBG (0.05mg kg$^{-1}$) + MCPP (0.1mg kg$^{-1}$) | 22.5 ± 3.2*+ |

Values (mean ± s.e. mean); (n = 10). *P<0.01 compared to vehicle + vehicle controls; +P<0.01 compared to vehicle + MCPBG (0.05 mg kg$^{-1}$/MCPP (0.05 mg kg$^{-1}$) (one way ANOVA followed by Dunnett's t test).

## 243P CHARACTERISATION OF THE 5-HT RECEPTOR MEDIATING THE BEHAVIOURAL INHIBITORY EFFECTS OF M-CHLOROPHENYLBIGUANIDE IN THE MOUSE LIGHT DARK TEST

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The relatively selective 5-$HT_3$ receptor agonist m-chlorophenylbiguanide (MCPBG) (Kilpatrick *et al.*, 1990) exerts an inhibitory or anxiogenic profile in the mouse light/dark test (Costall & Naylor, 1995). The present study uses selective 5-HT receptor antagonists to characterise the receptor mediating the effects of MCPBG.

Male albino BKW mice (Bradford strain) weighing 25-30g received an intraperitoneal injection of vehicle + vehicle/MCPBG/ondansetron/ritanserin/methysergide/GR113808 or MCPBG + ondansetron/ritanserin/methysergide, with 40 min between treatments and behavioural testing 40 min after the last treatment. Mice were placed into the centre of the light compartment of the test box and the latency of first movement from the light (L) to the dark (D) compartment and line crossings was video recorded over a 5 min period (for detailed methodology see Cheng *et al.*, 1994).

In the absence of non-specific changes in line crossings in the light and dark areas, the administration of MCPBG alone decreased the latency of first movement from the light to the dark compartment. The 5-$HT_1$, 5-$HT_2$ and 5-$HT_4$ receptor antagonists methysergide, ritanserin and GR113808 when administered alone failed to modify behaviour or the effects of MCPBG. In contrast, the 5-$HT_3$ receptor antagonist, ondansetron, disinhibited behaviour: the effects of a low dose of ondansetron were antagonised by MCPBG whereas the effects of higher doses of ondansetron when administered with MCPBG effectively negated the effect of either drug. The selective antagonistic interaction between ondansetron and MCPBG is supportive of the importance of a 5-$HT_3$ receptor involvement in the mediation of their effects.

Costall, B. & Naylor, R.J. (1995) *Br. J. Pharmacol.*, in press
Cheng, C.H.K., Costall, B., Kelly, M.E. *et al.* (1994) *Eur. J. Pharmacol.* 255, 39-49.
Kilpatrick, G.J., Butler, A., Burridge, J. *et al.* (1990) *Eur. J. Pharmacol.* 182, 193-197.

Table 1. Effect of MCPBG and its interaction with 5-HT receptor antagonists in the mouse light/dark test box

| Treatment | Latency L > D (s) | Treatment | Latency L > D (s) |
|---|---|---|---|
| Vehicle + vehicle | 11.4 ± 1.3 | GR113808 (10 μg $kg^{-1}$) + vehicle | 11.6 ± 1.2 |
| Vehicle + MCPBG (0.005 mg $kg^{-1}$) | 10.7 ± 1.6 | GR113808 + MCPBG (0.01 mg $kg^{-1}$) | 1.8 ± 0.3* |
| Vehicle + MCPBG (0.01 mg $kg^{-1}$) | 2.0 ± 0.3* | Ondansetron (0.01 μg $kg^{-1}$) + vehicle | 12.5 ± 1.8 |
| Vehicle + MCPBG (0.05 mg $kg^{-1}$) | 3.1 ± 0.4* | Ondansetron + MCPBG (0.01 mg $kg^{-1}$) | 2.2 ± 0.3* |
| Methysergide (1.0 mg $kg^{-1}$) + vehicle | 12.5 ± 1.6 | Ondansetron (0.1 μg $kg^{-1}$) + vehicle | 24.9 ± 2.8* |
| Methysergide + MCPBG (0.01 mg $kg^{-1}$) | 2.3 ± 0.5* | Ondansetron + MCPBG (0.01 mg $kg^{-1}$) | 1.8 ± 0.4*° |
| Ritanserin (1.0 mg $kg^{-1}$) + vehicle | 10.9 ± 1.1 | Ondansetron (1.0 μg $kg^{-1}$) + vehicle | 26.3 ± 2.2* |
| Ritanserin + MCPBG (0.01 mg $kg^{-1}$) | 1.5 ± 0.4 | Ondansetron + MCPBG (0.01 mg $kg^{-1}$) | 14.0 ± 1.9+ |

Values (mean ± s.e. mean); (n = 10). *P<0.01 compared to vehicle + vehicle controls; +P<0.01 compared to vehicle + MCPBG (0.01 mg $kg^{-1}$); °P<0.01 compared to ondansetron vehicle control (one way ANOVA followed by Dunnett's t test).

## 244P THE EFFECT OF MICRODIALYSIS PROBE IMPLANTATION ON BEHAVIOUR OF RATS IN THE LIGHT/DARK BOX

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Microdialysis in rats undergoing behavioural testing has been used to correlate extracellular neurotransmitter levels with behaviour (Westerink *et al.*, 1994). It is established that there is exocytotic release of neurotransmitters 24 h after surgery and implantation of a microdialysis probe. However, it is unclear whether normal behaviour is re-established on a similar time scale. Consequently, the use of guide cannulae, to enable longer post-surgical recovery, has been advocated (Drijfhout *et al.*, 1995). Here we have studied the activity of rats exposed to novel environments 1 and 7 days after experiencing surgery procedures associated with microdialysis.

The behaviour of 3 groups of male S.D. rats was studied. The 1st group comprised rats (270-300 g) naïve to surgery. The 2nd group (270-300 g) was anaesthetised with halothane and a microdialysis probe implanted into the frontal cortex (A/P 3.5 mm, L/M 1.5 mm, to bregma and V/D 5.0 mm to dura mater, surgery lasted ~30 min). Behaviour was tested the following day (day 1). A 3rd group (220-240 g) was anaesthetised with halothane and a stainless-steel guide cannula implanted above the frontal cortex (V/D 0.5 mm to dura mater, surgery lasted ~30 min). 6 days later they were briefly anaesthetised (3 min) and behaviour tested on day 7.

The behavioural test measured rats' response to a novel environment using a dark/light box modified from Costall *et al.* (1989). After an initial 100 min adaptation period in the central (neutral) zone of the test box, rats were exposed to either a brightly illuminated, or a dark, end box. The behaviour of the rats (each of which was only used once) was recorded for 2 h and scored for the following parameters: latency to leave; activity (lines crossed) in; total time spent in, and number of returns to, the box. Data were analysed by Kruskal-Wallis ANOVA with *post hoc* Mann-Whitney U test. The results are shown in Table 1.

Table 1

| | Latency to leave (seconds) | | activity (lines crossed) | | total time ($10^2$ seconds) | | number of returns | |
|---|---|---|---|---|---|---|---|---|
| group | dark | light | dark | light | dark | light | dark | light |
| 1 naïve | 17±6 | 14±6 | 310±60 | 87±36 * | 32.5±9 | 3.7±1.5 * | 44±8 | 16±6 * |
| 2 day 1 | 53±29 | 24±15 | 95±16 † | 20±11 * | 14.5±8 † | 0.6±0.3 * | 12±2 † | 4±2 * |
| 3 day 7 | 16±6 | 13±5 | 133±41 † | 9±2 * | 12.3±7 † | 0.2±0.1 * | 25±6 | 4±1 * |

(mean ± s.e.mean; n=8-10 rats per group). *p<0.05 *cf* dark and light boxes (within group)† p<0.05 *cf* naïve rats (between group).

All three groups of rats were more active in, spent more time in, and made more returns to, the dark box than the light box. This indicates that, even after surgery, the rats found the light, illuminated box, more aversive than the dark box. However, surgery did affect behaviour, even after 7 days. Thus, after surgery rats were less active, spent less time in, and made fewer returns to the dark box than did naïve rats. Although the number of returns to the dark compartment did recover after 7 days, other measures did not.

We conclude that when microdialysis is used in combination with behaviour, the effects of surgery should be taken into account. Adverse effects of surgery on behaviour cannot necessarily be eliminated by using guide cannulae.

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Costall, B., Jones, B., Kelly, L., *et al.*, (1989) *Pharm. Biochem. Behav.* 32, 777-785.
Drijfhout, W., Kemper, R., Meerlo, P, *et al.*, (1995) *J. Neurosc. Meth.* 61, 191-196.
Westerink, *B.* and Teisman, A., (1994) *Naunyn.-Schm-Arch-Pharmacol.* 349 (3), 230-235.

## 245P ANTAGONISM OF THE ANTI-IMMOBILITY EFFECTS OF 8-OH-DPAT IN THE MOUSE FORCED SWIM TEST BY (±)-PINDOLOL

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The mixed β-adrenoceptor and 5-$HT_{1A}$ receptor antagonist (±)-pindolol (Pin) enhances the onset of antidepressant action of selective serotonin re-uptake inhibitors (SSRIs; Artigas *et al.*, 1994). This effect is attributed to the ability of Pin to antagonise the somatodendritic $5HT_{1A}$ autoreceptors and reduce the "self inhibitory" action of the SSRIs. In preclinical studies Pin has been demonstrated to enhance the acute effects of SSRIs in the mouse forced swim test (FST; Jackson *et al.*, 1997), a putative test for antidepressant-like action (Porsolt *et al.*, 1977). The anti-immobility effects of compounds in the FST have been attributed to direct or indirect stimulation of the post-synaptic 5-$HT_{1A}$ receptors and it has been suggested that Pin can exert the enhancing effects because it is only active at the pre-synaptic, somatodendritic autoreceptors (Romero *et al.*, 1996). However, Pin is able to antagonise the post-synaptically mediated 5-HT syndrome which is observed following administration of 8-OH-DPAT (DPAT; Lucki, 1992). The aim of the present study was to investigate the effects of Pin against DPAT in the FST and compare it to the effects of the potent pre-and post-synaptic 5-$HT_{1A}$ receptor antagonist WAY 100.635 (Way; Forster *et al*, 1995).

Testing was performed according to published methodology, in brief: adult, male NMRI mice (Bomholtgård, Denmark) were housed in groups of 10 for at least 7 days prior to testing in 12:12 light:dark cycle (lights on 06:00). They were administered (s.c., 10 ml/kg) with DPAT alone (D/V) or in combination with Way or Pin, 30 min prior to being placed in the cylinder of water (at 23°C) for 6 min. The duration of immobility during the last 3 min of the session was assessed.

DPAT (3.8 μmol/kg) produced a significant inhibition in duration of immobility in the FST [P<0.05]. This effect was antagonised by all doses of Way (0.04-0.32μmol/kg; 0.02-0.16 mg/kg: fig 1). (±)-pindolol also antagonised the effects of DPAT (fig 1) though a dose of 40 μmol/kg (10 mg/kg) was required to give complete reversal.

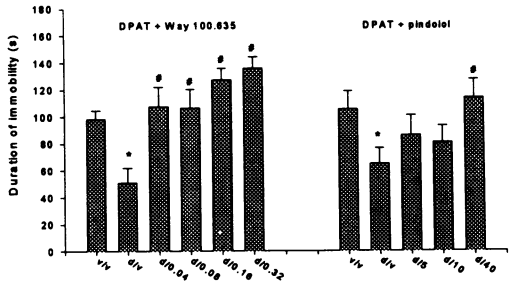


Figure 1: Duration of immobility following treatment with veh (v/v), DPAT (d/v) or DPAT + Way or Pin in the doses shown (μmol/kg). *P<0.01 vs v/v: t-test; #P<0.05 vs d/v: ANOVA followed by Dunnett. n = 9 / group

This study demonstrates the ability of both Way and Pin to antagonise the effects of DPAT in the FST. The dose of Way required to produce reversal is comparable to that required to reverse the effects of DPAT on body temperature (Forster *et al.*, 1995). The dose of Pin which will antagonise DPAT is greater than that which might be anticipated from comparison of its binding affinity at 5-$HT_{1A}$ receptors with that of Way. Whilst Pin does antagonise DPAT in this test, further evaluation would be required before this effect could be unequivocally attributed to action at post-synaptic 5-$HT_{1A}$ receptors.

Artigas F., Perez V. & Alvarez E.(1994)Arch, Gen. Psychiatry 51, 248-251
Forster E.A., Cliffe I.A *et al.* (1995) Eur. J. Pharmacol. 281, 81-88
Lucki I. (1992) Neurosci. Biobehav. Rev. (1992) 16, 83-93
Jackson H.C., Needham A.M., *et al.*, (1997) J. Psychopharm. 11(3): A43
Porsolt R.D., Bertin A. & Jalfre M. (1977) Arch. Int. Pharmacodyn. 229, 327-336
Romero L., Bel N *et al.* (1996) Neuropsychopharmacology 15(4), 349-360

## 246P DIFFERENTIAL REINFORCEMENT OF LOW RESPONSE RATE (DRL-72s) SCHEDULE: EFFECTS OF ANTIDEPRESSANTS

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The performance of rats maintained under a differential reinforcement of low response rate schedule (DRL) is sensitive to antidepressant drugs (AD). ADs typically increase the number of reinforcements earned, reduce the response rate and thereby increase performance efficiency (see Evenden *et al.*, 1993). The present study assessed a variety of ADs in this test; paroxetine (5-30 mg/kg po), fluoxetine (3-30mg/kg ip), mianserin (5-20 mg/kg ip), mirtazepine (5-20 mg/kg ip), venlafaxine (3-30 mg/kg sc) and desipramine (1-10 mg/kg ip). A combination of an SSRI with (±)pindolol (PIND)was also assessed following reports of clinical benefits with this combination (Artigas *et al.*, 1994).

Food restricted male Sprague Dawley rats (300-400g) were trained to lever press for a food pellet using a DRL schedule in which they had to wait at least 72s between lever responses (DRL-72s). Once performance was stable, rats were run for 60 min three times weekly. On the day before drug-testing, all rats were tested following vehicle (VEH) treatment (VEH test day); the mean values ranged from 13-17 pellets, 57-76 responses and 21-38% efficiency.

All data for the drug test day are shown as a percentage of the VEH test day. The response to the most effective dose for each of the ADs tested is shown in Table 1 (means ± SEM). All ADs tested improved performance, except venlafaxine. PIND (2 or 8 mg/kg ip) did not influence paroxetine's effects. However, PIND alone impaired performance (e.g., 8 mg/kg reduced %Efficiency in one experiment from 174 ±28% [VEH/VEH] to 112 ±18%[VEH/PIND], P<0.05).

**Table 1.** Effects of antidepressants upon DRL-72s performance (Data expressed as % of preceding vehicle test day).

| Drug | %Pellets | | %Responses | | %Efficiency | |
|---|---|---|---|---|---|---|
| | VEH | DRUG | VEH | DRUG | VEH | DRUG |
| paroxetine | 111 | 148 | 101 | 76 | 127 | 223 |
| 30 mg/kg p.o. | ±17 | ±18 | ±9 | ±7* | ±23 | ±30* |
| fluoxetine | 100 | 122 | 100 | 57 | 106 | 246 |
| 10 mg/kg ip | ±10 | ±21 | ±10 | ±8* | ±12 | ±35* |
| mianserin | 141 | 145 | 90 | 58 | 179 | 257 |
| 20mg/kg ip | ±13 | ±19 | ±5 | ±7* | ±28 | ±33 |
| mirtazepine | 117 | 191 | 101 | 98 | 135 | 201 |
| 5 mg/kg ip | ±22 | ±42* | ±7 | ±5 | ±35 | ±41 |
| venlafaxine | 141 | 197 | 90 | 80 | 164 | 255 |
| 30 mg/kg sc | ±12 | ±54 | ±3 | ±5 | ±19 | ±64 |
| desipramine | 114 | 141 | 91 | 48 | 149 | 293 |
| 3 mg/kg ip | ±9 | ±24 | ±6 | ±5* | ±29 | ±46* |

* = P<0.05 *cf* with VEH, (ANOVA or Kruskall-Wallis tests, n=17-18).

In summary, the DRL-72s task detected most of the ADs tested. The findings with paroxetine confirm our previous report (Bright *et al.*, 1997). In contrast, venlafaxine was a false negative. This may be explained by the relatively high baseline performance of rats in this study compared with reports by others. Evenden *et al.* (1993) have suggested that the ability to detect antidepressants may be sensitive to the baseline performance of rats. Further studies are required to test this suggestion

Artigas F., Perez, V. and Alvarez, E. (1994) *Arch. Gen Psychiatry* 51(3): 248-251
Bright, F, Hagan, J.J., Kennett, G.A. and Jones, D.N.C. (1997) *J. Psychopharm* 11 (3): A39.
Evenden, J.L., Ryan, C.N.and Matilla, M.E. in Sahgal (ed) Behavioural Neuroscience: Vol II, A Practical Approach, IRL Press, pp55-91, 1993.

## 247P DIFFERENCES IN THE *IN VIVO* AND *IN VITRO* EFFECTS OF THE ENDOGENOUS SLEEP-INDUCING AGENT OLEAMIDE ON 5-$HT_2$ RECEPTOR FUNCTION

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cis-9,10-Octadecenoamide (ODA) has been identified as a putative sleep-inducing agent in the cerebrospinal fluid of sleep-deprived cats (Cravatt *et al.*, 1995). In *Xenopus* oocytes expressing mouse 5-$HT_2$ receptors, ODA has been shown to potentiate selectively the responses to 5-HT leading to the suggestion that its mechanism might be related to the modulation of 5-$HT_2$ receptors (Huidobro-Toro & Harris, 1996). In the present study, we have examined the effects of ODA on 5-$HT_2$ receptor-mediated phoshoinositide (PI) hydrolysis in rat cerebral cortical slices and SHSY-5Y cells, transfected with human 5-$HT_{2A}$ receptors. In addition, the effects of ODA on two different 5-$HT_2$ receptor-mediated rat behaviours were monitored.

PI hydrolysis was measured by monitoring the accumulation of total [$^3$H]-inositol phosphates ([$^3$H]-IP) in the presence of 5mM lithium, in rat (male, Wistar, 180-250g) cerebral cortex slices and in human 5-$HT_{2A}$ receptor-transfected SHSY-5Y cells pre-labelled with [$^3$H]-myo-inositol for 1 hour or 48 hours respectively.

5-HT stimulated [$^3$H]-IP accumulation in both cerebral cortical slices and SHSY-5Y cells in a concentration-dependent manner which was unaffected by the presence of 100nM or 1μM ODA ($p>0.05$, Student's t-test, n=3).

In competition studies, racemic ODA competed with [$^3$H]-CP55,940 (0.5nM) binding to CB1 cannabinoid receptors on rat cerebellar membranes ($IC_{50}$ = 9.3 ± 0.15 μM, n = 3) (Herkenham et al., 1990). At the maximally employed concentration of 300μM, the trans isomer of ODA displaced only 20.3 ± 0.5% (n = 3) specific binding indicating stereoselectivity. ODA competition for [$^3$H]-CP55,940 (0.5nM) binding was unaffected by the presence of the amidohydrolase inhibitor phenyl-methylsulphonyl fluoride (150μM; $IC_{50}$ = 10.0 ± 0.2μM, n = 3).

ODA administration (2mg/kg, i.p.) 5 minutes after the the administration of the selective 5-$HT_2$ receptor agonist (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane hydrochloride (DOI 0.5mg/kg i.p.) significantly potentiated the number of back muscle contractions (control 50 ± 4, ODA 127 ± 6, $p<0.05$, Mann Witney U test, n = 5) but did not alter the number of wet dog shakes (control 18.6 ± 1.5, ODA 19.2 ± 0.8). Male Wistar rats were used.

We can, therefore, present no evidence to support a direct modulation by ODA of 5-$HT_2$ receptor function in mammalian neuronal cells *in vitro*. However, ODA has the properties of an amidohydrolase-resistant ligand at CB1 cannnabinoid receptors which could mediate its behavioural effects, perphaps in concert with 5-$HT_2$ receptors, *in vivo*.

