## 294P EFFECT OF ANTAGONISM OF 5-$HT_{1A}$ AND 5-$HT_{1B}$ RECEPTORS ON RESPONSE TO 5-$HT_1$ RECEPTOR AGONISTS AND IMIPRAMINE IN THE FORCED SWIM TEST IN MICE

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8-OH-DPAT induces antidepressant-like effects in the forced swim test (FST) in mice (Luscombe et al, 1993) while the mixed 5HT1A/B agonist RU24969 decreases immobility in the tails suspension test in mice (O'Neill et al, 1996). We set out to compare the effect of 8-OH-DPAT with the mixed 5HT1A/B agonist RU24969 and the more selective 5HT1B agonist anpirtoline in the FST in mice. We also set out to characterise the effects of antagonists selective for these subtypes alone and their interaction with the agonists described above. Finally we examined the effect of the WAY100635 and isamoltane on the actions of imipramine in this test. Female BKTO mice (25±3g) were housed under standard conditions. The animals received a subcutaneous injection 30 min prior to testing. Where a pretreatment was used it was given 30 min prior to the main treatment and the animals were tested 30 min later. Swimming was measured in a 1L beaker. Activity was measured for the last 4 min of a 5 min test session. N was minimum of 6 for all groups.

8-OH-DPAT induces a 40% decrease in immobility in the FST [minimum effective dose MED 0.25mgkg$^{-1}$($p < 0.01$)]. Pindolol reversed the effect of 8-OH-DPAT over a dose range that did not alter swimming when given alone (1-5mgkg$^{-1}$) as did buspirone (1-5mgkg$^{-1}$) and WAY100635 (0.1-0.5mgkg$^{-1}$) but not GR127935. Both the selective 5HT1B agonist anpirtoline (1.25-5mgkg$^{-1}$) and mixed 5HT1A/B agonist RU24969 (0.6-2.5mgkg$^{-1}$) significantly increased time spent swimming in the FST. The effect of anpirtoline (1.25mgkg$^{-1}$) was reversed by GR127935 (1.25mgkg$^{-1}$) and isamoltane (5mgkg$^{-1}$) but not by WAY100635. GR127935 (1.25mgkg$^{-1}$) also reversed the effect of the mixed agonist RU24969 (0.6mgkg$^{-1}$) but WAY 100635 had no effect. The effect of imipramine (5mgkg$^{-1}$) was significantly reversed by isamoltane (5mgkg$^{-1}$) while WAY100635 (0.1-0.5mgkg$^{-1}$) was without effect.

These results confirm previous findings that direct stimulation of 5HT1A and 5HT1B receptors induces antidepressant-like effects in the FST. These results further suggest that the effects of RU24969 in the forced swim test are mediated by 5HT1B rather than 5HT1A receptors. The doses of 5HT1B receptor agonists used increased locomotor activity in mice, while 8-OHDPAT does not (data not shown) suggesting that the effects of 5HT1B agonists in this test may be due to their effects on locomotor activity. Finally these results support previous findings that blockade of 5HT1B receptors but not 5HT1A receptors attenuates the anti-immobility effects of imipramine suggesting that activity at these receptors may be an important mediator of its behavioural effects in this test.

Luscombe G, Martin, KF, Hutchins, LJ, et al, (1993) Br J Pharmacol. 108, 669-677
O'Neill MF, Fernandez AG, and Palacios JM, (1996) Pharmacol Biochem Behav. 53, 535-539

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## 295P THE EFFECT OF CHRONIC PAROXETINE ON 20 kHz ULTRASOUND-INDUCED DEFENCE BEHAVIOUR IN THE RAT

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Aversive situations, such as predator threat, result in the production of ultrasonic vocalisations from rats in the range of 20-32kHz (Blanchard *et al* 1993). Rats exposed to artificially generated 20kHz ultrasound exhibit defence-like behaviour that is associated with activation of the brain aversive system (Beckett *et al* 1997). This behaviour has previously been shown to be sensitive to pharmacological manipulation (Beckett *et al* 1996), and may be a model of panic. The present study aims to look at the effect of chronic treatment with the selective serotonin reuptake inhibitor paroxetine, which is used clinically to treat panic disorder (Oehrberg *et al* 1995), on the expression of this response.

Male hooded Lister rats (190-250g, n=5-8) received daily I.P. injections of paroxetine (10mg/kg) or saline for 6 weeks, and were exposed to 20kHz ultrasound twice weekly during this period. On test days, 30 minutes post-injection, the rats were placed in an open field arena containing a wall-mounted speaker. After a 2 minute basal period they received a 1 minute ultrasound pulse (91, 98 or 101dB SPL) followed by 2 minutes without sound. This was repeated for each sound intensity with an inter-procedure interval of 1 minute. Animal behaviour was analysed using a computer tracking system as distance travelled and speed (Beckett *et al* 1995).

20kHz ultrasound exposure produced an intensity related defence response characterised by hyperlocomotion. Chronic administration of paroxetine (10mg/kg) had no significant effect on maximum speed values obtained during 12 exposures to ultrasound at the 91dB intensity when compared to saline treated controls. Paroxetine treatment increased this response at the 98dB intensity, reaching statistical significance on exposures 4, 7 and 8. Exposure to 101dB ultrasound also resulted in an augmentation of this response, with exposures 6 and 12 reaching statistical significance (figure 1).

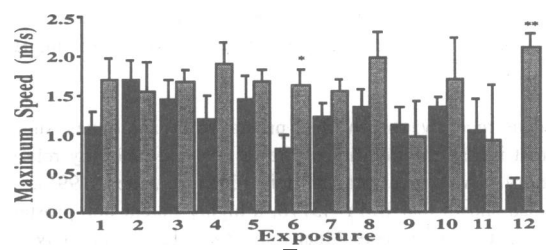


Figure 1: Effect of paroxetine (■ 10mg/kg) on maximum speed values obtained during 1 minute of 20kHz ultrasound (101dB) (mean ± s.e. mean) compared to saline (□) * $p<0.05$ vs saline ** $p<0.01$ vs saline. One-way ANOVA with post-hoc Duncan's NMR.

These results demonstrate that chronic treatment with paroxetine caused a small, but significant, increase in the response to 20kHz ultrasound. As paroxetine is an effective treatment for human panic disorder (Oehrberg *et al* 1995), this result may question the validity of the ultrasound paradigm as a model of panic disorder. It may be that 20kHz ultrasound-induced behaviour is analogous to a panic attack, and not the underlying panic disorder. The introduction of a conditioning procedure to this model may improve its value for the detection of potential drug treatments for panic disorder.

We thank SmithKline Beecham for financial support.

Beckett, S. & Marsden, C. (1995) J. Neurosci. Meths. **58** 157-61
Beckett, S., Aspley, S., Graham, M., *et al* (1996) Psychopharmacol. **127** 384-90
Beckett, S., Duxon, M., Aspley, S., *et al* (1997) Brain Res. Bull. **42** 421-26
Blanchard, R., Yudko, E., Rodgers, R., *et al* (1993) Behav. Brain Res. **58** 155-65
Oehrberg, S., Christiansen, P., Behnke, K., *et al* (1995) Br. J. Psychiatry **167** 374-9

## 296P EFFECT OF ACUTE FLUOXETINE AND YOHIMBINE ON BRAIN DEHYDROEPIANDROSTERONE (DHEA)

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Dehydroepiandrosterone (DHEA) is one of several neurosteroids released from the adrenal cortex and transported into the CNS, though recent studies have suggested that DHEA may also be synthesised in the brain (Robel & Baulieu, 1994). In addition Melchior & Ritzman (1994) have demonstrated that DHEA, has a dose dependent anxiolytic property in animals but there is little information about the effect of aversive situations and anxiogenic drug treatment on brain levels or cellular release of DHEA. The present study investigated the effects of :
1) exposure of rats to the elevated plus maze, an established animal model of anxiety (Lister, 1987) and 2) the acute administration of fluoxetine and yohimbine on the levels of DHEA in brain regions.

Lister hooded rats were used for all experiments. In the first experiment rats (n=8) were exposed to the plus maze for 5 minutes and then immediately killed and brain regions dissected and stored at -80$^0$C. Controls (n=8) were killed directly after removal from the home cage. The second experiment looked at changes in DHEA in response to fluoxetine (10mg/kg) and yohimbine (0.5mg/kg) administered acutely (1h and 20min pretreatment respectively) . Controls were given saline (0.9%). In the analysis of DHEA, the brain regions were sonicated first in ethanol and then extracted with an equal volume of acetone followed by chloroform/methanol (1:1) and finally ethyl acetate / water (1:1). In the last step of the extraction the organic layer containing the DHEA was dried, and this sample was resuspended in the radioimmunoassay (RIA) buffer and assayed by RIA using a specific DHEA antibody (Nayak et al 1997). The sensitivity of the assay is 0.19 ng/ml.

DHEA was measured in 4 brain regions,with the highest control levels in the hippocampus (3.3 ± 0.2 x $10^{-2}$ ng/mg tissue) followed by the hypothalamus (2.3 ± 0.2) , the striatum (1.9 ± 0.2 ) and the cortex (0.1 ± 0.3 ) . Exposure to the elevated plus maze had no effect on the levels of DHEA in any of the brain regions. Fluoxetine (n=6-8 ) significantly ($p< 0.05$, Student t test) decreased hippocampal DHEA (30%) and reduced hypothalamic levels by 38% but had no effect on DHEA in the striatum and cortex. Yohimbine (n=6-8) also tended to decrease DHEA levels in brain regions (hippocampus 17%; cortex 60% and striatum 40% ) but the changes were not significant.

While exposure to the elevated plus maze had no effect on DHEA, acute fluoxetine, which has been shown to produce anxiogenic behaviour, (Handley & McBlane, 1992), decreased DHEA. Since previous studies have shown that administration of DHEA produces anxiolytic behaviour (Melchior & Ritzman, 1994), it is possible that the present changes in DHEA may be associated with the anxiogenic effects of fluoxetine and yohimbine. Further studies are required to identify the mechanisms involved in the change in brain DHEA and to determine its role in aversive behaviour.

Robel, P. & Baulieu, E.E. Trends in Endocrine Metab., 1994, 5, 1-9.

Melchior, C.L., & Ritzman, R.F., Pharmacol. Biochem. and Behav., 1994, 47, 437-442.

Lister R.G., Psychopharmacology, 1987, 92, 180-185.

Nayak, S., Bennett, G.W. & Marsden, C.A., J. Psychopharmacol., 1997, 11, A51.

Handley, S.L. & McBlane, J.W., Br. J. Pharmacol., 1992, 107, 446P.

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## 297P HIGHER EFFICIENCY OF FLUOXETINE-INDUCED INCREASES IN EXTRACELLULAR 5-HT LEVELS IN THE VENTRAL HIPPOCAMPUS IN CONSCIOUS MICE LACKING 5-HT$_{1B}$ AUTORECEPTOR

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Various in vivo microdialysis studies have shown that 5-HT$_{1A}$ receptor antagonists potentiate the effects of antidepressant drugs such as selective serotonin reuptake inhibitors (SSRI) on extracellular levels of serotonin ([5-HT]$_{ext}$) at brain serotoninergic nerve terminals (Malagié et al., 1996). This potentiation, observed following the acute administration of SSRI to rats, is likely due to the prevention of a negative feedback action of 5-HT at somatodendritic level by the blockade of HT$_{1A}$ autoreceptors. To examine whether the blockade of another autoreceptor, of the 5-HT$_{1B}$ sub-type, could also potentiate the SSRI response, we monitored the effects of an acute administration of SSRI on [5-HT]$_{ext}$ in the frontal cortex (FC) and ventral hippocampus (VHPC) by using *in vivo* microdialysis in awake, freely moving homozygous mice ( body weight between 25 and 30 g ) lacking 5-HT$_{1B}$ receptor gene (5-HT$_{1B}$-/-) (Saudou et al., 1994). Animals were anaesthetized with chloral hydrate (400 mg/kg) and a concentric dialysis probe was implanted in each brain region; mice were allowed to recover for approximately 20 hours and then, probes were continuously perfused with an artificial cerebrospinal fluid pH (7,4±0,2) at a flow rate of 1,3 µl/min. Dialysate samples were collected every 15 min and were immediately analyzed for 5-HT by High Performance Liquid Chromatography. Usually, five basal fractions were collected to obtain basal values of [5-HT]$_{ext}$ (means ± SEM) before saline or fluoxetine administration. Then, response to drug injection was determined over a 3 hours period. Data were expressed as percentage of basal values of [5-HT]$_{ext}$ ( means ± SEM, n=7 ) and statistical analysis was carried out using a one-way analysis of variance ( ANOVA ) for repeated measures on the time followed by Fisher Protected Least Significance Difference (PLSD) post-hoc test. Furthermore, using percentage data, net changes in dialysate 5-HT were determined by calculating means of the area under the curve (AUC) for 180 min after injection. Statistical comparisons of these AUCs were made by applying a one-way ANOVA followed by Fisher PLSD post-hoc test. Thus, responses to an acute intraperitoneal (i.p.) fluoxetine injection ( Flx, 1, 5 and 10 mg/kg ) were compared between conscious 5-HT$_{1B}$-/- mice and their wild-type 129/Sv littermates (males of same age). Flx increased [5-HT]$_{ext}$ in a dose-dependent manner in the two strains of mice when compared to control-saline mice. In the VHPC of 5-HT$_{1B}$-/- mice, the increases in [5-HT]$_{ext}$ induced by Flx was dramatically potentiated following administration of the 5 and 10 mg/kg doses (maximal increases to 329±23% and 489±40% of basal, respectively), when compared to the wild-type mice (in which maximal increases were to 242±16% and 359±26% of basal, respectively). Furthermore, the maximal effects induced by Flx were higher in the VHPC than in the FC in the two strains of mice. In the FC of 5-HT$_{1B}$ -/- mice, increases in [5-HT]$_{ext}$ induced by Flx did not differ from those measured in the wild-type mice at any Flx dose tested. These data fully agree with the 5-HT$_{1B}$ receptor distribution found in autoradiographic studies performed in mice (Boschert et al., 1994). To confirm the modulatory role of these presynaptic terminal autoreceptors in the VHPC in the SSRI mechanism of action, further studies using the selective 5-HT$_{1B/1D}$ receptor antagonist GR 127,935 are needed.

Malagié I, Trillat A-C, Douvier E et al. (1996) *Naunyn-Schmiedeberg Arch Pharmacol* 354:785.
Boschert U, Amara D.A, Segu L et al. (1994) *Neurosciences* 58:167.
Saudou F, Aït Amara D, Dierich A et al (1994) *Sciences* 265:1875.

## 298P ENHANCEMENT OF SSRI'S EFFECTS BY 5-HT$_{1A}$ AUTORECEPTOR BLOCKADE IN RATS: *IN VIVO* MICRODIALYSIS AND BEHAVIOURAL STUDIES

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Recently, we (Malagié *et al.*, 1996) and others have demonstrated that 5-hydroxytryptamine (5-HT) 5-HT$_{1A}$ receptor antagonists potentiate the increase in extracellular serotonin levels (5-HT ext) induced by selective serotonin reuptake inhibitors (SSRI) such as fluoxetine (Flx) or citalopram by preventing a negative feedback mechanism exerted by somatodendritic 5-HT$_{1A}$ receptors located in the dorsal raphe nucleus in rats. Flx has also been shown to decrease food intake (Kim and Wurtman, 1988), a 5-HT-related behavioral effect. Here, we examined whether 5-HT$_{1A}$ receptor antagonists could also enhance the functional effects of Flx on 5-HT neurons. Two sets of experiments were performed following the same dose and acute drug administration to male Sprague Dawley rats (200-300 g). Flx (5 mg/kg, i.p.) or saline was injected 1 hour before the administration of the 5-HT$_{1A}$ receptor antagonists, either WAY 100635 (0.5 mg/kg i.v.) or a new compound (-)5-Me-8-OH-DPAT (3 mg/kg, i.v., Trillat et al., 1996). In the first one, rats were anaesthetized with chloral hydrate and a concentric dialysis probe was inserted in the ventral hippocampus (VHPC) and perfused with an artificial cerebrospinal fluid (pH 7.2; flow rate 1.3 µl min$^{-1}$). A jugular vein was cannulated for intravenous administration of 5-HT$_{1A}$ receptor antagonists. Twenty-four hours after the surgery, five dialysate samples were collected to determine basal levels of 5-HT in awake rats. Dialysates were collected for a total period of 180 min after the Flx or saline injection. Data were expressed as percentage of basal values (means ± SEM, n=5-8). Net changes in 5-HT ext induced by drug administration were determined by calculating the area under the curve (AUC) for a 0-180 min period. Statistical comparisons on AUCs were then made by a one-way ANOVA followed by Fisher PLSD *post-hoc* test. The maximal increase in 5-HT ext induced by Flx, was to 164±11% of the basal AUC value. AUC were further increased over the effect of Flx by +58±3% and +54±2%, when coadministered with WAY 100635 or (-)5-Me-8-OH-DPAT, respectively, P<0.05. This suggests that both 5-HT$_{1A}$ receptor antagonists potentiated the Flx-induced rise in 5-HT ext. In the second experiment, the rats were placed under a light-dark cycle (light on: 4:00 a.m.-4:00 p.m.). Three days before the test, the jugular vein was cannulated and the awake animals were then placed in individual wire-hanging cages where the anorexic test was conducted. Rats were food deprived for 24 h. The experiment started at a time (4:00 p.m) at which Flx was administered. Rats were provided with a known amount of lab chow immediatly, 1, 2, 3, 4 and 24 h after the Flx injection. The effects of the different treatments on cumulative food intake were analyzed by a one-way ANOVA, *post hoc* comparisons were carried out with a Fisher PLSD test. Flx significantly decreased food intake (P<0.05 vs. control group) by -33.4±3.3%, -24.1±2.8% and -17.6±1.7% at 1, 4 and 24 h after Flx injection, respectively. Food intake further significantly decreased over the effect of Flx by -33.2±2.9% and -37.8±3.7% when Flx was coadministered with WAY 100635 or (-) 5-Me-8-OH-DPAT 3 hours after Flx injection and by -37.2±2.1% and -37±3.5% 4 hours after Flx injection, respectively. These results indicate that 5-HT$_{1A}$ receptor antagonists potentiate the functional output of hippocampal 5-HT pathways mobilized by an acute systemic administration of Flx.

Malagié I, Trillat A-C, Douvier E et al. (1996) *Naunyn-Schmiedeberg Arch Pharmacol* 354:785
Kim S.H., Wurtman R.J. (1988) *Physiol. Behav.* 42:319-322
Trillat A-C., Malagié I., Langlois M et al. (1996) *Soc. Neurosci. Abst.* 22:2, 527.7 p 1327

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## 299P THE EFFECT OF 5-HT$_3$ AGONISTS ON YOUNG RAT LUMBAR DORSAL HORN NEURONES *IN VITRO*

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5-hydroxytryptamine (5-HT) modulates synaptic activity in spinal neurons (Lopez-Garcia and King, 1996). The 5-HT$_3$ ionotropic receptor subtype is present in spinal cord (Pratt, *et al.*, 1990) and is involved in modulation of spinal reflexes and nociception (Giordano, 1997). We have studied the effects of 5-HT$_3$ receptor activation on primary afferent-induced excitatory synaptic responses of single spinal cord dorsal horn neurons *in vitro*.

After urethane anaesthesia (2 g/kg i.p.), hemisected spinal cords from 10-12 day old rats were isolated and superfused with oxygenated artificial cerebrospinal fluid. High intensity (250 µs, 250 µA) electrical stimulation of dorsal roots (L4 - L6) was performed using glass suction electrodes. Sharp microelectrodes were used to record dorsal root-evoked excitatory post-synaptic potentials (DR-EPSPs) in 22 dorsal horn neurons. The 5-HT$_3$ agonist m-Chlorophenylbiguanide hydrochloride (m-ChPB) and Serotonin creatinine sulfate (5-HT) were applied at concentrations of 10 µM and 50 µM in the superfusate. The 5-HT$_3$ agonist 2 methyl-serotonin maleate (2-Met-5HT) was applied at 50 µM.

m-ChPB and 5-HT produced small but statistically significant changes in the resting membrane potential (P < 0.05). For example, 50 µM m-ChPB produced a depolarisation of 1.5 ± 0.3 mV (n=6) or a hyperpolarisation of 0.9 ± 0.2 mV (n=5). 5-HT at 50 µM produced a depolarisation of 3.1 ±1.2 mV (n=4).

The predominant effect of 5-HT (50 µM) and m-ChPB (10 and 50 µM) was a reduction in the amplitude and duration of DR-EPSPs. The quantified data for these DR-EPSP parameters are presented in Table 1. In a small population of neurons (n=5) the higher concentration of m-ChPB elicited an augmented DR-EPSP amplitude (13.7 ± 2.3 %, P < 0.05) although the duration did not increase. 2-Met-5HT (50 µM) produced modest reductions of the DR-EPSP (amplitude, 4.8 ± 2.1 %; duration 17.2 ± 8.3 %).

These data support the concept that 5-HT$_3$ receptors may play an important role in serotoninergic descending modulation of primary afferent input in the mammalian dorsal horn. The pre- or post-synaptic mechanisms by which 5-HT exerts these actions and the relative contribution of the 5-HT$_3$ receptor compared to other subtypes e.g. the 5-HT$_1$ subtype are as yet undetermined.

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Giordano, J. (1997) *Devel. Brain Res.* 98(1):142-144.
Lopez-Garcia, J.A., & King, A.E. (1996) *Eur. J. Neurosci.* 8, 2188-2197.
Pratt, G.D., Bowery, N.G., Kilpatrick, G.J., *et al.*, (1990) *Trends Pharmacol. Sci.* 11, 135-127.

Table 1: Effects of 10 µM and 50 µM m-ChPB and 5-HT on the DR-EPSP. All changes are presented as percentage reduction ± s.e.m. from the control. * P < 0.05 compared to control. Number of cells shown in parenthesis.

| | m-ChPB (10 µM) | m-ChPB (50 µM) | 5-HT (10 µM) | 5-HT (50 µM) |
|---|---|---|---|---|
| Amplitude | 5.9 ± 1.6* (4) | 5.7 ± 1.4* (7) | 20 ± 13.7 (3) | 22.5 ± 6.4* (6) |
| Duration | 15.0 ± 5.7* (5) | 18.7 ± 2.1* (12) | 29.2 ± 14.6 (3) | 37.1 ± 5.6* (7) |

## 300P RELATIONSHIP BETWEEN HIPPOCAMPAL FIELD ACTIVITIES AND MEDIAN RAPHE NEURONAL CELL DISCHARGES

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Hippocampal theta activity is a rhythmic, sinusoidal waveform with a frequency range of 3-10 Hz that occurs in alert, immobile rats presented with threatening stimuli (Bland, 1986). This type of theta is abolished by cholinergic receptor antagonists and is reliably modelled using urethane-anaesthetized rats (Smythe *et al.*, 1992). Considerable research has demonstrated the existance of ascending synchronizing systems that generate hippocampal theta activity; however, there is comparatively less known about afferents mediating desynchronized states such as large, irregular activity (LIA) (Vertes *et al.*, 1993). It appears as though serotonergic inputs to the hippocampus may induce LIA, since electrical stimulation of the median raphe (MnR) elicits hippocampal LIA, while MnR inhibition produces theta (Kocsis and Vertes, 1996). In the present study, we have examined MnR unit discharges to determine if these cells show a relationship to hippocampal field activities.

Adult male, Lister hooded rats (350-550g) served as subjects. Under isoflurane anesthesia, rats were implanted with jugular cannulae for delivery of i.v. urethane (0.8g/ml) anesthesia. A hippocampal recording electrode (tungsten; impedance of 0.2-0.5 MΩ) was positioned in the stratum moleculare (A-P -3.3; M-L +2.5; D-V 2.5mm) to record theta and LIA activities, and MnR cells (A-P -7.8; M-L + 0; D-V 8-9mm) were recorded with glass micropipettes (2-5 MΩ) backfilled with 2M sodium acetate/direct blue dye. Theta and LIA states were discriminated using a computer-based FFT calculation, and discharge rates (mean±SD), and patterns (rhythmic, phasic, tonic) during each condition were assessed by auto-correlation and cross-correlation functions. Independent t-tests were used to compare discharge rates during theta with those during LIA applying a randomization process (Edgington, 1987). At the conclusion of each experiment a 0.25mA D.C. current was passed through the cell electrode to eject dye and assist in verifying recording placements.

A total of 32 cells were recorded from 9 rats, 28 of which were localized to the MnR region. 13/28 cells (46%) were significantly more active during theta compared with LIA (theta-on) (θ-5.06±1.3; LIA-1.71±0.5, Ps<.01). 4/28 cells (14%) were less active during theta compared to LIA (theta-off) (θ-5.34±0.5; LIA-8.24±1.4, Ps<.05). 11/28 cells (39%) were unrelated to theta (θ-1.91±0.8; LIA-1.53±0.6). A number of cells (17/28) exhibited rhythmic firing and these included both theta-on and unrelated cells. Rhythmically-firing theta-on cells also showed evidence of being phase-locked to individual theta waves. The remaining unrelated cells, and all theta-off cells exhibited tonic firing only (11/28), and were not phasically-linked to hippocampal theta.

These data show that some MnR cells are related to hippocampal field activity. Previously, Kocsis and Vertes (1996) have reported theta-on cells in the raphe, but no evidence of theta-off cells. They suggest that the MnR receives descending afferents that signal non-serotonergic cells to discharge during theta. On the basis of our data, it is intriguing to speculate that ascending serotonin projections thought to desynchronize hippocampal field activity may exhibit theta-off firing parameters.

Bland, B.H. (1986) *Prog. Neurobiol.* 54, 1-54.
Edgington, E. Randomization Tests. Academic Press, N.Y. (1987)
Kocsis, B. and Vertes, R.P. (1996) *Neuroreport.* 7, 2867-2872.
Smythe, J.W.*et al.*, (1992) *Neurosci.BioBehav. Rev.*16, 289-308.
Vertes, R.P.*et al*,(1993)*Exp. Brain Research.* 96, 419-429.

## 301P THE INFLUENCE OF NICOTINE ON 5-HT OVERFLOW IN THE DORSAL HIPPOCAMPUS OF THE RAT

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Chronic nicotine administration reduces the concentration and biosynthesis of 5-hydroxytryptamine (5-HT) in the hippocampus of the rat (Benwell and Balfour 1979, 1982). The purpose of these experiments was to examine the possibility that these responses to nicotine are related to decreased 5-HT release in the hippocampus.

Groups (n=4-6) of male Sprague-Dawley rats were pretreated with daily subcutaneous injections of saline or nicotine (0.4mg $kg^{-1}$) for 21 days. Three hours after the last injection, dialysis probes (Benwell and Balfour 1992) were located in the dorsal hippocampus. Dialysis studies were performed on the following day. Following a period (60 min) of equilibration, 3 baseline dialysate samples were collected and analysed (HPLC with electrochemical detection) before the animals were injected with saline or nicotine (0.4mg $kg^{-1}$). Dialysate samples were collected for a further 2 hours. The basal concentration of 5-HT in the saline-pretreated rats (0.014±0.01 pmoles 20$\mu l^{-1}$) was unaffected by pretreatment with nicotine. The injection of nicotine suppressed 5-HT overflow ($F(1,16)=25.6$: $P<0.001$) in both the saline- and nicotine-pretreated rats (figure 1). The response to nicotine on the test day in the two pretreatment groups was not statistically different. In a second experiment, the effect of pretreatment on the response to a depolarising concentration of KCl (50mM), delivered through the probe, was examined. The KCl significantly increased ($F(2,9)=8.3$: $P<0.01$) 5-HT overflow to 290 ± 25 percent of baseline. This response was not influenced significantly by pretreatment with nicotine. The results suggest that acute nicotine injections suppress 5-HT release in the dorsal hippocampus and that chronic administration of the drug does not influence the response to nicotine or the total pool of 5-HT available for release in response to depolarisation of the nerve terminal membranes.

The support of the MRC is gratefully acknowledged

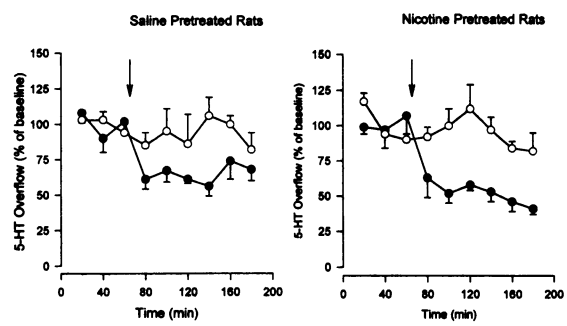


**Figure 1.** The effects of acute and chronic nicotine on 5-HT overflow in the dorsal hippocampus. Saline (open circles) or nicotine (0.4mg $kg^{-1}$; closed circles) was injected at the points indicated by the arrows. The results are expressed as means ± s.e. mean.

Benwell, M.E.M & Balfour, D.J.K. (1979) *Psychopharmacology* **63**, 7-11.
Benwell, M.E.M & Balfour, D.J.K. (1982) *Eur. J. Pharmacol.* **84**, 71-77.
Benwell, M.E.M & Balfour, D.J.K. (1992) *Br J. Pharmacol.* **105**, 849-856.

## 302P EFFECT OF CHRONIC TIANEPTINE TREATMENT ON THE RAT 5-HT TRANSPORTER

J.A. Lawrence, S.P. Butcher, P. Wren & H.J. Olverman. Department of Pharmacology and Fujisawa Institute of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ.

The hypothesis that disturbances in normal serotonergic transmission play an important role in the aetiology of depression is based predominantly on selective serotonergic reuptake inhibitors (SSRIs) being highly effective antidepressants. The delay between initiation of treatment and clinical onset suggests long-term neuroadaptive mechanisms which may involve alterations in density and function of the serotonergic transporter (SERT). The recent cloning of a cDNA encoding the rat SERT (Blakely *et al*, 1991) has allowed production of site-directed antibodies, raised against highly conserved sequences of SERT. A fifteen amino acid sequence in the fourth extracellular loop was used to produce the antibody SERT 387 and a similar length sequence from the intracellular carboxyl loop for SERT 612.

These antibodies have been used in conjunction with radioligand binding studies, to investigate the effects of chronic treatment with tianeptine, an atypical antidepressant, on the rat SERT and 5-$HT_{1A}$ receptors. Male Sprague-Dawley rats were injected with tianeptine (10mg/kg s.c. for 14 days).

Tianeptine treatment did not affect binding affinity ($K_D$) of [$^3$H]citalopram or [$^3$H]8-OHDPAT in cortical, hippocampal or striatal membranes. There was no change in [$^3$H]8-OHDPAT binding density ($B_{max}$) in any area investigated. [$^3$H]Citalopram binding site density was similarly unchanged in all brain areas investigated (see Table 1). Immunohistochemical analysis, using both intracellular and extracellular antibodies showed selective staining in the dorsal raphe nucleus together with specific staining in the terminal fields of the cortex, hippocampus and striatum. This staining pattern was unchanged by chronic tianeptine treatment.

The antidepressant, tianeptine, did not alter any of the serotonergic parameters investigated. It has been shown to enhance 5-HT reuptake (Ortiz *et al*, 1987) although it is devoid of significant actions on the imipramine binding site and on a number of other 5-HT receptor sites (Mennini *et al*, 1987). It would therefore also appear from our studies that tianeptine produces its antidepressant effects by actions other than those on the serotonergic transporter.

Supported by European Commission: BIO4 (CT96 0752).
P.W. is MRC Scholar. J.A.L. is R.C.P. SIM Fellow.

Blakely, R.D. *et al* (1991) *Nature*, 354, 66.
Mennini, T. *et al* (1987) *Naunyn-Schmiedeberg's Arch.Pharmacol.*, 336, 478.
Ortiz, J.O. *et al* (1987) *Eur.J.Pharmacol.*, 199, 335.

Table 1 The effect of tianeptine treatment on $K_D$ (nM) and $B_{max}$ (pmol/mg protein) values

| | Rostral Cortex | | Caudal Cortex | | Hippocampus | | Striatum | |
|---|---|---|---|---|---|---|---|---|
| [$^3$H]citalopram | Control | Tianeptine | Control | Tianeptine | Control | Tianeptine | Control | Tianeptine |
| $K_D$ | 0.91 ± 0.06 | 1.06 ± 0.05 | 1.33 ± 0.17 | 1.08 ± 0.06 | 1.01 ± 0.08 | 0.99 ± 0.05 | 1.18 ± 0.19 | 0.91 ± 0.18 |
| $B_{max}$ | 1.12 ± 0.06 | 1.20 ± 0.12 | 0.89 ± 0.12 | 0.73 ± 0.06 | 0.61 ± 0.06 | 0.70 ± 0.05 | 0.96 ± 0.14 | 0.77 ± 0.20 |
| [$^3$H]8-OH-DPAT | | | | | | | | |
| $K_D$ | 0.55 ± 0.09 | 0.58 ± 0.06 | 0.49 ± 0.05 | 1.01 ± 0.30 | 1.61 ± 0.33 | 1.95 ± 0.63 | | |
| $B_{max}$ | 0.24 ± 0.02 | 0.24 ± 0.02 | 0.14 ± 0.02 | 0.21 ± 0.03 | 0.56 ± 0.10 | 0.55 ± 0.07 | | |

## 303P RELATIONSHIP BETWEEN THE DOSE OF MDMA AND FREQUENCY OF ADMINISTRATION ON THE SUBSEQUENT NEURODEGENERATION OF RAT BRAIN 5-HT

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3,4-Metylenedioxymethamphetamine (MDMA or 'ecstasy') administration has been shown to induce damage to 5-HT nerve terminals in the brain of experimental animals (see Green *et al.*, 1995). However, doses used have often been far in excess of doses used recreationally by humans, weakening arguments that data are relevant to the clinical situation. It is known that, in rats, either one large single dose (20 mg $kg^{-1}$) or several repeated smaller doses (e.g. 5 mg $kg^{-1}$ x 4 over two days) produces damage (see McKenna & Peroutka, 1990). However, there are few systematic studies. We have now studied the effect of various dose protocols on the production of neurotoxic damage in the brains of male Dark Agouti rats (weight 150-170g at the start of treatment), measuring cerebral 5-HT content 7 days after the final dose of MDMA (given i.p.). Tissue 5-HT concentration was measured by h.p.l.c. with electrochemical detection as described previously (Colado *et al.*, 1997).

A single dose of MDMA of 15or10 mg $kg^{-1}$,but not 4 mg $kg^{-1}$, produced substantial neurotoxic loss of 5-HT in cerebral cortex 7 days later (Table). A dose of 4 mg $kg^{-1}$ given once daily for 4 days resulted in a non-significant decrease, however, when this dose was given twice daily (09.00h and 17.00h) for 4 days a major loss of 5-HT (39%)) was seen (Table). When a dose of MDMA of 4 mg $kg^{-1}$ was given 2 times weekly (Monday and Friday) for 8 weeks, no change in cortical 5-HT content was observed.

MDMA-induced damage probably results from increased free radical formation in the brain (see Colado *et al.*, 1997). Neurodegeneration presumably occurs when endogenous free radical scavenging mechanisms become overwhelmed or exhausted following respectively either a single high dose or repeated lower doses. What cannot be done is extrapolate our results to calculate "safe" doses since many other factors, including the CYP2D6 enzyme status of the user (see Tucker *et al.*, 1994) are probably also important in determining the production of neurotoxic damage.

Colado, M.I. *et al.*, (1997) *Br. J. Pharmacol.* **121**, 889-900.
Green, A.R *et al., (1995) Psychopharmacology* **119**, 247-260.
McKenna, D.J. & Peroutka, S.J. (1990) *J. Neurochem.* ***54***, 14-22
Tucker, G.T. *et al.*, (1994) *Biochem. Pharmacol.* **47**, 1151-1156

Table. Effect of various dose regimes of MDMA on 5-HT content of rat cerebral cortex 7 days later.

| Dose (mg $kg^{-1}$) of MDMA | Frequency of administration | 5-HT concentration (ng $g^{-1}$ wet weight) |
|---|---|---|
| Saline | once | 291± 12 (9) |
| 15 | once | 131± 6 (10)* |
| 10 | once | 205± 21 (8)* |
| 4 | once | 301± 12 (6) |
| Saline | 2 x daily (4d) | 283± 2 (5) |
| 4 | 1 x daily (4d) | 261± 9 (6) |
| 4 | 2 x daily (4d) | 172± 6 (6)*+ |
| Saline | 2 x weekly (8w) | 340± 7 (6) |
| 4 | 2 x weekly (8w) | 326± 13 (6) |

Different from the corresponding saline-treated group: *P< 0.01; Different from MDMA 4 mg $kg^{-1}$ (once daily): + P< 0.01 (Newman-Keuls test).

*MIC thanks CICYT (SAF 1560/95) and Astra Arcus for support.*

## 304P THE ROLE OF TEMPERATURE IN THE NEUROPROTECTIVE EFFECT OF CLOMETHIAZOLE AGAINST MDMA-INDUCED DEGENERATION IN RAT BRAIN

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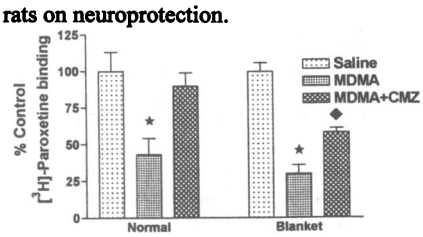
The role of changing temperature as the mechanism by which drugs protect against the neurodegeneration of 5-HT nerve terminals induced by 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') remains a vexed question. We have shown clomethiazole (CMZ) to be an effective neuroprotective agent against MDMA-induced neurodegeneration (Colado *et al.*, 1993; Hewitt & Green, 1994). However CMZ, whilst not producing overt hypothermia, nevertheless abolished the MDMA-induced hyperthermia. Such an effect on the temperature rise by other drugs has been reported by Farfel & Seiden (1995) to result in neuroprotection. We have now therefore re-examined the effect of CMZ on MDMA-induced damage.

Male Dark Agouti rats (150-180g) were injected with CMZ (50 mg $kg^{-1}$i.p.) 5min prior and 55 min post MDMA (15 mg $kg^{-1}$i.p.) administration. Rectal temperature was measured for the next 6h. Seven days later the rats were killed and brain cortical [$^3$H]-paroxetine binding measured as an index of neurodegeneration (Colado *et al.*, 1997). In other studies the MDMA/CMZ treated rats were placed in a cage with a homeothermic blanket to keep their temperature raised to that seen in the MDMA treated rats.

MDMA increased rectal temperature by approx 1.5°C above saline-injected controls during the 6h post-injection. This was abolished by CMZ administration. Seven days later binding data showed that CMZ had afforded a mean 83% protection from the MDMA-induced damage (Figure). When this experiment was repeated using the homeothermic blanket, the rectal temperature of the CMZ/MDMA treated rats remained elevated to that seen after MDMA alone. Binding data showed the CMZ had still produced neuroprotection in the MDMA treated rats, despite the elevated body temperature. However in this situation the mean % protection from damage was now only 40%. These data suggest that CMZ provides neuroprotection in MDMA treated rats in two ways: prevention of MDMA-induced hyperthermia, and a true neuroprotective effect as observed in animal models of acute ischaemic stroke (Green & Cross, 1994).

Colado, M.I. *et al.*, (1993) *Br. J. Pharmacol.* **108**, 583-589.
Colado, M.I. *et al.*, (1997) *Br.J.Pharmacol.* **121**, 889-900
Farfel, G.M. & Seiden, L.S. (1995) *J. Pharmacol. Exp. Ther.* **272**, 860-867.
Green, A.R. & Cross, A.J. (1994) *Progr. Neurobiol.* **44**, 463-484.
Hewitt, K. & Green, A.R. (1994) *Neuropharmacology* **33**, 1589-1595.

Figure. Effect of maintaining hyperthermia in CMZ/MDMA treated rats on neuroprotection.




'Blanket' means CMZ/MDMA treated rats kept hyperthermic with homeothermic blanket. *Diff. from saline-injected $p<0.01$; ◆ Diff. from saline and MDMA treated $p<0.01$ (Newman-Keuls test).

*MIC thanks CICYT (SAF 1560/95) and Astra Arcus for support.*

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## 305P SEROTONERGIC 5-HT$_{2A}$ RECEPTORS MEDIATE THE OESTRADIOL-INDUCED SURGE OF LUTEINISING HORMONE-RELEASING HORMONE IN THE RAT

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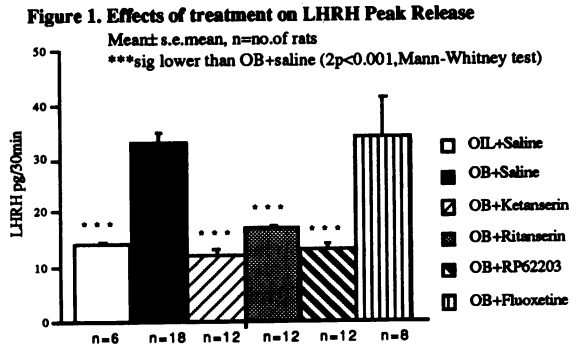
Serotonin (5-HT) mechanisms in brain play a key role in mediating the oestradiol-induced surge of luteinising hormone (LH) from the rat pituitary. Our previous *in situ* hybridisation and *in vivo* pharmacological studies suggested that the action of 5-HT involves 5-HT$_{2A}$ receptors in brain (Sumner & Fink, 1993 & 1995; Dow et al., 1994). The aim of the present study was to investigate this possibility directly by determining whether selective 5-HT$_{2A}$ receptor antagonists block the oestradiol-induced surge of luteinising hormone releasing hormone (LHRH). Adult female Wistar rats, wt 200-250g, which had shown at least two consecutive 4-day oestrous cycles, were ovariectomised under halothane anaesthesia on the morning of dioestrus and injected s.c. with 20µg oestradiol benzoate (OB) or 0.2ml arachis oil (OIL). At 1200h on the next day, presumptive pro-oestrus, the OB-treated rats were injected ip with ketanserin (Janssen 5mg$kg^{-1}$), ritanserin (Janssen 2mg$kg^{-1}$) or RP62203 (Rhone-Poulenc, 2mg$kg^{-1}$), or the selective serotonin reuptake inhibitor fluoxetine (Sigma 10mg$kg^{-1}$), or appropriate vehicle alone. The amount of LHRH released (pg/30min) was determined by radioimmunoassay of sequential hypophysial portal blood samples collected under alphaxalone anaesthesia between 1500-1900h (Sarkar & Fink 1979). Data for the peak release of LHRH are shown in Figure 1. Oestradiol induced a 2-3 fold increase in LHRH release into portal blood compared with oil controls. The OB-induced LHRH surge was abolished by the 5-HT$_{2A}$ receptor antagonists ketanserin, ritanserin and RP 62203, but not fluoxetine. The administration of vehicle alone had no significant effect on LHRH release. These results provide the first direct evidence that 5-HT$_{2A}$ receptors mediate the oestradiol-induced surge release of LHRH. As well as their fundamental importance, our findings may be of relevance to the clinical use of 5-HT$_{2A}$ receptor antagonists.

**Figure 1. Effects of treatment on LHRH Peak Release**




Dow, R.C., Williams, B.C., Bennie, J., *et al* (1994). *Psychoneuroendocrinology* **19**, 395-399.
Sarkar, K. & Fink, G. (1979). *J.Endo* **80**, 303-313.
Sumner, B.E.H. & Fink, G. (1993). *Mol Cell Neurosci* **4**, 83-92.
Sumner, B.E.H. & Fink, G. (1995) *J Steroid Biochem Mol Biol* **54**, 15-20

## 306P THE INHIBITORY EFFECT OF SOME MONOAMINES ON UPTAKE OF 5-HT BY EQUINE PLATELETS

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Equine laminitis is a common vasospastic disease involving ischaemia of the hoof laminae. 5-HT is a very potent vasoconstrictor of equine digital blood vessels and has been implicated in its pathogenesis. Gastrointestinal disturbances, such as carbohydrate overload, are the most common initiating factors in acute laminitis, leading to abnormal fermentation in the caecum. Oxidative decarboxylation of amino acids by bacteria involved in fermentation generates amines which may gain access to the circulation. The aim of this study was to determine the effect of such amines on 5-HT uptake by platelets.

Blood samples were obtained at a local abattoir from mixed native breed horses of both sexes. Equine platelets were separated by centrifugation and washed in phosphate buffered saline containing 0.1 g/ 100 ml glucose and resuspended in Hank's balanced salt solution. Platelet numbers were established by Coulter counter (approx. 3 $x10^8$/ml). After preincubating for 30 min at 37°C in the absence (control) and presence of the monoamines tryptamine (TRP; 10 µM), β-phenylethylamine (β-PEA; 10 µM), tyramine (TYR; 100 µM) and isoamylamine (IAA; 100 µM), [$^3$H] 5-HT (10 µCi/ µmole) was added to give concentrations in the range 10 nM to 20 µM. The samples were then incubated at 37°C for 7 min (giving optimum rate of uptake) and after washing, the platelets were osmolysed and freeze-thawed and the lysate counted by liquid scintillation. Uptake was expressed in pmoles of 5-HT /min /$10^7$ platelets. The $K_m$ and $V_{max}$ values for 5-HT uptake were calculated by the method of Wilkinson (1961) and are shown in Table 1 (mean ±s.e.mean; all n=3). All of the monoamines tested caused a significant decrease in the $V_{max}$ at the concentrations shown and tyramine alone caused a significant increase in the $K_m$ value.

These data demonstrate that amines which may be formed in the equine caecum interfere with uptake of 5-HT into platelets in a non-competitive manner. The mechanism of this effect warrants further investigation. The free plasma concentration of 5-HT in the horse has been estimated to be 1.12 ±0.5 x $10^{-8}$ M which is above the threshold for digital vasoconstriction (Bailey & Elliott, 1997). Circulating monoamines derived from the gut could further increase plasma 5-HT concentrations by inhibiting 5-HT uptake by platelets. These findings may have important implications for the pathogenesis of laminitis.

Table 1: Kinetics of 5-HT uptake by equine platelets in the presence of other monoamines

| | $K_m$ (µM) | $V_{max}$ (pmoles /min /$10^7$ platelets) | % decrease in $v_{max}$ |
|---|---|---|---|
| control | 3.58 ±0.7 | 11.72 ±1.0 | |
| β-PEA (10 µM) | 3.93 ±1.3 | 6.72 ±0.1* | 41.33 ±6.3 |
| control | 2.18 ±0.1 | 11.86 ±1.4 | |
| TRP (10 µM) | 2.29 ±0.3 | 7.46 ±0.2* | 34.33 ±6.7 |
| control | 3.69 ±0.8 | 7.40 ±0.5 | |
| TYR (100 µM) | 6.16 ±0.6* | 4.76 ±0.6* | 37.33 ±12.6 |
| control | 4.95 ±0.4 | 7.76 ±0.3 | |
| IAA (100 µM) | 6.33 ±0.3 | 6.40 ±0.1* | 17.66 ±3.81 |

*significant difference vs. control ($p<0.05$) paired Student's *t*-test

We thank the Home of Rest for Horses for their financial support.

Bailey S.R., Elliott J, (1997) *Equine Vet. J.* (in press).
Wilkinson (1961) *Biochem J.* 80, 324-332

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## 307P THE 5-$HT_{1A}$ ANTAGONIST WAY100635 DID NOT PREVENT THE ABILITY OF GR127935 TO INHIBIT THE HYPOTHERMIA INDUCED BY THE 5-$HT_{1B/1D}$ AGONIST GR46611 IN THE GUINEA-PIG

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There is now considerable interest in developing compounds which antagonise both 5-$HT_{1B}$ receptors and 5-$HT_{1A}$ receptors. Such drugs would be expected to increase 5-HT neurotransmission by blocking inhibitory 5-HT autoreceptors on cell bodies and nerve terminals. 5-$HT_{1B/1D}$ antagonists (e.g. GR127935; GR1) reverse the hypothermia induced by 5-$HT_{1B/1D}$ agonists (e.g. GR46611; GR4) in guinea pigs (Skingle *et al.*, 1994). Conversely, the 5-$HT_{1A}$ antagonist, WAY100635 (WAY) appears to potentiate the hypothermia induced by 5-$HT_{1B/1D}$ agonists (Hatcher *et al.*, 1995) suggesting that 5-$HT_{1A}$ receptor antagonism may mask the effects of 5-$HT_{1B/1D}$ antagonism in the guinea pig assay. This study explores the ability of GR127935 to inhibit GR46611-induced hypothermia in the presence of WAY100635.

Rectal temperatures of male Dunkin-Hartley guinea pigs (n=6; 200-400g; Charles River) were measured 60 min before, immediately before, and 30, 60, 90, 120 and 240 min after, s.c. admininistration of GR46611 and WAY100635. GR127935 was given p.o. 60 min before the two other drugs.

The hypothermia induced by GR46611 (10 mg/kg) was antagonised by GR127935 (3 mg/kg; Table 1) which had no effect on body temperature. This response was unaltered by WAY100635 (0.1 and 0.3 mg/kg). A high dose of WAY100635 (1 mg/kg) attenuated the effects of GR127935, but only to a minor extent and only in the first 60 min. WAY100635 (0.1 and 0.3 mg/kg) had no effect on body temperature. WAY100635 (1 mg/kg) produced weak hypothermia (e.g. at 120 min: vehicle 38.3±0.1 vs. WAY 38.0±0.1*). WAY100635 potentiated the effects of GR46611 3 mg/kg (at 120 min: vehicle 38.4±0.2; GR4 36.6±0.2*; GR4+WAY 0.1 mg/kg 35.8±0.2*†; GR4+WAY 0.3 mg/kg 36.1±0.2*†; GR4+WAY 1 mg/kg 35.4±0.2*†) confirming the report by Hatcher *et al.* (1995).

The results of this study suggest that the ability of GR127935 to antagonise the hypothermic effects of GR46611 in guinea pigs is not substantially altered by concurrent 5-$HT_{1A}$ receptor antagonism and that the guinea pig hypothermia model can be used to detect 5-$HT_{1B/1D}$ antagonist activity of drugs which also selectively antagonise 5-$HT_{1A}$ receptors.

Hatcher J.P. *et al.* (1995) J. Psychopharmacol. 9, 234-241.
Skingle M. *et al.* (1994) J. Psychopharmacol. 8, 14-21.

Table 1. Effect of WAY100635 on the ability of GR127935 (3 mg/kg) to inhibit GR46611 (10 mg/kg)-induced hypothermia in guinea pigs

| | 30 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|
| Vehicle | 38.4±0.2 | 38.5±0.3 | 38.6±0.2 | 38.7±0.2 | 38.6±0.1 |
| GR46611 | 37.7±0.1* | 36.6±0.1* | 36.5±0.1* | 36.6±0.1* | 36.7±0.1* |
| GR46611+GR127935 | 38.8±0.1† | 38.7±0.1† | 38.8±0.1† | 38.8±0.1† | 38.8±0.1† |
| GR4+GR1+WAY 0.1 mg/kg | 38.9±0.1† | 38.9±0.1† | 39.0±0.1† | 38.9±0.1† | 39.0±0.1† |
| GR4+GR1+WAY 0.3 mg/kg | 38.5±0.2† | 38.6±0.3 † | 38.7±0.2† | 38.7±0.1† | 38.6±0.1† |
| GR4+GR1+WAY 1 mg/kg | 38.1±0.1# | 38.1±0.1†# | 38.5±0.1† | 38.7±0.1† | 38.9±0.1† |

Results are mean rectal temperatures (°C) ± se.mean. *P<0.05 vs vehicle; †P<0.05 vs GR46611; #P<0.05 vs GR46611+GR127935; two-way analysis of covariance followed by Dunnett's test (* and †) or Williams' test (#). Body temperatures between the treatment groups were not significantly different at the -60 and 0 min readings. Ambient temperature of the test room was 21±1°C.

## 308P EFFECTS OF 5-$HT_{1A}$ AND 5-$HT_{1B}$ RECEPTOR AGONISTS UPON LOCAL CEREBRAL BLOOD FLOW AND GLUCOSE UTILISATION IN THE RAT

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Ascending mesencephalic serotonergic (5-HT) projections terminate diffusely in a wide variety of functionally diverse forebrain areas. These projections also supply innervation to cerebral blood vessels (Edvinsson *et al.*, 1983). The development of selective agonists which activate subpopulations of 5-$HT_1$ receptors, provides the opportunity to investigate the role of serotonergic projection neurones in the control of the cerebral circulation.

Studies were performed on conscious male Sprague-Dawley rats (300-355g). Rats were prepared for measurement of local cerebral blood flow (LCBF) or glucose use (LCGU) in neocortex using [$^{14}$C]-iodoantipyrine and [$^{14}$C]-2-deoxyglucose autoradiography, respectively, as described previously (McBean *et al.*, 1990). Briefly, both femoral arteries and veins were cannulated under general anaesthesia. The rats were restrained and allowed to recover from the anaesthesia for two hours before further manipulation. In the first part of the study rats were injected intravenously (i.v.) with either the 5-$HT_{1A}$ agonist 8-OH-DPAT (1mg.kg$^{-1}$) or the 5-$HT_{1B}$ agonist RU24969 (3mg.kg$^{-1}$). Control rats received 1ml.kg$^{-1}$saline (n=5 in each of the groups). Measurements of LCBF and LCGU were intiated 10 min and 1 min after drug injection, respectively.

In the second part of the study, the two agonists were injected directly into the sensory-motor cortex (i.c.) (0.5µl of 10µM) via an indwelling cannula inserted two weeks previously under general anaesthesia, as described previously (McCulloch & Kelly, 1983). Artificial CSF was injected as a control (n=5 in each of the groups).

Measurements of LCBF and LCGU were initiated 10 min and 1 min after drug injection, respectively. In both studies data (mean ± s.e.mean) were compared to a single control group in each case using a Bonferroni *t*-test with levels of significance set at $P<0.05$.

Following i.v. 8-OH-DPAT both increases (+34%) and decreases (-25%) in LCGU were found in neocortex (control, 103 ± 2 µmol.100g$^{-1}$.min$^{-1}$). In areas where LCGU was increased, LCBF was also increased (+123%), but even where LCGU was decreased LCBF was again increased (+34%) (control, 140 ± 6 ml.100g$^{-1}$.min$^{-1}$). Thus following 8-OH-DPAT LCBF throughout cortex was in excess of that which would be predicted on the basis of metabolic demand (LCBF/LCGU ratio 2.26 to 2.43, c.f. control ratio of 1.36 to 1.37). In contrast, i.c. 8-OH-DPAT had no significant effect upon either LCGU or LCBF. RU24969, given i.v., produced either increases (+38%) or no change in cortical LCGU (control, 103 ± 2 µmol.100g$^{-1}$.min$^{-1}$)., with a similar pattern (+72%) in LCBF (control, 140 ± 6 ml.100g$^{-1}$. min$^{-1}$). The ratio of LCBF/LCGU (1.70) was however much closer to control (1.36). Following i.c. RU24969 significant increases in LCBF (+31%) were again observed (control, 116 ± 6 ml.100g$^{-1}$.min$^{-1}$), without the parallel changes in LCGU observed following i.v. treatment, resulting in a change in the LCBF/LCGU ratio from 1.40 in controls to 2.00.

These results would be consistent with 5-$HT_{1A}$ receptors on raphé cell bodies and 5-$HT_{1B}$ receptors on terminals, both inhibiting release of 5-HT into the perivascular space thereby removing constrictor tone from the vessels.

Edvinsson, L. *et al.* (1983) *Nature*, **306**, 55-57.
McBean, D.E. *et al.* (1990) *Brain Res.*, **537**, 307-310.
McCulloch, J. & Kelly, P.A.T. (1983) *Nature*, **304**, 438-440.

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## 309P EFFECTS OF WAY100635 ON THE INCREASE OF HIPPOCAMPAL EXTRACELLULAR 5-HT AND INHIBITION OF 5-HT SYNTHESIS BY FLUOXETINE

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It is generally accepted that both 5-$HT_{1A}$ and $_{1B}$ receptors regulate the function of central serotoninergic neurones by acting as somatodendritic and terminal inhibitory autoreceptors respectively. Selective serotonin reuptake inhibitors (SSRI's) increase brain extracellular 5-HT concentration and inhibit 5-HT neuronal firing, synthesis and release presumably as a consequence of the indirect activation of autoreceptors. While it has been shown that 5-$HT_{1A}$ receptor blockade prevents the reduction of raphe unit firing and potentiates the increase of extracellular 5-HT concentration by SSRI's it is not clear if these receptors are involved in the inhibition of 5-HT synthesis by SSRI's.

Male Sprague-Dawley rats (275-300 g, B&K) were anaesthetised using isoflurane and implanted with a 5 mm concentric dialysis probe in the hippocampus (Paxinos & Watson, 1982: AP +5.8 mm, L 5.0 mm, V-7.0mm relative to bregma and dura). After overnight recovery, the probe was perfused with Ringer (150 mM Na, 3.0 mM K, 1.4 mM Ca, 1.0 mM $PO_4$, 0.8 mM Mg, 155 mM Cl, Harvard ), at a flow rate of 1 µl/min. Samples were collected at 30 minute intervals and analysed by HPLC with electrochemical detection. Values given are mean area under curve (A.U.C.) ± s.e.mean calculated for the 180 min following either vehicle (1 ml/kg i.p.) or fluoxetine (10 mg/kg i.p.).

Male Sprague Dawley rats (180-200g, B & K) were implanted with miniosmotic pumps delivering WAY100635, 1 mg/kg/day. After 7 days animals were administered either saline (1 ml/kg i.p. or s.c.), fluoxetine (10 mg/kg, i.p.) or 8-OH-DPAT (0.3 mg/kg s.c.) followed 90 mins later by the decarboxylase inhibitor NSD 1015 (100 mg/kg, i.p.). Thirty min later rats were humanely killed, the hippocampus dissected and analysed for 5-HTP by HPLC with electrochemical detection (Hutson *et. al.* 1991). Data from all experiments were subjected to two way analysis of variance followed where significant, by Tukey's Test. A value of $P<0.05$ was considered significant. Basal 5-HT efflux in rat hippocampus was 3.73 ± 0.35 fmol/20µl (mean ± s.e.mean., n=32). Fluoxetine (10 mg/kg, s.c.) increased hippocampal 5-HT A.U.C. above basal levels and this was further increased by pre-treatment of rats with WAY100635 (0.3 mg/kg, s.c.). In contrast, animals pretreated with WAY100635 (1 mg/kg/day, 7 days), a dose which prevented the inhibition of 5-HT synthesis by 8-OH-DPAT, did not prevent the inhibition of 5-HT synthesis induced by fluoxetine (10 mg/kg, i.p.) (Table 1).

Results indicate that while 5-$HT_{1A}$ receptor blockade enhanced the increase of hippocampal 5-HT efflux by fluoxetine, inhibition of hippocampal 5-HT synthesis, as indicated by the decrease of 5-HTP concentration, by fluoxetine may not be mediated by 5-$HT_{1A}$ receptors.