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Cravatt B.F., Prospero-Garcia O., Siuzdak G., Gilula N.B., Henriksen S.J., Boger D.L. and Lerner R.A. (1995). Science, 268, 1506-1509.
Herkenham M., Lynn A.B., Little M.D., Johnson M.R., Melvin L.S., de Costa B.R. and Rice K.C. (1990). Proc. Natl. Acad. Sci. USA, 87, 1932-1936.
Huidobro-Toro J.P. and Harris R.A. (1996). Proc. Natl. Acad. Sci. USA, 93, 8078-8082.

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## 248P A COMPARISON BETWEEN PLASMA CORTICOSTERONE CONCENTRATIONS FOLLOWING SOCIAL COMPETITION AND RESTRAINT IN THE RAT

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It has been shown previously (Woodall et al, 1996a) that corticosterone (CORT) is not elevated in plasma taken from rats in stable hierarchies (triads) as measured by competition for sweetened milk. In addition, CORT was elevated only in the dominant rat of the triad when exposed to an open field subsequent to a period of competition suggesting that intermediate and subordinate rats are less responsive to a novel stimulus (Woodall et al., 1996a). To investigate this further, the present study measured the plasma CORT of rats following either the first exposure to the competition procedure or following the development of stable levels of competition combined with a period of restraint.

Social competition was established during 5 min access to sweetened milk in triads of adult male Lister Hooded rats weighing 327±4g at the start of the study, according to the protocol of Woodall et al (1996b). For each triad (n=12) the social hierarchy was established by observing which animal had access to the spout at 5s intervals throughout the testing period (i.e. 60 observations for one 5 min trial). After decapitation, trunk blood was collected from dominant, intermediate and subordinate rats 10 min after the 9th period of competition with or without a 15 min period of restraint in a perspex cylinder (n=4/condition) or from triads of rats 10 min after the 1st period of competition (n=5). Following centifugation, plasma samples were analysed for CORT by radioimmunoassay (Gamma-B $I^{125}$ corticosterone, Immuno Diagnostic Systems Ltd). Data were analysed using two way ANOVA followed by Dunnett's t-test and are expressed as mean ± s.e.m.

Over a 5 week period the triads of rats developed stable hierarchies consisting of dominant, intermediate and subordinate animals which had access to the drinking spout for 42±1%, 34±1% and 25±1% of the 5 min testing period respectively (n=8). There was no significant difference in plasma CORT between dominant, intermediate and subordinate rats from stable hierarchies (9th exposure) (60±3ng/ml, 60±4ng/ml, and 51±5ng/ml respectively). The concentration of plasma CORT from rats exposed to the competition procedure for the first time was only significantly different to that of the subordinate rats from the stable triads (70±4ng/ml; $p<0.05$). Following a period of restraint, CORT was elevated in plasma from dominant, intermediate and subordinate rats (60±3ng/ml compared to 161±25ng/ml [$p<0.01$], 60±4ng/ml compared to 153±18ng/ml [$p<0.01$] and 51±5ng/ml compared to 160±16ng/ml [$p<0.01$] respectively).

These data confirm that competition for sweetened milk in stable hierarchies of rats does not elevate CORT probably reflecting the fact that the procedure is not stressful. Indeed, even following the first exposure to the competition procedure CORT levels were only significantly elevated when compared to the subordinate rats of the triad. However, a period of restraint increased the plasma concentration of CORT in dominant, intermediate and subordinate rats indicating that in this situation the lower ranked rats in a triad are not less responsive to the stressor.

Woodall, K.L., Domeney, A.M., Kelly, M.E. (1996a) Br. J. Pharmacol. 119 (Suppl): 65P
Woodall, K.L., Domeney, A.M., Kelly, M.E. (1996b) Pharmacol. Biochem. Behav. 54: 169-173

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## 249P PHARMACOLOGICAL CHARACTERIZATION OF GV191869X: A NOVEL, POTENT AND SELECTIVE CCK-B RECEPTOR ANTAGONIST

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Cholecystokinin (CCK) is a gut peptide which acts as neurotransmitter in CNS through two receptors subtypes, CCK-A and CCK-B. In previous studies we identified a N-5-phenyl 3 ureido 1,5 benzodiazepine showing antagonism at the CCK-B receptor level (Corsi et al., 1995). To improve the physiochemical properties of this class of compound alternative substituents were introduced at the N-5 position. Here we describe the pharmacological properties of GV191869X (-)-N-[(Adamantane-1-methyl)-2,4-dioxo-5-(2-(4-morpholino)ethyl)-2,3,4,5-tetrahydro-1H-1,5benzodiazepin-3-yl]-N'phenylurea (Fig. 1), a more soluble derivative bearing at N-5 an amino-based cationic group. Binding assays were performed as described by van Amsterdam et al., (1995). In these studies GV191869X potently displaced [$^3$H]-CCK8 from guinea pig (pKi=9.64±0.03; n=3) and rat (pKi=9.34±0.06; n=4) brain membrane receptors and from HeLa cells membranes expressing human temporal cortex CCK-B receptors (pKi=9.37±0.01; n=3). In contrast, GV191869X weakly antagonized CCK-A binding in rat pancreas membranes (pKi=5.69±0.04; n=3). The functional preparation of guinea pig ileum longitudinal myenteric plexus (GPILMP) was used to determine the *in vitro* antagonism of the compound. GPILMP was prepared as described by Dal Forno et al. (1992) and contractions were induced by the selective CCK-B agonist CCK-4 in the presence of the CCK-A antagonist L-364,718 (10nM). The equilibration time for GV191869X used was one hour. Contractions induced by CCK-4 (0.01- 300μM) were antagonized in a concentration dependent manner by GV191869X (1-30nM). The estimated $pK_B$ was 9.48±0.13 (n=7-10). Experiments carried out with 3nM of GV191869X indicated that the antagonism was reversible after the tissues were washed-out for 120min. The *in vivo* anxiolytic activity of GV191869X was tested in the mouse light/dark box and in the marmoset "human threat" test. Experiments were performed as described by Costall et al. (1991). In the light/dark box, 10 mice each group were used. GV191869X (0.001-10μg/kg po) showed a dose dependent anxiolytic activity. The first significantly effective dose was 0.01μg/kg ($P<0.05$, ANOVA followed by Dunnett test) and an $ED_{50}$ of 0.002μg/kg was calculated. Similar results were obtained in the "human threat test". Four marmosets for each group were used. GV191869X (0.001-10μg/kg sc) showed a dose dependent effect with the first significantly effective dose of 0.01μg/kg ($P<0.05$, ANOVA followed by Dunnett test). In both models GV191869X maintained a significant effect at all doses from 0.01 up to 10μg/Kg, i.e. up to a dose more than 3000 times the $ED_{50}$ in the same test. The present findings indicate that GV191869X is one of the most potent and selective CCK-B receptor antagonist discovered. The wide dose range of anxiolytic activity in animal models makes GV191869X an attractive candidate to test the hypothesis that CCK-B antagonists are anxiolytic in man.

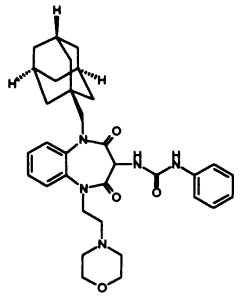

Fig.1 GV191869X

Corsi M., Dal Forno G., van Amsterdam F.Th.M et al., (1995). Br. J. Pharmacol., 114, 91P.

Costall B., Domeney A.M., Hughes J., et al., (1991). Neuropeptides 19, 65-73.

Dal Forno G., Pietra C., Urciuoli M. et al., (1992). J. Pharmacol. Exp. Ther., 261/3, 1056-1063.

Van Amsterdam F.Th.M., Oliosi B., Corsi M. et al., (1995). Br. J. Pharmacol., 116, 240P.

## 250P IONIC EFFECTS OF P2X$_7$ RECEPTOR-MEDIATED YO-PRO-1 INFLUX

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The P2X$_7$ receptor is the most recently identified member of the P2X receptor family and exhibits similar functional properties to the previously described cytolytic P2Z receptor (Surprenant *et al.*, 1996). P2X$_7$ receptors are differentiated from other P2X receptors by their ability to form a ligand-gated pore that allows molecules of MW up to 900 to enter cells. Previous studies have shown that receptor function may be studied by measuring influx of the DNA binding dye YO-PRO-1 into cells expressing P2X$_7$ receptors (Hickman *et al.*, 1994). In the present study we have determined if this methodology can be used to quantify P2X$_7$ receptor activation and have studied ionic effects on P2X$_7$ receptor-mediated function.

Studies were performed using CHO-K1 (Michel *et al.*, 1997) or human THP-1 (Grahames *et al.*, 1998) cells that express endogenous P2X$_7$ receptors, or in HEK293 cells expressing the human recombinant P2X$_7$ receptor. Influx of 1μM YO-PRO-1 was measured in cell suspensions in 96 well plates. The agonist used was Dibenzoyl-ATP (DbATP). Plates were centrifuged at 280g for 5 mins prior to reading fluorescence (excitation 490nm: emission 530nm). LDH release was measured in supernatants using a Promega Cytotox kit. A buffer of the following composition (mM) was utilised in most studies :- 10 Hepes, 10 glucose, 5 KCl, 280 sucrose (pH 7.4). Data are the mean ± s.e.mean of 3-5 experiments.

When studied at 37°C for 1h in the presence of 0.5mM $CaCl_2$, DbATP increased YO-PRO-1 fluorescence (YO-PRO-1 influx) in HEK293 ($pEC_{50}$=6.4±0.1; 4±1.5 fold increase), CHO-K1 ($pEC_{50}$ = 5.5±0.1; 11±0.4 fold increase) and THP-1 ($pEC_{50}$=5.7±0.2; 4±0.2 fold increase) cells. In all cases concentration-effect curve slope parameters were >1.5. DbATP also increased LDH release from CHO-K1 ($pEC_{50}$=5.5±0.1; 15±3 fold increase) and THP-1 ($pEC_{50}$=5.3±0.1; 6±0.8 fold increase) cells, but not HEK293 cells.

There were differences in the ionic sensitivity of YO-PRO-1 influx between the cell lines. In the absence of $Ca^{2+}$ (plus 0.1 mM EDTA) there was no specific DbATP-stimulated YO-PRO-1 influx in CHO-K1 cells. Increasing $Ca^{2+}$ from 0.03 to 1 mM resulted in an increase in DbATP-stimulated YO-PRO-1 influx with no marked effect on potency ($pEC_{50}$=5.4±0.1, 5.6±0.2 and 5.2±0.2 at 0.01, 0.1 and 1 mM $Ca^{2+}$, respectively). In contrast in THP-1 and HEK293 cells DbATP-stimulated YO-PRO-1 influx was maximal in the absence of $Ca^{2+}$. Increasing $Ca^{2+}$ from 0.03 to 1 mM reduced both basal and DbATP stimulated YO-PRO-1 influx but did not markedly affect DbATP potency ($pEC_{50}$ values in THP-1 cells were 5.7±0.1, 5.7±0.2 and 5.1±0.2 at $Ca^{2+}$ concentrations of 0.01, 0.1 and 1 mM, respectively).

In the presence of 0.5mM $Ca^{2+}$, replacement of 280mM sucrose with 140mM NaCl decreased YO-PRO-1 influx in all 3 cell lines by >95% and prevented quantitative studies. In the absence of added $Ca^{2+}$ (+0.1mM EDTA) substitution of 140mM NaCl for sucrose decreased both basal and DbATP-stimulated YO-PRO-1 influx in THP-1 and HEK293 cells and reduced the potency of DbATP (e.g. in THP-1 cells $pEC_{50}$ in sucrose=6.3±0.3; $pEC_{50}$ in 140mM NaCl=5.0±0.2). In THP-1 cells replacement of sucrose with 140mM NaCl increased DbATP-stimulated LDH release 1.8±0.2 fold ($p<0.05$; Dunnets-test).

These studies suggest that P2X$_7$ receptor-mediated function can be quantified by measuring changes in YO-PRO-1 fluorescence, although there are marked cell-specific effects of ions on the response. Changes in YO-PRO-1 fluorescence appear to be independent of cell lysis since YO-PRO-1 influx can occur in the absence of LDH release in HEK293 cells and because of the differential effects of sodium on YO-PRO-1 influx and LDH release in THP-1 cells.

Grahames, C.B.A. (1998). Br. J. Pharmacol., this meeting, in press.

Hickman, S.E., El Khoury, J., *et al.*, (1994). Blood, **84**, 2452-2456.

Michel, A.D., *et al.*, (1997). Br. J. Pharmacol., **122**, 113P.

Surprenant, A., Rassendren, F., *et al.*, (1996). Science, **272**, 735-738.

## 251P THE ACTIONS OF L-DOPA AND DOPAMINE AGONISTS FOLLOWING DOPAMINE DEPLETION IN MPTP-TREATED COMMON MARMOSETS

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In MPTP lesioned primates administration of the tyrosine hydroxylase inhibitor AMPT (α-methyl-para-tyrosine) diminishes the antiparkinsonian effect of $D_1$, $D_2$, and mixed $D_1/D_2$ dopamine (DA) agonists (Smith, 1991). We now investigate the effects of AMPT and the centrally acting DOPA decarboxylase inhibitor NSD-1015 (3-hydroxybenzyl hydrazine) on the actions of L-DOPA, the $D_1$ agonists A86929 ((-)- *trans* 9,10-hydroxy-2-propyl-4,5,5a,6,7,11b-hexahydro-3-thia-5-azacyclopent-1-ena[c]phenanthrene hydrochloride) or A77636 ({1R,3S},3(1-aminomethyl-3,4-dihydro-5,6-dihydroxy-1H-2-benzopyran hydrochloride), and the $D_2$ agonist quinpirole in the MPTP-treated marmoset.

Adult marmosets (n=24) were treated with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 10mg/kg sc over 5 days) until a stable parkinsonian state developed. They were randomised into 6 groups (n=4) and recieved the following treatments:

| Group | Pretreatment | Treatment |
|---|---|---|
| 1 | AMPT (100mg/kg sc) or vehicle | L-DOPA (5-25mg/kg po) |
| 2 | AMPT (150mg/kg sc) or vehicle | A77636 (0.1mg/kg sc) |
| 3 | AMPT (150mg/kg sc) or vehicle | quinpirole (0.03-0.3mg/kg sc) |
| 4 | NSD-1015 (25 mg/kg ip) or vehicle | L-DOPA methyl ester (ME) (6.25-22.5mg/kg sc) |
| 5 | NSD-1015 (25 mg/kg ip) or vehicle | A86929 (0.04mg/kg sc) |
| 6 | NSD-1015 (25 mg/kg ip) or vehicle | quinpirole (0.05-0.3mg/kg sc) |

AMPT or saline was administered 18hrs and again 1-2hrs prior to treatments, whereas NSD-1015 or saline was given 90 min prior to treatment.

Locomotor activity was measured automatically and behavioural disability assessed simultaneously using a rating scale of 0 (normal) to 18 (marked motor deficits). All ratings were carried out blind. Data (expressed mean ±s.e.mean) was analysed by one-way ANOVA followed by Mann Whitney test.

AMPT had no significant effect on L-DOPA's reversal of parkinsonian deficit. However, AMPT produced significant inhibition of A77636- and quinpirole- induced locomotor activities at all doses tested and attenuated the reversal of motor disability induced by these agonists ($p<0.05$). NSD-1015 also inhibited quinpirole- (figure), A86929- and L-DOPA ME-induced locomotor activities at all doses tested ($p<0.05$) and inhibited their reduction in Parkinsonian motor disability ($p<0.05$, all doses tested).

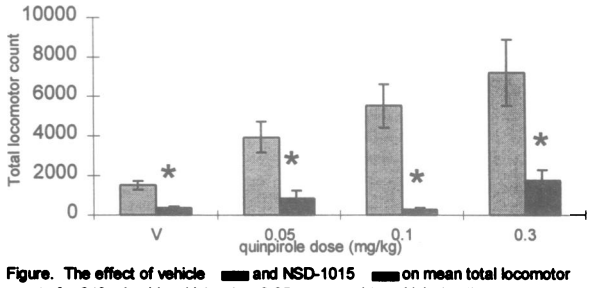


Figure. The effect of vehicle ■ and NSD-1015 ■ on mean total locomotor counts for 240 min. V=vehicle. * $p<0.05$ compared to vehicle (n=4).

The action of both $D_1$ and $D_2$ agonists depend on endogenous DA tone. This may explain why agonists lose their efficacy when DA levels are severely depleted in advanced Parkinson's disease. Endogenous L-DOPA accumulation does not potentiate quinpirole or A86929 action. Similarly, contrary to reports L-DOPA itself does not appear to have a neuromodulatory role in motor function and needs to be decarboxylated to DA.

Smith LA, Jenner P, Marsden CD (1991) Brit J Pharmac:(104)Suppl;284.

## 252P EFFECTS OF REPEATED TREATMENT WITH COMBINATIONS OF L-DOPA AND ROPINIROLE ON DYSKINESIA INDUCTION IN MPTP-TREATED COMMON MARMOSETS

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L-DOPA (LD) induces dyskinesias in patients with Parkinson's disease (PD) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated non-human primates (Bedard *et al* 1986). However, *de novo* administration of long-acting D2 agonists is not associated with the onset of dyskinesias. The novel non-ergoline D2/ D3 dopamine (DA) agonist ropinirole induces negligible amounts of dyskinesia in MPTP-treated drug-naive common marmosets (Pearce *et al* 1997). We now investigate the dyskinesogenic potential of ropinirole in combination with L-DOPA compared to these drugs administered alone. Two combinations of L-DOPA and ropinirole were investigated - one with ropinirole dominant, one with L-DOPA dominant.

Adult common marmosets (*Callithrix jacchus*, n=16) were treated with MPTP (2mg/kg/day s.c. for 5 days) resulting in a stable parkinsonian state and, after a recovery period of 8-15 weeks, assigned to treatment groups (n=4): LD alone (carbidopa (12.5mg/kg po gavage od) 45 mins prior to LD (12.5 mg/kg po gavage od)); ropinirole alone (0.5 mg/kg po reduced to 0.2mg/kg po od); 2 combinations (carbidopa (doses identical to LD) 45 minutes prior to dosing with ropinirole (0.1mg/kg po daily in LD dominant group; 0.2 mg/kg po daily in ropinirole dominant group) and LD (5mg/kg po bid increased to 7.5mg/kg po bid on day 8 in LD dominant group; 2.5mg/kg bid po in ropinirole dominant group)) for 28 consecutive days. Animals were visually scored for dyskinesia using a scale of 0 (none) to 4 (severe, disabling) and disability (0 normal - 16 severely parkinsonian) on a daily basis and locomotor activity was assessed in computer linked observation cages equipped with infrared diode monitors, as previously described (Pearce *et al* 1995). The results were analysed using the Kruskall-Wallis test followed by the Mann-Whitney U test.

All drug treatments alleviated the akinesia and motor disabilities induced by acute MPTP treatment. This was reflected in the locomotor and disability scores. LD rapidly induced moderate to severe levels of dyskinesia in contrast to ropinirole alone. In addition, both combination groups induced less dyskinesia than LD alone and there was a marked trend indicating that less dyskinesia was induced by the ropinirole-dominant combination than the LD-dominant therapy (Figure 1).

The results show that combining ropinirole and L-DOPA allows a significant reduction in the dose of L-DOPA required to elicit a reversal of parkinsonian symptoms. Findings suggest that ropinirole is an L-DOPA sparing agent, and that combinations of L-DOPA and ropinirole result in less dyskinesia than with L-DOPA alone.