Hutson, P. H., Bristow, L.J., Thorn, L *et al.*, (1991) Brit. J. Pharmacol. 103, 2037-2044.
Paxinos, G. & Watson, C. (1982) The rat brain in stereotaxic coordinates, Sydney: Academic Press.

Table 1. Effects of WAY 100635 on the increase of Hippocampal 5-HT Efflux and Inhibition of 5-HT Synthesis by Fluoxetine

| | Vehicle + Vehicle | WAY + Vehicle | Vehicle + Fluoxetine | WAY + Fluoxetine | Vehicle + Vehicle | WAY + Vehicle | Vehicle + 8-OH-DPAT | WAY + 8-OH-DPAT |
|---|---|---|---|---|---|---|---|---|
| 5-HT Efflux (A.U.C.) | 6.58 ± 2.48 | 8.63 ± 0.49 | 26.05 ± 3.49* | 42.67 ± 5.13* | | | | |
| 5-HT Synthesis | 122 ± 8 | 102 ± 8 | 70 ± 5* | 67 ± 2* | 92 ± 6 | 115 ± 18 | 65 ± 5 * | 106 ± 11 |

Dialysis values are mean A.U.C. (fmol/20µl/min) ± s.e.mean. n=5/8 per group. 5-HT synthesis values are mean 5-HTP (ng/g) ± s.e.mean n=8/16 per group.
* $p < 0.05$ compared with appropriate controls by ANOVA and Tukey's test.

## 310P THE EFFECTS OF PREVIOUS EXPOSURE TO METHYLENEDIOXY-METHAMPHETAMINE UPON CEREBROVASCULAR DYSFUNCTION INDUCED BY ACUTE TREATMENT WITH THE DRUG

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A link has been suggested between methylenedioxymethamphetamine (MDMA; "Ecstasy") and the occurrence of haemorrhagic stroke in man (Harries & DeSilva, 1992), and we have previously shown that a single exposure to the drug produces focal cerebrovascular dysfunction in rat (Kelly *et al.*, 1994). The purpose of the present study was to examine whether previous exposure to the drug, using a protocol known to deplete central serotonergic terminals (Sharkey *et al.*, 1991), might alter the acute cerebrovascular effects.

Male Sprague-Dawley rats (100g at the outset) were injected s.c. with MDMA (20mg.kg$^{-1}$; n = 12) or saline (0.2ml; n = 20) twice daily over four consecutive days. Four weeks later the efficacy of the MDMA treatment was assessed (n = 4) by radioligand [$^3$H]-paroxetine binding autoradiography (Sharkey et al., 1991). On the day of the final experiment, saline-pretreated rats were injected i.v. with either MDMA (5mg. kg$^{-1}$; n =8) or saline (0.5ml; n = 8). MDMA-pretreated rats were all injected with MDMA (5mg. kg$^{-1}$; n = 8). Equal numbers of rats from each treatment group were used for the measurement of either local cerebral glucose utilization (LCGU, n = 12), using [$^{14}$C]-2-deoxyglucose, or local cerebral blood flow (LCBF, n = 12), using [$^{14}$C]-iodoantipyrine, as described by us previously (Kelly et al., 1994). Measurements were initiated at 10 and 20min after the acute treatment respectively. Data (presented as mean ± s.e.m.) were analysed by ANOVA with *post hoc* Scheffé test. Acceptable levels of significance were set at $P < 0.05$.

Chronic MDMA produced a significant decrease in forebrain [$^3$H]-paroxetine binding (Bmax), from 19.1 ± 1.3fmol.mg$^{-1}$ tissue in controls to 1.5 ± 0.3 in treated rats ($P < 0.05$). Despite this pronounced difference in the density of 5-HT uptake sites, there was very little difference in the overt responses to acute MDMA between saline- and MDMA-pretreated rats. Irrespective of pretreatment, acute MDMA produced a transient, but pronounced increase in mean arterial blood pressure (from 136 ± 3 to 182 ± 6mmHg in saline pretreated rats; $P < 0.05$, vs. 132 ± 3 to 180 ± 2mmHg in MDMA pretreated rats; $P < 0.05$) which returned to normal within 5min (134 ± 4 and 133 ± 3mmHg in the two groups). There were no significant differences in blood gases between the groups.

Despite an increase in motor activity in both groups of rats treated acutely with MDMA, there were no significant differences in LCGU between controls and either of these two groups. However, LCBF in frontal (224 ± 26ml.100g$^{-1}$.min$^{-1}$) and parietal cortex (189 ± 4ml.100g$^{-1}$.min$^{-1}$) was significantly increased (by 87 and 22% respectively) following acute MDMA in saline-pretreated rats, and similar increases in LCBF were also observed in the MDMA-pretreated group (+71 and +19% in frontal and parietal cortex respectively). In the absence of any changes in LCGU, these increases in LCBF represent focal uncoupling of flow from metabolic demand, and suggest that the risk of stroke resulting from MDMA-induced cerebrovascular regulatory dysfunction is likely to be the same with every exposure to the drug, and does not diminish with persistent use.

Harries, D.P. & DeSilva, R. (1992) *Scot. Med. J.* **37**, 150-152.
Kelly, P.A.T. *et al.* (1994) *Brain Res.* **665**, 315-318.
Sharkey, J. *et al.* (1991) *Psychopharmacol.* **105**, 113-118.

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## 311P CHARACTERISATION OF THE 5-HT RECEPTORS MEDIATING THE CONTRACTILE EFFECT OF 5-HT IN THE TERMINAL REGION OF THE RAT SMALL INTESTINE

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In previous studies we have demonstrated that the contractile response to 5-HT in proximal segments of the rat intestine was antagonised by selective 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptor antagonists and atropine (Javid *et al*, 1997) . The aim of the present study was to characterise the 5-HT receptors mediating i the contractile effect of 5-HT in the terminal region of the rat small intestine.

Segments (1-1.5 cm long) taken from the intestine (1 cm proximal to the ilecaecal junction) of adult Hooded Lister rats (210-320 g) of either sex were mounted in 10 ml organ baths containing Krebs' solution (37°C, 95% $O_2$, 5% $CO_2$). The tissues were allowed to equilibrate for 60 min and washed every 20 min. The resting tension was maintained at 0.5 g and recorded isometrically. Non-cumulative concentration-response curves to 5-HT (1nM-30μM) were established with a 1 min contact time and 20 min intervals. The procedure was repeated in the presence of methysergide (methy, 1μM), ritanserin (rit, 0.1μM), atropine (atr, 1μM), ondansetron (ond, 1μM), SB204070 (SB, 1nM), a combination of methy (1μM) plus ond (1μM), SB (1nM) or atr (1μM). Tissues were allowed to equilibrate for 1 h in the presence of antagonist before the application of 5-HT. Tension changes were expressed as the mean±s.e.mean of n=7-10 and analysed using one-tail Dunnett's t-test.

5-HT (1nM-30μM) produced a concentration-dependent contraction. Methysergide and ritanserin significantly ($P<0.05$) shifted the concentration-response curve to 5-HT to the right without affecting the maximum response. Ondansetron significantly ($P<0.05$) reduced the responses to 5-HT at concentrations >30nM and atropine significantly ($P<0.05$) reduced the responses to 5-HT at concentrations >1μM. SB204070, a selective 5-HT$_4$ receptor antagonist (Wardle *et al*, 1994), significantly ($P<0.05$) reduced the response to 5-HT (0.1-3μM) without affecting the maximum response. A combination of methysergide plus atropine or ondansetron or SB204070 significantly ($P<0.05$) shifted the concentration-response curve to 5-HT to the right. The maximum response was also significantly ($P<0.05$) reduced except when methysergide was used in combination with SB204070 (Figs. 1.a-c).

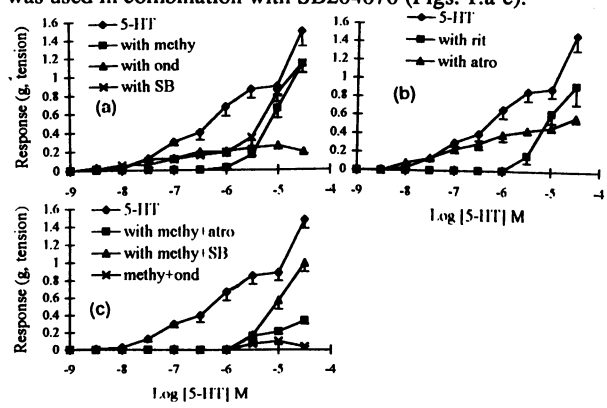


Figure 1. The effect of 5-HT receptor antagonists to inhibit the contractile response to 5-HT in the terminal part of the rat small intestine.

The data suggest that in the terminal part of the rat small intestine, as previously found in the proximal region, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors are involved in mediating the 5-HT response. However, in the terminal region and unlike the response obtained in the proximal segment, the involvement of 5-HT$_2$ and 5-HT$_4$ receptors in mediating the contraction induced by higher concentrations of 5-HT is unlikely.

Javid, F. A. & Naylor, R. J. (1997) *Br. J. Pharmacol.* in Press.
Wardle, K. A., Ellis, E. S., Baxter, G. S. *et al.* (1994) *Br. J. Pharmacol.* 112, 789-794.

## 312P ALTERATIONS IN CEREBRAL FUNCTIONAL ACTIVITY FOLLOWING ACUTE CITALOPRAM TREATMENT IN THE RAT

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We have previously shown that serotonergic (5-HT) lesions do little to alter cerebral function, as it is reflected in altered metabolic demand (McBean *et al.*, 1990). However, in those chronic studies, the possibility of adaptive mechanisms compensating for the loss of 5-HT, cannot be discounted. The purpose of the present study was to further investigate the role of 5-HT in cerebral function using acute treatment with the potent and selective 5-HT reuptake blocker citalopram (Hyttel, 1982).

The study was carried out using conscious male Sprague-Dawley rats (304-385g). The rats were prepared for the measurement of local cerebral glucose use (LCMRglu) using [$^{14}$C]-2-deoxyglucose (2-DG) autoradiography, as described in detail previously (McBean *et al.*, 1990). Briefly, both femoral arteries and veins were cannulated under general anaesthesia. The rats were lightly restrained and allowed to recover from the anaesthesia for two hours before further manipulation. Ten minutes prior to the start of the 2-DG administration the rats received an intravenous injection of either saline (0.75ml; n=6) or citalopram (10mg.kg$^{-1}$; n=5). Data were analysed using grouped t-test with acceptable levels of significance set at $P<0.05$.

Acute citlopram treatment resulted in significant reductions in LCMRglu in 13 of the 23 anatomically discrete and functionally diverse brain regions analysed. The most profound reductions were found in limbic areas (lateral amygdala, -40%; hippocampal dentate gyrus, -30%), but reductions were also found in motor areas (striatum, -18%; sensory motor cortex, -16%) and primary sensory areas (visual cortex, -16%) (Table 1).

Table 1 Effects of 10 mg.kg$^{-1}$ Citalopram on LCMRglu

| Structure | Saline | Citalopram |
|---|---|---|
| Visual cortex | 100 ± 5 | 84 ± 5 * |
| Sensory motor cortex | 105 ± 5 | 88 ± 4 * |
| Striatum | 93 ± 4 | 76 ± 2 * |
| Dentate gyrus | 67 ± 5 | 47 ± 4 * |
| Lateral amygdala | 57 ± 4 | 34 ± 2 * |

Data for LCMRglu (μmol.100g$^{-1}$.min$^{-1}$) presented as mean ± s.e. mean. *, $P<0.05$.

Increased levels of endogenous 5-HT in the brain, induced by the inhibition of re-uptake mechanisms (Hyttel, 1982), results in widespread depression of cerebral function. This is in keeping with the ubiquitous distribution of 5-HT projections from the midbrain (Steinbusch, 1981), although some brain areas, most notably in the limbic system, appear to be more susceptible to the acute actions of citalopram. Moreover, these results indicate that, in the conscious rat, 5-HT neurones exert a tonic influence in the control of cerebral function.

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McBean, D.E. *et al.* (1990) *Neuroscience* **38**, 271-275.
Hyttel J. (1982) *Prog. Neuropsychopharmacol. Biol. Psychiatr.* **6**, 277-295.
Steinbusch, H.W.M. (1981) *Neuroscience* **6**, 557-618.

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## 313P RECEPTOR SUBTYPES FOR 5-HT IN BOVINE PULMONARY SUPERNUMERARY ARTERIES

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Platelet-derived 5-hydroxytryptamine (5-HT) has been implicated in some forms of pulmonary hypertension (PH) (Herve, 1990). The pulmonary circulation consists of two populations of arteries; conventional arteries (CA) which divide with and accompany the airway and supernumerary arteries (SNA) which leave the conventional artery at 90° and are unaccompanied by an airway. Because the SNA are small muscular arteries accounting for 40% of the total cross sectional area of all CA branches they are likely to play a key role in regulating pulmonary vascular resistance. SNA also have a muscular sphincter (Hislop & Reid, 1973) or baffle valve (Bunton et al., 1996) at their origin. The present study investigated the receptor types mediating 5-HT-induced constriction in SNA.

Bovine lungs were obtained fresh from the abattoir. Segments of supernumerary arteries (diameter 0.5 - 1 mm) were dissected from the lung and freed of surrounding connective tissue. The vessels were then weighed and suspended between stainless steel hooks in Krebs buffer (37°C) under a tension of 1 g and gassed with a mixture of $O_2$ : $CO_2$ (95%/5% v/v). The tissues were allowed to equilibrate for 1 hour before carrying out concentration response curves (CRCs) to 5-HT or the 5-HT$_1$- and 5-HT$_2$-selective agonists. For antagonist studies two CRCs to 5-HT were conducted, the second CRC in the presence of the antagonist. Results are means ± s.e. mean. The significance of differences was determined using Student's t-test.

5-HT (1nM-1mM), the selective 5-HT$_2$ agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (0.1nM-1μM) and the 5-HT$_{1D}$ agonist sumatriptan (10nM-100μM) produced concentration-dependent contractile responses in SNA. pD$_2$ values were 6.3 ± 0.1 (n = 15, 5-HT), 8.1± 0.1 (n = 11, DOI), 6.6 ± 0.3 ( n = 9, sumatriptan). Maximum responses to DOI and sumatriptan were 52.5 ± 10%, and 45 ± 10% respectively, of the maximum response to 5-HT. In the presence of sumatriptan (10nM) the maximum response induced by DOI was 95% of the maximum response to 5-HT. The 5-HT$_2$ receptor antagonist ritanserin (0.1nM-100nM) produced a concentration-dependent reduction in the magnitude of the maximum response to 5-HT. The maximum inhibition was observed at 10nM ritanserin which reduced the maximum response by 60%. The 5-HT$_{1D}$ receptor antagonist GR127935T (Skingle et al., 1993) produced a rightward shift of the CRC to 5-HT with no change in the maximum response (estimated pA$_2$ = 7.63, n = 6).

The results suggest that both 5-HT$_1$ and 5-HT$_2$ receptor types are involved in mediating contraction to 5-HT in SNA. Moreover the study also suggests that there exists a positive interaction between 5-HT$_1$ and 5-HT$_2$ receptor types in SNA. SNA may be important in regulating PVR, consequently a clear understanding of the receptors types for 5-HT in these vessels may be important in the management of some forms of PH.

Bunton, D., Fisher, A. M., Shaw, A., *et al.*, (1996) *Br. J. Pharmacol.* 119, 323P.
Herve, P., Drouet, L., Dosquet, C., *et al.*, (1990) *Am. J. Med.* 89, 117-120.
Hislop, A. & Reid, L. (1973) *Thorax* 28, 129 - 135.
Skingle, M., Scopes, D.I.C., Feniuk, W., *et al.*, (1993) *Br. J. Pharmacol.* 110, 9P.

## 314P THE ATYPICAL ANTIPSYCHOTIC, ZOTEPINE, ELEVATES EXTRACELLULAR NORADRENALINE IN THE FRONTAL CORTEX OF FREELY-MOVING RATS

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Zotepine, a dibenzothiepine, is a proven tricyclic antipsychotic drug which has an atypical profile (Needham *et al.*, 1996). Its clinical profile indicates efficacy against positive and negative symptoms of schizophrenia and a low propensity to induce extrapyramidal side effects. In addition to zotepine's affinity for dopamine $D_2$- and $D_1$-like receptors, it also has affinity for the noradrenaline (NA) transporter site and has been shown to inhibit [$^3$H]NA uptake by rat frontal cortex synaptosomes *in vitro* (Needham *et al.*, 1997). In the present study, we have used *in vivo* microdialysis to determine the effects of zotepine on extracellular NA in the frontal cortex of freely-moving rats.

Male, CD rats (250-350 g; Charles River), were anaesthetised with isoflurane (6% to induce, 2% to maintain) in an $O_2/N_2O$ mixture and a concentric microdialysis probe (2 mm tip, Hospal AN 69 membrane) was stereotaxically implanted into the frontal cortex (co-ordinates: A +3.2 mm, L 2.5 mm relative to bregma; V -4.0 mm from the dural surface; Paxinos and Watson, 1986). Following surgery, rats were returned to a home cage and allowed to recover for at least 20 h with food and water available *ad libitum*. Probes were continuously perfused with an artificial cerebrospinal fluid (aCSF) at a rate of 1.0 µl/min and samples collected every 20 min into 5.0 µl 0.1 M perchloric acid. Three 'basal' samples were taken prior to a pharmacological challenge of either calcium-free aCSF, nisoxetine or zotepine. Dialysate NA was determined by reverse-phase HPLC with electrochemical detection. Values are mean ± s.e.mean. Statistical comparison was made between treatment groups and controls by ANOVA with post-hoc Dunnett's *t*-test.

Basal NA levels were 30.9 ± 1.7 fmol/20 µl ($n$ = 18). Local perfusion with calcium-free aCSF for 60 min resulted in an immediate and significant ($p<0.001$) reduction in basal NA levels. The maximal reduction observed was 70.5 ± 4.4% compared with controls ($n$ = 6). Levels returned to pre-intervention values 40 min after perfusion with aCSF of normal composition. Perfusion with the NA uptake inhibitor, nisoxetine (1, 10 and 100 µM for 60 min each), significantly ($p<0.001$) increased extracellular NA in a concentration-related manner ($n$ = 6). A maximal effect was observed 40 min after the onset of perfusion with 100 µM (587.8 ± 92.8%) and levels returned to basal 60 min after returning to the original perfusing medium. Systemic treatment with zotepine (0.5 mg/kg i.p.), significantly ($p<0.001$) increased extracellular NA compared to saline-treated controls. These increases in NA levels were biphasic with an initial increase of 93.8 ± 8.5% seen 60 min after injection and a second increase of 141.6 ± 33.9% seen 240 min after dosing.

The data demonstrate that up to 70% of basal extracellular NA measured by microdialysis in the frontal cortex is calcium-dependent and therefore likely to be released by depolarisation-induced exocytosis. Local blockade of the NA transporter elevates NA in a concentration-dependent manner. Furthermore, systemic treatment with zotepine also elevates extracellular NA which is in agreement with *in vitro* data (Needham *et al.*, 1997). The biphasic response is most likely due to the contribution of norzotepine, the desmethyl metabolite of zotepine, which also has a predicted atypical antipsychotic profile (Heal & Needham, 1996) and as the current data suggest, affinity for the NA transporter which may contribute to zotepine's overall clinical profile.

Heal, D.J. & Needham, P.L. (1996) *Eur. Neuropsychopharmacol.* **6** (Suppl. 3), P-9-9.
Needham, P.L., Atkinson, J., Skill, M.J. *et al.* (1996) *Psychopharmacol. Bull.* **32**, 123-128.
Needham, P.L., Skill, M.J., Cheetham, S.C. *et al.* (1997) *Biol. Psychiat.* (In press).
Paxinos, G. & Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates.* London: Academic Press.

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## 315P DIFFERENTIAL EFFECTS OF ROPINIROLE, BROMOCRIPTINE AND L-DOPA ON STRIATAL DOPAMINE D-2 RECEPTOR BINDING IN MPTP-TREATED COMMON MARMOSETS

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Repeated administration of L-DOPA induces dyskinesias in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride) treated primates (Bedard et al., 1986). In contrast, the dopamine (DA) D-2 agonist bromocriptine and the D-2/D-3 agonist ropinirole induce only mild or intermittent dyskinesias (Pearce et al., 1996). We now report on the effect of these drug treatments on DA uptake sites and DA D-1 and D-2 receptor density in brain slices measured using quantitative autoradiography.

Adult common marmosets (n=16, 280-360 g, Callithrix jacchus) were treated with MPTP 2 mg/kg (sc) once daily for 5 days. Animals were divided into 4 groups and received 10% sucrose solution, L-DOPA plus carbidopa (12.5 mg/kg plus 12.5 mg/kg, po) bromocriptine (0.5-1.0 mg/kg, po), or ropinirole (0.3-0.5 mg/kg, po) once daily for 4 weeks. A further four animals were used as normal controls. At the end of the study, brains were removed under high dose anaesthesia of pentobarbitone sodium and flash frozen. For assessment of D-1 and D-2 receptor density and DA uptake sites, coronal sections (20 µm) were incubated with [$^3$H]-SCH23390 (0.5 nM), [$^3$H]-spiperone (0.5 nM) and [$^3$H]-mazindol (4 nM) respectively. Quantitative evaluation of autoradiograms was undertaken by computerised densitometry and results (mean ± s.e.m.) were analysed by one way ANOVA followed by post hoc Dunnett's test.

There was a marked decrease in [$^3$H]-mazindol binding in MPTP-treated animals compared to controls (98-99%). None of the drug treatments had any significant effect on [$^3$H]-mazindol binding compared to the MPTP group. MPTP treatment had no effect on striatal D-1 receptor density and this was also unaffected by the drug treatments. However MPTP treatment increased D-2 receptor density but this was not reversed by L-DOPA administration. In contrast, both bromocriptine and ropinirole normalised D-2 receptor levels (Table 1).

The present study shows that chronic L-DOPA treatment had no effect on D-1 receptor density (direct pathway) but failed to attenuate the elevated D-2 receptor density (indirect pathway) caused by MPTP. By contrast, bromocriptine and ropinirole reversed the elevated D-2 receptor density. This is in agreement with our previous report that bromocriptine and ropinirole, but not L-DOPA, attenuated the elevated striatal preproenkephalin mRNA (indirect pathway) (Tel et al., 1997).

Bedard, P.J., DiPaolo, T., Falardeau, P. and Boucher, R. (1986) *Brain Res.* 379:294-299.
Pearce, R.K.B., Banerji, T., Jenner, P. and Marsden, C.D. (1996) *Br. J. Pharmacol.* 118 *(suppl.)* :37p.
Tel, B.C., Zeng, B.-Y., Pearce, R.K.B. et al. (1997) *Br. J. Pharmacol,* in press.

Table 1: Density of dopamine D-1 and D-2 receptors in the striatum of MPTP-treated marmosets (fmol/mg).

| | | Normal | MPTP | L-DOPA | Bromocriptine | Ropinirole |
|---|---|---|---|---|---|---|
| D-1 | Caudate | 1.6±0.1 | 1.9±0.6 | 2.1±0.4 | 1.8±0.2 | 1.8±0.3 |
| | Putamen | 1.4±0.1 | 1.8±0.4 | 1.9±0.3 | 1.6±0.3 | 1.5±0.2 |
| D-2 | Caudate | 10.0±1.5 | 11.8±1.9* | 11.9±1.9* | 8.1±0.4 | 9.1±1.5 |
| | Putamen | 9.6±2.2 | 12.9±1.2* | 12.7±1.7* | 10.2±1.5 | 10.7±1.5 |

*P<0.05 vs normal marmoset.

## 316P THE TIMECOURSE OF D2 AUTORECEPTOR ACTION IN THE NUCLEUS ACCUMBENS AND NEOSTRIATUM FOLLOWING SINGLE PULSE ELECTRICAL STIMULATION *IN VITRO*

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Dopamine (DA) release in the nucleus accumbens (NAc) and neostriatum (CPu) is under the local control of D2 and also D3 autoreceptors (Patel *et al.*, 1995). In this study we investigated the effect of D2 autoreceptors, activated by DA evoked from a single electrical pulse (pulse 1). In order to determine the timecourse of D2 inhibition of subsequent DA efflux, we applied a second electrical pulse (pulse 2) at a range of time intervals from 50ms to 50s. The amplitude of DA efflux following pulse 2 was assessed in the absence and presence of the D2 antagonist, metoclopramide. Some temporal aspects of D2 autoreceptors have previously been addressed (Limberger *et al.*, 1991). In this study we used amperometry to measure DA in real time.

350μm thick slices of NAc or dorsolateral CPu were prepared from male Wistar rats (150-200g) and kept in a holding chamber of artificial cerebral spinal fluid (aCSF) at room temperature for at least 1h. Slices were placed in the recording chamber, superfused with aCSF (in the presence or absence of 1.0μM metoclopramide) at 32°C for 1h before the first stimulation and throughout the experiment. DA efflux was measured at carbon fibre microelectrodes (7μm diameter) using amperometry (300mV vs Ag/AgCl). Efflux was evoked by electrical pulses (0.1ms, 10mA) across bipolar tungsten stimulating electrodes (300-400μm tip separation). The tissue was stimulated every 5mins with either a single pulse or two pulses separated by 50ms, 100ms, 200ms, 500ms, 1s, 2s, 5s, 10s, 20s or 50s. For short inter-pulse intervals, digital subtraction of single pulse DA efflux unmasked the size of the concealed response. Electrodes were calibrated post-experimentally with 1.0μM DA using a flow injection cell. In each region for each pulse interval, the normalised amplitude of pulse 2 (% of pulse 1) was compared between metoclopramide and control groups using the unpaired t-test.

Change in extracellular [DA] following pulse 1 (or single pulse) was 1.37 ± 0.11μM (mean ± s.e.mean) or 1.18 ± 0.31μM in NAc (control or metoclopramide respectively; n=4) and 0.75 ± 0.19μM or 1.01 ± 0.22μM in CPu (control or metoclopramide respectively; n=5). Metoclopramide had no significant effect on DA efflux for pulse 1 in either region ($p>0.05$, unpaired t-test). The effect of metoclopramide on pulse 2 is summarised below:

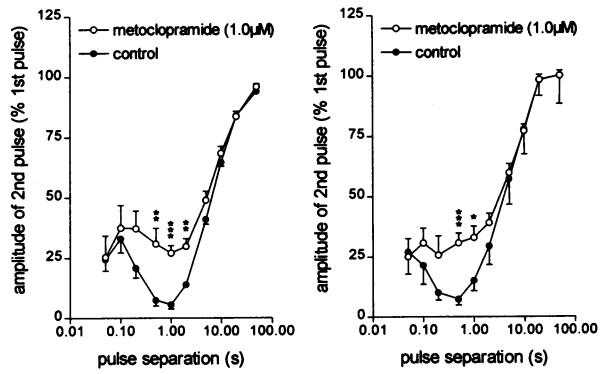


Figure 1: Amplitude of dopamine efflux (mean ± s.e.mean; n=5) for the second of 2 electrical pulses vs pulse separation for nucleus accumbens (left) and dorsolateral neostriatum (right), carried out in the presence (open circles) or absence (closed circles) of 1.0μM metoclopramide. *p<0.05, **p<0.01, ***p< 0.001 vs control.

In both NAc and CPu, autoreceptor activation was not significantly detectable when the second pulse occurred at 200ms or less after the first. Significant autoreceptor activation occurred at intervals of 500ms to 1s (CPu) and 2s (NAc). In both nuclei, autoreceptor inhibition of DA release was again undetectable 5s after the first pulse.

We conclude that, following a single electrical pulse *in vitro*, DA autoinhibition takes up to 500ms to become detectable and persists for less than 5s. The onset time may suggest either slow transduction of the autoreceptor activation event or a delay engendered by diffusion of DA from the release sites to the autoreceptors.

Limberger, N., Trout, S.J., Kruk, Z.L. *et al.* (1991) *Naunyn Schmiedebergs Arch. Pharmacol.* 344, 623-629.
Patel, J., Trout, S.J., Palij, P. *et al.* (1995) *Br. J. Pharmacol.* 115, 421-426.

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## 317P 7-OH-DPAT ATTENUATES THE HYPERACTIVITY RESPONSE TO INTRAACCUMBENS AMPHETAMINE IN THE RAT

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Previous research in the rat has shown that intraaccumbens (IACB) administration of d-amphetamine (AMPH) induces a locomotor hyperactivity response which may be mediated by elevated mesolimbic dopamine function (Costall & Naylor, 1975). The dopamine $D_3$ receptor has been located in the nucleus accumbens of the rat brain and has been proposed to have an inhibitory effect on locomotor behaviour (Daly & Waddington, 1993). Recently, the putative $D_3$ receptor agonist 7-OH-DPAT [7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin] has been shown to attenuate morphine-induced hyperlocomotion in mice (Suzuki *et al.*, 1995). Further experimental evidence is necessary to confirm the role of the $D_3$ receptor on behavioural parameters of mesolimbic dopaminergic function. The present studies investigated the effects of 7-OH-DPAT on the locomotor hyperactivity response induced by IACB AMPH in the rat.

Female Sprague-Dawley rats (250±10g) were subjected to standard stereotaxic surgery under sodium pentobarbitone (60 mg/kg i.p.) anaesthesia for implantation of chronically indwelling guide cannulae for subsequent bilateral IACB injection. Following a 14 day post-operative period, rats received a 15 min pretreatment (1 ml/kg s.c) with (±) 7-OH-DPAT (0.1-3.0 mg/kg) or vehicle (0.9 % saline) followed by bilateral IACB injection (1 μl/hemisphere/min) of AMPH (5-20 μg) or vehicle (artificial CSF). Locomotor activity (LMA) was immediately assessed in individual photocell cages over an 80 min test period. Data were analysed by a one-way ANOVA with post-hoc Dunnett's t-test and shown as mean±sem total LMA counts/80 min.

7-OH-DPAT alone (0.1-3.0 mg/kg) had a significant and biphasic effect on LMA ($F(4,35)=9.8$, $p<0.001$), with a significant reduction in LMA following 7-OH-DPAT (0.3 mg/kg) (116±21 counts/80 min; $p<0.001$ compared to vehicle 342±55 counts/80 min) and a significant increase in LMA following 7-OH-DPAT 3.0 mg/kg (508±36 counts/80 min; $p<0.05$ compared to vehicle). IACB AMPH alone (5-20 μg) induced a significant ($F(3,22)=6.4$, $p<0.01$) effect on LMA with a significant hyperactivity response following AMPH (10 and 20 μg) (867±208-1207±193 counts/80 min; $p<0.05$-$0.001$ compared to vehicle 399±53 counts/80 min). Pretreatment with 7-OH-DPAT (0.1-3.0 mg/kg) induced a significant and biphasic effect on the hyperactivity response to AMPH (20 μg IACB) ($F(4,26)=55.5$, $p<0.001$). 7-OH-DPAT (0.1-0.3 mg/kg) prior to AMPH (20 μg IACB) significantly reduced the hyperactivity (1034±21-395±37 counts/80 min; $p<0.01$-$0.001$ compared to 1228±62 counts/80 min for AMPH alone). In contrast, 7-OH-DPAT (3.0 mg/kg) prior to AMPH (20 μg IACB) significantly enhanced the hyperactivity (1413±60 counts/80 min, $p<0.05$ compared to AMPH alone)
The AMPH-induced hyperactivity response was thus inhibited by low doses and enhanced by high doses of 7-OH-DPAT in a manner corresponding with the biphasic effect of 7-OH-DPAT on LMA shown here and previously reported (Daly & Waddington, 1993). Activation of $D_3$ receptors in the limbic system by low doses of 7-OH-DPAT may attenuate expression of the hyperactivity, whilst higher doses may stimulate $D_2$ receptors enhancing the hyperactivity (Starr & Starr, 1995). These results provide further evidence for a functional role of the $D_3$ receptor in LMA in the rat; investigations with selective $D_3$ receptor antagonists are neccessary to confirm the receptor class involved.

Costall, B., Naylor, R.J. (1975) *Eur. J. Pharmacol.* 32; 87-93
Daly, S.A., Waddington, J.L. (1993) *Neuropharmacol.* 3; 509-510
Starr, M.S., Starr, B.S. (1995) *Eur. J. Pharmacol.* 277; 151-158
Suzuki, T., Maeda, J., Funada, M. *et al.* (1995) *Neurosci. Lett.* 187; 45-48

## 318P REPEATED MICRODIALYSIS EXPERIMENTS IN THE RAT STRIATUM OF CONSCIOUS RATS: EFFECTS OF QUINELORANE ON DOPAMINE RELEASE

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We have previously shown that quinelorane induced a dose-dependent decrease in dopamine levels in the rat nucleus accumbens and striatum ( Routledge et al., 1995). In the present study, we have investigated the reproducibility of repeat use microdialysis to measure drug-induced changes in dopamine release in vivo.

Male Sprague Dawley rats (250-350g) were anaesthetised and surgery performed as in Routledge et al., 1995. Guide cannulae (BAS) and microdialysis probes (4mm membrane) were used. Rats were singly housed and at least two weeks allowed for post-operative recovery and between consecutive experiments, the same experiment being repeated in the same rat so that comparison over time could be determined. On the day of an experiment, rats were anaesthetised with isoflurane to facilitate insertion of the microdialysis probe and allowed to recover consciousness. Probes were perfused with artificial cerebrospinal fluid ( NaCl, 125mM, KCl, 2.5mM, $MgCl_2$, 1.18mM, $CaCl_2$, 1.26mM) at a flow rate of 1µl/min. The first two hour perfusate was discarded and then samples were collected at one hourly intervals for five hours. After the second hour fraction collection, either quinelorane (30µg/kg, sc) or vehicle (saline, 1ml/kg, sc) were administered and samples collected for a further three hours. Samples were analysed using HPLC-ECD (Routledge et al, 1995). At the end of the experiment, following re-anaesthesia, the guide-pin was re-inserted and the rat returned to its home cage. The rats were used three times within 90 days.

The brains were removed following carbon dioxide euthanasia and fixed in 4% neutral-buffered formalin for subsequent staining of 50µm coronal sections with 1% cresylfast violet and processed for glial fibrillary acidic protein immunocytochemistry.

Basal levels of dopamine for the second hour fraction period were: (fmole/60µl fraction) 135 ± 26.0, 161.7 ± 37.1 and 107.2 ± 18.8, for the first, second and third challenges respectively, vehicle controls having maintained levels throughout the experiment. Quinelorane (30µg/kg, sc)-induced a significant inhibition of dopamine release for up to three challenges within a 90 day period. There was no significant difference ($p>0.05$) between the three challenges in the inhibition of dopamine release, although in the third challenge there was a trend towards significance. All three one hour time points were significantly different from vehicle controls on each challenge (ANOVA, $p<0.05$, $n=7$). The maximum inhibition of dopamine release induced by quinelorane was: first challenge, 59.9 ± 6.0%; second challenge, 71.0 ± 5.8%; third challenge, 75.9 ± 5.2%, (n=7). After 90 days there was ipsilateral reactive astrocytosis along the probe tract.

In conclusion, this data suggests that repetitive challenge with quinelorane in the rat striatum for at least three challenges yields reproducible results, thus reducing animal use. There is reactive astrocytosis, but this does not significantly affect dopamine levels or inhibition of dopamine release by quinelorane, suggesting that sampling is consistently from a neuronal cell type.

Routledge C, Thorn L, Ashmeade T and Taylor S (1995) Biochem. Soc. Trans., **24**, 199-201.

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## 319P EFFECTS OF THE METABOTROPIC GLUTAMATE RECEPTOR ANTAGONIST (S)-α-METHYL-4-CARBOXYPHENYLGLYCINE ON AMPHETAMINE-INDUCED DOPAMINE EFFLUX IN NUCLEUS ACCUMBENS

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Recent studies (Attarian and Almaric, 1997) have shown that local infusion of the metabotropic glutamate receptor (mGluR) antagonist (S)-α-methyl-4-carboxyphenylglycine ((S)-MCPG) into the nucleus accumbens (n.acc) attenuated the increase of locomotor activity following D-amphetamine without causing behavioural effects *per se*. However, the mechanism by which this occurs is unknown. Therefore, in the present study we have examined the effects of local infusion of (S)-MCPG on basal and amphetamine induced dopamine efflux in the n.acc. of conscious rats.

Male Sprague Dawley rats weighing 250-350g were implanted under isofluorane anaesthesia with a microdialysis probe (2mm Hospal membrane) in the nucleus accumbens (coordinates AP, +1.7mm ; L, +1.5mm from bregma ; DV -7.5mm from dura according to the atlas of Paxinos and Watson 1982). 24 hours later, animals were perfused (1µl/min) with ringer solution and basal samples collected at 20 minute intervals for 100 minutes after which animals were perfused with either ringer or ringer containing 5mM (S)-MCPG. 20 minutes later animals were either given D-amphetamine sulphate (1mg/kg s.c.) or saline (1ml/kg s.c) and samples collected for a further 180 minutes. All samples were analysed for dopamine by reversed phase HPLC using electrochemical detection (Hutson *et al.*, 1991). Results are expressed as mean ± s.e.mean dopamine area under the curve for pre (-100 - 0 min) and post amphetamine injection (0-180 min).

D-amphetamine (1mg/kg s.c.) significantly increased dopamine efflux (maximum 707% of basal) compared to saline treated animals. Treatment with (S)-MCPG failed to block the effect on dopamine efflux caused by D-amphetamine (maximum 642% of basal). (S)-MCPG caused an increase of basal dopamine efflux (maximum 199% of basal), although this was not statistically significant. Table 1 shows area under the curve values for all drug treatments.

Table 1. Effect of (S)-MCPG on amphetamine induced dopamine efflux.

| | Dopamine Area Under Curve (fg/µl/min) | |
|---|---|---|
| Treatment | Pre-injection | Post-injection |
| Ringer/Saline | 15.9 ± 3.04 | 23.6 ± 4.77 |
| Ringer/D-amphetamine | 28.9 ± 2.87 | 104.7 ± 17.91* |
| MCPG/Saline | 21.6 ± 3.78 | 40.9 ± 8.81 |
| MCPG/D-amphetamine | 28.9 ± 3.41 | 99.0 ± 17.40* |

Values are mean ± s.e.mean
*$p < 0.01$ compared with appropriate controls by Tukey test following 2 way ANOVA n = 5/6 per group.

Results show that the mGluR antagonist (S)-MCPG did not significantly affect either basal or D-amphetamine induced dopamine efflux in the n.acc suggesting that its previously reported inhibitory effects on D-amphetamine induced increase of locomotor activity may be mediated subsequent to the release of dopamine.

Attarian, S. and Amalric, M. (1997) *European Journal of Neuroscience*, Vol 9, pp 809-816.
Hutson, P.H., Bristow, L.J., Thorn, L. *et al* (1991) *Br J. Pharmacol.* 103, 2037-2044.
Paxinos, G. and Watson, C.(1982) *The Rat Brain in Stereotaxic Coordinates*, Sydney: Academic Press.

## 320P PUTATIVE NEUROTENSIN RECEPTOR SUBTYPES ARE INVOLVED IN NEUROTENSIN-INDUCED STIMULATION OF STRIATAL [3H]DOPAMINE RELEASE EVOKED BY K$^+$ *VERSUS* ELECTRICAL DEPOLARIZATION

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The effects of neurotensin (NT) agonists and antagonists (SR 142948A and SR 48692) on $K^+$ and electrically evoked [$^3$H] dopamine (DA) release from rat and guinea-pig striatal slices were investigated to determine whether different NT receptors are involved in these situations. Striatal slices (350 µm) from Sprague-Dawley rats (180-200 g) were incubated 30 min with 120 nM [$^3$H]DA in Krebs buffer containing 1 µM pargyline, 1 mM ascorbic acid and 0.1 µM desipramine, and then superfused in chambers at a rate of 0.5 ml/min. Depolarization was produced either electrically (1 msec, 22 mA, 7 Hz) or by superfusion for 3 min with buffer containing 20 mM KCl. NT agonists and antagonists were added respectively 9 min and 18 min before and throughout depolarization. Under $K^+$ depolarization, in the two species, NT(1-13), NT(8-13) and Eisai hexapeptide increased [$^3$H]DA release with $EC_{50}$ values in the nanomolar range and Emax values in the range of 100% of stimulated control. In contrast, NT(8-13) and Eisai hexapeptide did not exceed 40% of the NT(1-13) effect under electrical depolarization. D-Tyr$^{11}$-NT was inactive in both cases (Table 1). In rats, SR142948A was equally active, with $IC_{50}$ values in the nanomolar range, to antagonize both $K^+$ and electrically evoked [$^3$H]DA release. Conversely, SR48692 antagonized the NT(1-13) stimulated $K^+$ evoked release but failed to antagonize (up to $10^{-6}$M) the NT(1-13) enhancement of electrically stimulated [$^3$H]DA release (Table 2). Similar results were obtained in guinea-pigs. In rats, after a first NT($10^{-8}$M) application ($S_1$), the second NT effect ($S_2$, 45 min later) under electrical depolarization was not significantly reduced ($S_1$: 100 ± 10 % ; $S_2$: 86 ± 8 %, n = 3). In contrast, the second application of NT under $K^+$ depolarization evoked a markedly reduced response ($S_1$: 102 ± 12 % ; $S_2$: 16 ± 3 %, n = 3 ; $p < 0.01$, Student's t-test) indicating the development of desensitization to NT. More precise investigation showed that the desensitization process was primarily observed on Emax values whereas $EC_{50}$ values were only weakly reduced. We propose that NT action on each type of depolarization may be mediated through distinct receptors.

Table 1: Effects of NT agonists on rat striatal [$^3$H]DA release evoked by $K^+$ and electrical depolarization.

| | $K^+$ | | Electrical | |
|---|---|---|---|---|
| | $EC_{50}$ (nM) | Emax (%) | $EC_{50}$ (nM) | Emax (%) |
| NT(1-13) | 1.7 ± 0.1 | 96 ± 5 | 2.5 ± 0.6 | 108 ± 6 |
| NT(8-13) | 3.0 ± 0.4 | 102 ± 7 | 1.0 ± 0.4 | 43 ± 7 |
| Eisai hex. | 5.5 ± 0.7 | 113 ± 5 | inactive | |

Values are mean ± se.mean (n = 5 independent experiments) ; Emax: % stimulation above evoked DA release.

Table 2: Effects of SR 48692 and SR 142948A on the stimulation by NT of striatal [$^3$H]DA release evoked by $K^+$ and electrical depolarization.

| | $IC_{50}$ (nM) | |
|---|---|---|
| | $K^+$ | Electrical |
| SR 48692 | 0.45 ± 0.03 | > 1000 |
| SR 142948A | 0.80 ± 0.08 | 0.32 ± 0.07 |

Values are mean ± se.mean (n = 5 independent experiments)

## 321P EVIDENCE FOR THE REGULATION BY $NK_2$ RECEPTORS AND NO OF STRIATAL ACETYLCHOLINE RELEASE: AN *IN VIVO* MICRODIALYSIS STUDY IN ANAESTHETIZED RATS

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Previous studies suggested that stimulation of dopamine $D_1$ receptors induced the release of striatal acetylcholine (ACh) via the release of endogenous neurokinin A (NKA) and/ or Substance P (SP) (Steinberg et al., 1995). The present study was undertaken to define more precisely the local tachykinin-dependent circuitry involved in the action of $D_1$ agonists on striatal ACh release. We therefore investigated the effects of systemic and/or striatal injection of the new non-peptide $NK_2$ receptor antagonist SR144190 [(+)-3-{1-[2-(4-benzoyl-2-(3,4-difluorophenyl)-morpholin-2-yl)-ethyl]-4-phenylpiperidin-4-yl}-1-dimethylurea] on the increase in ACh release induced by (+)-SKF-38393, NKA or SP. The role of endogenous nitric oxide (NO) in these hetero-regulations was addressed using striatal application of the inhibitor of NO synthase, $N^G$-monomethyl-l-arginine acetate (L-NMMA).

Male Sprague Dawley rats (270-320g) were anaesthetized with urethane (1.4g/kg, i.p.) and placed in a stereotaxic frame. Microdialysis probes were implanted in the striatum (A 0.3mm; L 3mm; V 6.5) and perfused at a flow rate of 2µl/min with Ringer solution. Samples were collected at 20min intervals and analysed for ACh levels by HPLC with electrochemical detection (Steinberg et al., 1995). Each treatment was performed after 3 stable basal ACh values: agonists were perfused through the probe for 60min (except 80min for (+)-SKF-38393). For systemic administration SR 144190 was given i.p 30min before (+)-SKF-38393. For local application, SR 144190 and L-NMMA were perfused respectively 60 and 120min before and during perfusion with the agonists. The area under the curve during perfusion with the agonists was calculated for each treatment, and statistical analysis was carried out by the Kruskall-Wallis non parametric test. All results are expressed as mean ± s.e.mean (3-13 animals).

Systemic or striatal administration of SR 144190 prevented the enhancement of ACh release induced by 3µM of (+)-SKF-38393 (fig 1A). The local perfusion of SR 144190 (0.001µM) totally blocked the increase in ACh release induced by 1µM NKA (52.8±5 *vs* 8.7±2, $p<0.01$) and partially blocked that evoked by 1µM SP (44.5±2.9 *vs* 20±2, $p<0.01$). Finally the intrastriatal application of 1µM L-NMMA suppressed the increase in ACh release induced by 3µM (+)-SKF-38393 (fig 1B) and 1µM NKA (71±7 *vs* 17±2, $p<0.01$).

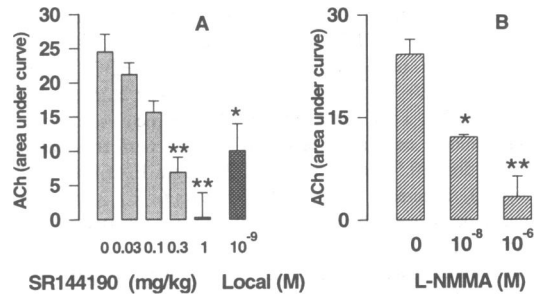


*Figure 1. Effect of local application of SR144190 (A) and L-NMMA (B) on (+)-SKF-38393 (3µM) -induced ACh release, **p<0.01, *p<0.05*

These results indicate endogenously-released NKA or SP, acting at $NK_2$ sites with NO as a mediator, control the striatal ACh release caused by dopamine $D_1$ receptor activation.

Steinberg et al, (1995) *J Neurochem.*, **65**, 2543-2548.

## 322P ACTION OF A NEUROPROTECTIVE DOSE OF FK506 ON A NOS-MEDIATED ACCUMULATION OF cGMP IN NEONATAL CEREBELLAR PRISMS

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The macrolide immunosuppressant FK506 is neuroprotective in a rat middle cerebral artery occlusion model of acute ischaemic stroke (Sharkey and Butcher 1994). It has been suggested from work in primary neuronal cultures (Dawson et al 1993) that this effect may be due to an inhibition of nitric oxide synthase activity caused by increased phosphorylation of NOS due to reduced calcineurin activity. We have used the neonatal rat cerebellar prism preparation to investigate the effect of FK506 on NOS activity. In this system stimulation of NMDA receptors leads to the rapid accumulation of cGMP (Garthwaite and Balazs 1978) through activation of NOS.

8 day old Sprague Dawley rats of either sex were killed by decapitation and their cerebella rapidly dissected. These were transfered to pre-warmed (37 °C) and pre-gassed (95% $O_2$ / 5% $CO_2$) Krebs buffer and crosschopped on a McIlwain tissue chopper to 400μ$M^2$ prisms. The prisms were transferred to a conical flask and incubated in a shaking water bath (Krebs buffer, 37 °C, 95% $O_2$ / 5% $CO_2$). The buffer was changed every 15 minutes for an hour. FK506 was added after the second buffer change. At the fourth buffer change 50μl aliquots of gravity packed prisms were transfered to flat bottomed tubes in the shaking water bath and exposed to experimental drugs in a final volume of 300μl. For each experiment 50μl aliquots were also taken for protein estimation (Bradford Assay) and Western blotting. After five minutes of exposure to N-methyl D-aspartate (NMDA) or sodium nitroprusside (SNP) in the presense or absense of inhibitors the reaction was stopped by the addition of 300μl ice cold 1M trichloroacetic acid and the samples left to stand on ice for 20 minutes. cGMP was extracted and stored at 4°C prior to radioimmunoassay. Results are the mean of 4-6 determinations and are expressed as pmol cGMP per mg protein. The significance of differences between treatments was assessed using analysis of variance.

NMDA caused a rapid accumulation of cGMP, peaking at 2 minutes and declining slowly thereafter.All subsequent experiments were performed at the five minute time point. The response to NMDA was concentration dependent, with an $ED_{50}$ of 50μM. This effect was mirrored by SNP. Both the NMDA antagonist, MK801 (10μM ; $p<0.05$) and the NOS inhibitor, L-NAME (10μM ; $p<0.05$) inhibited the effect of NMDA but not of SNP. FK506 at 100nM or 1μM added at the second buffer change had no effect on the maximal NMDA effect or on the shape of the NMDA dose response curve. Protein extracts from neonatal rat cerebellar tissue subjected to western blotting confimed the presense of both FKBP12 and calcineurin A.

FK506 has no effect on the NMDA stimulated NOS-mediated accumulation of cGMP in neonatal rat cerebellar prisms at immunosuppressive concentrations similar to those used in previous cell culture work (Dawson et al 1993). This data does not support the hypothesis that the neuroprotective effect of FK506 is mediated through an effect on the activity of NOS, and suggests that an alternative explanation is required.

M Macleod is an MRC Clinical Training Fellow

Sharkey and Butcher (1994) *Nature* 371 ; 336 - 339
Garthwaite and Balazs (1978) *Nature* 275; 328 - 329
Dawson et al (1993) *Proc. Natl. Acad. Sci. USA* 90 ; 9808 - 9812

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## 323P DELAYED NEUROPROTECTIVE EFFECT OF LOW DOSES OF BACTERIAL LIPOPOLYSACCHARIDE IN TRANSIENT FOCAL CEREBRAL ISCHAEMIA IN RATS

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The brain as well as myocardium can be protected against the consequences of ischaemia by a number of procedures given hours previously. Delayed cardioprotection by single doses of lipopolysaccharide (LPS) pretreatment was first demonstrated (Song *et al*, 1996) and a recent work (Tasaki *et al*, 1997) has shown that LPS pretreatment induces delayed neuroprotection in a model of permanent focal cerebral ischaemia in spontaneously hypertensive rats.
The aim of this study was (i) to evaluate the time course of neuroprotection by LPS in transient focal cerebral ischaemia in rats, (ii) to eliminate a possible early deleterious effect and (iii) to determine if LPS efficiency is dose-dependent.
Thirty-seven male Wistar rats (IFFA CREDO, France), weighting 280-320 g, were anaesthetised with chloral hydrate (300 mg/kg, i.p.). Intraluminal monofilament occlusion of right middle cerebral artery (MCA) was performed after a single dose (0.5 mg/kg, i.p.) of LPS (from E. coli 055 : B5, Sigma), injected 4 hours (n=4), 24 hours (n=6), 3 days (n=6) and 7 days (n=4) before. Control animals (n=8) were given vehicle (saline 0.5 ml, i.p.). Mean arterial blood pressure and blood gases were measured by cannulating tail artery. Twenty-four hours after a one-hour ischaemia, all animals were sacrificed and infarct volumes were quantified after cresyl violet staining. To assess the dose-dependent efficiency of LPS, 9 animals received 1 mg/kg (n=4) or 2 mg/kg (n=5) of LPS, 3 days prior MCA occlusion.
There was no significant difference between control and LPS animals in mean arterial blood pressure and blood gases before and during the one-hour ischaemia. Infarct volume (mean ± SEM), after brain edema correction, was significantly lower only in the 3-days LPS group (146±23 mm3) compared to control animals (264±10 mm3 ; ANOVA, $p < 0.0001$) and the neuroprotective effect disappeared when LPS doses were increased (fig. 1).
In conclusion, our data suggest that only low doses of LPS are able to induce a delayed neuroprotection on transient focal cerebral ischaemia without early protective or deleterious effect whereas higher doses probably activate other mechanisms who may blunt this protection.

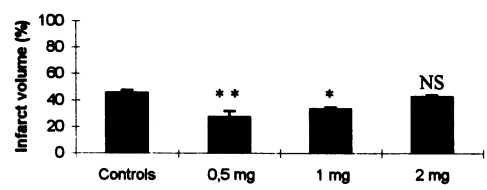


Figure 1 : Effect of middle cerebral artery occlusion on total cerebral necrotic volumes (% of left hemisphere after brain edema correction) developed by control rats and by rats treated with LPS 0.5 mg/kg, i.p. 1 mg/Kg, i.p. and 2 mg/kg, i.p. administered 3 days before induction of cerebral focal ischaemia. Statistical significance was assessed by ANOVA followed by a PLSD Fisher test. * $p < 0.05$, * * $p < 0.001$.

Song W., Furman B.L., Parratt J.R. (1996) *Br. J. Pharmacol.* 118, 2157-2163.
Tasaki K., Ruetzler C.A., Ohtsuki T., Martin D., Nawashiro H., Hallenbeck J.M. (1997) *Brain Res.* 748, 267-270.

## 324P SB-238592 (CP-0127) DOES NOT REDUCE INDICES OF ISCHAEMIC DAMAGE FOLLOWING PERMANENT OR TRANSIENT FOCAL CEREBRAL ISCHAEMIA IN NORMOTENSIVE RATS

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Neuroprotective efficacy of bradykinin receptor antagonists following permanent focal cerebral ischaemia in rats is variable (Niedermaier & Hacke, 1996: Relton, et al, 1996). SB-238592 (CP-0127) is a peptidic bradykinin receptor antagonist (Cheronis, et al, 1992) that has been shown to attenuate the effects of traumatic injury in animal models (Christopher, et al, 1994). As some of the underlying mechanisms of traumatic injury and cerebral ischaemia are thought to be similar, antagonism of the effects of bradykinin using SB-238592 may also be neuroprotective.

Under halothane anaesthesia, left middle cerebral artery (MCA) permanent or transient occlusions were performed in male Sprague Dawley rats (300-350g, n=7-11/group) using the intraluminal thread model (Longa, et al, 1989). Briefly, a 3/0 nylon filament, with a heat blunted tip 0.26-0.30mm in diameter, was advanced through an arteriotomy in the left external carotid artery until it occluded the MCA at its origin with the Circle of Willis. Transient MCA occlusion was instituted by compete removal of the filament 2h following induction of ischaemia. Silicone-tipped cannulae were positioned in the jugular vein, exteriorised between the scapulae and attached to a tethering system which allowed the rats free movement without interference with the patency of the intravenous cannulae. Following permanent MCA occlusion SB-238592 (1, 3, or 9μg/kg/min) or saline (1.2ml/h) were intravenously infused beginning 30 min post-MCA occlusion for the 23.5h. Following transient MCA occlusion SB-238592 (9μg/kg/min) or saline (1.2ml/h) was intravenously infused beginning 10 min post-reperfusion for 21.83h. Twenty four hours post-MCA occlusion the rats were neurologically assessed using a subjective 8 point scoring system. Following neurological assessment the rats were killed and transcardially perfused with neutral buffered formalin for brain fixation. The brains were sectioned and stained with Cresyl Fast Violet for histologically image analysis. Lesion size and hemispheric swelling data were analysed parametrically whereas neurological scores were analysed non-parametrically.

Following permanent MCA occlusion SB-238592 treatment did not significantly reduce, compared to the saline treated group, the lesion size (222±20, 227±9, 206±21 vs 231±14mm$^3$; mean±sem; p=0.762), hemispheric swelling (26±3, 28±3, 23±3 vs 28±3%; mean±sem; p=0.598) nor the neurological score (4±1, 4±0, 4±1 vs 3.5±1; median±iqr; p=0.578). Similarly, following transient MCA occlusion SB-238592 did not significantly reduce, compared to the saline treated group, the lesion volume (180±16 vs 196±20mm$^3$; p=0.529), hemispheric swelling (16±3 vs 18±2%; p=0.741) nor neurological score (4±3 vs 4±3; p=0.429).

Since SB-238592 did not significantly reduce indices of ischaemic damage these data suggest that antagonism of bradykinin receptors by SB-238592 does not significantly alter ischaemic proccesses within the first 24 hours following induction of permanent or transient focal cerebral ischaemia. Previous experience with this model has displayed efficacy of other neuroprotectants (Sydserf, et al, 1996; Mackay, et al, 1996).

SB-238592 (CP-0127) was supplied by Cortech Inc., Denver, CO, USA.

Cheronis J.C., Whalley E.T., Nguyen K.T., *et al.* (1992) J Med Chem 35, 1563-1572.
Christopher T.A., Ma X.L., Gauthier T.W., *et al.* (1994) Am J Physiol. 266, H867-873.
Longa E.Z., Weinstein P.R., Carlson S., *et al.* (1989) Stroke 20,84-91.
Mackay K.B., Bailey S.J., King P.D., *et al.* (1996) Neurodegeneration 5:319-323.
Niedermaier N., Hacke W. (1996) Cerebrovasc Dis 6 (suppl 2), 43.
Relton J.K., Beckey V.E., Rothwell N.J., *et al.* (1996) Br J Pharmacol 120(suppl), 75P.
Sydserff S.G., Green A.R., Cross A.J. (1996) Neurodegeneration. 5:81-5.

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## 325P POST-TREATMENT WITH SUMATRIPTAN DOES NOT REDUCE HISTOLOGICAL OR NEUROLOGICAL OUTCOME AFTER PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION IN NORMOTENSIVE RATS

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Peri-infarct spreading waves of depression occur in various experimental models of cerebral ischaemia, and are putatively involved in the genesis of ischaemic brain damage (Hossmann, 1996). A causal relationship between suppression of peri-infarct depolarisations and anti-ischaemic efficacy has been observed following administration of both MK-801 (Iijima *et al.*, 1992) and NBQX (Mies *et al.*, 1994) after permanent middle cerebral artery (MCA) occlusion in the rat. It has been suggested that the 5-HT$_{1B}$ agonist sumatriptan may have some neuroprotective effect by attenuating peri-infarct depolarisations after focal ischaemia (Hossmann, 1996). Thus, we have investigated the effect of sumatriptan on the neuropathological (infarction and brain swelling) and neurobehavioural sequelae following permanent MCA occlusion in the rat.