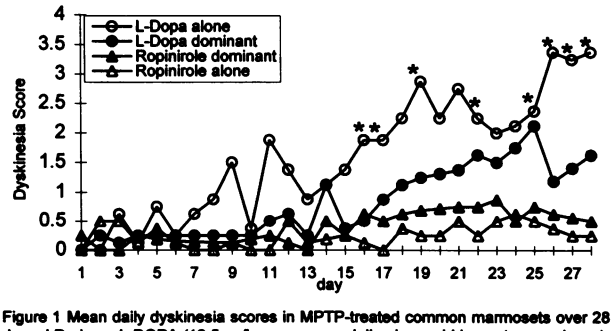


Figure 1 Mean daily dyskinesia scores in MPTP-treated common marmosets over 28 days. LD alone: L-DOPA (12.5mg/kg po gavage daily plus carbidopa at same doses). LD dominant: L-DOPA (5mg/kg x7days then 7.5mg/kg po bid plus carbidopa at same doses) combined with ropinirole (0.1mg/kg po daily). Ropinirole dominant: L-DOPA (2.5mg/kg po bid plus carbidopa at same doses) combined with ropinirole (0.2mg/kg po daily). Ropinirole alone: ropinirole (0.5mg/kg x12 days then 0.3mg/kg x5 days then 0.2mg/kg po gavage. *$p< 0.05$ vs. ropinirole alone group.

Bedard P.J., diPaolo T., Falardeau P & Boucher R (1986) *Brain Res.* **379 (2)** 294-299
Pearce R.K.B., Jackson M., Smith L., *et al* (1995) *Mov. Disord.* **10**, 731-740
Pearce R.K.B., Banerji T, Jenner P & Marsden C.D. (1997) *in press*

## 253P BTS 74 398 REVERSES MOTOR DEFICITS IN MPTP-TREATED COMMON MARMOSETS WITHOUT INDUCING DYSKINESIAS

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L-DOPA remains the mainstay of treatment for Parkinson's disease (PD) but its prolonged use is associated with the development of unwanted side effects such as loss of drug efficacy and dyskinesia. Dopamine (DA) agonists such as bromocriptine and ropinirole alleviate parkinsonian symptoms in humans and non-human primates without inducing significant dyskinesia when compared with L-DOPA (Bédard,1986; Pearce, 1996). A new approach to the treatment of parkinsonian disability has been the introduction of DA/noradrenaline reuptake inhibitors. Previously we showed that the DA reuptake inhibitor NS 2214 reverses MPTP-induced motor deficits in the common marmoset without inducing significant dyskinesia (Pearce et al., 1995). We now report on the antiparkinsonian effects of the DA/noradrenaline reuptake inhibitor BTS 74 398 (1-[1-(3,4-dichlorophenyl) cyclobutyl]-2-(3-dimethylaminoproplythio) ethanone monocitrate ) on locomotor activity and disability scores in MPTP-treated common marmosets.

Adult common marmosets (*Callithrix jacchus*, n=4) were treated with MPTP (2.0mg/kg/day for 5 days) to develop a clear parkinsonian state. Eight weeks later the animals were administered BTS 74 398 (5.0-20.0mg/kg po, dissolved in 10% sucrose) on a once weekly basis. They were rated for disability using a scale of 0-14 (normal-marked motor deficits). Motility counts were measured automatically in test cages fitted with infrared photocell emitters and their corresponding light detectors. Each interruption of a light beam by a moving animal was recorded as a movement count.

Oral administration of BTS 74 398 (5.0-20.0mg/kg) produced a reversal of parkinsonian motor deficits and a corresponding reduction in disability scores (Table 1).

Table 1 :Total locomotor counts or disability scores (± s.e.m, n=4) following oral administration of BTS 74 398 (5.0-20.0mg/kg) (*p<0.05, compared to vehicle: Mann Whitney test).

| Dose (mg/kg) | Locomotor Counts/10h | Disability Scores/8h |
|---|---|---|
| Vehicle | 2,285±301 | 189.3±7.1 |
| 5.0 | 4,414±1,017 | 129.3±17* |
| 10.0 | 6,074±641* | 94.0±5.4* |
| 20.0 | 7,900±1,086* | 75.8±10.5* |

In conclusion, the DA reuptake inhibitor BTS 74 398 (5.0-20.0mg/kg) produced a prolonged but mild, dose-related reversal of MPTP-induced motor deficits in common marmosets. These results add further to a role for DA/noradrenaline reuptake inhibitors in the treatment of PD.

Bédard PJ et al., Brain Res (1986);379:294-299.
Pearce R. et al., Brit J. Pharmacol. (1995);116:304P
Pearce R. Exp Opin Ther Patents (1996); 6(10):949-953.

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## 254P EVIDENCE THAT FOOD-INDUCED CONDITIONED PLACE-PREFERENCE CANNOT BE DEPLOYED AS A TEST IN RATS TO DISCRIMINATE BETWEEN TYPICAL AND ATYPICAL ANTIPSYCHOTIC DRUGS

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The enhanced incentive value of food that is induced by some selective dopamine $D_2$ (and $D_3$) receptor antagonists using a version of the conditioned place-preference paradigm (CPP), may be mediated via the $D_2$ autoreceptor (Guyon *et al.* 1993). In that study, antipsychotic drugs (APDs) were chosen for their reportedly high autoreceptor affinity (sulpiride, amisulpiride, pimozide) as well as a reduced selectivity versus postsynaptic $D_2$ receptors (metoclopramide, haloperidol). The enhancing actions were evoked both by sulpiride and amisulpiride, APDs that are regarded as atypical (Gerlach, 1993). We have therefore examined whether clozapine, a recognised atypical APD, may also produce an increase in the incentive value of food.

Experimental procedures were adapted from those of Guyon *et al.* (1993) and consisted of repeated pairings of food to male CD rats (110-130 g initially) using a reversed 12 h light-dark cycle. Using the floor covering of an open field as the environmental cue, the data agreed with those of Guyon *et al.* (1993) in that only sulpiride produced an increase in the reward value of food and then only at one dose (7.5 mg/kg i.p.; Table 1). The factor common to clozapine, haloperidol and sulpiride is that all three agents reduced CPP at the highest doses tested (Table 1).

The present study has shown that the atypical APD, clozapine, cannot be distinguished from the classical APD, haloperidol, using this behavioural approach. Equally, the study of Guyon *et al.* (1993) revealed that the use of a similar paradigm was unable to differentiate between the atypical APDs, sulpiride and amisulpiride and the typical APD, pimozide. Thus, food-induced CPP does not appear to be an appropriate tool with which to separate typical from atypical APDs.

Gerlach, J. (1993) in *Antipsychotic Drugs and their Side-Effects* (ed. Barnes, T.) pp 45-63. Academic Press, London.

Guyon, A., Assouly-Besse, F., Biala, G. *et al.* (1993) *Psychopharmacology* **110**, 460-466.

Table 1. Effects of clozapine, sulpiride and haloperidol on food-induced conditioned place-preference

| | Doses # | | | | | |
|---|---|---|---|---|---|---|
| | (Vehicle) | 1 | 2 | 3 | 4 | 5 |
| Clozapine | 654 ± 29 | 614 ± 15 | 667 ± 16 | 636 ± 25 | 579 ± 31 | 460 ± 31*** |
| Sulpiride | 620 ± 21 | 621 ± 30 | 612 ± 35 | 702 ± 21* | 620 ± 31 | 529 ± 20* |
| Haloperidol | 678 ± 19 | 648 ± 22 | 648 ± 19 | 638 ± 16 | 612 ± 31 | 502 ± 37*** |

Values are the adjusted mean time in seconds (± s.e.mean) which rats spent during the 20 min test on the textured floor associated with drug/food. # The following doses (1-5) in mg/kg i.p. were used: clozapine (1.25, 2.5, 5, 10, 30) sulpiride (1.88, 3.75, 7.5, 15, 30) haloperidol (0.02, 0.04, 0.08, 0.16, 0.32). Three-way analysis of variance was used for monotonic dose-response relationships (clozapine and haloperidol) with comparisons to control made by Williams' test. If not monotonic (sulpiride), comparisons were made by Fisher's LSD test. $n$ = 10-14; *$p$ <0.05 and ***$p$ <0.01 versus vehicle control.

## 255P VARIABLES OF MOVEMENT AMPLITUDE AND FREQUENCY AS EMETIC STIMULI IN *SUNCUS MURINUS*

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*Suncus murinus* is an insectivore which is sensitive to the emetic effects of a number of drugs including copper sulphate, nicotine, dopamine agonists, chemotherapeutic agents and other compounds (Matsuki *et al.*, 1992). In addition, it has been reported that *Suncus murinus* is susceptible to motion sickness (Ueno *et al.*, 1988). The aim of the present study was to investigate the effect of different frequencies and amplitude of movement to induce motion sickness in *Suncus murinus* .

Adult Japanese House Musk shrew, *Suncus murinus* (38-88 g) of either sex were used. Each animal was placed individually in a transparent cage (100Wx150Lx150H mm) of 6 linked units. After 3 min, a horizontal motion stimulus was commenced with various amplitudes (peak-to peak displacement: 7, 13 or 40 mm) and frequencies (0.5, 1, 2 or 3 Hz). The number of vomits and the latency of onset (s, the time from the start of the shaking to the first vomit) were recorded over a 10 min period. Data were expressed as the mean±s.e.mean of n=6 and analysed using one-way ANOVA which was followed by Bonferronni-Dunnett's t-test.

Different amplitudes of shaking (7, 13 or 40 mm) caused a similar number of vomits and onset of response using 0.5, 1, 2 or 3 Hz frequency of stimulation. The data obtained using the 13 and 40 mm amplitudes is shown in Figure 1. The number of vomits obtained at 1 and 2 Hz were approximately 3 fold and significantly ($p<0.001$) higher than those at 0.5 and 3 Hz using an amplitude of either 13 or 40 mm. The onset of the emetic response in animals that were exposed to the motion stimuli at 1 and 2 Hz (40 mm amplitude) was significantly ($p<0.001$) shorter than that at either 0.5 or 3 Hz. The onset of vomiting in animals that experienced the motion stimuli at 1 and 2 Hz (amplitude of 13 mm) was also significantly ($p<0.001$) less than that at 0.5 Hz, Figure 1.

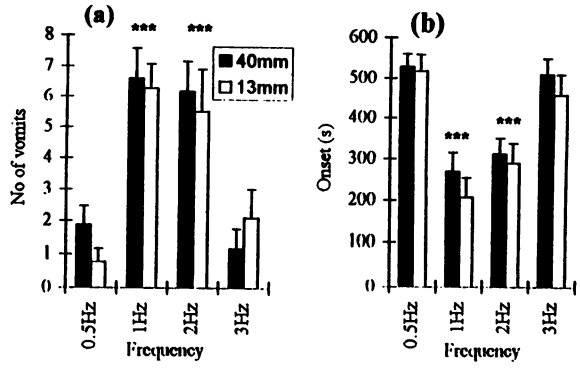


Figure 1- A comparison of the intensity of motion sickness induced by 13 mm and 40 mm displacement amplitudes at different frequencies (0.5-3 Hz) in adult male *Suncus murinus*: (a) the number of vomits and (b) the latency to first vomit during repeated shaking over a 10 min period. Each histogram represents the mean±s.e.mean; n=6. *** $p<0.001$ compared to the data obtained at 0.5 or 3 Hz.

The data suggest that the optimal shaking conditions for *Suncus murinus* to develop motion sickness consists of a frequency of 1 or 2 Hz although the amplitude of shaking is not critical.

Matsuki, N., Torii, Y., Ueno, S. & Saito, H. (1992) *Mechanisms and control of emesis.* Colloque Inserm. vol. 223, 323-329.
Ueno, S., Matsuki, N. & Saito, H. (1988) *Life Sci.* 43, 413-420.

## 256P BEHAVIOURAL EFFECTS OF THE MONOAMINE REUPTAKE INHIBITOR, BTS 74 398, IN RATS AND MICE

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BTS 74 398 (1-[1-(3,4-dichlorophenyl)cyclobutyl]-2-(3-dimethyl aminopropylthio)ethanone monocitrate) potently inhibits the reuptake of dopamine, noradrenaline and 5-hydroxytryptamine (5-HT) *in vitro* (Cheetham *et al.*, 1997). This study evaluates the effects of BTS 74 398 in the following animal models of monoamine reuptake inhibition: unilateral nigrostriatal lesioned or circling rats; d-amphetamine-cued drug discrimination; prevention of reserpine-induced ptosis and the Porsolt test.

Male CD1 mice (20-30g; n=10) were used in the Porsolt test (Luscombe *et al.*, 1989) and male Sprague-Dawley rats were used in the reserpine test (140-190g; n=6; Luscombe *et al.*, 1989) and the circling rat test (280-300g; n=6; Heal *et al.*, 1992). Female PVG rats (200-280 g; n=5-10) were trained to discriminate (≥75% correct lever choice) between d-amphetamine (0.5 mg/kg i.p.) and saline (i.p.) in a 2-choice lever-pressing model (Heal *et al.*, 1992). In addition, observations of the general behavioural effects of BTS 74 398 in male Sprague-Dawley rats (120-140g; n=6) were made using a time-sampling procedure (Jackson, 1990). Individually-housed rats were acclimatised to the observation boxes (42 x 25 x 20 cm) for 1h before drug treatment. BTS 74 398 was dissolved in water. Doses refer to the free base.

BTS 74 398 (32 mg/kg p.o.) induced ipsilateral circling in 4/6 unilateral nigrostriatal lesioned rats in the hour following drug administration. However, this response was not significant as 2/6 animals did not turn during this period (mean turns/min ± s.e.mean 2.0±0.9; paired Student's t-test; each animal was its own control). On the other hand, significant increases in circling behaviour were observed 4-5h following drug administration (4.3±0.7 turns/min; **P<0.01). BTS 74 398 (6.5 mg/kg i.p.) produced generalisation to saline in 5/9 rats 15 min following drug administration. Four rats showed no preference for the saline or d-amphetamine lever. When re-tested 5h later, 5 of the rats recognised BTS 74 398 as d-amphetamine, one recognised it as saline and 3 rats had no preference. BTS 74 398 potently prevented the ptosis induced by reserpine (0.75 mg/kg i.v.; 3 h pretreatment) in rats ($ED_{50}$ (95% confidence limits) mg/kg p.o. 3.2 (2.0-5.0)). BTS 74 398 (10, 30 and 100 mg/kg p.o.; 60 min pretreatment) significantly increased the mobility of mice in the Porsolt test (vehicle 153±24 counts; 1 mg/kg 223±42; 3 mg/kg 194±18; 10 mg/kg 344±52*; 30 mg/kg 495±56*; 100 mg/kg 635±24*; **P<0.01; ANOVA and Dunnett's test). BTS 74 398 (30 mg/kg p.o.) produced small, significant increases in general activity in the 2h following drug administration (median scores (range) 0-60 min vehicle 0 (0-2), BTS 74 398 3 (0-7)*; 60-120 min vehicle 0 (0-2), BTS 74 398 7.5 (0-23)*; maximal score 60; *P<0.05; Cochran-Mantel-Haenszel test) and significantly increased sniffing behaviour (0-60 min vehicle 0.5 (0-4), BTS 74 398 3.5 (2-16)*; 60-120 min vehicle 0 (0-5), BTS 74 398 30 (0-49)*). Lower doses of BTS 74 398 (3 and 10 mg/kg) were inactive.

This study demonstrates that BTS 74 398 produces similar behavioural effects in rats and mice to other dopamine and/or noradrenaline reuptake inhibitors (Luscombe *et al.*, 1989; Heal *et al.*, 1992; Nakachi *et al.*, 1995). These results are consistent with its *in vitro* profile as a potent monoamine reuptake inhibitor (Cheetham *et al.*, 1997). The ability of BTS 74 398 to inhibit dopamine and noradrenaline reuptake *in vivo* suggests that it may be of value in the treatment of the motor dysfunction in Parkinson's Disease while its potent antidepressant-like effects in the Porsolt test, indicate that it may also help to alleviate the symptoms of depression which occur in many of these patients.

Cheetham, S.C. *et al.* (1997) This meeting
Heal, D.J. *et al.* (1992) Psychopharmacology 107, 303-309.
Jackson, H.C. (1990) Peptides 11, 897-901.
Luscombe, G.P. *et al.* (1989) Neuropharmacology 28, 129-134.
Nakachi, N. *et al.*(1995) Eur. J. Pharmacol. 281, 195-203.

## 257P ACUTE THYROXINE ADMINISTRATION ENHANCES COGNITIVE ABILITIES IN THE RAT

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Central cholinergic blockade with scopolamine produces profound cognitive impairments in human and animal subjects (Fibiger, 1991). Recent reports suggest that thyroid hormones augment cholinergic function as revealed by neurochemical changes (Schwegler, 1995) In the present study we have investigated whether or not peripheral administration of thyroxine (T4; tetra-iodothyronine), one of the principal thyroid hromones, would effect cognitive performance in a water maze task.
Adult male, Lister hooded rats (350-500 g) were used as subjects. On the first test day, rats were injected with vehicle (VEH; physiological saline+5% ethanol; n=7), or 10 mg/kg of T4 (n=4), both given IP, and tested 20 min later in a water maze. Rats were given 8 trials of training to locate the platform, with each trial limited to a 60 sec maximum. On the next day, rats were tested for their retention of the task. Data (latencies to locate the platform, aggregates of 2 trials blocked together) were assessed by ANOVA (applying a Greenhouse-Gieser correction) and *post hoc* testing was performed using Bonferroni corrected t-tests.
ANOVA on the latency data revealed a significant interaction between drug treatment and trial block $F(3,9)=3.93$, $P<.02$. As shown in Fig. 1A, T4 treatment significantly improved acquisition of the task compared to the VEH group, an effect significant on trial blocks 3 and 4 (P's<.05). Analysis of retention data revealed a significant effect of drug only $F(3,9)=24.5$, $P<.001$. As illustrated in Fig. 1B, rats treated with T4 on day 1 displayed improved retention performance over all trial blocks compared to VEH controls (P's<.05). There was no effect of VEH or T4 on swim speeds. Thus, acute T4 administration improved both the acquisition and retention phases of the water maze task compared to VEH animals.

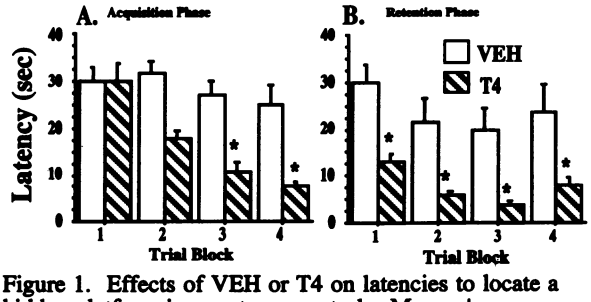


Figure 1. Effects of VEH or T4 on latencies to locate a hidden platform in a water maze task. Means ± sem are shown.
*significantly different from corresponding VEH group

Chronic thyroxine treatment has been shown to enhance cholinergic innervation of the hippocampus, a structure with a well-known role in spatial behaviour (Morris *et al.*, 1982). It is possible that acute T4 injections may transiently elevate cholinergic function and act to augment cognitive performance. In conclusion, acute thyroid hormone administration increases cognitive performance in rats, possibly by elevating cholinergic function in the brain.

Fibiger, H.C. (1991) *Trends Neurosci.* 14, 220-223.
Morris, R.G.M.*et al.*, (1982) *Nature.* 297, 681-683.
Schwegler, H. (1995) *Neurosci. Lett.* 198, 197-200.

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## 258P HIPPOCAMPAL THETA ACTIVITY IS REGULATED BY CORTICOTROPIN-RELEASING HORMONE IN THE RAT

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Hippocampal cholinergic theta is a rhythmic, sinusoidal waveform that occurs in alert, immobile rats presented with threatening stimuli (Bland, 1986). Corticotropin-releasing-hormone (CRH) increases anxiety and fearfulness when administered intracerebroventricularly (Dunn *et al.*, 1990). In the present study, we have examined the effects of direct intrahippocampal infusions of CRH on the expression of theta activity in urethane-anaesthetized rats, who display only cholinergic theta or large, irregular activity (LIA) (Smythe, 1992).
Adult male, Lister hooded rats weighing between 350-500 g, were anaesthetized with isofluorane and implanted with jugular catheters. They were then switched to urethane (0.8 g/ml) anaesthesia and placed in a stereotaxic frame. A theta recording electrode was placed in the stratum moleculare of the hippocampus (from bregma: A-P -3.3, M-L 2.5, D-V 2.7 mm). A 30 gauge cannula was positioned 1-2 mm from the theta recording electrode for local drug infusions. Rats were administered either vehicle (VEH; artificial CSF; n=4) or 2 μg of CRH (n=5) in 2 μl VEH. Spontaneous theta activity was monitored for 60 min. Changes in frequencies (Hz) and amplitude (mV) of the theta signals over time were analyzed using ANOVA (employing a Greenhouse-Geiser correction), followed by Bonferroni corrected, t-tests.
ANOVA revealed no effects of CRH treatment on theta frequency with $F(1,8)=0.58$, ns. These data are shown in Figure 1A. ANOVA revealed a significant drug x time effect of CRH treatment on amplitude values $F(1,8)=18.9$, $P<.001$. Overall, CRH-treated rats displayed higher theta amplitudes compared to the VEH animals, an effect that was significant by 35 min post-CRH administration (P's<.05).