Focal cerebral ischaemia was induced in male Sprague-Dawley rats (300-350g, n=7-10 per group) by permanent occlusion of the left middle cerebral artery under halothane anaesthesia, using the intraluminal filament technique (Longa et al, 1989). Immediately prior to arterial occlusion, the left jugular vein was cannulated and exteriorised between the scapulae to permit continuous intravenous drug/vehicle infusion (1.2ml/hr). Sumatriptan or vehicle (group A: 0.9% saline) was administered as a bolus intravenous injection (1ml/kg) at 15 minutes post-MCAO followed by continuous intravenous infusion thereafter (group B: 30μg/kg bolus + 30μg/kg/hour infusion; group C: 100μg/kg + 100μg/kg/hr; group D: 300μg/kg + 300μg/kg/hr) until twenty-four hours post MCAO. Rats were neurologically assessed using a seven-point scale, and transcardially perfused with neutral buffered formalin. Brains were serially sectioned (50μm) and stained with Cresyl Fast Violet for quantification of infarct volume and brain swelling using image analysis. Infarct volume, hemispheric swelling and neurological score data were analysed using one-way ANOVA with one-way Dunnett's test. Results are expressed as mean infarct volume ± s.e.m., mean percentage change in hemispheric swelling ± s.e.m., and as median neurological score ± interquartile range.

There was no significant reduction (p>0.05) in the volume of total hemispheric infarct at 24hours, following post-ischaemic administration of sumatriptan (group B: 230±39mm$^3$, group C: 258±11mm$^3$, group D: 239±20mm$^3$), relative to vehicle-treated control animals (group A: 205±20mm$^3$). Sumatriptan also had no significant effect (p>0.05) on hemispheric swelling (group B: 29±5%, group C: 28±3%, group D: 29±4%), compared to vehicle-treated rats (22±3%). There was no significant effect (p>0.05) on neurological score (3±0) after treatment with sumatriptan at any dose, compared with vehicle-treated rats (3±0).

These results demonstrate that sumatriptan has no significant neuroprotective effects in this model of focal cerebral ischaemia.

Hossmann K.A. (1996) *Cerebrovasc Brain Metab Rev* 8, 195-208.
Iijima T., Mies G., & Hossmann K.A. (1992) *J Cereb Blood Flow Metab* 12, 727-33.
Longa E.Z., Weinstein P.R., Carlson S., *et al.* (1989) *Stroke* 20, 84-91.
Mies G., Kohno K., & Hossmann K.A. (1994) *J Cereb Blood Flow Metab* 14, 802-7.

## 326P PRESERVATION OF CORTICAL NICOTINE BINDING SITES BUT LOSS OF ChAT ACTIVITY AFTER HUMAN HEAD INJURY

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Impairment of cognition and memory function are common sequalae in patients who survive a head injury and one of the neurochemial events underlying this has been proposed to be dysfunction of cholinergic transmission. In support of this we have previously reported deficits of choline acetyltransferase (ChAT) activity, a marker of presynaptic cholinergic terminals, in the cortex of postmortem brain from patients who died following head injury (1). Nicotine binding sites were reduced in concert with ChAT activity in various progressive neurodegenerative disorders (2), but nicotine receptor integrity after head injury is not known. The purpose of the present study was to determine nicotine binding and ChAT activity in the cerebral cortex of postmortem brain from head-injured patients and control cases.

Membrane preparations were incubated at room temperature with 4nM L-(N-methyl)-$^3$H-Nicotine for 1 hour then filtered through GF/C filters in a Bradford cell harvester. Non-specific binding was determined in the presence of 0.1mM carbachol. ChAT activity was determined in the same samples as described previously (1). Homogenates were prepared from frozen postmortem human brain tissue, from 16 head injured patients (age 48 ±4 yrs; postmortem delay 58±7 h, mean ± sem) and 8 control cases (age 58±5 yrs; postmortem delay 43.6±6 h, mean ± sem). The survival periods after injury for the head-injured patients ranged from 3-300 h; the majority having sustained injury due to a fall or road traffic accident.

| Cortical Area | | Control | Head -Injured |
|---|---|---|---|
| **Cingulate** | Nicotine | 8.9±2.0 | 11.2±1.6 |
| | ChAT | 9.9±1.0 | 4.5±0.9* |
| **Parietal** | Nicotine | 7.6±1.9 | 7.0±1.0 |
| | ChAT | 8.9±0.5 | 5.6±0.4* |
| **Temporal** | Nicotine | 9.9±2.7 | 9.7±1.3 |
| | ChAT | 8.9±1.0 | 4.3±0.7* |

Data are mean ± sem nicotine binding (fmol/mg protein) and ChAT activity (nmol/mg protein/h).
*$p<0.05$ unpaired t-test compared to equivalent control region.

Nicotine binding was similar in head-injured and control cases in all 3 cortical regions, although the level of ChAT activity was reduced.

The results indicate selective cortical cholinergic abnormalities after human head injury.

1. Dewar, D. & Graham, D.I. (1996) *J. Neurotrauma* 13, 181-187.
2. Perry, E.K., Morris, C.M., Court, J.A., *et al.* (1995) *Neuroscience* 64 (2), 385-395.

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## 327P ENDOTHELIN-1 IN PERIAQUEDUCTAL GRAY AREA OF MICE INDUCES ANTINOCICEPTIVE EFFECTS VIA BOTH $ET_A$ AND $ET_B$ RECEPTORS

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Endothelin-1 (ET-1) injected into dorsolateral periaqueductal gray (PAG) area of mice produces a dose related antinociceptive effect (D'Amico et al., 1996). However, no study has been done on which receptor mediate(s) this response to endothelin-1 into the PAG area. Thus, using Swiss-Webster mice we have examined the effects of the endothelin receptor antagonists, FR 139317 ($ET_A$ receptor selective), bosentan ($ET_A$/$ET_B$ receptor non-selective) and BQ-788 ($ET_B$ receptor selective) on the antinociceptive response following administration of endothelin-1 at a dose of 2 pmol/mouse to the PAG area.

The mice, under ketamine anaesthesia (100 mg/kg *i.p.*), had a stainless steel guide cannula implanted in the dorsolateral PAG in accordance to the following coordinates measured in mm from the bregma AP:-4.36; L:0.2; V:2.0. The intra-PAG microinjections were conducted with a Hamilton 1 microlitre syringe, each intra-PAG injection being delivered in a total volume of 50 nl at a rate of 50 nl/5 sec. Antagonist studies were performed microinjecting each antagonist into the PAG area 10 min prior the injection of ET-1. Each drug used has been tested in separate series of experiments.

The method of Eddy and Lembach (1953) was used with slight modifications. Mice were placed on a hot plate (constant temperature of 56.0 ± 0.5 C°) before and at 5 - 10 - 15 - 30 - 60 - 90 - 120 min after ET-1 administration. Licking of the hind paws, as well as jumping, were considered as nociceptive response and the latency time was measured in seconds. The mean latency values of each time period were obtained and compared with the control data by ANOVA and Newman Keul test for multiple comparisons. P values less than 0.05 and 0.01 were considered significant. The control reaction time of the mice measured prior or after the injection of vehicle (saline) into the PAG area was 7.0 ± 0.8 s, n=10; mice showing a control reaction time over 10 sec were excluded. These animals were then randomised for drugs or vehicle administration.

The administration of ET-1 into the PAG area showed an antinociceptive effect. The latency time for the reaction to a hot plate began to be significant 15 min after the administration of ET-1 (e.g. 15 min, 10 ± 1.4 s, $p<0.05$, n=10). Peak values were achieved 30 min after administration of ET-1 (13.5 ± 1.9 s, $p<0.01$, n=10) and maintained constant 60 min later. 90 min after the administration of ET-1, the latency time for the reaction was 9.5 ± 1.0 s ($p<0.05$) while almost recovered 120 min later (7.6 ± 1.1 s, $p>0.05$, n=10). Pretreatment of the PAG area with bosentan (10 nmol), FR 139317 (5 nmol) or BQ-788 (5 nmol) greatly reduced the increases in the latency time for the reaction to the hot plate induced by ET-1. For instance, at 30 min the mean latency values were 7.6 ± 1.2 s, $p<0.01$, n=10; 9.0 ± 1.5 s, $p<0.05$, n=10; 10 ± 0.8 s, $p<0.05$, n=10; respectively for bosentan, FR139317 and BQ-788. These drugs lowered *per se* the control reaction time of the mice when administered alone to the PAG area (e.g. 30 min; bosentan, 84 ± 8%, $p<0.01$, n=10; FR139317, 48 ± 5%, $p<0.05$, n=10; BQ-788, 45 ± 6%, $p<0.05$, n=10).

ET-1, thus, appears to produce its antinociceptive effects through an action on both endothelin receptor types $ET_A$ and $ET_B$. In addition, since ET-antagonists lowered *per se* the control reaction time of the mice when administered alone to the PAG area, we would suggest that exogenous/endogenous ET-1 acting within the PAG area modulates the perception of pain exerting an antinociceptive effect.

Eddy, N.B., Lembach, D.J., *J Pharmacol Exp Ther* (1953). 107:385-93
D'Amico, M. et al., *Pain* (1996). 65:205-209.

## 328P *IN VITRO* STUDIES ON THE INTERACTION OF CLOMETHIAZOLE WITH GLUTAMATE RECEPTOR SUBTYPES IN RAT BRAIN

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Clomethiazole (CMZ) is an effective neuroprotective agent in models of acute ischaemic stroke (Green & Cross, 1994). It enhances cerebral GABA function, an action possibly associated with its neuroprotective action (Green & Cross, 1994; Lyden, 1997). However, there are few data on interactions of CMZ with glutamatergic function. Ligand binding studies have shown that CMZ is not an NMDA antagonist (Cross *et al.*, 1993), but certain NMDA receptor-mediated effects *in vivo* are attenuated by the drug (see Green & Cross, 1994). This study examined whether CMZ interacts with other glutamate receptor sub-types.

Using rat cortical tissue and standard ligand-receptor binding techniques we examined the effect of CMZ on the binding of [$^3$H]-dizocilpine (NMDA receptor), [$^3$H]-CNQX, [$^3$H]-AMPA and [$^3$H]-fluorowillardiine (the AMPA/ kainate receptor). While reference compounds gave $IC_{50}$ values similar to published values, no evidence was obtained for CMZ interacting at any sites (Table). We also studied the effect of CMZ at metabotropic glutamate receptors by prelabelling cortical tissue prisms with [$^3$H]-myo-inositol and measuring accumulation of [$^3$H]-$IP_1$, in the presence of LiCl (see Court *et al.*, 1986). PI hydrolysis was stimulated by ACPD in a concentration dependent manner (105 ± 13% at 330μM), an effect partially antagonised by L-AP3 (43 ± 6% at 1mM). CMZ partially antagonised the ACPD-stimulated PI turnover at 1mM (40%), but lower concentrations were without effect.

In conclusion, we found no interaction of CMZ with glutamate receptor sub-types at pharmacologically relevant concentrations, suggesting that CMZ is not neuroprotective because it antagonises glutamate function.

Court, J. *et al.* (1986) *N-S Arch. Pharmacol.*, **334**, 10-16..
Cross, A.J. *et al.* (1993) *Psychopharmacology*, **112**, 403-406.
Green, A.R. & Cross A.J. (1994) *Progr.Neurobiol.*, **44**, 463-484.
Lyden, P.(1997) *Int. Rev. Neurobiol.*, **40**, 233-258.

Table 1: Effect of clomethiazole on binding of radioligands to glutamate related receptor sites.

| Glutamate receptor | Ligand | Reference Compound | $IC_{50}$ (M) | Clomethiazole $IC_{50}$(M) |
|---|---|---|---|---|
| NMDA | [$^3$H]-Dizocilpine | Dizocilpine | $2 \times 10^{-9}$ | $> 10^{-3}$ |
| AMPA/Kainate | [$^3$H]-CNQX | AMPA (agonist) | $9.5 \times 10^{-7}$ | $> 10^{-3}$ |
| | | CNQX (antagonist) | $8 \times 10^{-8}$ | $> 10^{-3}$ |
| | [$^3$H]-AMPA | AMPA | $2 \times 10^{-8}$ | $> 10^{-3}$ |
| | [$^3$H]-Fluorowillardiine | L-Glutamate | $3 \times 10^{-6}$ | $> 10^{-3}$ |

## 329P BRAIN LEVELS OF FK506 AND NEUROPROTECTION IN EXPERIMENTAL STROKE

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FK506 (Tacrolimus) is a potent immunosuppressant presently in clinical use for the treatment of allograft rejection. Recent studies have shown FK506 to be neuroprotective in various animal models of cerebral ischaemia (Sharkey *et al.*, 1996). In the present studies we examine the limits of the neuroprotective window for FK506 in a rat model of endothelin-induced middle cerebral artery (MCA) occlusion (Sharkey *et al.*, 1993).

Male Sprague-Dawley rats (280-320g; n = 130) were anaesthetised by halothane in $N_2O$ and $O_2$ (80:20 v/v). The femoral vessels were exposed to permit the insertion of a polythene catheter into the femoral vein and FK506 (1mg/kg) or polyoxyl 60 hydrogenated castor oil vehicle, was administered as a single intravenous injection. The catheter was removed, the vessel tied and the wound sutured. MCA occlusion was performed by placing the anaesthetised rat into a stereotaxic frame (incisor =-3.7mm) and injecting endothelin-1 (ET-1: 150pmols in 3μl) via a 28g needle into piriform cortex immediately dorsal to the MCA (AP = 0.9mm; L = 5.2mm and V = 8.7mm relative to Bregma). The cannula was left in situ for 5 mins before being slowly withdrawn and the wounds sutured. Rats were killed, and the brain processed for quantitative histopathology, 72 hours post MCA occlusion (Osborne *et al.*, 1987).

For pharmacokinetic studies, groups of 4 rats received an iv injection of FK506 (1mg/kg) or vehicle at specified timepoints (15, 30 min, 1, 3, 12, 24, 48, 72 hours) prior to tissue and blood sampling. Samples were rapidly frozen, then stored at -60°C prior to analysis of FK506 content by competitive immunoassay. Additional groups of rats received FK506 5 min post MCA occlusion. These animals were then killed 1 or 3 hours post ET-1 and samples of blood and cerebral cortex taken. Data, presented as mean ± s.e. mean, were analysed using analysis of variance with post hoc Dunnett's test.

A single i.v. injection of FK506 (1mg/kg) was significantly neuroprotective from 72 hours pre- to 2 hours post MCA occlusion, but afforded no protection when injected 3 hrs post occlusion. Pharmacokinetic studies revealed that 15 min after injection of FK506, the concentration in whole blood was 164 ± 23ng/ml . The blood concentration fell over the next 24 hrs to 3ng/g and was below the detection limits at 72 hrs. Brain concentrations of FK506 were 51 ± 10ng/g in cortex, 15 min post injection and remained essentially the same throughout the entire 72 hour measurement period (49 ± 10ng/g @ 72hr). Furthermore, there were no significant differences in FK506 levels between ischaemic and non-ischaemic cortex when examined 1- or 3 hours post MCA occlusion.

These data confirm that brain levels of FK506 rise rapidly and remain at the same level for at least 72hrs following a single i.v. injection and that the presence of FK506 in the brain tissue is neuroprotective.

Osborne *et al* (1987) *J. Neurol. Neurosurg. Psychiatry* 50: 402-410
Sharkey *et al* (1993) *J. Cereb. Blood Flow Metab.* 13: 865-871
Sharkey *et al* (1996) In *Clinical Pharmacology of Cerebral Ischaemia.* ed. Ter Horst & Korf, pp 235-262. Humana Press.

## 330P β-AMYLOID PRODUCES A SELECTIVE BLOCK OF LATE-PHASE LONG-TERM POTENTIATION IN RAT HIPPOCAMPUS *IN VIVO*

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The brains of patients with Alzheimer's disease contain large deposits of ß-amyloid (Aß), a 39-43 amino acid peptide derived from ß-amyloid precursor protein (ß-APP) (Fraser *et al.*, 1997; Hardy, 1997). Since there is extensive synaptic disruption in brain areas such as the hippocampus in Alzheimer's disease the present study examined the effects of Aß on synaptic plasticity in the hippocampus of the rat.

We have assessed the effects of i.c.v. injection of Aß1-40 on long-term potentiation (LTP) in the CA1 area of urethane (1.5 g/kg, i.p.) anaesthetized male Wistar rats (250-350g). In vehicle (5 µl artificial cerebrospinal fluid) injected animals high frequency stimulation (HFS: 10 trains of 20 stimuli, inter-stimulus interval of 5 ms, inter-train interval of 2 s) of Schaffer collateral/commissural fibers induced LTP of field excitatory postsynaptic potentials (EPSPs) recorded in the stratum radiatum which was stable over a 5 hour recording period (168±15% baseline at 5 h after HFS, n=8, $P<0.05$, mean ± s.e.m., t-test). Doses of the peptide (Aß1-40, 0.4 and 3.5 nmol) which did not affect baseline transmission during this period were found to abolish late-phase LTP. For example, in animals which received Aß1-40 (3.5 nmol, i.c.v., n=9) HFS induced an increase in the EPSP amplitude which had returned to the baseline level by 4 h, the potentiation being significant only up to 3 h post-HFS ($P<0.05$). However, the magnitude of potentiation in the Aß1-40 (3.5 nmol) treated rats at 10 min (159±26%) and 1 h (148±15%) was not significantly different from controls (204±27 and 188±18%, respectively). At the lower doses of 0.1 and 0.025 nmol Aß1-40 there was no significant difference from controls up to 5 h after the HFS (n=4 per group).

The block of late-phase LTP was prevented by pretreatment with the cholinesterase inhibitor physostigmine (0.1 mg/kg, i.p.) 20 min prior to Aß1-40 (3.5 nmol, i.c.v.). Thus the EPSP amplitude at 5 h after the HFS was 171±7% (n=5, $P<0.05$ compared to baseline or Aß1-40 alone). This level of LTP was not significantly different from that observed at 5 h post-HFS in animals which received physostigmine (0.1 mg/kg, i.p.) in combination with an i.c.v. injection of vehicle (156±20% at 5 h, n=6). This dose of physostigmine on its own did not have a significant effect on baseline transmission or the magnitude of LTP induced by HFS, there being no difference from the group treated with vehicle alone.

Given that the stabilization of late-phase LTP is a model for the consolidation of memory in the hippocampus (Frey and Morris, 1997) it is possible that the observed selective block of this form of synaptic plasticity may contribute to the cognitive deficits in Alzheimer's disease. The antagonism of the effect by physostigmine indicates the possible involvement of cholinergic mechanisms in the action of the peptide.

Hardy, J. (1997) Trends Neurosci., 20, 154-159.
Fraser, S.P., Suh, Y.-H., Djamgoz, M.B.A. (1997) Trends Neurosci. 20, 67-72.
Frey, U., Morris, R.G.M. (1997) Nature 385, 533-536

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## 331P EFFECTS OF PKA AND PKC MODULATORS ON HIGH AFFINITY GLUTAMATE UPTAKE IN PRIMARY NEURONAL CELL CULTURES

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It is now assumed that extracellular concentrations of excitatory amino-acids are regulated by sodium-dependant high affinity transporters. Actually, three subtypes of these transporters have been cloned in rats (neuronal $EAAC_1$, glial $GLT_1$ and GLAST) and present putative phosphorylation sites indicating that they may be phosphorylated by protein kinases. The aim of this study was to examine the effects of various agents known to alter protein phosphorylation on the high affinity glutamate uptake rate measured on primary cultures of rat cortical neurons which are supposed to express only the neuronal transporter subtype.

Primary cultures of cortical cells from rat brain cortex were prepared from 17 days pregnant Wistar rats. Cells were suspended in 5ml ofDMEM-F12 supplemented with glucose,insulin, transferrin, putrescein, progesterone, selenium, glutamine, estradiol, arachidonicacid, decosahexaenoïc acid, penicillin and streptomycin. The experiments were performed after the cells were maintened for 6 days in culture ($10^5$ cells /$cm^2$) and glutamate uptake was measured on the cell plates after 30 min incubation with PKA or PKC modulators according to Pisano et al. For estimating statistical differences the data were compared using Student's-t-test ($p<0,05$). Neither the PKA (forskolin and dibutyrylcyclic AMP or dbcAMP,98% and 119% vs control respectively) nor the PKC (phorbol 12,13 myristate acetate or PMA, 115% vs control) activators significantly modified the glutamate uptake rate.

In contrast, the PKA (H89 : isoquinoline sulphonamide) and PKC (chelerythrin) inhibitors induced a dose-dependant decrease in glutamate transport process. The respective concentrations producing 50% inhibition of glutamate uptake ($Cl_{50}$) were 5 ± 1.2 µM for chelerythrin and 40 ±5.8µM for H89.Moreover the effect of 5µM chelerythrin and 50µM H89 were respectively counteracted by 10µM PMAand 50µM forskolin respectively, indicating that the chelerythrin and H89-induced inhibition of glutamate uptake is a specific process (5µM chelerythrin : 42.2% inhibition of glutamate uptake rate; 5µM chelerythrin + 10µM PMA : 19% inhibition of glutamate uptake rate; 50µM H89 : 57.4% inhibition of glutamate uptake rate; 50µM H89 + 50µM forskolin : 15% inhibition of glutamate uptake rate).

These results suggest :

1) that the basal phosphorylation state of the neuronal glutamate transporter is rather high since the protein kinase activators tested lacked to stimulate the glutamate uptake

2) that PKA and PKC may be involved in the posttranslational regulation of neuronal glutamate uptake on primary neuronal cells culture.

Saturation kinetic analysis of the decrease of transport activity observed with PKA and PKC inhibitors are now being performed.

References :
Pisano P. et al. Neuropharmacol. 1996, 25 : 541-547.

## 332P THE EFFECT OF NGF IN REGULATING CALCIUM CONDUCTANCES OF SYMPATHETIC NEURONES IN CELL CULTURE IS MIMICKED BY ANTI-TRK RECEPTOR ANTIBODY

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Nerve growth factor (NGF) is known to be necessary for the differentiation and maintenance of peripheral sympathetic neurones. Its neurotrophic activity is attained through binding to the high affinity Trk A receptor and to the low affinity $p75^{NGFR}$ receptor. The Trk A receptor contains a cytoplasmic domain with tyrosine kinase activity, and we have recently reported that the NGF mediated maintenance and enhancement of $Ca^{2+}$ conductance in isolated sympathetic neurones in culture involves components of a tyrosine kinase dependent cascade leading to the *ras* pathway and mitogen activated protein kinase (MAP kinase) (Lei *et al.*, 1996). The present study attempts to confirm that this aspect of NGF neurotrophism is mediated by a Trk receptor.

Bullfrog paravertebral sympathetic ganglia were placed in explant culture as described by Traynor *et al.* (1992). Isolated neurones were obtained by collagenase disaggregation of ganglia, and maintained in serum free culture for up to 15 days. Explants were observed microscopically for evidence of neurite outgrowth, and isolated cells were subjected to examination by whole cell patch clamp techniques after at least six days in culture. Cells were held at -80 mV, and subjected to a series of 20 ms depolarising steps. Peak current at 0 mV was used as the standard and normalised against the integrated capacitative transient current elicited by a 10 mV depolarising step from -80 mV. Cultures were exposed to NGF or antibodies throughout the culture period, but for recording, a sodium free medium containing $Ba^{2+}$ instead of $Ca^{2+}$ was used. Data were derived from groups of at least 20 cells.

Anti-Trk IgG alone (20μg/ml) evoked a vigorous neurite outgrowth, comparable to that seen with NGF (Kelly *et al.*, 1989), and contrasted with the outgrowth produced in the presence of control IgG (20μg/ml). Anti-Trk IgG treatment maintained the $Ba^{2+}$ current density at 72% greater than control values which decline with culture (64.4 ± 5.9 pA/pF vs. 37.4 ± 5.9 pA/pF, $P<0.01$)) while NGF maintained it at 90% greater than control (71 ± 6.2 pA/pF). Cell capacitance alone was not affected (177.5 ± 10.3 pF vs. 166.8 ± 12.3 pF). Anti-p75 IgG (20μg/ml) did not affect $Ba^{2+}$ current density (35 ± 4.2 pA/pF), while the current density of cells grown in the presence of NGF (200 ng/ml) and control IgG was not significantly different from that of cells grown in the presence of NGF and anti-p75 IgG (70 ± 13.75 pA/pF vs 68 ± 6.7 pA/pF).

As anti-p75 IgG is known to inhibit the binding of NGF to the p75 receptor, these data suggest that the effect of NGF on $Ba^{2+}$ current density is independent of the p75 receptor. An agonist action of anti-Trk IgG has been demonstrated by neurite extension and receptor activation in PC12 cells (Clary *et al.*, 1994), and explained in terms of receptor oligomerisation after cross linking by the divalent IgG. Our results extend these conclusions to differentiated adult neurones, and confirm that NGF, acting on the Trk-A receptor influences the maintenance of $Ca^{2+}$ conductance in sympathetic neurones.

Clary, D.O. *et al.*(1994) Mol. Biol. Cell 5, 549-563
Kelly, M.E.. *et al.* (1989) Comp. Biochem .Physiol. 93A, 133-140
Lei, S. *et al.*(1996) Soc. Neurosci.Abstracts 22, no.300.22
Traynor, P. *et al.* (1992) Can.J.Physiol. Pharmacol. 70,826-834

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## 333P CHARACTERISATION OF BRADYKININ RECEPTORS THAT AFFECT C-FIBRE AFFERENT ACTIVITY FROM THE RAT ANKLE JOINT

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Bradykinin (BK) is one of the most potent algogenic substances known, and has been shown to excite articular C-fibre afferents in the rat (Grubb *et al.*, 1991). The principal receptors mediating the actions of kinins are the $B_1$ and $B_2$ subtypes (Regoli *et al.*, 1994). The aim of this study was to determine which of the BK receptor subtypes affects neural discharge from high threshold mechanonociceptor afferent fibres innervating the rat ankle joint. In order to investigate this, we determined the actions on these sensory receptors of BK, the selective $B_1$ receptor agonist des-$Arg^9$-BK, the selective $B_1$ receptor antagonist des-$Arg^9$-$Leu^8$-BK, and the $B_2$ receptor antagonist, icatibant (Hoe 140).

Male Wistar rats (250-350g) were anaesthetised with urethane (25% w/v, 0.6 $ml.kg^{-1}$ i.p.) and basal discharge (BD) along with the response to a mechanical stimulus (MS, applied for 2s to the joint capsule every 2 min) recorded from articular C-fibres innervating the ankle joint according to the methods of Birrell *et al.* (1993). All mechanonociceptor units studied had low levels of BD (1.1 ± 0.5 impulses. $s^{-1}$), conduction velocities within the C-fibre range (0.7 ± 0.1 $ms^{-1}$) and were excited by capsaicin (3 - 10 nmoles i.a.). BK and BK analogues were injected i.a. (catheter tip at iliac bifurcation, introduced via the contralateral femoral artery).

Quantitative data are given in Table 1. BK (0.9 - 94 nmol) caused a dose-dependent increase in both BD and the response to the MS, whereas des-$Arg^9$-BK (0.9 - 94 nmol) had no effect on neural discharge. BK-induced elevations in BD or in the MS were not significantly affected by des-$Arg^9$-$Leu^8$-BK. In contrast, Hoe 140 significantly antagonised both BK-induced BD and the increased responsiveness to the MS. Moreover, a differing profile of antagonism was observed with Hoe 140 : whereas BK-induced enhancement in the responsiveness to the MS appeared to be surmountable, the BK-induced increase in BD was insurmountable.

These data suggest that the BK receptors mediating the increase in both BD and the MS are of the $B_2$ subtype; $B_1$ receptors do not appear to activate or modulate mechanonocicepter discharge in the normal joint. In addition, the $B_2$ receptor antagonist Hoe 140 had differential actions between BK-induced BD and the increased responsiveness of the MS.

Birrell, G.J., McQueen, D.S., Iggo, A. *et al.* (1993). *Neuroscience*, 54, 537-544.
Grubb, B.D., Birrell, G.J., McQueen, D.S., *et al.* (1991). *Exp. Brain Res.*, 84, 383-392.
Regoli, D., Gobeil, F., Nguyen, Q.T. *et al.* (1994). *Life Sci.*, 55, 735-749.

Table 1: Effects of bradykinin and bradykinin analogues on mechanonociceptor discharge from the rat ankle joint.

| | Peak increase in BD (impulses $.s^{-1}$) | | | | | Peak increase in the MS (impulses) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.9 nmol | 2.8 nmol | 9.4 nmol | 28 nmol | 94 nmol | 0.9 nmol | 2.8 nmol | 9.4 nmol | 28 nmol | 94 nmol |
| BK | 1.3 ± 0.3 | 4.7 ± 1.1 | 7.9 ± 2.9 | 9.9 ± 1.6 | 9.3 ± 3.8 | 6 ± 1 | 16 ± 2 | 25 ± 2 | 29 ± 9 | 33 ± 12 |
| des-$Arg^9$-BK | 0.3 ± 0.1 | ND | 0.1 ± 0.1 | ND | 0.4 ± 0.2 | 2 ± 3 | ND | 2 ± 1 | ND | 5 ± 2 |
| BK (des-$Arg^9$-$Leu^8$-BK, 1$mgkg^{-1}$) | 1.7 ± 0.6 | 4.0 ± 1.1 | 6.0 ± 1.4 | 9.2 ± 2.5 | ND | 6 ± 3 | 12 ± 7 | 19 ± 7 | 23 ± 11 | ND |
| BK (Hoe 140 100$\mu gkg^{-1}$) | ND | 2.5 ± 1.4* | 1.9 ± 0.7* | 1.0 ± 0.2* | 1.1 ± 0.4* | ND | 5 ± 2* | 9 ± 1* | 17 ± 6* | 24 ± 2 |

All data are mean ± s.e.m. of n = 5-10 observations. * significant reduction, $P < 0.05$ Mann Whitney U-test versus BK response. ND = not determined.

## 334P A METHOD FOR DETERMINING THERMAL HYPERALGESIA AND INFLAMMATION IN THE MOUSE HIND PAW

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Carrageenan has been used to investigate inflammatory and nociceptive mechanisms in the rat (Coderre and Melzack, 1987). The aim of the present study was to characterize thermal hyperalgesia in the mouse where inflammation has been induced by an intraplantar injection of carrageenan.

Male ICR mice (20-35g; Harlan Olac UK) were used in all experiments. Following two periods of habituation, paw withdrawal latencies (PWL; s) to a thermal stimulus were measured using the Hargreaves plantar apparatus (Hargreaves *et al.* 1988). PWL and paw depth (PD; mm) for both hind paws were measured 24 hours before administration of carrageenan. Inflammation was induced by intraplantar (i.pl.) injection of carrageenan (25µl, 2%) into a randomly selected hindpaw, and PD and PWL assessed at 1, 2, 3, 4, 5, 6 and 24 hours. The effect of indomethacin (0.3, 1 and 10 mg/kg s.c., 30mins pre-carrageenan) on inflammation was assessed at 4 and 24h. All data are presented as mean ± sem. Significance was assessed between treatment groups using analysis of variance (ANOVA) followed by post hoc Dunnetts T-test with a significance level of $p<0.05$.

Intraplantar carrageenan elicited a time-dependant increase in PD, and reduced thermal nociceptive threshold. A significant increase in paw depth was observed 1 hour post carrageenan (baseline 1.75 ± 0.03 mm; 1h 2.36 ± 0.07 mm), with maximum inflammation occurring at 4 h (3.79 + 0.21 mm). Significant thermal hyperalgesia was not observed until 3 hours post carrageenan (baseline 8.6 ± 1.5 s; 3 hours 3.3 ± 0.4 s), with the maximum reduction of PWL at 4 hours (2.7 ± 0.4 s). Hyperalgesia was still present at 24h (4.9 ± 0.5 s). Thermal nociceptive threshold in the contralateral paw was unaffected following carrageenan treatment.

Indomethacin (0.3, 1 and 10mg/kg; 30mins pre-carrageenan) dose dependantly inhibited inflammation at all doses compared to vehicle (PD 3.68 ± 0.17 mm), with 10mg/kg decreasing PD to 2.97 ± 0.15 mm.

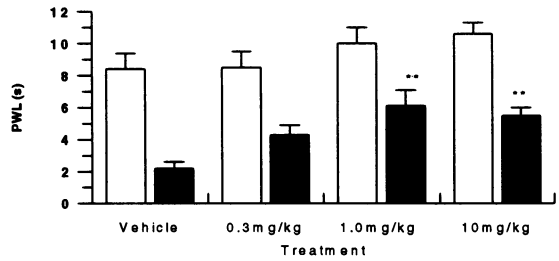


Figure 1: Effect of indomethacin (0.3, 1 and 10mg/kg s.c.; 30mins pre-carrageenan) on paw withdrawal latencies. Data represents mean ± sem. (n=8). Open columns 0h; filled columns 4h. **(ANOVA F=7.61, post hoc Dunnetts T-test $p<0.05$).

Thermal nociceptive threshold was similarly affected, with PWL significantly increased to 6.1 ± 1.0 s at 1mg/kg and to 5.5 ± 0.6 s at 10mg/kg, compared to vehicle (2.2 ± 0.4 s) (Figure 1).

Following intraplantar carrageenan administration in to the hind paw of the mouse, thermal hyperalgesia develops with time. The COX-1 inhibitor, indomethacin (Li et al., 1995), dose-dependantly inhibits carrageenan-induced hyperalgesia. These observations are similar to those observed in the ratand demonstrates that this technique can be readily applied to the mouse.

Hargreaves, K.M., et al. 1988a. *Pain* 32, 77-88.
Coderre, T.J. & Melzack, R., 1987. *Brain Res* 404, 95-106
Li, C.S. et al. 1995. *J.Med.Chem.* 38, 4897-905.

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## 335P CI988, AN ANTAGONIST OF CHOLECYSTOKININ TYPE B RECEPTOR AND 1DME, AN ANALOGUE OF NEUROPEPTIDE FF, ENHANCE THE ANALGESIC EFFECT OF MORPHINE IN DIABETIC RATS

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The analgesic effect of morphine is decreased in streptozocin (STZ)-diabetic rats (Courteix *et al.*, 1994). Since evidence demonstrates the involvement of cholecystokinin (CCK) in nociception and neuropeptide FF (NPFF) in antinociception and morphine (M) activity (Stanfa *et al.*, 1994; Gouardères *et al.*, 1996), we have examined the effects of intrathecal (i.t.) CI988, (4-{[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.$1^{3,7}$]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenyl]amino}-4-oxo-[R-(R*,R*)]-butanoate, (an antagonist of CCKB receptors), 1DME ([D-Tyr[1], (NMe) Phe[3]]NPFF, an analog of NPFF) and of the combination of CI988 (i.t.)+morphine (i.v.) and 1DME (i.t.)+M (i.v.) on mechanical hyperalgesia in diabetic rats, previously described as a model of chronic pain, (Courteix *et al.*, 1993).

Male Sprague Dawley rats (200-250 g, Charles River, France) were rendered diabetic by an injection of STZ (75 mg/kg i.p., 0.1 ml/100g, Upjohn). Diabetic (D) rats had a blood glucose>14 mM. Weight-matched normal (N) rats received i.p. distilled water. Pharmacological experiment took place 4 weeks later:

- experiment I: CI988 (0.1-1-10-100 µg) or 1DME (0.1-1-7.5 µg) or saline was i.t. (10 µl) injected in N and D rats.
- experiment II: M (0.1-0.5-1 or 4 mg/kg) or saline was injected i.v. (0.1 ml/100g) just before the i.t. injection of CI988 (10 or 100 µg) or 1DME (0.3 or 2.3 µg) or saline in D rats. The i.t. injections were performed as described by Mestre *et al.* (1994).Vocalization thresholds induced by the paw pressure were determined before and up to 120 min after drug injections (n=6 to 11 per group).

CI988 (0.1-1-10-100 µg), ineffective in N rats, caused a dose-dependent antinociceptive effect in D rats with a maximal score elevation (MSE) of +140±21g, and +290±20g for 10 and 100 µg, respectively. In D rats, the combination of CI988 (10 µg) with an ineffective dose of M (0.1 mg/kg) induced a marked antinociceptive effect (MSE:+358±21g) significantly higher than that induced by CI988 (10 µg) (MSE:+140±21g) alone. This potentiation, confirmed by an isobolographic analysis was not found when CI988 (100µg) and M (1 mg/kg, ineffective dose) were combined (MSE:+312±12g).

In D rats, only 0.1 and 1 µg of 1DME reduced hyperalgesia (MSE:+63±34g for 0.1 µg and +111±38g for 1 µg). All the tested doses (0.1-1-7.5 µg) were ineffective in N rats. The combination of 1DME 0.3 µg and M 0.5 mg/kg, an ineffective dose) resulted in antinociception (MSE:+154±62g) significantly higher than that observed with 1DME 1 µg injected alone, suggesting a potentiation of the two drugs. This interaction was confirmed with an active dose (4 mg/kg) of M combined with 2.3 µg of 1DME. The resulting effect (MSE:+363±177g) was significantly higher than that (MSE:+182±52g) of M (4mg/kg) alone. Moreover, the duration of this effect was longer (90 min).

These results show that:

(1) a CCKB receptor antagonist and a neuropeptide FF analog, both ineffective in N rats, induced antinociception in D rats, suggesting an involvement of these two systems in neurogenic pain conditions,

(2) the blockade of the CCKB mediated effects, an anti-opioid system exacerbated by diabetes, revealed the antinociceptive action of M.

(3) conversely, the activation of the NPFF system increased M-induced analgesia.

Courteix C, Eschalier A., Lavarenne J., *Pain*, **53**: 81-88, 1993.
Courteix C., Bardin M., Chantelauze C. et al., *Pain*, **57**: 153-160, 1994.
Gouardères C., Jhanandas K., Sutak M. et al., *Br. J. Pharmacol.*, **117**: 493-501, 1996.
Mestre C., Pélissier T., Fialip J., et al., *J. Pharmacol. Toxicol. Methods*, **32**:197-200, 1994.
Stanfa L., Dickenson A., Xu X.-J. et al., *T.I.P.S.*, **15**:65-66, 1994.

## 336P STRAIN DIFFERENCES IN THE EFFECTS OF LOSARTAN IN THE MOUSE ELEVATED PLUS-MAZE TEST AND THE RELATIONSHIP TO CONTRACTILE RESPONSES TO ANGIOTENSIN II *IN VITRO*

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Using BKW strain mice, Barnes *et al.*, (1990) reported that the selective angiotensin II, $AT_1$ receptor antagonist losartan elicited anxiolytic-like responses in a light-dark aversion test. Using TO strain mice, however, Shepherd *et al.*, (1996), failed to demonstrate such activity. The aim of this study was to investigate the relationship between the anxiolytic-like activity of losartan in different strains of mouse and the contractile responses to angiotensin II of isolated colon taken from those strains.

Anxiolytic-like activity was assessed using the elevated plus-maze which consists of an elevated cruciform runway, the four arms of which are 8 cm wide and 15 cm long. The outer limits of two of the opposing arms are also enclosed by 15cm walls, but are open-topped. The animal is placed in the centre of the maze and has a natural tendency to move into one of the enclosed arms; over a 5 minute test period, anxiolytic drugs increase the amount of time spent on the open runways (Stephens *et al.*, 1986). Losartan (10 or 20 $mg.kg^{-1}$ i.p.) or diazepam (1 $mg.kg^{-1}$ i.p.) were administered 30 minutes prior to testing. The results obtained were compared to those obtained from a parallel vehicle control group using Student's independent t-test and are expressed as the percentage (± s.e.mean) of the mean control value; the animals selected for study were male mice of the CD (27-33g), BKW (26-31g), C57 (18-23g) and DBA/2 strains (18-22g), the group size was 6 or greater in all cases.

For determination of the contractile responses to angiotensin II, ascending colon from the same mouse strains was suspended in Krebs' solution, at 32°C, under a resting tension of 0.5g. Isometric contractile responses to angiotensin II ($10^{-12}$ - $10^{-7}$ M, non-cumulative) were recorded using a contact time of 60s and a 3-minute dose cycle (n = 7-9).

Diazepam significantly increased the amount of time that the mice spent on the open arms in C57 mice (435 ± 71% of control, $p < 0.002$) and in BKW mice (370 ± 101% of control, $p < 0.03$) but had no significant effect in DBA/2 mice (166 ± 32%, $p > 0.1$) nor in CD mice (118 ± 53%, $p > 0.7$). Losartan ($10mg.kg^{-1}$) similarly produced a significant effect in BKW mice (329 ± 60%, $p < 0.01$), but even at $20mg.kg^{-1}$ had no significant effect in either C57 (85 ± 29%, $p > 0.8$); DBA/2 (92 ± 33, $p > 0.8$) or CD mice (93 ± 33%, $p > 0.8$).

Assessment of angiotensin activity in vitro also highlighted differences between the strains. The maximal tension developed in response to angiotensin in tissues from BKW mice was 6.4 ± 0.6 g per g of tissue, with an $EC_{50}$ of $6.76 \times 10^{-10}$ M, the maximal responses from tissues from the other strains were 8.44 ± 0.91; 10.15 ± 1.57 and 12.57 ± 1.50 g per g of tissue for DBA/2, C57 and CD strains with $EC_{50}$ values of $10.96 \times 10^{-10}$, $7.59 \times 10^{-10}$ and $1.12 \times 10^{-10}$M respectively.

These results reiterate the importance of strain in studies of this nature and extend and support the work of Barnes et al., (1990) by demonstrating an anxiolytic-like action of losartan, albeit strain dependent. Furthermore, these results indicate that there are strain differences in the contactile responses of ascending colon to angiotensin II, and suggest that the susceptibility of the BKW strain of mouse to the behavioural effects of losartan may be related to a more generalized decreased responsiveness to angiotensin II in this strain.

Barnes, N.M., Costall, B., Kelly, M.E. *et al.* (1990) *Neuroreport* 1, 15-16
Shepherd, J., Bill, D.J., Dourish, C.T. *et al.* (1996) *Psychopharmacology* 126, 206-218
Stephens, D.N., Meldrum, B.S., Weidmann, R. *et al.* (1986) *Psychopharmacology* 90, 166-169

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## 337P PROLONGED CHANGES IN MONOAMINES AND METABOLITES IN THE VENTRAL TEGMENTAL AREA AFTER CHRONIC ETHANOL TREATMENT

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Long-term changes in brain function may be responsible for the frequent relapses back into drug taking which are a major problem in addicts. We have been examining prolonged behavioural and neuronal changes that might be involved in such relapses. We showed prolonged increases in the effects of amphetamine and cocaine on locomotor activity after chronic ethanol administration (Manley and Little, 1997). The behavioural changes were seen 24h, 6 days and 2 months after cessation of chronic ethanol consumption. Electrophysiological recordings showed decreases in firing rate of neurones in the ventral tegmental area at 24h and at 6 days, but not at 2 months after cessation of ethanol intake (Bailey et al., 1997). We now investigate the effects of chronic ethanol administration on the monoamine content and turnover in the ventral tegmental area (VTA), an area considered to play a major role in drug dependence and relapse, and for comparison, in the striatum.

Male TO mice (20-25g) were given ethanol by liquid diet. Ethanol-treated mice received control diet for 3 days, followed by 3.5% v/v ethanol diet for 2 days, rising to 5%, then 8% ethanol, each for 9 days (average daily intake was 22-30 g/kg). Controls were pair-fed equi-calorific control diet. N values were 7-9 per treatment group. At the end of the ethanol treatment, laboratory chow was given to all animals. Tissue samples were taken at 24h, 6 days and 2 months after the end of the ethanol treatment. The brains were dissected, frozen immediately on dry-ice, homogenised in 0.1M perchloric acid, and spun at 12000g for 15 minutes. Levels of noradrenaline (NA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed by HPLC (Leung &Tsao, 1992). Protein was assayed by the Lowry method.

Increases in NA and DA content, compared with controls, were seen in VTA samples at the 24h interval after alcohol treatment and decreases in the ratios of DOPAC/DA and HVA/DA.The increase in NA was still there at 6 days, but the other changes were not. At the 2 month interval, tissues from ethanol-treated animals showed a decrease in DOPAC/DA ratio. Corresponding changes were not seen in striatal tissue, except a decrease in DA, DOPAC and HVA content 24h after ethanol withdrawal. The results indicate changes in monoamine turnover are still seen up to 2 months after cessation of chronic ethanol consumption. The majority of the changes were found only in the VTA suggesting a possible link with relapse drinking

Leung, P. Y. & Tsao, C. S. (1992) *J. Chromatography,* 576, 245-254
Manley, S.J. & Little, H.J. (1996) *J. Pharmacol. Exp There.* In Press
Bailey, C.P., Molleman, A. & Little, H.J. 1997) *Br. J. Pharmacol.* In Press

Table 1.Mean ± s.e.m. for VTA samples; DA & NA are ng/mg protein, other values are ratios, *P<0.05; **P<0.01 cf controls

| Chronic treatment | DA 24h | NA 24h | DOPAC/DA 24h | HVA/DA.24h | DA 6 day | NA 6 day |
|---|---|---|---|---|---|---|
| Control diet | 2.6 ± 0.1 | 13.3 ± 0.7 | 0.77 ± 0.05 | 0.85 ± 0.06 | 3.3 ± 0.2 | 15.3 ± 0.6 |
| Ethanol diet | 3.4 ± 0.2* | 15.7 ± 0.6* | 0.61 ± 0.02* | 0.66 ± 0.04** | 3.8 ± 0.3 | 19.2 ± 1.3* |
| Chronic treatment | DOPAC/DA 6 day | HVA/DA 6 day | DA 2 month | NA 2 month | DOPAC/DA 2 month | HVA/DA 2month |
| Control diet | 0.78 ± 0.03 | 0.43 ± 0.07 | 5.4 ± 0.6 | 28.9 ± 3.4 | 0.75 ± 0.03 | 0.64 ± 0.06 |
| Ethanol diet | 0.74 ± 0.05 | 0.77 ± 0.2 | 5.6 ± 0.7 | 22.7 ± 2.9 | 0.52 ± 0.04** | 0.50 ± 0.04 |

## 338P AUGMENTATION OF THE QUANTAL RELEASE OF ACETYLCHOLINE FROM RAT MOTOR NERVE TERMINALS BY THE CHOLINOCEPTOR AGONIST, DMPP

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Nicotinic acetylcholine (ACh) receptor antagonists produce a wide variety of effects on ACh release from motor nerve terminals (Prior *et al.*, 1995). These are mostly interpreted in terms of an action of the compounds on prejunctional nicotinic ACh autoreceptors. Considering this hypothesis, it should be possible to produce the opposite effects to the antagonists using nicotinic ACh agonists. Indeed, radiolabel [$^3$H]ACh overflow studies have been able to show an augmenting effect of nicotinic ACh receptor agonists on ACh release (Wessler, 1989). However, agonist effects on evoked quantal ACh release determined using electrophysiological analysis have not been reported. The purpose of the present study was to determine if, as would be predicted from the autoreceptor hypothesis, a nicotinic ACh receptor agonist 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) has an effect on the evoked quantal release of ACh determined from electrophysiologically recorded endplate currents (e.p.cs) and miniature endplate currents (m.e.p.cs).

All experiments were performed on the rat hemidiaphragm/phrenic nerve preparation (Sprague-Dawley rats, 150 - 200 g) using a standard two-microelectrode voltage clamp technique. At each motor endplate studied, spontaneoulsy occurring m.e.p.cs and evoked e.p.cs (elicited at 50 Hz for 2 s) were recorded before and after exposure of the preparation to 1 μM DMPP for 5 minutes. Evoked quantal ACh release (the e.p.c. quantal content) was determined from the ratio of the amplitudes of e.p.cs and m.e.p.cs.

DMPP (1 μM) had no effect on the time course or frequency of occurrence of m.e.p.cs (Table 1). However, there was a small, but consistent and statistically significant, decrease in the peak amplitude of m.e.p.cs after exposure to DMPP.

**Table 1:** Effect of 1 μM DMPP on m.e.p.cs. (All data, n=8. **P*<0.05, control versus 1 μM DMPP).

| | Control | 1 μM DMPP |
|---|---|---|
| Peak amplitude (nA) | 2.1 ± 0.2 | 1.7 ± 0.1* |
| Time constant of decay (ms) | 0.49 ± 0.03 | 0.48 ± 0.03 |
| Frequency of occurrence (Hz) | 2.8 ± 0.6 | 2.5 ± 0.4 |

In spite of its effect on m.e.p.c. amplitude, 1 μM DMPP had no effect on e.p.c. amplitude at any point in the 2 s train of responses. These imply that there was an increase in the e.p.c. quantal content throughout the period of stimulation (Table 2). Binomial analysis of e.p.c amplitude variance revealed that this increase in e.p.c. quantal content was due to changes in the size of the pool of quanta available for release rather that the probability of release of each quantum.

**Table 2:** Effect of DMPP on e.p.c. amplitude and quantal content. All data are percent control, n=8. **P*<0.05 versus 100%).

| | 1$^{st}$ e.p.c. | 21$^{st}$ - 100$^{th}$ e.p.cs |
|---|---|---|
| E.p.c. amplitude | 103 ± 10 | 102 ± 10 |
| E.p.c. quantal content | 122.0 ± 7.6* | 120.6 ±8.6* |

In conclusion, our data confirm that, in agreement with overflow studies and the autoreceptor hypothesis, nicotinic agonists such as DMPP can augment the electrophysiologically determined release of ACh from rat motor nerve terminals. However, further studies need to be conducted to determine the nature of this augmentation.

Prior, C. *et al.* (1995). *Gen. Pharmacol.*, **26,** 659-666.
Wessler, I. (1989). *Trends Pharmacol. Sci.*, **10,** 110-114.

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## 339P MODULATION OF VECURO-NIUM-INDUCED TETANIC FADE IN THE MOUSE HEMIDIAPHRAGM BY THE PHOSPHODIESTERASE INHIBITOR, RO20,1724

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Nicotinic acetylcholine (ACh) receptor antagonists cause waning of tetanic responses in skeletal muscle. This is a prejunctional effect which has been widely interpreted in terms of an inhibitory action at nerve terminal nicotinic autoreceptors (Bowman *et al.*, 1990). However, inhibitors of adenosine $A_1$ receptors attenuate nicotinic antagonist-induced tetanic fade (Prior *et al.*, 1997), suggesting that a component of fade may be due to a prejunctional inhibitory effect of endogenous adenosine. The activation of adenosine $A_1$ receptors is linked to decreased adenylyl cyclase activity, and hence reduced intracellular cAMP. Therefore, we decided to study directly the link between intracellular cAMP levels and the prejunctional effects of nicotinic antagonists by determining the effects of the phosphodiesterase inhibitor 4-[(3-butoxy-4-methoxyphenyl) methyl]-2-imidazolidinone (RO-20,1724) on vecuronium-induced tetanic fade.

Tetanic (50 Hz for 2 s) and twitch (0.1 Hz) responses were recorded from the mouse hemidiaphragm/phrenic nerve preparation using a standard *in vitro* isometric tension recording setup (32°C, Krebs-Henseliet solution, Balb/c mice, 20 - 25 g). Each muscle preparation was exposed (for a minimum of 30 min) to a concentration of vecuronium that produced approximately 40 - 50 % fade of the tetanic responses (range: 0.18 - 0.23 μM). Subsequently, preparations were exposed to RO-20,1724 (5 μM) for 25 minutes and the effect that this had on the vecuronium-induced tetanic fade was determined.

**Table 1:** Effects of RO-20,1724 on muscle tension parameters in the vecuronium-treated mouse hemidiaphragm preparation.

| | Twitch (% control) | Peak tetanus (% control) | Tetanic fade (%) |
|---|---|---|---|
| VEC | 96.2 ± 3.5 | 84.3 ± 10.3 | 42.3 ± 2.4 |
| VEC + RO | 90.8 ± 4.2 | 83.8 ± 9.4 | 34.0 ± 3.3* |

Table 1 shows the effects of vecuronium alone (VEC) and vecuronium with 5 μM RO-20,1724 (VEC + RO) on the measured twitch and tetanic parameters (mean and s.e.mean, n=7). Vecuronium alone produced an approximate 5 - 10 % decrease in twitch force, a 15 - 20% decrease in the peak force of the tetanus and around 40 % tetanic fade. RO-20,1724 had no further effect on the peak force of twitch responses at 0.1 Hz or on the peak force of the 50 Hz tetanic contractions. However, RO-20,1724 produced a significant (**P* < 0.05, paired Student's *t* test) attenuation of vecuronium-induced tetanic fade.

Our current results are similar to those previously reported for the adenosine $A_1$ receptor antagonist DPCPX (Prior *et al.*, 1997). Thus they are consistent with the hypothesis that a component of vecuronium-induced tetanic fade is due to a depression of intracellular levels of cAMP following the activation of prejunctional adenosine $A_1$ receptors by endogenous adenosine.

Bowman, W. C. *et al.* (1990). *Ann. N. Y. Acad. Sci.*, **604,** 69-81.
Prior, C. *et al.* (1997). *Eur. J. Pharmacol.*, **327,** 103-108.

## 340P OPTIMISATION OF CONDITIONS FOR BINDING OF $^3$H-QNB TO MUSCARINIC RECEPTORS IN SHEEP CILIARY MUSCLE AND IRIS SPHINCTER

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Binding studies of muscarinic receptors on ciliary muscle (CM) and iris sphincter (IS) have been hampered by low specific binding (SB) and high non-specific binding (NSB), caused by melanin pigment. Furthermore, few studies have compared the IS and CM under identical experimental conditions (Gupta *et al*, 1994). In the present study we have examined the influence of experimental conditions upon muscarinic receptor binding in order to establish a protocol for further studies on these intra-ocular tissues.

CMs and ISs were separately dissected from adult female sheep (≤6 hours post mortem), homogenised on ice, centrifuged at 4°C and the pellets resuspended in 25mM Hepes-Krebs' buffer (pH 7.4), to an approximate concentration of 1CM/10ml or 1IS/2ml. Membranes were incubated at 37°C with $^3$H-quinuclidinyl-benzilate (QNB, 0.16-5.0nM, 47Ci/mmol), buffer and 10μM atropine (to define NSB) in a final assay volume of 1ml. Binding was terminated by rapid filtration through a Brandel cell harvester and bound radioactivity was counted by liquid scintillation spectrometry. The influence of tissue preparation, conditions for binding and counting upon CM binding characteristics were investigated, and confirmed in several cases for IS. Definitive $K_D$, $B_{max}$ and $n_H$ values for CM and IS tissue were obtained once appropriate conditions were established. Relative binding levels are expressed as mean dpm/mg protein (for $^3$H-QNB=5nM) or % change in relative binding levels where indicated (n≥3). Analyses for saturation are expressed as mean±s.e.m. Differences were tested for significance by 1-way ANOVA or t-tests.

Definitive SB of $^3$H-QNB was 28.9% of total in both tissues, typically 57558 and 29823 dpm/mg protein (for CM and IS respectively). Overnight soaking (16 hours) of CM and IS tissue in 10mM chloroquine (reported to reduce melanin-related NSB; Matsuo & Cynader, 1992) had no significant effect upon SB levels, whereas NSB levels were significantly reduced by 67% and 95% respectively ($p<0.001$). The chloroquine concentration had no significant effect on specific binding in CM and IS, but NSB increased in CM by 21% where 5mM was used instead of 10mM (15mM was not significantly different from 10mM). All subsequent results were thus based on pre-soaking the tissue in 10mM chloroquine before use. Homogenisation time was optimum at 2x5 secs, since increasing the homogenisation time to a maximum 4x20 secs significantly reduced SB by 58% ($p<0.01$) and had no effect on NSB. The optimal centrifugation procedure was for 30 mins at 100,000g (4°C). Where centrifugation was prolonged or the pellet was resuspended and centrifuged for a second time, the SB of the pellet was reduced by 56% ($p<0.05$) and the NSB increased by 16% ($p<0.05$). The final protein concentration in the assay was limited to 0.08mg/ml, where the total assay volume is 1ml. Although an increase in protein concentration exhibited a small increase in relative SB ($p<0.05$), the filtration time of the assay was significantly increased for final concentrations >0.08mg/ml (from 2.75±0.28 secs to 8.58±0.53 secs, $p<0.001$).

Hepes-Krebs' buffer was selected for use since it produced significantly less NSB (96026) than other aqueous substitutes: Barany's solution (116722), Balanced Salt Solution (Alcon) (149449) ($p<0.001$). There was no difference in SB between these buffers. Binding was optimal if the assay incubation period was ≥40 mins, since shorter times reduced SB by 69% ($p<0.001$). NSB was significantly reduced by 69% with incubation times <60 mins ($p<0.001$). Although not significant, SB was 36% higher and NSB 22% lower when the filter washing temperature was 4°C rather than 20°C. Filters were washed twice at the most, since it was found that more washes increased NSB by 54% ($p<0.001$). Presoaking the filters in 0.0003% polyethylenimine significantly reduced binding to the filters by 0.15% ($p<0.005$). When preparing filters for counting, storage in ≥10ml scintillant was considered optimal since smaller volumes increased the NSB levels by ,12%, $p<0.05$). Leaving the samples for less than 8 hours before counting resulted in reduced SB (by 36%) and NSB (by 25%, $p<0.05$). Utilising the above optimal conditions, definitive binding studies (n=10) indicated saturable SB for both tissues. Further analysis calculated $K_D$s of 0.55±0.11nM and 0.48±0.05nM for IS and CM respectively, $B_{max}$ of 320±85fmol/mg for IS and 623±73 for CM. Hill coefficients were not significantly different from unity (0.98±0.05 and 0.99±0.01 respectively). The experiments described here allowed identification of the optimum conditions for future radioligand binding analysis of intra-ocular muscle tissue (German *et al*, 1997).

Gupta, N., *et al* (1994) *Br. J. Ophthalmol.* 78:555-559
Matsuo, T., Cynader, M.S., (1992) *Ophthalmic Res.* 24:213-219
German, E.J., Wood, D., Hurst, M.A., (1997) *This meeting.*

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## 341P ASSESSMENT OF MUSCARINIC RECEPTOR SUBTYPES IN THE SHEEP IRIS SPHINCTER AND CILIARY MUSCLE BY RADIOLIGAND BINDING TECHNIQUES

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In current ophthalmic and optometric practice anti-muscarinic agents are frequently employed for mydriasis and cycloplegia. Although their actions are required in quite different clinical circumstances, these compounds produce both effects simultaneously (albeit in varying degrees) often causing discomfort and inconvenience. If cycloplegic and mydriatic effects could be separated, many unnecessary side effects could be avoided. In the current study the receptor subtype in the target tissues (sheep iris sphincter (IS) and ciliary muscle (CM), believed to be the $M_3$ subtype: Gabelt & Kaufman, 1994) was investigated under identical conditions using a radioligand binding technique adapted for intra-ocular muscle tissue (German *et al.*, this meeting).