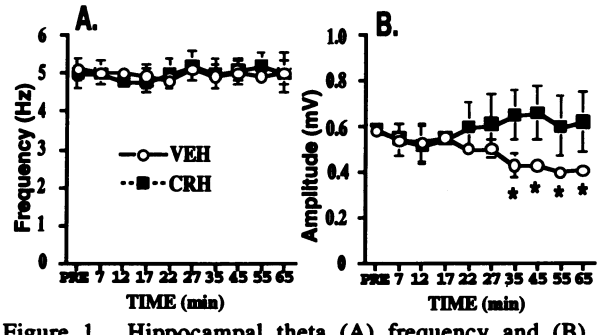


Figure 1. Hippocampal theta (A) frequency and (B) amplitude under spontaneous conditions at various times following VEH or CRH infusions (Means ±SEM are shown).
Significantly different from corresponding group at same time point *(P<.05).

Amplitude, but not frequency, of hippocampal theta was affected by CRH. Increased amplitudes may reflect enhanced acetylcholine release (Monmaur *et al.*, 1997). The significance of this effect for the behavioural responses produced by CRH remains to be determined. In conclusion, ongoing hippocampal electrical activity is regulated by CRH probably acting locally.

Bland, B.H. (1986) *Prog. Neurobiol.* 54, 1-54.
Dunn, A. *et al.*, (1990) *Brain Res. Rev.*, 15, 71-100.
Monmaur, P.*et al.*, (1997) *Brain Res. Bull.* 42, 141-146.
Smythe, J.W.(1992) *Neurosci.BioBehav.Rev.*16, 289-308.

## 259P FURTHER EVIDENCE THAT NMDA ANTAGONISTS PROTECT AGAINST MDMA ('ECSTASY')-INDUCED NEURODEGENERATION BECAUSE OF HYPOTHERMIA

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NMDA antagonists have been reported to protect against 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy')-induced neurodegeneration of 5-HT neurones in rat brain (Finnegan *et al.*, 1990; Colado *et al.*, 1993; Hewitt & Green, 1994). Recently Farfel & Seiden (1995) showed that dizocilpine and CGS 19755, in combination with MDMA, produced hypothermia and found that when the body temperature was kept elevated, these NMDA antagonists were not neuroprotective. They proposed therefore that NMDA antagonists do not have a true neuroprotective action against MDMA-induced damage. We have now extended these studies by examining the effect of AR-R15896AR, a novel NMDA receptor ion channel antagonist (Palmer *et al.*, 1997), on MDMA-induced neurodegeneration.

Male Dark Agouti rats (160-180g) were kept at normal ambient temperature (21°±2°C) and injected with saline or AR-R15896AR (20 $mgkg^{-1}$i.p) 5 min pre and 55 min post MDMA(15 $mgkg^{-1}$i.p.). AR-R15896AR had no effect on MDMA-induced hyperthermia ($F(1,10)=0.71$,Figure).When given to saline injected rats it produced a modest rise in temperature ($F(1,8)=5.84$, $P<0.05$, drug x time interaction). Seven days later the rats were killed and neurodegeneration of 5-HT nerve terminals examined by measurement of [$^3$H]-paroxetine binding in cortex (see Hewitt & Green, 1994). MDMA produced the expected loss of [$^3$H]-paroxetine binding which was unaltered by treatment with AR-R15896AR [saline: 58 ± 4(6); MDMA: 29 ± 3(6); MDMA + AR-R15896AR: 26 ± 6(6); AR-R15896AR: 60 ± 8(5); results (in fmol $mg^{-1}$ protein) shown as mean ± s.e.mean (n), MDMA group different from saline $p<0.01$ (ANOVA followed by Neuman-Keuls)].

These data support the proposal of Farfel & Seiden (1995) that NMDA antagonists only protect against MDMA-induced damage when they lower body temperature. AR-R15896AR did not alter the hyperthermia produced by MDMA administration and did not prevent neurodegeneration.

Colado, M.I. *et al.*, (1993) *Br. J. Pharmacol*, **108**, 583-589.
Farfel, G.M. & Seiden L.S. (1995) *J. Pharmacol. Exp. Ther* **272**, 860-867
Finnegan, K.T. *et al.*, (1990) *Neurosci Letts* **105**, 300-306
Hewitt, K.E. & Green, A.R. (1994) *Neuropharmacology* **33**, 1589-1595
Palmer, G.C. *et al.*, (1997) *Ann. NY Acad. Sci.* (In Press).

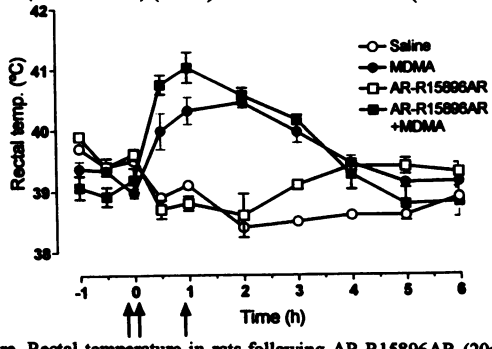


Figure. Rectal temperature in rats following AR-R15896AR (20$mgkg^{-1}$) and MDMA (20$mgkg^{-1}$). The rats were given saline or AR-R15896AR at -5min and +55min with MDMA or saline at time 0min. Results shown as mean ± s.e.mean (n=5-6)

M.I.C. thanks CICYT (SAF 1560/95) and Astra Arcus for support and Astra Charnwood for AR-R15896AR.

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## 260P IPRATROPIUM BROMIDE MAY PREVENT SALBUTAMOL-INDUCED DOWN-REGULATION OF $\beta_2$ RECEPTORS IN GUINEA-PIG TRACHEA

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Beta$_2$ agonists produce bronchodilation by directly stimulation $\beta_2$ receptors in airway smooth muscles, which have a great value in asthmatic attacks. However, continuous use of a $\beta_2$ against often leads to subsensitivity, which may be due to down-regulation of β-receptors. Some experimental studies have shown that concomitant use of some drugs e.g. corticosteroids with β-agonists may prevent the development of down regulation of these receptors (Malolepszy *et al.* 1993). In this study similar effect of Ipratropium bromide (IPB) has been investigated in guinea pig trachea.

Groups of guinea-pigs (Per Bright) of either sex weighing 500-700g were used during the study. First group of animals did not received any treatment. In order to induce down regulation in respiratory β-receptors, second group of animals (n=6) were nebulised by salbutamol (4mg/kg) three times a day for a period of 3 weeks. Third group of animals (n=6) were nebulised with vehicle (distilled water) for the same period of time. Animals of treated and control groups were killed with pentobarbitone overdose, tracheal spiral was prepared and placed in an organ bath containing krebs solution and aerated with 95% $O_2$ & 5% $CO_2$ at 37°C. A resting tension of 1g was applied to the tissue, the responses being recorded isometrically through force-displacement transducers.

Non cumulative dose-response curve for acetyl choline(Ach) was obtained in tissues of control groups. Incubation of control tissues with salbutamol ($10^{-8}$ M) for 20 minutes, caused a significant ($p<0.05$) reduction in Ach-induced contractions.

Such reduction was less pronounced in tissues from animals previously nebulised with salbutamol. This may indicate the possible β-receptor down regulation in treated animals. However incubation of salbutamol treated tissues with a combination of IPB ipratropium ($5\times10^{-9}$ M) and salbutamol ($10^{-8}$ M) for 20 minutes, caused significant reduction of Ach induced contractions compared with salbutamol incubation alone.(Fig 1)

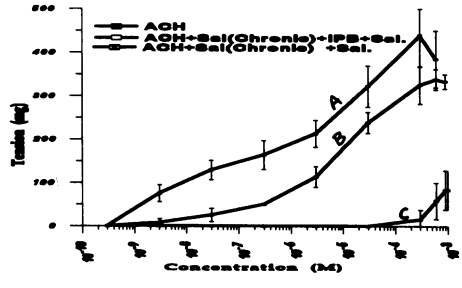


*Fig 1: Dose response curve for Ach in trachea of control animals(A) and salbutamol treated animals in presence of Salbutamol alone(B) and in presence of Salbutamol + IPB(C). Values significantly different from control are indicated with *($p<0.05$) and **($p<0.01$)*

The results of this study showed that although continuous therapy with β-agonists can elicit down-regulation of β-receptors in respiratory smooth muscles. The down regulated receptors may recover with the concomitant use of IPB and salbutamol. Such phenomenon can be attributed to the inhibitory effect of IPB on muscarinic receptors which leads to activation of adenylate cyclase and subsequent increase in intracellular cAMP may induce relaxation in respiratory smooth muscles.

Malolepszy, J. *et al.* (1993) Pneumonol. Alergol. pul. 61, 400-404

## 261P β-ADRENOCEPTOR ANTAGONIST-MEDIATED RELAXATION IN ISOLATED PORCINE OCULAR ARTERIES

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Increased intraocular pressure (IOP) is a major risk factor associated with optic nerve head damage in patients with glaucoma (Quigley & Addicks, 1980). β-adrenergic antagonists are widely used as ocular hypotensive drugs and lower IOP by reducing aqueous humour formation (Frishman *et al.*, 1994). Some β-adrenergic antagonists also improve visual field performance in these patients, possibly by increasing ocular blood flow. The mechanisms responsible for this are unclear, as much of the ocular vasculature is devoid of functional β-adrenoceptors (Nielson & Nyborg, 1989), but direct antagonism of voltage-dependent $Ca^{2+}$ channels has been implicated (Bessho *et al.*, 1991; Hester *et al.*, 1994). This study aimed to determine whether β-adrenoceptor antagonists could actively dilate precontracted arteries and inhibit agonist-induced contraction in porcine posterior ciliary arteries. Two non-selective β-adrenoceptor antagonists (propranolol and timolol) and a $\beta_1$-adrenoceptor selective antagonist (betaxolol) were compared with a calcium antagonist (nifedipine).

Segments of porcine posterior ciliary artery were mounted in a myograph for isometric force measurement. Vessels were equilibrated in physiological salt solution (PSS) maintained at 37°C and gassed with 95% $O_2$; 5% $CO_2$ before normalisation (Mulvany & Halpern, 1977). All vessels had an intact endothelium as confirmed by relaxation with bradykinin (87.24 ± 1.77%) and had a mean normalised lumen diameter of 331.6 ± 11.4μm (n=56). Relaxant effects of β-adrenoceptor antagonists, and nifedipine, were assessed following contraction of the vessels with $PGF_{2\alpha}$ ($3x10^{-6}$M) or depolarisation with $K^+$PSS (40mM). The effect of β-adrenoceptor antagonists ($10^{-4}$M) or nifedipine ($10^{-5}$M) on cumulative concentration-response curves to KCl and endothelin-1 (ET-1) was investigated following 10mins preincubation.

Propranolol, timolol and betaxolol ($10^{-7}$ - $10^{-3}$M) all produced concentration-dependent relaxations in arteries precontracted with either $PGF_{2\alpha}$ (approximate $pD_2$ values 3.81 ± 0.07, 3.53 ± 0.07, 3.50 ± 0.08 respectively) or KCl (approximate $pD_2$ values 3.60 ± 0.06, 3.28 ± 0.09, 4.02 ± 0.12 respectively).

KCl and ET-1 produced concentration-dependent contractions with $pD_2$ values of 1.42 ± 0.02 and 8.15 ± 0.18 and maximum responses of 6.88 ± 0.65 and 7.51 ± 0.71mN/mm respectively. Preincubation with propranolol or timolol resulted in both a reduction in maximum contractile response (3.34 ± 0.67 and 4.22 ± 0.60mN/mm respectively) and a reduced sensitivity ($pD_2$s 1.52 ± 0.03 and 1.57 ± 0.05 respectively) to KCl whereas betaxolol only resulted in a reduced sensitivity ($pD_2$ 1.52 ± 0.05). The maximum response to ET-1 was reduced following incubation with all three β-adrenoceptor antagonists (1.02 ± 0.31, 4.69 ± 0.81 and 4.76 ± 0.77mN/mm for propranolol, timolol and betaxolol respectively) but the sensitivity was not altered.

Nifedipine induced relaxation in $PGF_{2\alpha}$ and KCl precontracted arteries with $pD_2$ values of 7.05 ± 0.16 and 7.68 ± 0.20 respectively. KCl-induced contraction was completely abolished by nifedipine and the maximum response to ET-1 was significantly attenuated (2.95 ± 0.39mN/mm) but sensitivity was unaltered.

These results show that β-adrenoceptor antagonists have a direct relaxing effect on porcine isolated ocular arteries. The similarities between the effects of β-adrenoceptor antagonists and nifedipine shown in this study support the view that an inhibitory effect on $Ca^{2+}$ channels may contribute to the relaxant effect of β-adrenoceptor antagonists. This additional action of β-adrenoceptor antagonists in the eye may result in increased ocular blood flow and contribute to their beneficial effectiveness when used in the treatment of glaucoma.

Bessho, H., Suzuki, J. & Tobe, A. (1991) *Jpn. J. Pharmacol.* **55**, 351-358.
Frishman, W.H. *et al.* (1994) *J. Clin. Pharmacol.* **34**, 795-803.
Hester, R.K. *et al.* (1994) *Surv. Ophthalmol.* **38**, S125-S134.
Mulvany, M.J. & Halpern, W. (1977) *Circ. Res.* **411**,19-26.
Nielson, P.J. & Nyborg, N.C.B. (1989) *Int. Ophthalmol.* **13**, 103-107.
Quigley, H.A. & Addicks, E.M. (1980) *Invest. Ophthalmol. Vis. Sci.* **19**, 137-152.

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## 262P Ro 05-4864, A PERIPHERALLY ACTING BENZODIAZEPINE, STABILIZES MUSCLE MEMBRANE ARCHITECTURE

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Ro 05-4864 (4'-chlorodiazepam) is a benzodiazepine which has been reported to act exclusively peripherally. In a previous study (VanMarle et al., 1992) it has been demonstrated that a related benzodiazepine, midazolam, stabilizes the membrane architecture of the muscle fibres of rat diaphragm, the amount of inter membraneous particles is increased but the aquaporin waterchannels are not affected (no effect of midazolam on the orthogonally arrayed particles was observed).In this abstract preliminary results of the effects of Ro 05-4864, on the muscle membrane architecture are presented.

Rat phrenic nerve-diaphragm preparations were fixed in a vessel containing Krebs-Ringer solution (35 °C, pH 7.0), gassed with 5 % $CO_2$ in $O_2$, and equilibrated for 60 min. Preparations were stimulated indirectly with supramaximal rectangular pulses (pulse duration 0.2 msec,10 V, 0.1 Hz). Contractions were evoked for 60 min with Ro 05-4864 ($5.10^{-5}$ M) added to the organ bath. Controls were treated likewise but in the absence of Ro 05-4864. For subsequent freeze-fracture electron microscopy, the diaphragms were fixed in 2.5% glutaraldehyde, rinsed and cryoprotected. After cryoprotection the samples were processed for routine freeze-fracture. For comparison of the effects only the p-fracture faces were used since on the p-fracture faces the membrane architecture is most clearly observed.

Incubation for 120 min in Krebs Ringer solution in the absence of Ro 05-4864 induced a marked deterioration of the membrane architecture: the amount of intermembraneous particles, representative for the integral membrane proteins, was decreased. They were not as distinct from their surroundings as in untreated controls (i.e. fixed and processed for freeze fracture directly after excision), and sometimes the membranes did not fracture in the normal way, which is indicative for phase separation in the muscle fibre membrane. The presence of Ro 05-4864 in the incubation medium prevented this deterioration of the membranes. The membranes of the muscle fibres could not be distinguished from the control situation.

In conclusion, this effect of Ro 05-4864 closely resembles the effect of midozalam which was described earlier. Its interpretation remains obscure. Although the maintenance of the membrane seems to be an energy-related process (Woods and Williams, 1996), an effect of the peripherally acting benzodiazepine on the mitochondria appears unlikely, just as a direct effect on the muscle fibre membrane. An explanation, not related to the binding site, is also possible, since Ro 05-4864 is known to bind to calmodulin (Morgan et al., 1987), which may interfere with the calmodulin-induced activation of protein breakdown during the prolonged incubation under, obviously non-optimal, incubation conditions.

Morgan, P.F., Patel, I., Marangos, P.J. et al. (1987). Biochem. Pharmacol., 36, 4257-4262
VanMarle, J., Eigenhuis, J.J., Bokkinga, A., et al.(1992). Comp.Biochem.Physiol., 101C,579-582
Woods, M.J., Williams, D.C. (1996). Biochem. Pharmacol., 52, 1805-1814

## 263P THAPSIGARGIN-INDUCED CONTRACTIONS OF THE MOUSE ANOCOCCYGEUS: CALCIUM DEPENDENCE AND PHARMACOLOGY

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In the mouse anococcygeus, the Ca-ATPase inhibitor cyclopiazonic acid (CPA) produces contractions which have a nifedipine-sensitive and nifedipine-insensitive component (Gibson *et al.*, 1994). We have proposed that the latter component (which is reduced by Ca omission, SKF96365 and Cd, but is resistant to La) results from capacitative calcium entry through a non-selective cation channel opened by depletion of the sarcoplasmic reticulum (SR; Wayman *et al.*, 1996a,b). The object of the present study was to determine whether another commonly used SR Ca-ATPase inhibitor, thapsigargin (Tg), would also produce contractions with a similar pharmacology.

Anococcygeus muscles were dissected from male mice (LACA; 25-35g) and set up for the recording of isometric tension responses as described previously (Gibson *et al.*, 1994); two preparations were obtained from each animal. The Krebs solution contained phentolamine (1μM) and L-$N^G$-nitroarginine (50μM). Results are expressed as mean±sem.

Tg (0.5-100nM) produced concentration-related contractions of the mouse anococcygeus muscle. In the case of 100nM Tg, the time to onset of contraction was 90±9s and the time to peak tension was 14.2±2min. The magnitude of the contraction (543± 58mg) was similar to that produced by 50μM carbachol (566± 60mg), which is often used to produce a just-sub-maximal contraction of this preparation. Having reached peak tension, the contractile response to 100nM Tg showed a slow decline, although even by 90min it was still 49.3±6.9% of the peak response. The pharmacology of the response to Tg was studied by contracting the muscle with 100nM Tg and then, at the plateau phase (12min), adding the test drugs; the effect of the test drug was then measured 18min later (30min after addition of Tg). In control tissues, the tone remaining at 30min after addition of Tg was 83.9±7.2% of the peak response. However, this residual tone was abolished by changing to Ca-free Krebs solution (with 0.5mM EGTA), or by addition of the general Ca entry blocker SKF96365 (20μM), 12min after application of Tg. Tg-induced tone was also greatly reduced by Cd (23.9±8.5% tone remaining at 30min), but La had no effect on the tone remaining at 30min (85.4±4.7%; $P>0.05$ compared with 30min control). Verapamil (1-100μM) produced concentration-related relaxations of Tg-induced tone, but even at 100μM the relaxation was only partial (46.8±3.1% peak tone at 30min).

In conclusion, Tg produced strong, long-lasting contractions of the mouse anococcygeus. These contractions were dependent on extracellular Ca, but were only partially inhibited by verapamil. Like CPA, the contractions to Tg appear to involve both voltage-dependent (via L-type channels) and non-voltage-dependent mechanisms; this latter mechanism may involve capacitative Ca entry.

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Gibson, A., McFadzean, I., Tucker, J.F. *et al.* (1994) *Br. J. Pharmacol.* 113, 1494-1500.
Wayman, C.P., McFadzean, I., Gibson, A. *et al.* (1996a) *Br. J. Pharmacol.* 117, 566-572.
Wayman, C.P., McFadzean, I., Gibson, A. *et al.* (1996b) *Br. J. Pharmacol.* 118, 2001-2008.

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## 264P CONTRIBUTION OF Na/Ca EXCHANGE TO $Ca^{2+}$ OUTFLOW AND ENTRY IN THE RAT PANCREATIC B CELL: STUDIES WITH ANTISENSE OLIGONUCLEOTIDES

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In cardiomyocytes, Na/Ca exchange (NCX) appears to be the predominant mechanism for $Ca^{2+}$ extrusion, and has been proposed to mediate also $Ca^{2+}$ entry during the action potential. However, the latter view remains controversial (Leblanc & Hume 1990; Sham *et al.*, 1992). In other cell types, the exact contribution of Na/Ca exchange to $Ca^{2+}$ extrusion and entry during physiological cell signalling remains unknown.

The aim of the present study was to determine the contribution of Na/Ca exchange to both $Ca^{2+}$ outflow and entry in a non cardiac cell under physiological conditions. To do so phosphorothioated antisense oligonucleotides (AS-oligos) were used to blockade the exchanger in rat (Wistar) pancreatic β cells. AS-oligos were designed to target the region encompassing the ATG start codon of the rat NCX1 mRNA (Van Eylen *et al.*, 1997). Na/Ca exchange activity was evaluated by measuring cytosolic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in single cells ( 37 ° C) by microspectrofluorimetry, using fura-2.
Exposure of β cells to 500 nM of the AS-oligos for 24 hours inhibited the increase in $[Ca^{2+}]_i$ induced by extracellular $Na^+$ removal (area under the curve: 72.7 ± 7.6 μM.sec) by 77±7 % (n=30, $P<0.05$), providing functional evidence of Na/Ca exchange blockade. In contrast, control oligonucleotides (scrambled and mismatched) did not affect Na/Ca exchange activity (n=30, $P>0.2$). In AS-oligos-treated cells, the increase in $[Ca^{2+}]_i$ induced by membrane depolarisation [$K^+$ (512 ± 19 nM, n=54)] or the hypoglycaemic sulfonylurea, tolbutamide (156 ± 5, n=87)] was reduced by 28% ($p<0.0001$) and 40% ($p<0.01$), respectively. Likewise, the rate of $[Ca^{2+}]_i$ decrease after removal of $K^+$ (151 ± 10 nM/min, n=54) or tolbutamide (36±2 nM/min, n=87) was reduced by 72±5% ($p<0.0001$) and 40±2% ($P<0.001$), respectively. AS-oligos treatment also abolished (97±3%, n=50, $P<0.01$) the nifedipine (20μM) resistant increase in $[Ca^{2+}]_i$ induced by $K^+$ and profoundly altered the oscillatory or sustained increases in $[Ca^{2+}]_i$ induced by 11.1 mM glucose (n=50).
The present study shows that AS-oligos may specifically inhibit Na/Ca exchange in pancreatic β cells. In the latter cell, Na/Ca exchange appears to mediate $Ca^{2+}$ entry in response to membrane depolarisation and to be responsible for up to 70 % of $Ca^{2+}$ removal from the cytoplasm upon membrane repolarization.

Leblanc N & Hume JR (1990) Science, 248, 372-376.
Sham JSK, Cleemann L & Morad M (1992) Science, 255, 850-853.
Van Eylen F, Svoboda M & Herchuelz A (1997) Cell Calcium, 21, 185-193.

## 265P COMPARISON OF THE POTENCIES OF OPIOID AGONISTS AT THE HUMAN RECOMBINANT AND MOUSE DELTA OPIOID RECEPTORS

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The electrically-stimulated isolated mouse vas deferens preparation is used routinely to evaluate the functional efficacy of delta opioid receptor agonists (Falconieri Erspamer & Severini 1992). A single human delta opioid receptor has been cloned and expressed in a Chinese hamster ovary (CHO) cell line (Malatynska et al., 1995). The Cytosensor microphysiometer measures cellular acidification rate and can be used to investigate the pharmacology of recombinant receptors in intact cells (McConnell et al., 1992). Therefore, the aim of this study was to compare the relative activities of a range of opioid agonists at the mouse and recombinant human delta opioid receptors (rhδCHO) to determine whether the pharmacological profile of this receptor is conserved across species and assay techniques.

CD-1 Mice (over 35 g) were killed by stunning followed by cervical dislocation and the vasa deferentia removed and suspended between platinum electrodes in tissue baths containing aerated $Mg^{2+}$-free Krebs' buffer at 32°C. The tissues were attached to isometric force transducers under 0.5g tension and stimulated every 30 s at supramaximal voltage (45 V) with a frequency of 1 Hz and a pulse width of 0.5 ms. Cumulative concentration-response curves were constructed for the determination of agonist $pEC_{50}$ values in the presence of 0.3 μM CTOP, to inhibit μ-opioid receptors. For microphysiometry experiments, rhδCHO cells were plated at a density of $2x10^5$ per Cytosensor capsule and perfused with modified RPMI 1640. Acidification rate was determined over a 25 second period at 2 minute intervals. Serial concentration-response curves were constructed at 30 minute intervals with agonists included in the perfusate for 4 minutes, for determination of the $pEC_{50}$. Data are expressed as mean ± s.e. mean values (n=6).

There was good agreement between vas deferens $pEC_{50}$ values and microphysiometer $pEC_{50}$ values for a range of synthetic opioid receptor agonists; Table 1. These results demonstrate the utility of the microphysiometer for studying the agonist pharmacology of the human delta opioid receptor expressed in a CHO cell line. In addition, it is clear that a range of synthetic peptide and non-peptide opioid agonists show similar activity at the mouse vas deferens and rhδCHO cell delta opioid receptors. This is confirmed by a correlation co-efficient (r) of 0.92.

Falconieri Erspamer, G. & Severini, C. (1992) *Pharmacol. Res.* 26, 109-121.
Malatynska, E. et al, (1995) *NeuroReport* 6, 613-616.
McConnell, H.M et al, (1992) *Science* 257, 1906-1912

Table 1: The relative potencies of opioid agonists in the mouse vas deferens ($pEC_{50}$, n=6) and rhδCHO cells ($pEC_{50}$, n=6).

| Compound | Mouse Vas Deferens | rhδCHO cells | Compound | Mouse Vas Deferens | rhδCHO cells |
|---|---|---|---|---|---|
| Deltorphin 1 | 9.82 ± 0.05 | 9.29 ± 0.06 | BW373U86 | 9.26 ± 0.22 | 9.31 ± 0.07 |
| Cl-DPDPE | 9.48 ± 0.05 | 9.30 ± 0.13 | DPDPE | 8.85 ± 0.06 | 8.90 ± 0.09 |
| DSLET | 9.46 ± 0.07 | 9.90 ± 0.02 | SNC 80 | 7.92 ± 0.16 | 8.87 ± 0.06 |
| Deltrophin II | 9.42 ± 0.09 | 9.54 ± 0.06 | DAMGO | 6.41 ± 0.11 | 5.77 ± 0.06 |
| DADLE | 9.41 ± 0.07 | 9.78 ± 0.04 | U69593 | <5* | 4.78 ± 0.14 |

*In the presence of 100nM *nor*-binaltorphimine to inhibit κ-receptors

## 266P CHARACTERISATION OF [$^3$H]SNC-80 AND [$^3$H]NALTRINDOLE BINDING TO THE HUMAN DELTA OPIOID RECEPTOR EXPRESSED IN CHINESE HAMSTER OVARY CELLS

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Studies evaluating the binding domain of the human delta opioid receptor have been reported using [$^3$H]naltrindole, a selective delta opioid receptor antagonist (Knapp et al., 1994). However, it is possible that the binding domain for antagonists and agonists may be different for this receptor. Therefore, the aim of this study was to compare binding affinities for a range of standard opioid agonists and antagonists using both [$^3$H]naltrindole and, for the first time, the delta opioid receptor agonist [$^3$H]SNC-80 (Bilsky et al., 1995) on a Chinese hamster ovary cell line giving stable expression of the human delta opioid receptor (Malatynska et al., 1995).

Membranes were prepared by homogenisation (polytron mixer, 3 min full speed) followed by centrifugation (13,000g, 20 min, 4°C). Membrane pellets were resuspended in wash buffer (50 mM Tris, pH 7.4, 4°C). Radioligand binding experiments were performed in 96 well plates using [$^3$H]naltrindole (0.5 nM, 150 min, 25°C, 10 μg/well protein concentration) or [$^3$H]SNC-80 (0.5 nM, 120 min, 25°C, 12.5 μg/well protein concentration). The pKi values were determined using 12 concentrations of competing opioid compounds. The non specific binding was defined using 10 μM naltrexone. The reaction was terminated by rapid vacuum filtration (Wallac Big spot filters pre-soaked in 0.5% P.E.I.) and bound radioactivity was measured using scintillation counting. Data from competition studies were analysed using an iterative non-linear curve fitting programme (GraphPad Prism).

Saturation analysis yielded a $B_{max}$ value of 9.04 fmol/mg protein and a $K_d$ of 0.5 nM for [$^3$H]SNC-80, and a $B_{max}$ value of 9.08 fmol/mg protein and a $K_d$ of 0.1 nM for [$^3$H]naltrindole.

Table 1.The pKi values (± s.e.mean) derived from competition experiments for standard opioid compounds.

| Ligand | [$^3$H]SNC-80 | [$^3$H]naltrindole |
|---|---|---|
| BNTX | 8.20 ± 0.04 (n=6) | 8.16 ± 0.05 (n=6) |
| Cl-DPDPE | 8.46 ± 0.01 (n=10) | 8.47 ± 0.07 (n=7) |
| DAMGO | 6.12 ± 0.11 (n=7) | 6.11 ± 0.08 (n=7) |
| Deltorphin II | 8.30 ± 0.02 (n=7) | 8.23 ± 0.05 (n=8) |
| DPDPE | 8.01 ± 0.02 (n=10) | 7.84 ± 0.08 (n=7) |
| Naloxone | 7.01 ± 0.01 (n=8) | 6.99 ± 0.05 (n=7) |
| Naltrindole | 9.17 ± 0.01 (n=10) | 9.80 ± 0.04 (n=6)* |
| Nor-BNI | 7.48 ± 0.06 (n=8) | 7.53 ± 0.04 (n=7) |
| SNC-80 | 8.98 ± 0.03 (n=9) | 8.42 ± 0.01 (n=7)* |
| TAN-67 | 8.43 ± 0.01 (n=7) | 8.65 ± 0.03 (n=7) |
| U69593 | 5.61 ± 0.09 (n=9) | 5.12 ± 0.03 (n=7) |

*= $p < 0.01$ versus [$^3$H]SNC-80 value (Student's unpaired t test)

The pKi values for displacement of [$^3$H]SNC-80 are not significantly different from those determined using [$^3$H]naltrindole and are in close agreement with published data using [$^3$H]diprenorphine (Simonin et al., 1994). Only the unlabelled version of the ligands give significantly different pKi values, both showing apparently higher affinity in displacing their labelled counterparts. Regression analysis indicated a high degree of correlation between the two sets of data ($r^2 = 0.95$). Therefore, we conclude that the binding domain for agonists and antagonists at the human delta opioid receptor are not pharmacologically distinguishable using a range of standard agents.

Bilsky, E., et al (1995). J. Pharmacol. Exp. Ther., 273(1), 359-366.
Knapp, R. J., et al (1994). Life-Sci., 54(25), PL463-469.
Malatynska, E., et al (1995). NeuroReport., 6, 613-616.
Simonin, F., et al (1994). Mol. Pharmacol., 46, 1015-1021.

## 267P XEROSTOMIA AND MYDRIASIS: TWO POSSIBLE MUSCARINIC PERIPHERAL SIDE EFFECTS ASSOCIATED WITH DESCARBOETHOXYLORATIDINE, THE MAIN METABOLITE OF LORATADINE

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Recent studies on human cloned muscarinic receptor subtypes have revealed that descarboethoxyloratadine (DCL), the principal metabolite of loratadine, exhibits affinities for $M_1$ and $M_3$ receptors similar to that for $H_1$ receptors, $IC_{50}$=48, 125 and 51 nM respectively, (Handley D. *et al.*, 1997). Metabolites of several widely used $H_1$ antagonists are currently being developed or marketed, and it is of interest to compare them *in vivo*, in terms of undesired effects related to such muscarinic antagonism.

In the present study, fexofenadine (FXF), DCL, carebastine (CAR), and their respective parent compounds terfenadine, loratadine and ebastine have been tested in mice on: a) Salivary secretion induced by pilocarpine (1 mg/kg s.c.), a functional model for $M_3$ receptors, involving with equal relevance central and peripheral receptors (Sánchez *et al.*,1994), b) topical induced mydriasis and c) Oxotremorine (OXO)-induced (0.3 mg/kg s.c.) hypothermia (driven either by $M_2$ and $M_3$ receptors) and OXO-induced tremor (an $M_3$ mediated effect). The two latter muscarinic effects have been described to be "mainly central and with a minor peripheral component" and "exclusively central", respectively (Sánchez *et al.*,1993).

In contrast to the other antihistamines tested, DCL was the only one that showed a dose response inhibition of pilocarpine-induced salivation in mice ($ID_{50}$=10.8± 0.3 mg/kg p.o. and 3.2 mg/kg s.c., n=10), while LOR showed a significant inhibition only at the highest dose tested (24% at 30 mg/kg p.o., n=10). Histological sections of mice salivary glands, following pilocarpine treatment, revealed a dose response degranulation of acinar cells of submandibular glands, without affecting the sublingual and parotid ones. These glands have been shown to contain muscarinic receptors which can be stimulated by cholinomimetic drugs (Peter *et al.*, 1995). DCL (10 mg/kg s.c.) and atropine (1 mg/kg s.c.) showed a partial protection of acinar cell degranulation induced by pilocarpine, while FXF and CAR were virtually inactive. Moreover, only DCL produced a potent and long lasting (>120 min) mydriasis after topical administration, ($ED_{50}$=2.7± 0.2 mg/ml, n=10). None of antihistamines tested exerted any significant effect on OXO-induced hypothermia and tremor.

In conclusion, DCL showed peripheral antimuscarinic activity thereby inducing undesirable effects such as xerostomia and mydriasis in mice. The low CNS penetration capacity of DCL might be responsible for the lack of centrally-mediated antimuscarinic effects.

Handley D. *et al.*, (1997) An. All. Asth. Imm. **78(1)**, 143.
Sánchez C. & Lembol HL., (1994). Pharm. Toxicol., **74**,35-39.
Sánchez C. & Meier E., (1993). Pharm. Toxicol., **72**, 262-267.
Peter B. *et al.*, (1995). Clin. Exp. Pharm. Phys., **22**, 330-336.

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## 268P COMPARATIVE PHARMACOLOGY OF HUMAN MUSCARINIC M3 AND M5 CHOLINO- CEPTORS EXPRESSED IN CHINESE HAMSTER OVARY (CHO) CELLS

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Muscarinic receptors have been divided into five subtypes based upon the identification of 5 gene products (m1 - m5). While the m1 - m4 gene products correspond to the pharmacologically defined $M_1$ - $M_4$ receptors, an endogenous correlate for the m5 gene product is yet to be characterized. It is the lack of m5 selective antagonists that has hampered the search for an endogenous functional correlate ($M_5$). Expression of the human gene products (m1-m5) in CHO cells allows investigators to examine homogeneous receptor populations using radioligand binding and functional pharmacological techniques. In this way compounds with differential selectivity for the m5 subtype over the other subtypes might be identified which would aid in the isolation of an endogenous functional correlate ($M_5$). The aim of the present study was to pharmacologically characterize the human m3 and m5 receptors expressed in CHO-K1 cells by means of the carbachol-induced accumulation of [$^3$H]inositol phosphates, using a range of antagonists.

CHO-K1 cells expressing human muscarinic m3 and m5 receptors were cultured to confluence in Ham's F-12 nutrient media supplemented with 10% fetal bovine serum, geneticin (150 µg/ml) and penicillin/streptomycin (30 $\mu gml^{-1}$, 30 $\mu gml^{-1}$) at 37°C in 7% $CO_2$. Measurement of inositol phosphates accumulation was performed using well established techniques (Brown *et al.*, 1984). Antagonists were preincubated for 20 min and agonist stimulation was for 5 min (37°C).

Carbachol produced 6.5 ± 0.4 fold stimulation of inositol phosphates accumulation with a potency ($pEC_{50}$) of 5.9 ± 0.1 in cells expressing the m3 receptor subtype and a 2.9 ± 0.2 fold stimulation in cells expressing the m5 receptor subtype, with a potency of 4.8 ± 0.1. There was no carbachol stimulation of inositol phosphates accumulation in native CHO-K1 cells and no evidence of either positive or negative coupling of m5 receptors to adenylyl cyclase. Antagonist affinities ($pK_B$ see Table 1) were determined from antagonist inhibition curves (Leff & Dougall, 1993).

Table 1: Antagonist affinity estimates

| | m3 | | | | m5 | | | |
|---|---|---|---|---|---|---|---|---|
| **Antagonist** | **$pK_i$** | **$pK_B$** | **sem** | **n** | **$pK_i$** | **$pK_B$** | **sem** | **n** |
| Atropine | 9.5 | 8.57 | 0.12 | 4 | 9.1 | 8.73 | 0.05 | 4 |
| Pirenzepine | 6.8 | 6.38 | 0.08 | 4 | 6.9 | 6.38 | 0.13 | 4 |
| Methoctramine | 6.1 | 6.76 | 0.08 | 3 | 6.4 | 6.33 | 0.23 | 3 |
| AQ-RA 741 | 7.2 | 7.06 | 0.07 | 3 | 6.1 | 6.07 | 0.19 | 3 |
| Himbacine | 6.9 | 7.20 | 0.06 | 3 | 6.1 | 6.25 | 0.16 | 3 |
| 4-DAMP | 9.3 | 8.82 | 0.13 | 4 | 8.9 | 8.55 | 0.22 | 4 |
| p-f-HHSiD | 7.5 | 7.59 | 0.04 | 3 | 6.7 | 6.55 | 0.11 | 3 |
| Darifenacin | 8.9 | 8.88 | 0.10 | 3 | 8.1 | 7.65 | 0.05 | 3 |
| Zamifenacin | 7.9 | 7.89 | 0.03 | 3 | 7.4 | 7.64 | 0.10 | 3 |
| Tolterodine | 8.5 | 8.46 | 0.25 | 4 | 8.6 | 8.56 | 0.18 | 4 |

$pK_i$ estimates from Eglen et al., 1997, except AQ RA 741 (Dörje et al., 1991) sem/s.e.mean = standard error of the mean, n = number of experiments

These values correlate well with published binding affinities ($pK_i$) for these antagonists (m3: $pK_i$ vs $pK_B$; r = 0.94, sum of squares = 1.84; m5: $pK_i$ vs $pK_B$; r = 0.98, sum of squares = 0.84). The affinity profile of the m3 and m5 receptors are very similar with only darifenacin, p-f-HHSiD, AQ-RA 741 and himbacine demonstrating approximately 10 fold selectivity for one subtype (m3) over the other (m5). These four compounds may be useful tools in the identification of native $M_5$ receptor populations.

Brown, E., Kendall, D.A, & Nahorski, S.P. (1984) J. Neurochem. **42**, 1379.
Leff, P. & Dougall, I.G. (1993). TIPS, **14**, 110.
Dörje, F. Wess, J. Lambrecht, G. *et al.*, (1991) J. Pharmacol. Exp. Ther., **256**, 727.
Eglen, R.M., Bonhaus, D., Calixto, J.J., *et al.* (1997) Br. J. Pharmacol. **120**, 63P.