A protocol previously described (German *et al.*, this meeting) for membrane preparation and experimental conditions was followed, using ciliary muscles (CM) and iris sphincters (IS) from adult female sheep. ISs and CMs were dissected (≤6 hours post mortem) and soaked in 10mM chloroquine at 4°C overnight (16 hours). Tissues were then rinsed twice in 25mM Hepes-Krebs' (HK) buffer (pH7.4, 4°C), homogenised on ice for 2x5secs, centrifuged for 30mins at 100,000g (4°C) and resuspended in the same buffer to an approximate initial protein concentration of 0.32mg/ml (1CM/10ml or 1IS/2ml). For saturation studies, membranes were incubated for 45 mins at 37°C with $^3$H-quinuclidinyl-benzilate (QNB, 0.16-5.0nM, 47Ci/mmol), HK buffer and 10μM atropine (to define NSB) in a final assay volume of 1ml. In competition studies, HHSiD (hexahydrosiladifenidol), pfHHSiD (p-fluoro analogue of HHSiD), methoctramine, pirenzepine, AFDX-116 (11-({2-[(diethylamino)-methyl]-1-piperidyl}acetyl)-5,11-dihydro-6H-pyridol[2,3-b][1,4]benzodiazepine-6-one), telenzepine, 4-DAMP (4-diphenylacetoxy-N-methyl-piperidine methiodide) and gallamine were used as competing drugs; tissue was incubated with $^3$H-QNB (0.5nM), HK buffer and the competing drug ($10^{-9}$-$10^{-4}$M). Binding was halted by rapid filtration through filters presoaked in 0.0003% polyethylenimine, followed by washing with HK buffer twice at 4°C, using a Brandel cell harvester. Filters were immersed in 10ml scintillant and left for a minimum of 8 hours before the bound radioactivity was counted by scintillation spectrometry. Saturation, Scatchard and Hill plots/analysis of data derived $K_D$, $B_{max}$ and $n_H$ values for CM and IS tissue, which are expressed as mean±s.e.m (n=10). Analysis of competition curves yielded affinity estimates (mean pKi ± s.e.m., n≥2) for all compounds.

Initial binding studies indicated the specific binding of $^3$H-QNB to receptors was saturable (28.9% of total in both tissues). Dissociation constants were similar for both tissues ($K_D$ = 0.55±0.11nM (IS), 0.48±0.05nM (CM)) but $B_{max}$ was almost 2-fold greater in the CM (623±73fmol/mg) compared with the IS (320±85fmol/mg). Binding to a single population of sites was likely since Hill coefficients were not significantly different from unity (0.98±0.05 and 0.99±0.01 respectively). Competition between various drugs and $^3$H-QNB for muscarinic binding sites is outlined in Table 1. Telenzepine displayed a 1.4-fold higher affinity for CM receptors, and pfHHSiD showed a 2.5-fold greater affinity for IS tissue. However, there was a high correlation between affinities for IS and CM tissue ($r=0.96$, $p<0.05$), and the rank order of potencies was identical for both tissues.

Table 1. Affinity estimates of muscarinic antagonists for receptors in sheep IS and CM tissue (mean ± s.e.m, n≥2).

| | IS pKi | CM pKi | $Ki_{IS}/Ki_{CM}$ |
|---|---|---|---|
| 4-DAMP | 7.35 ± 0.09 | 7.21 ± 0.11 | 0.74 |
| telenzepine | 6.86 ± 0.03 | 7.00 ± 0.07 | 1.41 |
| HHSiD | 6.44 ± 0.03 | 6.23 ± 0.12 | 0.67 |
| pfHHSiD | 6.18 ± 0.08 | 5.79 ± 0.01 | 0.41 |
| pirenzepine | 5.84 ± 0.24 | 5.64 ± 0.07 | 0.68 |
| AFDX-116 | 5.49 ± 0.08 | 5.45 ± 0.06 | 0.97 |
| methoctramine | 5.46 ± 0.11 | 5.34 ± 0.08 | 0.77 |
| gallamine | 5.09 ± 0.10 | 5.17 ± 0.05 | 1.28 |

The results suggest that the receptor subtype in both the IS and CM is likely to be the same ($M_3$ subtype), although the number of binding sites is much greater in the CM.

Gabelt, B.T., Kaufman, P.L. (1994) *Exp. Eye Res.* 58:623-630
German, E., Wood, D., Hurst, M. (1997) *This meeting.*
Matsuo, T., Cynader, M.S., (1992) *Ophthalmic Res.* 24:213-219

## 342P POSTSYNAPTIC MODULATION OF DEPOLARIZING $GABA_A$ RECEPTOR-MEDIATED IPSPs IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONES

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During periods of repetitive synaptic activation both hyperpolarizing ($IPSP_H$) and depolarizing ($IPSP_D$) $GABA_A$ receptor - mediated responses can be evoked (Staley *et al.*, 1995). Previously, we have demonstrated that these responses are susceptible to similar regulation by presynaptic receptors (Manuel & Davies, 1997). However, it is uncertain whether both responses result from activation of identical or different $GABA_A$ receptors. In an attempt to address this question we have investigated the effects on $IPSP_D$ of a number of agents that are known to affect $IPSP_H$.

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

Alternate stimulation in s. oriens and s. radiatum every minute evoked reproducible $IPSP_D$s. Application of the GABA uptake inhibitor diphenylmethanone, 0-[2-(3-carboxy-1,2,5,6-tetrahydro-1-pyridinyl) ethyl]oxime, HCl (NNC 05-0711, 10 μM), zolpidem (30 μM), propofol (50 μM) and CGP55845A (1 μM) increased the amplitude and duration of the $IPSP_D$. Thus, NNC 05-0711 increased control $IPSP_D$s evoked by stimulation in s. oriens and s. radiatum to (mean ± s.e.mean) 227 ± 23 (n = 4, $p<0.01$) and 232 ± 40% (n = 3, $p<0.05$), respectively, zolpidem to 143 ± 11 (n = 3, $p<0.05$) and 159 ± 18 % (n = 3, $p<0.05$), propofol to 141 ± 12 (n = 4, $p<0.05$) and 138 ± 14% (n = 4, $p<0.05$), and CGP55845A to 156 ± 8 (n = 4, $p<0.01$) and 162 ± 16% (n = 4, $p<0.05$). In contrast, the benzodiazepine inverse agonist methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (1 - 10 μM) reduced the amplitude of both $IPSP_D$s, reducing s. oriens and s. radiatum responses by 26 ± 10 (n = 3) and 17 ± 4 % (n = 4, $p<0.01$), respectively.

These data indicate that $IPSP_D$ evoked by repetitive stimulation in s.oriens and s. radiatum is regulated by positive and negative allosteric modulators that affect $IPSP_H$.

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Manuel, N.A. and Davies, C.H. (1997) *Br. J. Pharmacol.* (In press)
Staley, K.J., Soldo, B.L. & Proctor, W.R. (1995) *Science*, **269**, 977-981.

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## 343P MECHANISMS FOR POTENTIATION OF $GABA_A$ RECEPTORS BY CIS-9,10-OCTADECENOAMIDE (COA) IN CULTURED RAT CORTICAL NEURONES

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cOA was first isolated from the cerebrospinal fluid of sleep deprived cats and induced physiological sleep when injected, i.p., into laboratory rats (Cravatt *et* al, 1995). The mechanism of action of cOA is unknown although it has been shown to modulate recombinant $5HT_2$ expressed in *Xenopus* oocytes (Huidobro-Toro & Harris, 1996). In the current study we have characterised the mechanism for enhancement of GABA-activated chloride currents by cOA.

Whole-cell recordings were obtained from pyramidal cells in cultured cortical neurones (22-24°C): exogenous GABA was applied via a Y-tube as 0.1-1s pulses using established techniques and saline solutions (Lees & Leach, 1993). cOA was dissolved initially in dimethylsulphoxide (DMSO). All extracellular solutions contained 0.1% DMSO, 0.033% bovine serum albumin (to facilitate dissolution of the lipid) with, or without, 20μM cOA. Curves were fitted by non-linear regression to a three-term logistic equation (Graph Pad software). Data are cited as mean ± s.e.mean and were analysed statistically as indicated below. $p<0.05$ was taken as significant.

At a holding potential of -45mV, GABA evoked fast outward currents in all cells which saturated across the range 1-500μM (n= 46: max. current 48.9 ± 2.89 pA/pF). Superfusion of 20μM cOA directly evoked small reversible outward current shifts (0.61 ± 0.16 pA/pF) in only 3 of these cells. Responses to subsaturating GABA concentrations were markedly enhanced by cOA (maximum enhancement *circa* 2 fold, at 3.2μM GABA). This fully reversible modulatory effect peaked within 10-12 minutes of superfusion and was characterised at equilibrium. cOA significantly ($p<0.05$) enhanced the affinity of GABA for its receptor: in control saline GABA $EC_{50}$ was 26.5μM (95% C.I., 22.7-31.0); in the presence of 20μM cOA it was reduced to 17.0μM (C.I., 11.5-25.2). The Hill coefficient for GABA was *circa* 1 and was not significantly altered by cOA. The maximal response to GABA (500μM) was marginally but not significantly (two-tailed, unpaired t-test on maxima from curve fits) enhanced by cOA.

Cells were voltage-clamped between -90 and 0mV (30mV increments) and currents were evoked in response to 3.2μM GABA in control saline then in the presence of 20μM cOA. The reversal potential for outwardly rectifying GABA-evoked currents (*circa* -58mV) was not altered by cOA in any of the cells examined (n=4, $p>0.05$, paired t-test). The extent of enhancement of $Cl^-$ current at 3.2 μM GABA at -90 (1.63x), -30 (1.87x) and at 0mV (1.25x) was not voltage dependent (one-way ANOVA).

The brain lipid cOA enhances chloride currents through the $GABA_A$ receptor by enhancing agonist affinity but without affecting the driving force for the permeant ion. These modulatory effects were independent of transmembrane voltage and may, at least in part, underpin the hypnotic effects of this molecule.

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Cravatt, BF, et al (1995) Science 268, 1506-1509.
Huidobro-Toro, JP & Harris, RA (1996) PNAS, USA 93, 8078-8082.
Lees, G & Leach, MJ (1993) Brain Res., 612, 190-199.

## 344P $GABA_B$ RECEPTORS IN A RAT MODEL OF ABSENCE EPILEPSY: AN AUTORADIOGRAPHIC STUDY USING [3H]-CGP 62349

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The Genetic Absence Epilepsy Rat from Strasbourg (GAERS) has been validated as a model of human absence epilepsy. There is convincing evidence that excessive GABA-mediated inhibition may underlie absence seizures, with the effectiveness of $GABA_B$ receptor antagonists at blocking these seizures suggesting that this receptor sub-type, in particular, may play an important role. Using [$^3$H]GABA as a ligand, we have previously shown no change in either $GABA_A$ or $GABA_B$ binding affinity or receptor number in GAERS as opposed to non-epileptic controls (Knight and Bowery, 1992) although the low affinity of the ligand may not have detected more subtle changes. Recently, a high affinity $GABA_B$ receptor antagonist radioligand has been described, [$^3$H]CGP 62349 (Bittiger et al., 1996) and the present study has employed this new ligand to investigate $GABA_B$ receptor binding characteristics in several brain regions of GAERS.

Female adult GAERS (n=6) and non-epileptic controls (n=6) were sacrificed by decapitation and the brains immediately removed and stored at -80°C. Coronal sections (10 μm), -2.1 to -2.8 mm posterior to bregma, were prepared at -15°C to -20°C. Sections were pre-incubated (20 then 60 minutes) in assay buffer (50 mM TRIS/HCl, pH 7.4, 2.5 mM $CaCl_2$) before incubation for 60 min at 25°C with [$^3$H]-CGP 62349 (0.125 - 8nM). Non-specific binding was determined with 10 μM CGP 54626 (Bittiger et al., 1992). Slides were washed for 2 x 1 minute in assay buffer at 25 °C, dipped briefly in distilled water, air dried and apposed to [$^3$H]-sensitive film for 3 weeks. Resulting images were analysed on an MCID M4, and optical density converted to fmol/mg using calibrated polymer standards. Seven brain regions (centromedial thalamus (CMT), ventrolateral thalamus (VLT), reticular thalamic nucleus (Rt), striatum (STR), hippocampus (CA1), inner cortex (laminae V-VI) (IC) and outer cortex (laminae I-IV) (OC)). were identified using a stereotaxic atlas of the rat brain and analysed. Values of Kd and Bmax were determined using non linear regression (GraphPad Prism).

Table: [$^3$H]CGP 62349 binding in GAERS and controls.

| Region | Bmax (fmol/mg) | Kd (nM) | Bmax (fmol/mg) | Kd (nM) |
|---|---|---|---|---|
| | GAERS | GAERS | Controls | Controls |
| CMT | 585 ± 51 | 4.34 ± 0.64 | 692 ± 35 | 4.77 ± 0.81 |
| VLT | 656 ± 50 | 5.26 ± 0.45 | 671 ± 20 | 4.86 ± 0.57 |
| Rt | 92 ± 6 | 1.16 ± 0.09 | 115 ± 13 | 1.39 ± 0.19 |
| STR | 243 ± 37 | 2.00 ± 0.35 | 261 ± 28 | 2.58 ± 0.67 |
| CA1 | 301 ± 12 | 1.89 ± 0.09 | 315 ± 13 | 2.01 ± 0.48 |
| IC | 325 ± 22 | 2.40 ± 0.19 | 321 ± 25 | 2.42 ± 0.42 |
| OC | 417 ± 33 | 2.01 ± 0.11 | 476 ± 29 | 2.80 ± 0.96 |

Data are expressed as mean ± s.e.mean. No significant differences were found, in either Bmax or Kd, between GAERS and controls in any of the brain regions investigated (Student's unpaired t-test). However, it should be noted that our Kd values were higher than those previously reported using cortical synaptosomal membrane fractions (Bittiger et al., 1996).

Our previous finding that $GABA_B$ receptor density and affinity were unchanged in GAERS (Knight and Bowery, 1992) has been confirmed by the present study which employed a higher affinity ligand which additionally, as an antagonist, would detect all receptor affinity states.

Bittiger, H. *et al*, (1992) *Pharmacol. Commun.*2, 23.
Bittiger, H. *et al*, (1996) *Pharmacol. Rev. Commun.* 8, 97-98.
Knight, A.R. and Bowery, N.G. (1992) *J. Neural. Transm.* 35, 189-196.

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## 345P $GABA_B$ RECEPTORS IN SPINAL CORD AND DORSAL ROOT GANGLIA OF SCIATIC NERVE AXOTOMISED AND CONTROL RATS: LOCALIZATION OF RECEPTOR PROTEIN BY AUTORADIOGRAPHY USING [3H]-CGP62349

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$GABA_B$ receptor activation of pain fibre primary afferent terminals within the spinal cord reduces the release of nociceptive transmitters and this may, in part, be the basis for the analgesic action of baclofen in acute pain models. However, in chronic pain $GABA_B$ receptor agonists have only limited antinociceptive activity and one reason for this may be the plasticity in the $GABA_B$ receptor system. To examine this we have initially chosen a neuropathic pain model to determine the level of $GABA_B$ receptor binding in spinal cord (SC) and dorsal root ganglia (DRG) using an autoradiography technique with the tritiated $GABA_B$ antagonist [$^3$H]-CGP62349 (Bittiger, *et al.*, 1996). Previous studies have demonstrated that the $GABA_B$ receptor is highly localised in superficial laminae (laminae I-III of the dorsal horn), of which 50% appear to be associated with presynaptic terminals (Price *et al.*, 1984).

Lumbar SC and DRG (ipsilateral and contralateral) were obtained from male Wistar rats (200-250g) 17 days after complete section (mid thigh level) of the left sciatic nerve and frozen in liquid nitrogen in isopentane over dry ice. Tissue was then stored at -80°C. Cryostat sections (10 μm) were prepared at -15°C to -20°C, and stored at -80°C until assay. Sections were pre-incubated (20 then 60 minutes) in assay buffer (50 mM TRIS/HCl, pH 7.4, 2.5 mM $CaCl_2$) before incubation for 60 min at 25°C with [$^3$H]-CGP62349 (0.08 nM - 8 nM). Non-specific binding was determined with 10 μM of another $GABA_B$ antagonist, CGP54626 (Bittiger, *et al.*, 1992). Slides were washed for 2 x 1 minute in assay buffer at 25 °C, dipped briefly in distilled water and air dried. Microscope coverglasses dipped in Ilford K-5 emulsion / 2% glycerol solution (0.67 g emulsion / 1ml 2% glycerol) were dry apposed to the sections for 2 weeks before development. Sections were then stained in 0.1% methylene blue and resulting silver grains / stained sections analysed under oil immersed light field conditions using Polyvar microscope / MCID M4. Values of Kd and Bmax were determined using non linear regression analysis (GraphPad Prism). Data are expressed as mean ± s.e.mean. ($^*$P<0.05 using unpaired t-test)

Table 1; [$^3$H]-CGP623349 binding in SC sections

| Spinal cord Laminae | Mean Bmax (silver grains/1000μm$^2$) *n=3* | | Mean Kd (nM) *n=3* | |
|---|---|---|---|---|
| | Control | Axotomy | Control | Axotomy |
| Superficial (ipsilateral) | 441.1 ± 32.8 | 519.0 ± 38.3 | 1.16 ± 0.19 | 1.16 ± 0.51 |
| Superficial (Contralateral) | 418.1 ± 46.5 | 445.3 ± 43.1 | 0.97 ± 0.22 | 1.13 ± 0.23 |
| Deep Ventral | 48.5 ± 3.5 | 38.8 ± 18.4 | 1.44 ± 0.57 | 2.14 ± 0.59 |

$GABA_B$ receptor binding in SC was highly localised in superficial laminae, with a much lower density observed in the deeper, ventral, laminae. No difference in $GABA_B$ receptor Bmax or Kd values was observed between control and axotomised animals either ipsilateral or contralateral to the nerve section (Table 1).

Table 2; [$^3$H]-CGP623349 binding in DRG sections

| Dorsal root ganglia | Mean Bmax (silver grains/1000μm$^2$) *n=3* | Mean Kd (nM) *n=3* |
|---|---|---|
| Control | 764.3 ± 17.1 | 0.69 ± 0.05 |
| Ipsilateral | 640.9 ± 26.3$^*$ | 0.35 ± 0.03$^*$ |

However, in DRG, $GABA_B$ receptor density and Kd values were significantly (P<0.05) reduced ipsilateral to nerve section when compared to control tissue (Table 2). This small reduction in receptor number in DRG does not appear to be reflected by a change in the dorsal horn and the reason(s) for this have yet to be established.

Bittiger, H. *et al.*, (1996). *Pharmacol. Rev. Comm.*, 8, 97-98.
Bittiger, H. *et al.*, (1992). *Pharmacol. Comm.*, 2, 23.
Price *et al.*, (1984). *Nature.*, 307, 71-74.

## 346P ACTIONS OF THE REMACEMIDE METABOLITES, AR-R12859AA AND AR-R12860AA, ON DORSAL ROOT-EVOKED VENTRAL ROOT POTENTIALS IN THE RAT ISOLATED SPINAL CORD

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*des*-glycinyl remacemide [(±)-1-methyl-1,2-diphenylethylamine] is a major active metabolite of the novel anticonvulsant drug, remacemide (Wamil *et al.*, 1996). Electrophysiological studies using the S(+) and R(-) forms of *des*-glycinyl remacemide, AR-R12859AA and AR-R12860AA respectively, have suggested that they both possess NMDA receptor antagonist activity (Subramaniam *et al.*, 1996). The aim of the current study was to determine the effects of these compounds on the slow component of the dorsal root-evoked ventral root potential and the cumulative depolarisation produced by repetitive stimulation. Both of these synaptically-mediated phenomena are partly dependent on NMDA receptor activation (Thompson *et al.*, 1992).

Hemisected spinal cords, obtained from 10-14 day old Wistar rats anaesthetised with urethane (2 $g.kg^{-1}$), were superfused with oxygenated artificial cerebrospinal fluid (ACSF). A dorsal root (L3 - L6) was sucked into a glass electrode and electrically-stimulated at high intensity (200 μs, 200 μA) to recruit A- and C-fibres. The dorsal root was stimulated either once to obtain a dorsal root-evoked ventral root potential (DR-VRP) or repetitively (2 Hz for 20 s) for a cumulative ventral root depolarisation (CVRD). Synaptically-evoked potentials were recorded from a corresponding segmental ventral root in another suction electrode. AR-R12859AA or AR-R12860AA was superfused for 20min. Quantitative data are presented in Table 1. Neither AR-R12859AA nor AR-R12860AA (50 & 100 μM) had any significant effect on the slow peak amplitude but both caused significant reductions in the duration and the half time to decay of the slow DR-VRP. Both enantiomers (50 & 100 μM) caused similar significant reductions in the amplitude of the CVRD.

In conclusion both the enantiomers, AR-R12859AA and AR-R12860AA, significantly reduced the slow phase DR-VRP and the amplitude of CVRD. Since summation of the NMDA-dependent slow potential is implicated in central sensitisation and hyperalgesia (Woolf and Thompson, 1991) further evaluation of these drugs as potential analgesics is warranted.

*We acknowledge Astra Charnwood for financial support and supplying compounds.*

Subramaniam, S., Donevan, S.D. & Rogawski, M.A. (1996). *J. Pharmacol. Exp. Ther.*, 276, 161-168.
Thompson, S.W.N., Gerber, G., Sivilotti, L.G. *et al.* (1992). *Brain Res.*, 595, 87-97.
Wamil, A.W., Cheung, H., Harris., E.W. *et al.* (1996). *Epilepsy Res.*, 23, 1-14.
Woolf, C. J. & Thompson, S.W.N. (1991). *Pain*, 44, 293-299.

Table 1: Suppression of dorsal root-evoked potentials by the enantiomers AR-R12859AA and AR-R12860AA

| | slow peak amplitude | slow peak half time decay | slow phase duration | CVRD |
|---|---|---|---|---|
| AR-R12859AA 50 μM | 0.8 ± 4.1 | -51.4 ± 4.4* | -51.7 ± 5.7* | -23.9 ± 6.1* |
| AR-R12859AA 100 μM | -0.5 ± 4.8 | -57.1 ± 8.3* | -69.4 ± 7.9* | -35.3 ± 6.7* |
| AR-R12860AA 50 μM | 3.2 ± 6.5 | -25.9 ± 6.7* | -50.6 ± 9.0* | -21.9 ± 2.8* |
| AR-R12860AA 100 μM | -4.2 ± 4.2 | -28.3 ± 4.3* | -59.9 ± 4.7* | -26.8 ± 5.4* |

All values (n = 6) represent percentage change ± s.e.m. from the control.* $P < 0.05$ compared to drug vehicle (ACSF).

## 347P THE EFFECT OF AN ADENOSINE A1 RECEPTOR AGONIST AND ANTAGONIST ON EPSCs EVOKED BY PAIRED STIMULI IN HIPPOCAMPAL CA1 NEURONES

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When pairs of stimuli were applied to single CA3 neurones in slice culture the amplitude of the second excitatory post-synaptic current (EPSC2) recorded in a CA1 neurone was inversely related to the amplitude of the first (EPSC1, Debanne *et al.*, 1996) suggesting that short term depletion of transmitter quanta may occur. In this study the properties of release evoked by single and pairs of stimuli were examined in conventional hippocampal slices before and after exposure to either the adenosine A1 receptor antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX, 30nM) which enhanced transmitter release by blocking the inhibitory action of endogenous adenosine or the agonist 2-Chloro-$N^6$-cyclopentyladenosine (CCPA, 100nM).

EPSCs evoked by minimal stimulation of the stratum radiatum were recorded with the 'blind patch' configuration in rat hippocampal CA1 neurones kept submerged in aCSF containing picrotoxin (100μM) and voltage clamped at -70mV. In 5/5 CA1 neurones DPCPX significantly increased EPSC amplitude ($P < 0.05$, paired t-test) and quantal content $m_f$ (= ln number of stimuli/ number of failures) ($P < 0.02$) evoked by single stimuli. In a further 15 CA1 neurones pairs of stimuli (separation 90 ms) were delivered at a rate of 0.16 - 0.25 Hz. In the presence of DPCPX, the mean amplitude of EPSC1 increased in 5/9 neurones but fell in 4/9 so that the overall increase from 29.5 ± 6.22 pA (s.e.m., n = 9) to 32.6 ± 8.6 pA was not significant. When looking at individual pairs of records, the second response could be larger or smaller than the first and an analysis of all 15 cells showed that the proportion of responses exhibiting paired pulse depression was 25.2 %. In a more detailed analysis of individual cells, the mean amplitudes of the second responses (EPSC2) associated with either EPSC1 greater than the mean or smaller than the mean, were not significantly different. Furthermore, even when the analysis was restricted to the mean amplitudes of EPSC2 associated with EPSC1 in either the top or bottom third of the amplitude distribution, the mean amplitudes did not differ ($P > 0.05$, Mann-Whitney U-test). Similar results were obtained in all 15 neurones. The outcome of a further analysis of paired responses was the same when a second series were recorded in the presence of DPCPX in 9 cells and in a further 6 cells in which the mean amplitude of EPSC1 was significantly decreased in the presence of CCPA ($p < 0.01$, n = 6) from 27.1 ± 3.67pA. to 16.7 ± 3.22pA. The proportion of responses showing paired pulse depression was 25.0 ± 8.2% in DPCPX and 23.8 ± 4.7% in CCPA and neither value was significantly different from control ($P > 0.05$, paired t-test). Furthermore, when the paired-pulse ratio EPSC2/EPSC1 was plotted against EPSC1 for all three treatments in all 15 cells the distributions for CCPA and DPCPX were simply shifted along EPSC1 axis with no clear shift along the EPSC2/EPSC1 axis.

Thus our data are similar to these of Turner *et al* (1997) and show no evidence for an inverse relationship between EPSC1 amplitude and EPSC2 amplitude. This was unaltered when transmitter release was enhanced or depressed by modulation of the adenosine A1 receptor.

References

Debanne, D.*et al* (1996) *J.Physiol.* 491, 163-176.
Turner, D.A.*et al* (1997) *J.Physiol.* 500, 441-461.

## 348P NOCICEPTORS OF THE CAT AND RAT CORNEA ARE NOT EXCITED BY P2X PURINOCEPTOR AGONISTS

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ATP causes pain when applied to a blister base in humans (Bleehan and Keele, 1977) and the recent finding that six of the seven known ATP-gated ion-channel receptors ($P2X_{1-6}$) are expressed in the trigeminal and dorsal root ganglia (Collo *et al.*, 1996) suggests that ATP may play a role in peripheral nociception. The aim of our studies was to establish electrophysiologically whether P2X purinoceptor agonists activate polymodal nociceptors in an avascular preparation, the cat cornea, and to determine if there is a behavioural correlate when the drugs are applied to the rat eye.

Five adult cats were anaesthetised with pentobarbitone (40mg.kg$^{-1}$ i.p., supplemented hourly i.v.) and the trachea and right saphenous vein cannulated. Extracellular recordings from filaments of mixed ciliary nerves with receptive fields in the cornea were performed as described previously (Belmonte *et al.*, 1991). Drugs were applied to the cornea for 30s via solution-soaked tissue. Data are expressed as mean change in action potential frequency ± s.e.mean. For behavioural studies, drug solutions (5µl drop) were instilled into the eyes of conscious male Wistar rats (283±7g; n=4) and the number of blinks and forepaw wipes of the eye, behaviours associated with nociception, were counted in the first minute post-instillation.

Recordings were made from C-fibre polymodal nociceptors (conduction velocity 0.77±0.08ms$^{-1}$, basal discharge 0.20±0.50 impulses.s$^{-1}$, n=9) all of which were excited by a 30s jet of $CO_2$ (2.86±0.40 impulses.s$^{-1}$) and 4 of which were also excited by capsaicin 0.1µM (2.48±0.89 impulses.s$^{-1}$). Application of αβmeATP (30-100µM), ATPγS (100-1000µM) or ATP (100µM) did not cause any significant change in action potential frequency (αβmeATP 100µM; 0.20±0.04 impulses.s$^{-1}$). Similar results were obtained with 29 Aδ fibres (conduction velocity 5.80±0.70ms$^{-1}$). In conscious rats, threshold concentrations of capsaicin and nicotine caused a significant blink response whereas αβmeATP did not (Table 1).

Table 1. Number of blinks and wipes in the first minute post-instillation of vehicles, capsaicin, nicotine and αβmeATP into the rat eye. Mean±s.e.mean. *P<0.01 Mann-Whitney versus vehicles. n = instillations.

| | Blinks | Wipes | n |
|---|---|---|---|
| Basal (events.min$^{-1}$) | 2.3±0.9 | 0±0 | 4 |
| Capsaicin vehicle (EtOH/PBS) | 2.8±2.0 | 0±0 | 5 |
| Capsaicin 10µM | 21.1±2.8* | 2.4±1.1 | 10 |
| Phosphate buffered saline (PBS) | 4.0±1.2 | 0.4±0.4 | 5 |
| Nicotine 1000µM in PBS | 13.5±1.7* | 0.6±0.3 | 12 |
| αβmeATP 10 µM in PBS | 2.3±1.2 | 0±0 | 6 |
| αβmeATP 100 µM in PBS | 3.7±1.7 | 0±0 | 6 |
| αβmeATP 1000 µM in PBS | 5.0±1.5 | 0±0 | 6 |

In conclusion, nociceptors of the cornea were not excited by P2X purinoceptor agonists in our experiments. Our data suggests that functional P2X receptors are not expressed on peripheral terminals of trigeminal afferents in the cat cornea and αβmeATP sensitive P2X receptors, if present, do not stimulate nociceptive responses in the rat cornea. Thus it is unlikely that P2X receptors are involved in pain sensation from the cornea of the cat or rat.

Belmonte, C., Gallar, J., Pozo, M.A. *et al.* (1991) *J. Physiol.*, 437, 709-725.
Bleehan, T. & Keele, C.A. (1977) *Pain*, 3, 367-377.
Collo, G., North, R.A., Kawashima, E. *et al.* (1996) *J. Neurosci.*, 16, 2495-2507.