## 269P DESENSITIZATION BY TWO P2X RECEPTOR AGONISTS IN THE GUINEA-PIG VAS DEFERENS

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The guinea-pig vas deferens contains P2X receptors (Burnstock and Kennedy, 1985) which may consist of subtypes (Bailey and Hourani, 1995). Cloned P2X receptors exhibit subtypes, some which desensitize and some which do not (Fredholm *et al.*, 1997). The guinea-pig vas deferens P2X receptors can be desensitized, a useful property for possible subclassification.

The aim of this study was to determine the characteristics of desensitization by two P2X agonists, adenosine 5'-triphosphate (ATP) and α,β-methylene adenosine 5'-triphosphate (α,β-methylene ATP) to provide further evidence to test the hypothesis of more than one P2X receptor in the guinea-pig vas deferens.

Isolated vas deferentia from male tri-colour guinea-pigs (350 - 650 g) were set up in tissue baths with Krebs' solution at 37 °C and gassed with 95% $O_2$ and 5% $CO_2$. Initial tension was 1 g and after equilibration for 1 h they were exposed to KCl (50 mM) for 3 min every 15 min until constant spasms were obtained. In control tissues two non-cummulative concentration-effect curves were constructed to 3.3-fold increments of agonist using a contact time of 30 s and a time cycle of 15 min. In test tissues, in the second concentration-effect curves these agonists were preceded by desensitizing exposures (13 min) to the same or to another agonist (1 mM ATP or 10 μM α,β-methylene ATP) in self- and cross-desensitization studies. In other experiments, tissues were repeatedly exposed to ATP (100 μM) or α,β-methylene ATP (1 μM) for 30 s every 15 mins before and after prior desensitization with 13 min exposures to increasing concentrations of ATP (10 μM - 1 mM) or α,β-methylene ATP (30 nM - 3 μM), again in self and cross-desensitization studies. Similar experiments were carried out using carbachol as agonist.

It was found that α,β-methylene ATP was 87-fold more potent than ATP as a spasmogen but 3,550-fold more potent as a self desensitizing agent. ATP and α,β-methylene ATP caused self desensitization with -log $EC_{40}$ of 4.42 ± 0.09 (n = 9) and 7.97 ± 0.44 (n = 8) respectively. ATP and α,β-methylene ATP produced cross desensitization with -log $EC_{40}$ of 3.60 ± 0.27 (n = 7) and 7.26 ± 0.19 (n =10) respectively. Hence, ATP was 6.7-fold more potent as a self than cross desensitizing agent while α,β-methylene ATP was equi-potent for the two actions. The effects increased with desensitizing concentration. Neither agent affected responses to carbachol demonstrating that the densensitization was P2X receptor selective.

The data suggest that ATP and α,β-methylene ATP interact with a common receptor but produce desensitization to different extents.

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Bailey, S.J. & Hourani, S.M.O. (1995) *Br. J. Pharmacol.* **114**, 1125 - 1132.

Burnstock, G. & Kennedy, C. (1985) *Gen. Pharmacol.* **16**, 433 - 440.

Fredholm, B.B., Abbracchio, M.P., Burnstock, G. *et al.*, (1997) *Trends Pharmacol. Sci.* **18**, 79 - 82.

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## 270P SUBTYPES OF PREJUNCTIONAL $\alpha_2$-ADRENOCEPTOR IN RAT ATRIUM AND CEREBRAL CORTEX

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We have previously suggested that the prejunctional $\alpha_2$-adrenoceptors in rat vas deferens and rat submandibular gland resemble the $\alpha_{2D}$-adrenoceptor ligand binding site of rat submandibular gland (Smith & Docherty, 1992), whereas the prejunctional $\alpha_2$-adrenoceptor of rat atrium differed (Smith et al., 1992). In this study, we have re-investigated the subtype of $\alpha_2$-adrenoceptor mediating prejunctional inhibition of neurotransmission in rat atrium in comparison with the $\alpha_2$-adrenoceptor mediating prejunctional inhibition in rat cerebral cortex.

Prejunctional $\alpha_2$-adrenoceptors were investigated in terms of the ability of $\alpha_2$-adrenoceptor antagonists to increase the stimulation-evoked overflow of tritium in tissues pre-incubated with [$^3$H]-noradrenaline. Stimulation-evoked overflow at $S_1$ was 5.22±0.44%, n=19, and 12.0±1.22%, n=20, of tissue content in atrium and cortex, respectively. The relatively non-selective $\alpha_2$-adrenoceptor antagonist yohimbine and the $\alpha_{2D}$-adrenoceptor selective antagonist BRL 44408 had potencies in rat atrium which were similar to their potencies in rat cerebral cortex. The antagonists ARC 239, HV 723, WB 4101, prazosin and chlorpromazine, which have low affinity for $\alpha_{2D}$-adrenoceptors, significantly increased stimulation-evoked overflow at lower concentrations in rat atrium than rat cerebral cortex. For example, HV 723 (0.1 μM) increased stimulation-evoked release of tritium to 102.0±6.6% and 131.8±6.0% of control, and WB 4101 (1μM) increased stimulation-evoked release of tritium to 116.1±3.7% and 172.8±12.0% of control in cortex and atrium, respectively (n=4) (effects in atrium significantly greater than effects of vehicle). In particular, the potency of WB 4101 in atrium was close to its potency at $\alpha_{2C}$-adrenoceptors.

The correlation between ligand binding sites (Gavin et al., 1997) and the functional receptor in the rat cerebral cortex was significant only for the $\alpha_{2D}$-adrenoceptor ligand binding site of rat submandibular gland ($r=0.88$, $n=7$, $P<0.01$) as compared to the $\alpha_{2B}$-adrenoceptor of rat kidney ($r=0.45$, n.s.) or human recombinant $\alpha_{2C}$-adrenoceptor ($r=0.33$, n.s.). The correlation between ligand binding sites and the functional receptor in the rat atrium was not significant for any ligand binding site, with r= 0.67, 0.64 and 0.74 for the $\alpha_{2D}$-, the $\alpha_{2B}$- and the $\alpha_{2C}$-adrenoceptor sites, respectively.

It is concluded that the functional prejunctional $\alpha_2$-adrenoceptor of rat cerebral cortex closely resembles the $\alpha_{2D}$-adrenoceptor ligand binding site of rat submandibular gland, but the rat atrium may contain two subypes of prejunctional $\alpha_2$-adrenoceptor, $\alpha_{2D}$- and possibly $\alpha_{2B}$ or $\alpha_{2C}$.

Smith, K. & Docherty, J.R. (1992). Eur. J. Pharmacol. 219: 203-210.

Gavin, K.T. et al. (1997). Naunyn-Schmiedeberg's Arch. Pharmacol. 355: 406-411

## 271P ENHANCED ROLE FOR PREJUNCTIONAL NPY RECEPTORS IN THE CONTROL OF EXERCISE-INDUCED NORADRENALINE OVERFLOW IN THE VASCULATURE OF SPONTANEOUSLY HYPERTENSIVE RATS

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The putative interaction between prejunctional NPY receptors and $\alpha_2$-adrenoceptors in the modulation of exercise-induced noradrenaline (NA) overflow in permanently instrumented normotensive Wistar rats (WR) and SHR (in which the prejunctional $\alpha_2$-adrenoceptors have been found (partially) dysfunctional (Remie et al., 1992)) was investigated. Exercise was performed by forcing the animals to swim against a counter current (0.22 m $s^{-1}$) for 10 min. Blood samples were taken before and during exercise for determination of catecholamines (HPLC-ECD). By monitoring blood pressure simultaneously the postjunctional effects were studied as well. All drugs were administered intravenously.

During swimming, plasma NA levels increased 5.01-fold (to 922 ± 90 pg $ml^{-1}$ after 1 min) in WR and 10.4-fold (to 1792 ± 295 pg $ml^{-1}$) in SHR. In WR, NPY (0.2 and 2 μg $kg^{-1}$ $min^{-1}$) dose-dependently inhibited total exercise-induced NA overflow up to 50 ± 8% ($p<0.01$) of control. In SHR, the effect of NPY was more pronounced yielding a maximum inhibition of 72 ± 3% ($p<0.01$) of control overflow. Remarkably, when WR were pretreated with the $\alpha_2$-adrenoceptor antagonist yohimbine (0.25 mg $kg^{-1}$), NPY (2 μg $kg^{-1}$ $min^{-1}$) inhibited the potentiated exercise-induced NA overflow (which was 252 ± 33% ($p<0.01$) of control overflow) by 77 ± 4% ($p<0.05$), which was strikingly similar to the effect found - in the absence of yohimbine - in SHR. Furthermore, NPY (2 μg $kg^{-1}$ $min^{-1}$) inhibited the exercise-induced adrenaline overflow in WR by 46 ± 10% ($p<0.05$), but not at all in yohimbine-pretreated WR, and less marked (with 35 ± 7%) in SHR.

At the postjunctional level, SHR were shown to be more responsive to NPY than WR: 0.2 μg $kg^{-1}$ $min^{-1}$ already led to a significant increase in blood pressure, both at rest (2.8 ± 0.8 mm Hg; $p<0.05$) and during exercise (16 - 25 mm Hg at different time points; $p<0.05$), in contrast to normotensive WR. After pretreatment with yohimbine (0.25 mg $kg^{-1}$) responsiveness of WR for NPY increased. However, the maximum rise of blood pressure by NPY (2 μg $kg^{-1}$ $min^{-1}$) reached before and during the whole episode of exercise was not higher than with NPY alone. Thus, in SHR, compared to WR, an enhanced responsiveness of NPY receptors is present, both prejunctionally - inhibiting NA overflow - and postjunctionally, mediating vasoconstriction. In contrast, NPY receptors in the adrenal medulla, inhibiting exercise-induced adrenaline overflow, appeared to be more responsive in WR than in SHR, and not responsive in yohimbine-treated WR.

The results showed that (1) the increased vasoconstriction in SHR induced by NPY before and during exercise is related to hyperactive postjunctional NPY receptors rather than to a positive interaction between postjunctional NPY and α-adrenoceptors, (2) the enhanced prejunctional effects of NPY were related to the diminished $\alpha_2$-adrenoceptor function in SHR indicating a negative cooperative interaction between prejunctional NPY and $\alpha_2$-adrenoceptors at the sympathetic nerve terminal, and (3) a synergistic interaction between these receptors may exist in the adrenal medulla.

Remie, R., Van Rossum, J.X.M., Coppes, R.P. *et al.* (1992). Eur. J. Pharmacol. 211, 257-261

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## 272P NO EVIDENCE FOR A NERVE-DERIVED HYPERPOLARISING FACTOR IN THE MOUSE ANOCOCCYGEUS

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In a recent paper, Selemidis & Cocks (1997) have proposed the existence of a nerve-derived hyperpolarising factor (NDHF) which is involved, along with nitric oxide, in non-adrenergic, non-cholinergic (NANC) relaxations of the rat anococcygeus muscle. NDHF becomes the dominant relaxant transmitter when submaximal concentrations of contractile agents are used to raise the tone of the muscle, and when lower frequencies of stimulation are applied. The object of the present study was to determine whether a similar NDHF contributes to NANC relaxations of the mouse anococcygeus, since these have previously been considered to be solely nitrergic.

Anococcygeus muscles from male mice (LACA strain; 25-35g) were set up for the recording of isometric tension responses to drugs and field stimulation as described previously (Gibson & Wedmore, 1981). Results are given as mean±s.e.m.

In the first series of experiments, tone was raised with carbachol. To negate sympathetic responses, phentolamine (1μM) was included in the Krebs solution and each muscle was pre-incubated with 30μM guanethidine for 10min during the initial equilibration period. Carbachol (0.2-75μM) produced concentration-related contractions ($pD_2$ 6.03±0.06; maximum response 964±59 mg tension; n=8); 1μM carbachol produced a contraction of 54±5% of the maximum response and was used to raise the tone to investigate NANC relaxations. Field stimulation (1ms pulse width; 70V; 10s train) caused frequency-related relaxations of carbachol (1μM)-induced tone; 14±8% relaxation at 0.5Hz, 30±15% at 1Hz, and 80±8% at 5Hz (n=4). In the presence of the nitric oxide synthase inhibitor L-$N^G$-nitroarginine (L-NOARG; 100μM), these relaxations were negated (0%, 3± 3%, and 12±5% respectively; n=4). L-NOARG also completely inhibited relaxations to 20s and 60s trains of field stimulation.

In a second series of experiments, tone was raised with phenylephrine, which was used by Selemidis & Cocks (1997) in the rat anococcygeus. Here, phentolamine was omitted from the Krebs solution, but the muscles were still pre-incubated with guanethidine. Phenylephrine (0.1-75μM) also caused concentration-related contractions of the mouse anococcygeus ($pD_2$ 6.12±0.24; maximum response 615±38 mg tension; n=4); 0.2μM phenylephrine produced a contraction of 43±7% of the maximum response and was used to raise tone to investigate NANC relaxations. Again, field stimulation produced frequency-related relaxations (18±7% relaxation at 0.5Hz, 31±9% at 1Hz, and 67±10% at 5Hz; n=6). In the presence of L-NOARG (100μM), these relaxations were again negated (0%, 0%, and 5±3% respectively; n=6).

In conclusion, the present results provide no evidence for the involvement of a NDHF in NANC relaxations of the mouse anococcygeus, and support the view that the major NANC innervation in this tissue is nitrergic.

Gibson, A. & Wedmore, C.V. (1981) *J. Auton. Pharmacol.* **1**, 225-234.

Selemidis, S. & Cocks, T.M. (1997) *Br. J. Pharmacol.* **120**, 662-666.

## 273P CHARACTERISATION OF TACHYKININ $NK_1$ RECEPTORS IN THE RABBIT ISOLATED IRIS WITH SB223412

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The tachykinin $NK_3$ receptors in the rabbit iris have been reported to be either atypical or a heterogeneous population based upon data comparing agonist concentration-effect curves (CEC) and the inhibitory profile of selective and non-selective $NK_3$ receptor antagonists (Medhurst *et al*, 1997). Recently Sarau *et al*, (1997) described the *in vitro* pharmacology of the novel $NK_3$ receptor antagonist, SB 223412. In this study we have used this antagonist to characterise further the $NK_3$ receptor in the rabbit isolated iris.

Whole rings of iris were isolated from male New Zealand White rabbits (2.5-3.5kg), mounted for isometric tension recording in Krebs'-Henseleit solution (37°C, aerated with 95% $O_2$, 5% $CO_2$) and allowed to reach a resting tension of between 100 and 200mg. Following a one hour equilibration, repeat contractions to 10μM carbachol were obtained until responses were consistent. Experiments were carried out in the presence of the tachykinin $NK_1$ receptor antagonist CP-99,994 (1μM). Cumulative CEC to a range of neurokinin agonists were constructed and expressed as percentages of the maximum response to carbachol. Tissues were incubated with SB 223412 or vehicle (0.1% DMSO) for one hour before constructing agonist CEC. Agonist activity ($pEC_{50}$±SEM) is defined as the negative log of the concentration of agonist which produces 50% of the response to 10μM carbachol. Antagonist potency ($pK_B$±SEM) was calculated according to the Gaddum equation: $pK_B$=log(CR-1)-log[B], or the method of Schild ($pA_2$±SEM; slope±SEM).

All neurokinin receptor agonists studied evoked monophasic concentration-dependent contractions of the rabbit isolated iris. The rank order of potency was: Senktide > NKB ≥ [MePhe$^7$]NKB >> [β-Ala$^8$]NKA-4–10 (BANA), consistent with the presence of $NK_3$ receptors (see Table 1).

Table 1: Neurokinin Receptor Agonist Activitiy (n≥7)

| Agonist | $pEC_{50}$ | $E_{max}$ (% Carbachol Max) |
|---|---|---|
| Senktide | 8.61 ± 0.04 | 97.1 ± 2.2 |
| NKB | 8.17 ± 0.04 | 88.2 ± 0.9 |
| [MePhe$^7$]NKB | 8.07 ± 0.07 | 90.3 ± 1.7 |
| BANA | 6.07 ± 0.10 | 109 ± 2.6 |

The CEC to BANA was unaffected by an $NK_2$ blocking concentration of SR 48968 (300nM; Emonds-Alt *et al*, 1992). A parallel rightward displacement of the BANA CEC was observed in the presence of 100nM SB 223412 ($pK_B$=8.64±0.15, n=4). SB 223412 (0.1 - 1μM) demonstrated competitive antagonism of the senktide CEC ($pA_2$=8.4±0.18; slope=1.05±0.14 n=3). In contrast, SB 223412 caused non-concentration related parallel displacement of the CEC to [MePhe$^7$]NKB and NKB, $pK_B$ values at 100nM were 7.5±0.17 and 8.83±0.1, respectively, whereas at 1μM, were 7.03±0.17 and 7.87±0.05, respectively (n=4-6).

The inhibition of BANA-induced contractions by SB 223412 but not SR 48968, suggests the contractile actions of this peptide are mediated via $NK_3$ receptors. Data obtained with senktide suggests that these contractions are mediated through a single population of $NK_3$ receptors. In contrast, the contractile actions of [MePhe$^7$]NKB and NKB may be mediated via more than one receptor type in this tissue.

Emonds-Alt, X., Vilain, P., Goulaouic, P., *et al* (1992) *Life Sciences* 50, PL-101 - PL-106.
Medhurst, A.D., Parsons, A.A., Roberts, J.C., *et al* (1997) *Br. J. Pharmacol.* 120, 93-101.
Sarau, H.M., Griswold, D.E., Potts, W., *et al* (1997) *J. Pharmacol. Exp. Ther.* 281, (3), 1301-1311.

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## 274P NEUROPEPTIDE Y (NPY) RECEPTOR EXPRESSION IN THE EPIDIDYMIS OF THE GUINEA-PIG

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The vasa deferentia of the guinea-pig contains NPY immunoreactive fibres (Dhami & Mitchell, 1994), and NPY inhibits neurotransmitter release and electrically-evoked contractions in this tissue (Ellis & Burnstock, 1990; Selbie *et al.*, 1997). We have recently demonstrated that in the vas deferens and associated epididymis, NPY and the related peptide peptide YY (PYY) act post-junctionally to augment contractions. However, in the epididymis, as compared to the vas deferens where these peptides are equipotent, PYY is more effective than NPY in inhibiting electrically-evoked contractions, possibly via an atypical prejunctional receptor (Selbie, *et al.*, 1997). To date there has been no description of the NPY receptor subtypes expressed in epididymis of the guinea-pig using reverse transcriptase-polymerase chain reaction (RT-PCR).

To determine the NPY receptor subtypes expressed in the epididymis, this tissue was isolated from male Duncan-Hartley strain guinea-pigs (600-1000g) and total RNA prepared using the Qiagen Total RNA kit. First strand cDNA was prepared using random primers, specific oligonucleotide primers and/or oligo dT using the Gibco cDNA synthesis kit. Degenerate oligonucleotides (26-30mers) were designed corresponding to the DNA sequences of the putative transmembrane II (TMII) and transmembrane VI (TMVI) domains of cloned NPY $Y_1$, $Y_2$ $Y_4$ and $Y_5$ receptors from human, rat, mouse and bovine. Inosine residues (I) were included in some positions. cDNA was then amplified in PCR with the cycling conditions of: 5min at 95°C, then 30 cycles of 1min at 92°C, 1min at 60°C, and 1min at 74°C, then a final extension time of 10min at 74°C. Amplified products were analysed on a 1% agarose gel. Products of approximately 550-700bp in size were cut from the gel, purified and reamplified for subcloning into the Invitrogen TA Cloning vector for sequence analysis. To confirm that the degenerate oligonucleotides could amplify NPY receptor cDNA, we intitially used the oligonucleotides to amplify DNA from a cloned human NPY $Y_1$ receptor cDNA. The amplified product was approximately 700bp, representing the expected size of the TMII-TMVI domain.

The DNA sequences of two clones amplified from epididymal cDNA were related to the cloned NPY $Y_1$ and $Y_2$ receptors from other species. The putative guinea-pig NPY $Y_1$ receptor DNA sequence was 90.2% homologous to the human and 87.8% homologous to the rat $Y_1$ receptor sequences. The guinea-pig $Y_1$-like receptor amino acid sequence was even more conserved, being 94.6% homologous to human and 92.4% homologous to rat. The putative guinea-pig NPY $Y_2$ receptor DNA sequence was 87.3% homologous to human and 90.4% to rat, while the amino acid sequence was 94.1% homologous to human and 95.2% to rat.

These results indicate that in one region of the guinea-pig male reproductive tract, the epididymis, at least two different NPY receptor subtypes are expressed. This finding is consistent with our previous results showing that NPY $Y_1$-like receptors are involved in NPY-mediated post-junctional contractile effects in both the vas deferens and epididymis, and that NPY $Y_2$-like receptors are involved in NPY-inhibition of electrically-evoked contractions in the vas deferens.This approach may be useful in identifying whether an additional receptor mediates the atypical pharmacology observed with PYY-mediated inhibition of electrically-evoked contractions in the epididymis of the guinea-pig.

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Dhami , D. & Mitchell, B.J. 1994. *Histochemical Journal*, **26,** 262-270.
Ellis , L. & Burnstock, G. 1990. *Br. J. Pharmacol.*, **100,** 457-462.
Selbie, L.A., Hill, S.J., & Haynes, J.M. 1997. *Br. J. Pharmacol.* **120** , P44. and *in press.*

## 275P CHARACTERISATION OF THE CONTRACTILE AND NEUROMODULATORY ACTIONS OF BRADYKININ ON RAT BLADDER

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Bradykinin (BK) has well documented contractile activities on bladder smooth muscle which are reported to be mediated via $B_2$ receptors, with an inducible time-dependant $B_1$ component (Marceau *et al.*, 1980; Maggi *et al.*, 1989). The aim of the present study was to investigate the effects of BK on nerve-stimulated bladder contraction and characterise the receptor subtype(s) involved.

Male Sprague Dawley rats (250-350g) were killed by a blow to the head. The bladders were removed, quartered and mounted for isometric recording under 1 g tension in organ baths, containing Krebs-Henseleit solution, aerated with 95% $O_2$ / 5% $CO_2$, at 37°C. The tissues were equilibrated for 75 min prior to commencing the experiment. Electrical field stimulation (EFS)-induced bladder contractions were evoked using square wave pulses (5Hz, 40V, 0.1msec for 5sec). The cholinergic component of the EFS response was studied following desensitisation of purinergic receptors using α,β mATP ($3x10^{-5}$ M) and the purinergic component was studied in the presence of atropine ($10^{-6}$M). For all experiments, n = 5-6.

BK directly contracted rat bladder strips ($pEC_{50}$ 7.38 ± 0.24). In contrast the $BK_1$-selective agonist des-$Arg^9$-BK had no significant contractile effect at concentrations up to $10^{-6}$M ($P > 0.05$). BK also potentiated both the purinergic and cholinergic components of the nerve-evoked contractile response ($pEC_{50}$'s 8.1 ± 0.46 and 7.8 ± 0.26, respectively). Des-$Arg^9$-BK had no significant contractile effect on either response at concentrations up to $10^{-6}$ M ($P > 0.05$). BK ($10^{-8}$ M) signficantly potentiated the direct contractile effects of $10^{-7}$ M, α,β-mATP (from 0.47 ± 0.08 to 1.07 ± 0.13 g; $P < 0.05$) but had no significant effect on the contraction evoked by $10^{-4}$ M ACh (from 4.17 ± 1.03 to 4.52 ± 1.03 g, $P > 0.05$). Following a 5h equilibration of bladder strips in Krebs at 37°C, des-$Arg^9$-BK ($10^{-7}$ M) induced a significant increase in basal contractile activity (1.54 ± 0.36 g; $P < 0.01$). Under these conditions, des-$Arg^9$-BK ($10^{-7}$ M) also significantly enhanced the contractile response to $10^{-7}$ M α,β mATP (from 1.80 ± 0.67 to 3.05 ± 0.65 g; $P < 0.05$) but not $10^{-4}$ M ACh (from 3.87 ± 0.80 to 4.49 ± 0.73 g ; $P > 0.05$).

BK contracts rat bladder and potentiates the purinergic and cholinergic components of the nerve-evoked contraction via $B_2$ receptors. The enhancement of the cholinergic component of the neuronal contraction is mediated pre-junctionally whereas the enhancement of the purinergic response has a post-junctional component. In addition, there is a time-inducible $B_1$ response, resulting in direct tissue contraction, and enhancement of the contractile response to exogenous α,β-mATP, by des-$Arg^9$-BK.

Maggi, C.A., Pattacchini, R., Santicioli, P. *et al.*, (1989) *Br. J. Pharmacol.*, 98, 619-629.

Marceau, F., Barabe, J., St-Pierre, S *et al.*, (1980) *Can. J. Physiol. Pharmacol.* 58, 536 - 542.

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## 276P INVESTIGATION OF THE 5-HT RECEPTORS MEDIATING CONTRACTION IN THE RABBIT ISOLATED RENAL ARTERY

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The vasoconstrictor effects of 5-Hydroxytryptamine (5-HT) are predominately mediate by 5-$HT_{2A}$ and 5-$HT_{1B/1D}$ receptors. In the majority of vessels, including the rabbit isolated renal artery elicitation of a contractile response to 5-$HT_{1B/1D}$ receptor stimulation requires prestimulation of the vessel with another contractile agonist or prior depolarisation with raised extracellular potassium (Choppin & O'Connor, 1994). The aim of the present study was to use RT-PCR techniques to identify the presence of 5-$HT_{1B}$ and 5-$HT_{1D}$ receptor mRNA in this artery and to use novel drugs to further characterise the contractile response mediated by these receptors.

Female New Zealand white rabbits (1.5-2kg) were anaesthetised (sodium pentobarbitone; 60mg.$kg^{-1}$, i.v.) and killed by rapid exsanguination. Renal arteries were dissected, cleared of adhering connective tissue and used for RT-PCR and contractile studies.

Total RNA was extracted, and expression of mRNA for 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors demonstrated by RT-PCR using the following primers: 5-$HT_{1B}$ receptor, sense primer, 5'-gctgtcgtcggatatcacct-3', antisense primer, 5'-cccaccgtggagtagacagt-3'; 5-$HT_{1D}$ receptor, sense primer, 5'-catgtgtggagtgttccag-3', antisense primer, 5'-agatagttggcaggggtgtg-3'. PCR products were analysed on an ethidium bromide stained agrose gel and photographed.

Isolated segments (2mm) of renal artery were mounted in a Mulvany-Halpern myograph for isometric tension recordings at 37°C in oxygenated Krebs buffer. Arterial segments were incubated for 30 min. with ketanserin, prazosin (each 1μM) and in some cases GR127935. Vessels were prestimulated by exchanging the Krebs buffer with 20mM equimolar potassium buffer, prior to the cummulative addition of 5-HT (1nM-10μM) or the selective 5-$HT_{1B}$ agonist CP-93129 (1nM-1mM; Matasubara, *et al.*, 1991). All responses are expressed as a percentage of the contraction to 100μM phenylephrine. Differences between mean values were calculated using Students t-test.

PCR products for both 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors were obtained from rabbit renal artery.

In the presence of 4.7mM potassium Krebs containing ketanserin and prazosin (both 1μM) 5-HT (1nM-10μM) failed to evoke a contractile response. However, when the tissues were prestimulated with 20mM potassium Krebs, 5-HT (1nM-10μM) evoked concentration dependent contractions (max.=59.18 ± 3.53%). 5-HT-induced contractions were significantly attenuated by the selective 5-$HT_{1B/1D}$ receptor antagonist GR127935 (n=4, $P<0.05$; Skingle, *et al.*, 1996). 1nM and 10nM GR127935 reduced the contractile response to 10μM 5-HT from 59.18 ± 3.53 % to 41.4 ± 2.98% and 16.72 ± 5.97% respectively. The antagonism by GR127935 appeared to be insurmountable, possibly due to high lipophilicity and slow dissociation from the receptor (Skingle, *et al.* 1996).

In arteries prestimulated with 20mM potassium Krebs, CP-93129 evoked a concentration dependent contraction (100nM-1mM).

Since the rabbit isolated renal artery expressed mRNA for both 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors, the contractile response may be mediated by either, or both of these receptors. Although antagonism by GR127935 confirms a 5-$HT_{1B/1D}$ receptor mediated contraction, this antagonist is not able to distinguish between the receptor subtypes. The ability of CP-93129 to induce contraction indicates that the 5-$HT_{1B}$ receptor can mediate a contraction in this vessel. However, due to the lack of selective 5-$HT_{1D}$ agonists or antagonists which distinguish between 5-$HT_{1B}$ or 5-$HT_{1D}$ receptors it is not possible to assess the involvement of the 5-$HT_{1D}$ receptor in 5-HT-evoked contraction.

This work was supported by the Wellcome Trust.

Choppin A. & O'Connor, S. E., (1994). *J. Pharm. Exp. Ther.*, 270, 650-655.

Matasubara, T., *et al.* (1991). *Br. J. Pharmacol.*, 104. 3-4.

Skingle, M. *et al.* (1993). *Behav. Brain Res.* 73, 157-161.

## 277P EVIDENCE FOR 5-HT$_{1B/1D}$ RECEPTORS IN PRIMARY CULTURES OF RABBIT RENAL VASCULAR SMOOTH MUSCLE CELLS

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In the majority of blood vessels, 5-hydroxytryptamine (5-HT) mediated vasoconstriction is via activation of ketanserin-sensitive 5-HT$_2$ receptors. However, in the renal artery the 5-HT$_{1B/1D}$ receptor has been shown to be linked to contraction but requires prior partial depolarization (Choppin & O'Conner, 1994). The 5-HT$_{1B/1D}$ receptor is negatively coupled to adenylyl cyclase via a $G_i/G_o$ G-protein (Sumner & Humphrey, 1990). Both of the receptor subtypes may be involved in cardiovascular function, but the precise distribution and function of each receptor subtype remains to be defined. In this study we have investigated the 5-HT receptors present in a primary culture of rabbit renal artery vascular smooth muscle cells (VSMC's).

VSMC's were obtained by a modification of the explant method (Ross, 1971). In order to characterise the cultured VSMC's and exclude contamination by epithelial cells and fibroblasts the cells were identified using a monoclonal antibody for smooth muscle specific α-actin. Cyclic AMP (cAMP) was measured by radio-immunoassay (RIA) in the presence of ketanserin and prazosin (each 1 μM). Total RNA was extracted from the cell cultures and the expression of mRNA for the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors was demonstrated by RT-PCR using the following primers: 5-HT$_{1B}$ receptor, sense primer, 5'-gctgtcgtcggatatcacct-3', antisense primer, 5'-cccaccgtggagtagacagt-3'; 5-HT$_{1D}$ receptor, sense primer, 5'-catgtgtggagtgttccag-3', antisense primer, 5'-agatagttggcaggggtgtg-3'. PCR products were analysed on an ethidium bromide stained agarose gel and photographed.

Cultured VSMC's displayed the characteristic 'hill and valley' pattern described by Chamley-Campbell (1979). All of the cells prepared from these cultures displayed smooth muscle α-actin as shown by direct immunofluorescence. Application of 5-HT to the cultured cells caused a concentration-dependent reduction in cAMP. A maximal reduction was obtained with 10 μM 5-HT, which reduced the cAMP level to 65.53 ± 3.33 % of basal ($n$= 5, $p$>0.005). However, stimulation with $K^+$ (20 mM) before the application of 5-HT (10 μM) increased the reduction of cAMP to a maximal 82.50 ± 2.50 % of the basal level ($n$= 5, $p$>0.005). The results obtained from the RT-PCR demonstrated the presence of mRNA for both the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. However, the PCR product which corresponded to the 5-HT$_{1B}$ receptor showed more intense staining than the 5-HT$_{1D}$ receptor.

These data demonstrate that cultured VSMC's from the rabbit renal artery express mRNA for both the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors and that the 5-HT$_{1B/1D}$ receptors expressed are negatively coupled to the formation of cAMP. These cell cultures provide a model which will allow us to further characterise the second messenger systems which mediate vascular smooth muscle contraction to 5HT$_{1B/1D}$ receptor stimulation.

This work was supported by the Wellcome Trust.

Chamley-Campbell, J. (1979) *Physiol. Rev.* **59**, 1-61.
Choppin, A. & O'Conner, S.E. (1994) *J. Pharm. Exp. Ther.* **270**, 650-655.
Ross, R. (1971) *J. Cell Biol.* **50**, 172-186.
Sumner, M.J. & Humphrey, P.P.A. (1990) *Br. J. Pharmacol.* **99**, 219-220.

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## 278P EFFECTS OF INHIBITION OF UPTAKE AND METABOLISM ON PRESSOR RESPONSE TO 5-HYDROXYTRYPTAMINE OF THE ISOLATED PERFUSED EQUINE DIGIT

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Equine laminitis is a disease where the sensitive laminae of the foot are starved of nutrient blood flow due to haemodynamic disturbances in the digital circulation (Hood et al., 1993) and corticosteroid administration is known to be a predisposing factor for this condition. 5-HT is a potent vasoconstrictor of isolated equine digital blood vessels (Bailey & Elliott 1996). Isolated equine digital arteries (EDAs) have been shown to be more sensitive to 5-HT than tail and facial arteries and the estimated concentration of 5-HT in equine plasma (10 nM) has been found to be above the threshold for constriction of EDAs (Bailey & Elliott 1997). The aim of this study was to determine the sensitivity of an isolated Krebs perfused hoof preparation to 5-HT and investigate the effects of inhibitors of 5-HT uptake and metabolism.

Hind limbs were obtained from mixed breed healthy adult horses killed at an abattoir. The digital circulation was perfused at constant flow rate of 100 ml min$^{-1}$ with oxygenated modified Krebs Henseleit solution (KHS) and the arterial perfusion pressure recorded. After a 60 minute equilibration period, bolus infusions of 1 litre KHS containing 5-HT (1 to 100 nM) were made and the peak increase in the perfusion pressure recorded. In a second series of experiments, responses to an infusion of 1 litre of KHS containing 30 nM 5-HT were obtained and, 15 min after the perfusion pressure returned to baseline, fluvonoxamine maleate (1 mM), corticosterone (3 mM), pargyline (1 mM) or clomipramine (0.1 mM) were added to the perfusing fluid 10 min prior to, during and for 10 min following a second bolus infusion of 1 litre of KHS containing 30 nM 5-HT. The area under the response curves (AUCs) were measured and the responses compared using a paired Student's $t$-test.

The baseline perfusion pressure of the digital circulation was 107.8 ± 4.3 mm Hg (n = 7). Infusion of 5-HT for 10 min caused a dose related increase in perfusion pressure (mm Hg) of 11.0 ± 0.9 (1 nM); 27.3 ± 5.1 (10 nM); 84.0 ± 9.6 (30 nM) and 326 ± 14.5 (100 nM). The AUC to an infusion of 30 nM 5-HT was significantly increased in the presence of fluvonoxamine (85.5 ± 17.6% increase; P=0.02) and corticosterone (95.1 ± 28.3% increase P=0.01) whereas pargyline had no significant effect and clomipramine caused a significant reduction (46.9 ± 9.2% decrease; P=0.04) in the responses recorded (n=4 in all cases).

5-HT caused an increase in vascular resistance of the digital circulation when infused at concentrations close to those estimated to be present free in the plasma of normal horses. Inhibitors of 5-HT uptake processes (fluvonoxamine and corticosterone) potentiated the pressor effect of 5-HT, suggesting that uptake of 5-HT in the digital vascular bed, probably by endothelial cells, is important in limiting the pressor effects of this amine. Inhibition of 5-HT deamination by pargyline had no effect. The mechanism by which clomipramine inhibited the pressor response to 5-HT remains to be determined. Small changes in circulating concentrations of 5-HT will have profound effects on the blood flow in the digital vascular bed and might contribute to the development of equine laminitis.

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Bailey, SR & Elliott J (1996) *Br. J. Pharmacol.*, 119, 23P.
Bailey, SR & Elliott J (1997) *Equine Vet J.* (in press)
Hood, DM, Grosenbaugh, DA, Mostafa, MB *et al.*, (1993) *J. Vet. Int. Med.* 7, 228-233.

## 279P ROLE OF ENDOGENOUS ADENOSINE AND $A_3$ RECEPTOR STIMULATION IN HYPOXIA-INDUCED MYOCARDIAL STUNNING AND CONTRACTURE

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Adenosine is cardioprotective against myocardial damage after ischaemia (Sekili *et al.*, 1994). During myocardial ischaemia or hypoxia, contractility is reduced and when function recovers on reoxygenation this is myocardial stunning. We have shown that an adenosine $A_3$ receptor agonist, IB-MECA ($N^6$-(3-iodobenzyl) adenosine-5'-N-methylcarboxamide), improves contractile recovery of isolated atria and papillary muscles when added at reoxygenation (Gardner & Broadley, 1997). Here we examine the role of endogenous adenosine in stunning and whether the $A_3$ agonist is still protective when adenosine is removed by adenosine deaminase.

Left atria and a left ventricular papillary muscle from male Dunkin-Hartley guinea-pigs (250-300g) were set up in 50ml organ baths, containing Krebs-bicarbonate solution (composition mM : NaCl 118.4, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4.7H_2O$, $NaHCO_3$ 24.9, $KH_2PO_4.2H_2O$ 1.2, glucose 11.6) maintained at 37± 0.5°C and gassed with 5% $CO_2$ in oxygen. Tissues were paced throughout at 2Hz with threshold voltage+50%. Resting tensions of 0.5-1.0g were applied and isometric tension recorded. Means of at least four experiments ±SEM are shown. The results were taken to be significantly different if $p<0.05$ by an unpaired Student's t-test. After 15 min equilibration, hypoxia was induced by gassing with 5% $CO_2$ in nitrogen, followed by several changes of bathing solution. Hypoxia was maintained for 30 min before reoxygenation with 5% $CO_2$ in oxygen.

In control left atria and papillary muscles, hypoxia caused a fall in developed tension to 16.9±3.4% and 20.3±1.6% of the pre-hypoxic levels, respectively. During hypoxia, a contracture was observed which was measured as an increase in diastolic tension. The onset of contracture occurred at 14.0±0.7 and 14.3±1.0 min into the hypoxic period and reached peaks of 41.8±11.5% and 17.7±6.2% above the pre-hypoxia resting diastolic tension in left atria and papillary muscles, respectively. On reoxygenation, recovery of developed tension was significantly below pre-hypoxia levels at 15min after reoxygenation, being 74.8±4.1 and 79.7±7.6% of pre-hypoxic values in left atria and papillary muscles, respectively.

When adenosine deaminase (AD, 1 IU $ml^{-1}$) was added immediately before hypoxia, the onset of contracture in left atria and papillary muscles was not significantly different from controls (12.8±0.7 and 11.1±1.4 mins, respectively). The extent of contracture was not affected in left atria (47.2±12.6%) but in papillary muscles it was significantly increased to 46.1±7.5% above pre-hypoxia resting diastolic tension. Recovery of developed tension was unaffected by the presence of AD in the left atria but it was significantly impaired in papillary muscles (70.7±2.8 and 50.5±8.1% at 15 mins after reoxygenation, respectively).

IB-MECA ($3x10^{-7}$M) was added at reoxygenation in the presence of AD. It caused a significant improvement in recovery from hypoxia in both left atria and papillary muscles. The developed tension at 15 mins post-reoxygenation was 87.6±1.5% and 84.5±0.7% of the pre-hypoxic value in left atria and papillary muscle, respectively.

Thus, endogenous adenosine appears to protect from myocardial stunning in papillary muscle but not atria since its removal with AD worsens contracture and recovery from hypoxia. In the absence of endogenous adenosine, the $A_3$ receptor agonist IB-MECA added at reoxygenation improved the recovery from hypoxia in both left atria and papillary muscle and thus attenuates the myocardial stunning.

Gardner, N.M. & Broadley K.J. (1997) Br. J. Pharmacol. 120, 179P.
Sekili, S. *et al.* (1994) Circ. Res. 76, 82-94

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## 280P GENERATION OF SUPEROXIDE IN RINGS OF ABDOMINAL AORTAE FROM WISTAR KYOTO AND STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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It has previously been shown that hypertension in the stroke prone spontaneously hypertensive rat (SHRSP) may be associated with a reduced availability of nitric oxide (NO) possibly due to an increased generation of superoxide anions ($O_2^{\cdot-}$) leading to an increased breakdown of NO (McIntyre et al, 1997). As it has also been shown that $O_2^{\cdot-}$ generation is affected by gender (Brandes and Mugge 1997) this study was designed to assess whether $O_2^{\cdot-}$ generation is altered by hypertension and what role, if any, gender plays.

Abdominal aortae were obtained from 12-15 week old male (n=10) and female (n=10) Wistar Kyoto rats (WKY) and 12-15 week old male (n=8) and female (n=9) SHRSP. Aortic rings (5 mm) were placed in Krebs buffer and basal superoxide generation measured by lucigenin chemiluminesence (Chara et al, 1993) in a Packard LSS50 liquid scintillation counter. Some rings were pre-incubated with diethyldithiocarbamate ($10^{-2}$M DTCA) which inhibits SOD, and others were pre-incubated with carbachol ($3x10^{-6}$M) to assess any effects of stimulated NO release. In additional groups of animals (WKY n=6, SHRSP n=4) $O_2^{\cdot-}$ generation was studied in intact rings and after removal of endothelium by rubbing. A group of Sprague Dawley rats (males only n=6) was also examined. All data shown are expressed as nmoles $O_2^{\cdot-}$ generated/mgtissue/min and as mean ± SEM for each group. Statistical analysis was by unpaired t-tests with corrections for multiple comparisons. A p value of less than 0.05 was accepted as statistically significant.

Basal $O_2^{\cdot-}$ generation was significantly greater in aortic rings from male SHRSP than male WKY rats (3.80 ± 0.27 vs 1.17 ± 0.09 $p<0.005$, and in aortic rings from female SHRSP compared to female WKY rats (3.23 ± 0.42 vs 0.77 ± 0.08 $p<0.005$). The gender difference observed in $O_2^{\cdot-}$ generation, with males producing more $O_2^{\cdot-}$ than females, was found to be statistically significant in WKY rats ($p<0.005$) but not in SHRSP. The addition of DTCA increased $O_2^{\cdot-}$ generation in all animal groups. In male and female WKY rats $O_2^{\cdot-}$ generation after addition of DTCA was 2.63 ± 0.16 and 2.17 ± 0.29 and in male and female SHRSP was 5.69 ± 0.46 and 4.89 ± 0.43. Incubation with carbachol decreased $O_2^{\cdot-}$ generation to 0.79 ± 0.07, 0.36 ± 0.04, 1.9 ± 0.29 and 1.54 ± 0.21 in male and female WKY and male and female SHRSP respectively. Removal of the endothelium had little effect on $O_2^{\cdot-}$ generation in WKY but significantly reduced $O_2^{\cdot-}$ levels in SHRSP (WKY- endothelium 1.31 ± 0.14, SHRSP- endothelium 1.29 ± 0.20. The $O_2^{\cdot-}$ generation from the male Sprague-Dawley rats (1.2 ± 0.08) was found to be comparable to that from WKY males.

These results suggest that increased $O_2^{\cdot-}$ generation by the endothelium leading to decreased NO availability, may be an important contributing factor to hypertension in SHRSP.

McIntyre M., Hamilton C.A., Rees DR, Reid JL, Dominiczak AF (1997) Hypertension In press.
Brandes R.P., Mugge A. (1997) Life Sci. 60; 6: 391-396.
Chara Y, Peterson T.E. and Harrison D.G. (1993) J. Clin. Invest. 91: 2546-2551.

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## 281P THE OFFSET OF THE CARDIAC ACTIONS OF β-ADRENOCEPTOR ANTAGONISTS IS RELATED TO THEIR LIPOPHILICITY

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It is widely assumed that the effects of competitive reversible antagonists are rapidly terminated by the removal of the antagonist from the bathing solution although there have been few studies of this. We have tested the hypothesis that the offset of antagonist action on the heart is related to lipophilicity of the antagonist, by studying the effects of atenolol, celiprolol, propranolol and bopindolol on the isoprenaline concentration-response curves obtained on the rat isolated right ventricle. Atenolol has low (octanol-water coefficient of 0.23), celiprolol has moderate and propranolol (3.65) and bopindolol (4.86) have high lipophilicity. After a challenge to isoprenaline, some ventricle strips were not treated, while others were superfused with 500 ml of a single concentration of antagonist containing Krebs solution over 60min. All strips were then washed with 500 ml of β-blocker-free Krebs for 60 min prior to another challenge to isoprenaline (1st wash). A further 500 ml β-blocker-free Krebs solution preceded a final challenge to isoprenaline (2nd wash). Atenolol at $10^{-6}$ and $10^{-5}$ M, celiprolol at $10^{-7}$ and $10^{-6}$ M, propranolol at $10^{-7}$ and $10^{-6}$ M and bopindolol at $2 \times 10^{-9}$ and $10^{-8}$ M caused parallel rightward shifts of the isoprenaline concentration response curves and the $pK_B$ values, using the formula $pK_B = pK_x + \log (x - 1)$, were 7.21 ± 0.15 (8) and 7.00 ± 0.14 (6) for atenolol, 7.70 ± 0.05 (7) and 7.64 ± 0.17 (8) for celiprolol, 8.97 ± 0.07 (6) and 9.15 ± 0.20 (5) for bopindolol, and 9.43 ± 0.16 (6) and 9.80 ± 0.09 (6) for propranolol, respectively. The inhibitory effects of atenolol and celiprolol were readily reversible. The concentration-ratios with atenolol at $10^{-6}$ and $10^{-5}$ M were 23 ± 5 (8) and 141 ± 40 (6), respectively, and the β-adrenoceptor blocking action at $10^{-6}$ and $10^{-5}$ M was completely reversed after the 1st and 2nd wash, respectively. The concentration-ratios with celiprolol at $10^{-7}$ and $10^{-6}$ M were 7 ± 2 (8) and 54 ± 6 (7), respectively, and the action at $10^{-7}$ and $10^{-6}$ M was completely reversed after the 1st and 2nd wash, respectively. The inhibitory effects of propranolol and bopindolol were slowly reversible. The inhibitory concentration-ratios were 100 ± 10, 45 ± 13 and 6 ± 1 (6) in the presence of propranolol at $10^{-7}$ M and after the 1st and 2nd washes, and, in a separate experiment, were 2074 ± 592, 103 ± 17 and 33 ± 5 (5) in the presence of propranolol at $10^{-6}$ M and after the 1st and 2nd washes, respectively. The inhibitory concentration-ratios were 5 ± 2, 8 ± 3, and 6 ± 2 (5) in the presence of bopindolol at $2 \times 10^{-9}$ M and after the 1st and 2nd washes, and 70 ± 9, 28 ± 8 and 21 ± 5 (6) in the presence of bopindolol at $10^{-8}$ M and after the 1st and 2nd washes, respectively. The hypothesis was supported as the β-adrenoceptor blocking activities of atenolol and celiprolol were readily reversible whereas that of bopindolol and propranolol was slowly reversible. It is suggested that highly lipophilic drugs are retained by the heart and during washing in β-blocker-free solution are slowly released from the heart and subsequently able to bind to β-adrenoceptors.

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## 282P COMPARISON OF THE EFFECTS OF INHIBITORS OF POLY(ADP-RIBOSE) SYNTHETASE AND RADICAL SCAVENGERS IN A RAT MODEL OF HEPATIC ISCHAEMIA AND REPERFUSION

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Reactive oxygen species (ROS) contribute to the pathophysiology of ischaemia-reperfusion injury of the liver and other organs. In cultured cells, ROS cause strand breaks in DNA which leads to activation of the nuclear enzyme poly (ADP-ribose) synthetase (PARS). This results in depletion of the intracellular levels of NAD and ATP, and ultimately cell death. Inhibitors of PARS activity reverse the cytotoxic effects of ROS (Stubberfield *et al.*, 1988). We have recently discovered that inhibition of PARS activity during reperfusion, reduces the degree of necrosis caused by ischaemia-reperfusion of the heart and skeletal muscle of the rabbit (Thiemermann *et al.*, 1997). Here we compare the effects of PARS inhibitors with anti-radical interventions on the hepatic injury caused by ischaemia-reperfusion in the anaesthetised rat.

Male Wistar rats (250-300 g) were anaesthetised with thiopentone sodium (120 $mg \cdot kg^{-1}$ i.p.). A transverse laparotomy was performed, and the hepatic artery, portal vein and bile duct supplying the left lobes of the liver were isolated. Following a stabilisation period (30 min.) the vessels were occluded for 60 min followed by 2 h reperfusion. Different groups of animals received, 1 min prior to reperfusion, a bolus injection (0.2 ml, i.v.) of (i) vehicle I (saline, n=6), (ii) vehicle II (10% DMSO, n=6), (iii) 3-aminobenzamide (3-AB, 10 $mg \cdot kg^{-1}$, n=6), (iv) 3-AB (30 $mg \cdot kg^{-1}$, n=4), (v) 1,5-dihydroxyisoquinoline (ISO, 1 $mg \cdot kg^{-1}$, n=6), (vi) 4,5–dihydroxy-1,3-benzene-disulfonic acid (Tiron, 300 $mg \cdot kg^{-1}$, n=6), (vii) deferoxamine (Def, 40 $mg \cdot kg^{-1}$, n=6). Blood samples were taken prior to ischaemia (time 0 = baseline), and at 180 min.(end of reperfusion period), and analysed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in serum as markers of liver injury. Results are expressed as mean±s.e.m. and analysed using ANOVA followed by a Dunnett's post-hoc test (*$p < 0.05$).

Baseline values ranged from 157 to 232 $IU \cdot ml^{-1}$ for AST, and 147 to 255 $IU \cdot L^{-1}$ for ALT, and were not different between groups. Hepatic ischaemia and reperfusion resulted in a substantial rise in the serum levels of AST and ALT. Administration of the PARS inhibitors had no effect, while Tiron (intracellular $O_2^-$ scavenger) or Def. (iron chelator) attenuated the rise in the serum levels of AST and ALT caused by ischaemia-reperfusion (Table 1).

*Table 1*

| Drug | Dose | End Rep AST (IU/L) | End Rep ALT (IU/L) |
|---|---|---|---|
| Vehicle I | saline | 3596±354 | 3522±480 |
| Vehicle II | 10% DMSO | 3488±315 | 3441±354 |
| 3-AB | 10 $mg \cdot kg^{-1}$ | 3687±451 | 3264±334 |
| 3-AB | 30 $mg \cdot kg^{-1}$ | 3226±376 | 2481±392 |
| ISO | 1 $mg \cdot kg^{-1}$ | 3487±370 | 2875±366 |
| Tiron | 300 $mg \cdot kg^{-1}$ | 1920±239* | 1840±396* |
| Def | 40 $mg \cdot kg^{-1}$ | 1677±280* | 2124±249* |

Thus, the generation of ROS ($OH^-$ & $O_2^-$) upon reperfusion, results in hepatocellular injury which is not due to activation of PARS.

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Stubberfield *et al.*, (1988). *Biochem. Pharmacol.* 37, 3967-3974.
Thiemermann, C. *et al.*, (1997). *Proc. Natl. Acad. Sci.*, 94, 979-683.

## 283P EFFECTS OF DIETARY ANTIOXIDANTS ON ORGANIC NITRATE TOLERANCE IN VIVO IN OBESE/LEAN ZUCKER RATS

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Oxidant stress in insulin resistance and diabetes (Giugliano *et al.*, 1995a) may adversely affect vascular reactivity to exogenous nitrovasodilators such as organic nitrates (McVeigh *et al.*, 1994; Giugliano *et al.*, 1995b). Vascular superoxide anion in particular, might be expected to aggravate nitrate tolerance via the inactivation of nitric oxide (see Münzel *et al.*, 1995). We therefore studied the effects of dietary antioxidants on vasodepressor reactivity to glyceryl trinitrate (GTN) and the development of GTN tolerance *in vivo* in the anaesthetised Zucker rat, the obese strain of which is a model of insulin resistance (metabolic Syndrome X) in man.

Male, 9-week old, obese/lean Zucker rats were maintained for 4 weeks on control diet or diets supplemented with the superoxide anion scavenger tiron (1 % $wv^{-1}$, in drinking water), vitamin E ((±)-α -tocopherol acetate, 0.5 % $ww^{-1}$ in chow ) or probucol (0.5 % $ww^{-1}$ in chow). Rats were subsequently anaesthetised with thiopentone sodium (120 mg $kg^{-1}$ i.p.). Percentage decreases in mean arterial pressure (MAP) to bolus dose GTN (0.1-100 μg $kg^{-1}$ i.v.) were evaluated before and after the rapid induction of tolerance by a 1 h infusion of GTN (40 μg $kg^{-1}$ i.v.) (Newman *et al.*, 1990). Data are mean±s.e. mean. AUC=area under dose-response curve.

Basal MAP was higher in obese rats irrespective of diet (Table 1).

Table 1. Basal MAP (mm Hg) in anaesthetised Zucker rats

| Diet | obese | n | lean | n |
|---|---|---|---|---|
| control | 153.1±4.6* | 6 | 135.5±5.7 | 6 |
| tiron | 141.5±5.1* | 6 | 126.9±3.9 | 5 |
| vitamin E | 152.1±2.9* | 6 | 119.7±5.4 | 6 |
| probucol | 153.5±1.9* | 6 | 133.2±3.9 | 6 |

*P<0.05 vs corresponding lean group (Student's unpaired t-test).

Non-tolerant vasodepression to GTN was depressed in obese relative to lean rats on control diet and enhanced by antioxidant diets (Table 2). In contrast, antioxidant diets had little effect on rapid tolerance development in either obese or lean groups (Table 3).

Table 2. Non-tolerant GTN vasodepression *in vivo* (AUC)

| Diet | obese | n | lean | n |
|---|---|---|---|---|
| control | 47.6±4.7* | 6 | 62.4±3.6 | 6 |
| tiron | 68.5±7.3‡ | 6 | 59.7±5.8 | 5 |
| vitamin E | 65.0±6.4 | 6 | 51.7±4.4 | 6 |
| probucol | 59.8±3.3 | 6 | 59.5±5.8 | 6 |

*P<0.05 vs lean control (Student's unpaired t-test); ‡P<0.05 vs obese control (Dunnett's test).

Table 3. GTN tolerance *in vivo* (tolerant AUC/non-tolerant AUC, %)

| Diet | obese | n | lean | n |
|---|---|---|---|---|
| control | 51.7±6.4 | 6 | 64.7±6.5 | 6 |
| tiron | 55.8±3.0 | 6 | 63.8±9.8 | 5 |
| vitamin E | 56.6±12.3 | 6 | 53.8±3.5 | 6 |
| probucol | 57.7±8.3 | 6 | 55.6±5.9 | 6 |

In conclusion, while vasodepression to GTN may be affected by oxidant stress in the obese Zucker rat, there is no evidence of such a modulation of rapid nitrate tolerance *in vivo* in Zucker rats.

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Giugliano, D., Ceriello, A. & Paolisso, G. (1995a) *Diabetes Care* 19, 257-267.

Giugliano, D., Marfella, R., Verrazzo, G. *et al.* (1995b) *Ann. Intern. Med.* 123, 338-343.

McVeigh, G., Brennan, G.M., Hayes, R. *et al.* (1994) *Diabetologia* 37, 115-117.

Münzel, T., Sayegh, H., Freeman, B.A. *et al.* (1995) *J. Clin. Invest.* 95, 187-194.

Newman, C.M., Warren, J.B., Taylor, G.W. *et al.* (1990) *Br. J. Pharmacol.* 99, 825-829.

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## 284P EFFECTS OF DIETARY ANTIOXIDANTS ON ORGANIC NITRATE TOLERANCE *IN VITRO* IN OBESE/LEAN ZUCKER RATS

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Insulin resistance and diabetes are associated with oxidant stress and vascular dysfuncton (Giugliano *et al.*, 1995a). Reactive oxygen species (ROS) may adversely affect responses to organic nitrates in diabetic man (McVeigh *et al.*, 1994; Giugliano *et al.*, 1995b); while superoxide anion may promote nitrate tolerance development via the inactivation of nitric oxide (see Münzel *et al.*, 1995). We therefore assessed a role for ROS in tolerance to glyceryl trinitrate (GTN) *in vitro* in the isolated aorta of the Zucker rat, the obese strain of which is a model of insulin resistance (metabolic Syndrome X) in man.

Male, 9-week old, obese and lean Zucker rats were maintained for 4 weeks on standard diet (control) or diets supplemented with the intracellular superoxide anion scavenger tiron ( 1% $wv^{-1}$, in drinking water), vitamin E ((±)-α-tocopherol acetate, 0.5 % $ww^{-1}$ in chow ) or probucol (0.5 % $ww^{-1}$ in chow). Rats were anaesthetised with pentobarbitone sodium (60 mg $kg^{-1}$ i.p.), euthanised and endothelium-intact, thoracic aortic rings prepared and mounted under isometric conditions in Krebs-Henseleit solution (37 °C, carbogen-gassed). Nitrate tolerance was induced by incubating with GTN (100 μM) for 30 min followed by 30 min washout (Laight *et al.*, 1996). Rings were then submaximally precontracted with noradrenaline (100 nM) and relaxation to GTN (1 nM-1 μM) assessed. Data are mean±s.e. mean.

Non-tolerant relaxation to GTN after control diet was not significantly different in obese (area under curve (AUC)=136.2±19.5; $pD_2$=7.53±0.04, n=5) and lean (AUC=125.3±7.9; $pD_2$=7.40±0.05, n=5) groups. Antioxidant diets did not significantly affect non-tolerant GTN responses in the obese group (tiron: AUC=132.3±7.5; $pD_2$=7.45±0.05, n=5; vitamin E: AUC=151.5±7.4; $pD_2$=7.57±0.07, n=6; probucol: AUC=142.6±7.4; $pD_2$=7.54±0.04, n=6). In contrast, non-tolerant GTN responses in the lean group were enhanced by vitamin E (AUC=154.6±6.8*; $pD_2$=7.60±0.05, n=6) (*P<0.05) and probucol (AUC=151.7±6.8*; $pD_2$=7.60±0.06, n=6) (*P=0.05), but not tiron (AUC=118.7±6.7; $pD_2$=7.37±0.08, n=5) (P>0.05). Tolerance in the lean group, which was more severe than in the obese group after control diet, was improved by antioxidants (Table 1).

Table 1. GTN tolerance *in vitro* (tolerant AUC/non-tolerant AUC, %)

| Diet | obese | n | lean | n |
|---|---|---|---|---|
| control | 72.9±4.9 | 5 | 45.2±6.8** | 5 |
| tiron | 69.4±10.6 | 6 | 68.8±10.9 | 6 |
| vitamin E | 85.0±13.0 | 5 | 67.6±6.8 | 5 |
| probucol | 77.1±6.6 | 6 | 82.3±8.4‡ | 6 |

**P<0.02 vs obese control (Student's unpaired t-test); ‡P<0.05 vs lean control (Dunnett's test).

In conclusion, dietary lipophilic antioxidants improved non-tolerant relaxation to GTN and, together with tiron, reduced GTN tolerance, selectively in the lean group. This may suggest a role for ROS in the modulation of both non-tolerant reactivity to GTN and the aggravation of cellular nitrate tolerance in the isolated aorta of lean Zucker rats.

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Giugliano, D., Ceriello, A. & Paolisso, G. (1995a) *Diabetes Care* 19, 257-267.

Giugliano, D., Marfella, R., Verrazzo, G. *et al.* (1995b) *Ann. Intern. Med.* 123, 338-343.

Laight, D.W., Carrier, M.J. & Änggård, E.E. (1996) *Br. J. Pharmacol.* 120, 1477-1482.

McVeigh, G., Brennan, G.M., Hayes, R. *et al.* (1994) *Diabetologia* 37, 115-117.

Münzel, T., Sayegh, H., Freeman, B.A. *et al.* (1995) *J. Clin. Invest.* 95, 187-194.