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## 349P PROTECTION AGAINST KAINATE NEUROTOXICITY BY AN ADENOSINE $A_2$ RECEPTOR ANTAGONIST

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Systemic injection of kainate has been widely used as an effective model for excitotoxic neurodegeneration in specific areas of the brain. We have previously shown that systemic injection of the $A_{2a}$ agonist CGS21680 (2-[p-(2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamido adenosine) protects the hippocampus from kainate-induced excitotoxic damage[1]. This study examines the effects of intrahippocampal injections of kainate alone or in conjunction with CGS21680, adenosine, the $A_{2a}$ receptor antagonist 4-(2-[7-amino-2-{2-furyl} {1,2,4}triazolo{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl)phenol (ZM241385) and the $A_1$ receptor agonist R-N6-phenylisopropyladenosine (R-PIA).

Male Wistar rats (270-310g) were anaesthetised with Equithesin before being placed into a stereotaxic frame. Injections were made in a volume of 1µl through a fine needle into the hippocampus (-3.6mm anterior, +3.2mm lateral, +3.2mm ventral to the bregma). Animals were allowed to recover for seven days before perfusion fixing. The brains were removed, wax embedded and sliced at 6µm. Sections were stained with haematoxylin and eosin. Damage was assessed by quantifying the extent of neuronal loss on a ten-point scale. Results are presented as the percentage damage ± s.e.mean.

Kainate, injected at 0.25nmols in 1µl (0.25mM) showed the highest degree of damage in the CA3a region of the hippocampus (98.8%±1.25, n=4), with a more moderate amount in the CA3b region (50.0%±22.0). The CA1, CA2 and CA4 regions were unaffected or showed negligible neurodegeneration. CGS21680 showed no neuroprotection at concentrations of 2.5, 0.25 or 0.01mM, in either the CA3a (100%±0, n=3, 95%±2.9, n=3, 75%±25.0, n=4 respectively) or CA3b regions (68.3%±8.8, 36.7%±31.8, 71.3%±23.8 respectively). Similarly adenosine at 2.5, 0.25 and 0.01mM, and R-PIA at 0.25 and 0.01mM also failed to prevent damage associated with kainate excitotoxicity in either area. ZM241385 significantly protected neurones in the CA3a region at a concentration of 2.5mM (32.9%±15.8, n=7) and reduced neurodegeneration in the CA3b (9.3%±4.7). Doses of 0.25 and 0.05mM had little or no effect in either the CA3a or CA3b regions.

The protective effects seen previously with systemic injections of CGS21680 against kainic acid excitotoxicity appear not to be duplicated with intrahippocampal administration, suggesting a peripheral, beneficial effect. Protection seen with ZM241385 does mirror our earlier results and gives further credence for the inhibition of $A_1$ receptors by $A_{2a}$ receptor activation. This is supported by the lack of effect observed with adenosine at a concentration high enough to activate $A_1$ and $A_{2a}$ receptors.

[1] Jones P.A. *et. al.* (1996) *Br. J. Pharmacol.* **119:** 337.

## 350P DIRECT ACTIONS OF L-ENKEPHALIN ON NEONATAL RAT SYMPATHETIC PREGANGLIONIC NEURONES VIA DELTA OPIOID RECEPTORS *IN VITRO*

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Sympathetic preganglionic neurones (SPN) represent the final central site of integration for information that influences the output of the sympathetic nervous system. Immunohistochemical labelling of leu-enkephalin (L-Enk) containing neurones in the thoracic spinal cord demonstrated synaptic contacts and direct apposition between L-Enk-immunoreactive neurones and retrogradely labelled SPN (Vera *et al.*, 1990). Evidence for opioidergic modulation of SPN activity is provided by the inhibitory effects of iontophoretically applied L-Enk upon blood pressure (Franz *et al.*, 1982). Hence we sought to investigate the direct effects of L-Enk on SPN.

Transverse slices of thoracic spinal cord (thickness 300-400μm) were obtained from 7-15 day old Sprague-Dawley rats of either sex. Whole-cell patch-clamp recordings were obtained in current-clamp mode from SPN. Sixty-five neurones were identified electrophysiologically as SPN as described previously (Pickering *et al.*, 1991). Thirty-six of these were confirmed as SPN by their characteristic morphology; namely their position within the intermediolateral cell column and axons exiting ventrally from the cord (Pickering *et al.*, 1990). The SPN had a mean resting membrane potential of 50±5.6mV and a mean input resistance of 954±653MΩ (n=17). Drugs were applied to the preparation by inclusion in the perfused artificial cerebrospinal fluid.

Leu-enkephalin (500nM-4μM) induced concentration-dependent hyperpolarising responses (6±3.7mV with 4μM) in 15 of the 65 (23%) neurones tested. Naloxone (1μM) reversibly blocked the L-Enk induced hyperpolarisations in all neurones tested (n=6).

Application of tetrodotoxin (500nM) resulted in the total loss of action potential firing, however the L-Enk response was unaffected (n=5) suggesting that it was a direct effect on the neurone.

To determine the opioid receptors responsible for the response, three selective agonists were applied to L-Enk responding cells. These agonists being [D-Pen$^2$,D-Pen$^5$]-enkephalin (DPDPE; 1-4μM; delta opioid receptor selective), [D-Ala$^2$,N-Methyl-Phe$^4$,Gly$^5$-ol]-enkephalin (DAMGO; 1-4μM; mu receptor selective) and 4-[(3,4-Dichlorophenyl ) acetyl ] - 3 - ( 1 - pyrrolidnylmethyl ) - 1-piperazinecarboxylic acid methyl ester fumarate (GR-89696 fumarate; 1-4μM; kappa receptor selective).

Leu-enkephalin responsive neurones did not respond to the application of either GR89696 or DAMGO. DPDPE (1-4μM) mimicked the hyperpolarising responses of L-enkephalin producing dose-dependent hyperpolarisations (5.2±2.5mV at 4μM, n=5).

These data suggest that leu-enkephalin directly inhibits a subset of sympathetic preganglionic neurones by selectively activating delta opioid receptors.

PG is a Wellcome Trust Prize Student

Franz, D.N., Hare B.D., McCloskey, K.L. (1982) Science 215. 215, 1643-1645.
Vera, P.L., Holets, V.R. & Miller K.E. (1990) Synapse. 6, 221-229.
Pickering, A.E., Spanswick, D. & Logan S.D. (1991) Neuroscience Letters. 130, 237-242.

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## 351P THE SHAPES OF CONCENTRATION (DOSE)-RESPONSE CURVES: AN APPEAL

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In 1971 Parker & Waud showed how a least-squares fit of response (R) to concentration ([A]) could be made by computer to the equation:

$$R = \frac{M[A]^P}{[A]^P + [A_{50}]^P} \quad \ldots\ldots\ldots 1$$

where M is the maximum response, $[A_{50}]$ produces a half-maximum response and P is an exponent which determines the steepness of the curve. This empirical model they referred to as "logistic" but it has the same form as the equation used by Hill (1913) to describe the binding of oxygen to haemoglobin and P is the same as the Hill coefficient ($n_H$). When P=1 the equation becomes a rectangular hyperbola and describes binding according to the law of mass action. A direct relation between response, R, and agonist concentration, [A], obtained by combining the binding relation between Y and [A] and a logistic equation between R and Y, has been given by Black & Leff (1983):

$$R = \frac{MT^n[A]^n}{(K_D+[A])^n + T^n[A]^n} \quad \ldots\ldots\ldots 2$$

where $n$ is the Hill coefficient for a logistic relation between R and Y, M is the maximum, T is the "transducer-ratio (tau)", which is the receptor concentration divided by the receptor concentration required to produce a response of M/2, and $K_D$ is the agonist dissociation constant. With a potent agonist $K_D >> [A]$ and the equation has the same form as equation 1. With a weaker agonist, however, the curve is not logistic; the symmetry between the upper and lower portions is lost (Fig 1).

With flat curves (P<1) the asymmetry makes it possible to fit the data to a 2-site binding curve:

$$R = \frac{M_1[A]}{[A]+[A_1]_{50}} + \frac{M_2[A]}{[A]+[A_2]_{50}} \quad \ldots\ldots\ldots 3$$

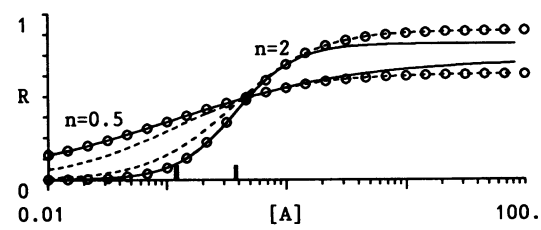


Figure 1 Values of R and [A] from equation 2 with M=1, K=1, T=3 and n=0.5 and 2. The full lines show the fit of the lower half of the values to equation 1 and have P=0.56 (for n=0.5) and 1.75 (for n=2): the broken lines show the fit of the upper half and the corresponding values of P are 0.89 and 1.24. Values of $[A_{50}]$ are marked on X-axis.

but the values of $M_2/M_1$ and $[A_2]_{50}/[A_1]_{50}$ can be used to estimate the probability that two processes are really involved: they are likely to be if $M_1 < M_2$

With flat (n=0.5) or steep (n=2) curves it is possible to fit theoretical data directly to equation 2. With experimental values the fitting process often fails to converge because errors produce similar effects on $K_D$ and T but a fit can be achieved with some curves (e.g. results obtained by Black et al, 1985) and it is better than that to the logistic equation (1). It ought to be possible to see whether this is generally true. Does anyone have suitable data (weak agonists and n<>1) which might be checked?

I wish to thank Dr Paul Leff for helpful discussion.

Black, J.M. & Leff, P. (1983) Proc.R.Soc. B, 220, 141-162.
Black, J.M., Leff, P., & Shankley, N.P (1985) Br.J.Pharmacol., 84, 561-571.
Hill, A.V. (1913) Biochem.J., 7, 471-480.
Parker, R.B., & Waud, D.R. (1971) J.Pharmacol. Exp Ther., 177, 1-12.

## 352P EFFECTS OF THE NONPEPTIDE BRADYKININ ANTAGONIST, FR173657, IN EXPERIMENTAL MODELS OF ACUTE PANCREATITIS AND CYSTITIS IN RATS

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The nonpeptide compound (E)-3-(6-acetamido-3-pyridyl)-N-[N-[2,4-dichloro-3-[(2-methyl-8-quinolinyl) oxymethyl] phenyl]-N-methylaminocarbonylmethyl] acrylamide (FR173657; Asano *et al.*, 1997) is a selective bradykinin $B_2$ antagonist both *in vitro* and *in vivo* (Griesbacher *et al.*, 1997, Griesbacher & Legat, 1997). We report here the activity of FR173657 in experimental models of acute inflammatory diseases involving endogenous kinin release.

Female Sprague-Dawley rats (200–300 g) were anaesthetized with pentobarbitone and phenobarbitone (40 mg $kg^{-1}$ each, i.p.) Acute pancreatitis was induced by i.v. infusion of caerulein (8 nmol $kg^{-1}$ within 120 min). This treatment increased the water content of the pancreatic tissue from 2.9±0.2 g $g^{-1}$ dry wt in control animals (infused with 4 ml $kg^{-1}$ 154 mM NaCl, $n$=6) to 8.3±1.0 g $g^{-1}$ ($n$=8). Plasma protein extravasation, determined by measurement of the protein marker, Evans blue (5 mg $kg^{-1}$, given i.v. 5 min before caerulein) increased from 18±9 µg E.blue $g^{-1}$ dry wt to 408±23 µg $g^{-1}$ ($P$<0.01).

FR173657 (300 nmol $kg^{-1}$, given s.c. 60 min prior to the caerulein infusion) inhibited the oedema formation significantly. Both the water content (3.5±0.8 g $g^{-1}$, $P$<0.05, nonparametric comparisons) and E.blue content (25±18 µg $g^{-1}$, $n$=6, $P$<0.05) of the tissue were reduced to values comparable to those of rats not infused with caerulein (see above). The caerulein-induced rise in serum lipase activity (4.9±0.8 iu $ml^{-1}$, $n$=8) was further increased (9.0±2.9 iu $ml^{-1}$, $P$<0.05) by FR173657 at 300 nmol $kg^{-1}$. A similar effect (10.2±1.2 iu $ml^{-1}$, $P$<0.05) was seen with the peptide $B_2$ antagonist, icatibant (Hoe-140, D-Arg-Hyp$^3$-Thi$^5$-D-Tic$^7$-Oic$^8$-bradykinin), albeit at only 10 nmol $kg^{-1}$. Similar to effects reported previously for icatibant (Griesbacher *et al.*, 1993), lipase release into the pancreatic tissue during pancreatitis (4.2±0.4 iu $mg^{-1}$, $n$=6) was reduced by FR173657 to 0.4±0.2 iu $mg^{-1}$ ($n$=6, $P$<0.05).

Acute cystitis was induced in anaesthetized rats by intravesical instillation of 30% v/v xylene. Control animals received silicone oil (0.3 ml) instead. Plasma proteins were stained with Evans blue (20 mg $kg^{-1}$ i.v. 5 min before xylene). Evans blue content of the tissue was increased from 265±49 µg $g^{-1}$ dry wt in control rats ($n$=5) to 579±87 µg $g^{-1}$ ($n$=8, $P$<0.01) 2 h after the application of xylene. Pretreatment with FR173657 at 300 nmol $kg^{-1}$ s.c. 60 min before the experiment prevented the effect of xylene completely (271±68 µg E.blue $g^{-1}$ dry wt, $n$=6, $P$<0.01). FR173657 had no effect on plasma protein extravasation in silicone oil-treated rats (293±33 µg $g^{-1}$, $n$=4).

Thus, FR173657 is demonstrated to be effective as inhibitor of the effects of endogenously released kinins and, due to its nonpeptide nature, may open possibilities for the treatment of inflammatory diseases.

Asano, M., Inamura, N., Hatori, C. *et al.* (1997) *Br. J. Pharmacol.*, 120, 617–624.
Griesbacher, T. & Legat, F.J. (1997) *Br. J. Pharmacol.*, 120, 933–939.
Griesbacher, T., Sametz, W., Legat, F.J. *et al.* (1997) *Br. J. Pharmacol.*, 121, 469–476.
Griesbacher, T., Tiran, B. & Lembeck, F. (1993) *Br. J. Pharmacol.*, 108, 405–411.

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## 353P EFFECT OF *TRANS* ARACHIDONIC ACID (AA) ON NEUTROPHIL ACTIVATION *IN VITRO*

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Little is known about the biological effects of *trans* polyunsaturated fatty acids, formed during the deodorisation of processed vegetable oils. *Trans* isomers of AA and EPA have been detected in platelet lipids (Chardigny *et al.*, 1993 and unpublished data) and initial experiments suggest that *trans* AA inhibits aggregation and eicosanoid biosynthesis by AA, consistent with inhibition of cyclo-oxygenase (Berdeaux *et al.*, 1996). The aim of this study was to investigate the effects of *trans* AA (20:4 Δ14t) on neutrophil activation *in vitro*.

Neutrophils were isolated from blood by dextran sedimentation followed by centrifugation using a discontinuous percoll gradient. Superoxide anion generation (SAG) was assayed by spectrophotometric evaluation of the reduction of ferricytochrome C to ferrocytochrome C (A550nm). Myeloperoxidase (MPO) release was determined using tetramethylbenzidine (0.1%) and $H_2SO_4$ (4 M) for spectrophotometric evaluation (A450nm). Neutrophil SAG produced by n-formyl-methionyl-leucyl-phenyl alanine (fMLP) (1-300 nM) was not altered in the presence of AA or *trans* AA (10-30 µM, n=4). Cells ($10^6$/ml) were suspended in RPMI containing fatty acid (or vehicle) and autologous plasma (ratio of 0.3 for fatty acid/albumin) for 24 hr at 37°C, 5%$CO_2$, and then washed for subsequent SAG or MPO determination. Results are shown in Table 1.

*Trans* AA significantly augmented fMLP induced SAG (Anova, p<0.001), but did not alter $EC_{50}$ values. This effect was greater than observed with AA, where only the maximum response to fMLP was increased (p=0.01). AA pre-incubation significantly (Anova, p<0.05) reduced A 23187-induced MPO release, whereas *trans* AA augmented it. MPO release was significantly greater after preincubation with *trans* AA, than with AA (Anova, p<0.01).

In conclusion, incorporation of *trans* AA into neutrophil phospholipids, results in increased neutrophil activation in response to fMLP or A23187.

Chardigny, JM, Sebedio, JL, Juaneda, P *et al.*, (1993). *Nutr. Res.*, **13**, 1105-1111.
Berdeaux, O, Chardigny, JM, Sebedio, JL, *et al.*, (1996). *J.Lipid.Res*, **37**, 2244-2250.

Table 1. Effect of pre-incubation (24 hr, 37°C, 5%$CO_2$) of neutrophils with *trans* AA and AA (10-30 µM), n=4.

| | Control | AA (30 µM) | *trans* AA (30 µM) |
|---|---|---|---|
| $EC_{50}$ for fMLP SAG (nM) | 17.6 ± 5.7 | 13.3 ± 2.6 | 12.2 ± 1.6 |
| max response (nmol $O_2^-$/$10^6$cells/10 min) | 26.3 ± 0.6 | 27.3 ± 0.6 | 28.9 ± 0.6 |
| | Control | AA (10 µM) | *trans* AA (10 µM) |
| $EC_{50}$ for A 23187 MPO (µM) | 1.6 ± 0.5 | 1.6 ± 0.6 | 1.0 ± 0.2 |
| % control max response | 100 ± 0 | 79.6 ± 6.1 | 105.2 ± 6.1 |

## 354P EICOSANOID PRODUCTION BY PLATELETS FROM NORMAL AND ALLERGIC HORSES

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Platelets from patients with atopic dermatitis have been reported to produce larger amounts of the lipoxygenase product 12-HETE than platelets from normal donors (Hilger et al. 1991; Neuber et al. 1992). An increase in the release of cyclooxygenase, but not lipoxygenase, products by platelets from atopic subjects with allergic rhinitis and/or asthma has also been reported (Audera et al. 1988). The present study has compared thromboxane (Tx) and 12-HETE production by equine platelets from horses with the seasonally recurrent, allergic, skin disease, sweet itch, with eicosanoid release by platelets from normal horses.

Blood samples were obtained during the active phase of the disease from 6 horses with clinical signs of sweet itch (SI) and 6 normal (N) horses with no clinical history of skin disease. Washed platelets were prepared and resuspended at approximately $10^8$ $ml^{-1}$. PAF ($10^{-11}$M - $10^{-9}$M) or ADP ($2.5x10^{-7}$M - $1.25x10^{-4}$M) were added to 0.5ml aliquots of stirred platelets in aggregometer cuvettes at 37°C and supernatants collected at the time of the maximal aggregation response to each of the agonist concentrations tested. Supernatant $TxB_2$, the stable breakdown product of $TxA_2$, and 12-HETE were measured by RIA. Results have been expressed as mean ± s.e. mean ng $TxB_2$ or 12-HETE/$10^8$ platelets. ANOVA was used to determine if there were significant differences between the amounts of eicosanoids produced by platelets from the allergic and normal animals.

PAF caused release of both Tx and 12-HETE by equine platelets. There was no significant difference between the amounts of the eicosanoids produced by platelets from SI when compared to N horses. The amounts of Tx and 12-HETE measured after aggregation of platelets from either SI or N horses with ADP were no different to the amounts measured in supernatants from unstimulated platelets.

Table: Tx and 12-HETE production by PAF- or ADP-stimulated platelets from N or SI horses.

| Stimulus | $TxB_2$ (ng/$10^8$ platelets) | | 12-HETE (ng/$10^8$ platelets) | |
|---|---|---|---|---|
| | N | SI | N | SI |
| PAF ($10^{-9}$M) | 91.5±8.4 | 97.8±11.2 | 17.4±1.8 | 15.4±1.5 |
| Unstimulated | 0.5±0.3 | 0.8± 0.8 | 4.6±2.4 | 3.1±1.4 |
| ADP ($1.25x10^{-4}$M) | 2.1±0.5 | 1.4± 0.7 | 9.4±1.7 | 4.9±0.8 |
| Unstimulated | 1.0±0.5 | 1.2± 0.6 | 8.8±3.1 | 3.9±1.2 |

These findings contrast with reports demonstrating differences in eicosanoid production by platelets from human atopic subjects (Audera et al. 1988; Hilger et al. 1991; Neuber et al. 1992). Stimulus dependent eicosanoid production by equine platelets has also been identified.

We are grateful to Pet Plan Charitable Trust for financial support

Audera C., Rocklin R., Vaillancourt R., Jakubowski J. & Deykin D. (1988). *Clin Immunol Immunopathol*, 46, 352-359.
Hilger R., Neuber K. & Konig W. (1991). *Immunology* 74, 689-695.
Neuber K., Hilger, RA, Konig W. (1992). *Int Arch Allergy Immunol* 98, 339-342

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## 355P EICOSAPENTAENOIC ACID ATTENUATES THE PRODUCTION OF PRO-INFLAMMATORY CYTOKINES IN HUMAN BLOOD

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Eicosapentaenoic acid (EPA) is a C20 fatty acid (5 double bonds) similar in structure to arachidonic acid (C20:4) and can be converted to 3-series prostaglandins. Many studies have reported that fish oils rich in EPA can alter inflammatory responses and various immune cell functions including cytokine release. However, these oils contain numerous other fatty acids, with very little information on the effect of EPA alone. In the present study we investigated the effect of EPA on the *ex vivo* production of the pro-inflammatory cytokines interleukin-1β, (IL-1), interleukin-8 (IL-8) and tumour necrosis factor-α (TNFα) in response to bacterial endotoxin (lipopolysaccharide, LPS) in human blood following administration of EPA.

Subjects (male 24 - 38 years old) were given EPA (1g orally per day) for 42 days and blood samples were taken at various intervals. Unfractionated blood was incubated with various concentrations of LPS for 20 hours after which the samples were centrifuged, 400 g for 10 min and the immunoreactive cytokines present in the plasma were measured by ELISA assays specific for the appropriate cytokine. All values are expressed as means ± s.e. mean of n = 6.

The levels of IL-1β, IL-8 produced in blood samples on day 42 in response to the highest level of LPS (1 $mg.ml^{-1}$) following EPA administration were less than the levels immediately preceding the administration of EPA (day 0). The levels of IL-1β and IL-8 were reduced to 63.5 % ± 6.5 and 54.1 % ± 21.5 respectively of the pretreatment levels of 100 % ± 15.8 for IL-1β and 100 % ± 22.3 for IL-8 (both $P < 0.05$). There were small decreases following EPA (not significant) in the blood levels of both IL-1β and IL-8 in samples incubated without LPS. The levels of IL-1β and IL-8 were also reduced in samples from EPA-treated subjects (day 42) incubated with a lower LPS concentration (100 $\mu g.ml^{-1}$). The levels of TNFα were also reduced in samples from EPA-treated subjects but this was only observed in blood incubated with 100 $\mu g.ml^{-1}$ LPS. The TNFα level was reduced from 100% ± 9.4, prior to EPA treatment, to 60.6% ± 10.1 ($P < 0.05$) on day 42. The level of TNFα in samples incubated with a higher level of LPS (1 $mg.ml^{-1}$) was not significantly decreased following EPA treatment.

The data obtained shows that administration of EPA can alter cytokine production and thus would be able to modulate immune/ inflammatory responses *in vivo*. It is not clear, however, if this is a direct effect or is related to the modulation of prostaglandin biosynthesis.

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## 356P EICOSAPENTAENOIC ACID SUPPRESSES THE ACUTE PHASE FEVER RESPONSE AND INCREASES IN BLOOD $PGE_2$ LEVELS

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Fish oils, which consist of a heterogeneous mixture of polyunsaturated fatty acids (PUFAs) suppress the release of pro-inflammatory cytokines from immunocompetent cells following stimulation *ex vivo* (Endres *et al.*, 1992) and have antiinflammatory effects in autoimmune disease *in vivo* (Robinson *et al.*, 1993). It has been suggested that *n* - 3 PUFAs such as the 20 carbon fatty acid eicosapentaenoic acid (EPA, 20:5 *n* - 3) are the active constituents. The aim of the present study therefore was to determine whether purified EPA could modulate a prominent symptomatic manifestation of the acute phase of the immune response in *vivo*, namely fever, and also whether EPA, a precursor PUFA of the 3-series prostaglandins, had any effect on blood levels of $PGE_2$ which is thought to be the final mediator of the fever response.

Dutch rabbits (1.7 - 2.3 kg) were given 100 $mg.kg^{-1}$ of 96 % pure EPA orally every day for a period of 42 days. Immediately prior to EPA supplementation (control) and at weekly intervals thereafter for 73 days, animals were challenged with polyinosinic:polycytidylic acid (poly I:C, 2.5 $\mu g.kg^{-1}$ i.v.) and body temperature monitored continuously for 5 h. Results are expressed either as the change in body temperature from basal, $\Delta T$, or as $TRI_5$ values which represent the change in body temperature over 5 h where a TRI value of 1 represents an increase of 1 °C for 1 h. In some experiments blood samples were taken and plasma levels of $PGE_2$ determined by RIA. All values are expressed as means ± s.e.mean for n = 8. Data was analysed using a paired students t-test, each rabbit acting as its own control.

Poly I:C produced a biphasic increase in body temperature, the first peak occurring 90 min and and the second peak at 210 min. EPA attenuated both peaks of the febrile response, $\Delta T$ values for the first and second peaks were reduced from 0.81 ± 0.12 °C and 1.50 ± 0.12 °C respectively prior to supplementation (control) to 0.58 ± 0.13 °C and 0.82 ± 0.14 °C after 28 days supplementation and to 0.49 ± 0.14 °C and 0.57 ± 0.14 °C after 42 days supplementation. The magnitude of suppression of the response by EPA increased with increasing duration of supplementation, reaching a maximum at 42 days. $TRI_5$ values were significantly reduced from 3.69 ± 0.32 °Ch before EPA (control) to 2.54 ± 0.34 °Ch ($P < 0.05$) after 28 days and to 1.71 ± 0.22 °Ch ($P < 0.001$) after 42 days. Suppression by EPA appeared to be maintained after supplementation was discontinued, $TRI_5$ values of 1.87 ± 0.21 °Ch obtained on day 73 were lower than controls ($P < 0.001$). EPA also attenuated poly I:C-induced increases in plasma levels of $PGE_2$. $PGE_2$ levels were reduced from 190 ± 31 $pg.ml^{-1}$ at 90 min and 200 ± 37 $pg.ml^{-1}$ at 210 min in control animals to 105 ± 10 $pg.ml^{-1}$ and 112 ± 10 $pg.ml^{-1}$ respectively following 42 days of EPA supplementation (both $P < 0.05$).

The data obtained shows that EPA reduces both the acute phase fever response and increases in plasma levels of $PGE_2$ during fever. It remains unclear, however, whether the decrease in plasma $PGE_2$ is secondary to a decrease in cytokine levels.

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Endres, S., Ghorbani, R., Kelley, V., *et al.* (1989) *N Engl J Med.* 320, 265 - 271.
Robinson, D.R., Xu, L., Tateno, S., *et al.* (1993) *J Lipid Res.* 34, 1435 - 1444.

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## 357P INDOMETHACIN PREVENTS THE IL-1β-INDUCED INCREASE IN NEURAL DISCHARGE AND ENHANCEMENT OF KININ-MEDIATED ACTIVATION OF C-FIBRE MECHANOCICEPTORS INNERVATING THE RAT KNEE JOINT

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We have previously shown that injection of the inflammatory cytokine IL-1β into the rat knee increases background neural discharge from joint mechanonociceptors, sensitises these afferents to bradykinin (Bk), and also induces a $B_1$ receptor mediated activation of these sensors (Kelly *et al.*, 1996). Since IL-1β can activate production of prostanoids, and prostanoids sensitise nociceptors to Bk (Schepelmann *et al.*, 1992), we have investigated the effects of indomethacin on the IL-1β-mediated increase in neural discharge and the modulation of kinin $B_1$ and $B_2$-responses in rat articular mechanonociceptors.

Male Wistar rats were anaesthetised with urethane (25%w/v, 0.6ml $100g.^{-1}$ weight i.p.). The trachea, carotid artery (for monitoring blood pressure) and right femoral artery (i.a. drug administration) were cannulated. Recording of afferent neural activity from high threshold mechanonociceptors was as described previously (Birrell *et al.*, 1993) except the medial articular nerve innervating the knee joint was used. IL-1β was injected intra-articular (100 units, i.art.) into the joint from which recordings were made. The mean ± s.e.mean peak increase in discharge following i.a. injection of drugs was calculated.

The mechanonociceptor units studied had a mean basal discharge of 0.5 ± 0.2 (n=5) impulses per second (i.p.s.), were activated by capsaicin (3.3 - 9.8 nmol i.a.) and had conduction velocities within the C-fibre range (0.46 ± 0.1 $ms^{-1}$, range 0.1 - 1.2 $ms^{-1}$). IL-1β failed to increase neural discharge (peak: 0.64 ± 0.25 i.p.s., n=5) following pre-treatment (30 min) with indomethacin (1 $mg.kg^{-1}$, i.a.) ($P>0.05$, Mann Whitney test). Indomethacin (1 $mg.kg^{-1}$, i.a.) prevented the IL-1β-induced enhancement of Bk-mediated increase in neural discharge (Table 1), and also prevented the IL-1β-induced kinin $B_1$-mediated increase in neural discharge from knee joint afferents (Table 1).

In conclusion, these data show that the IL-1β-induced increase in neural discharge from C-fibres is prevented by indomethacin and is therefore probably mediated by prostanoids. IL-1β-induced enhancement of the Bk-mediated increase in neural discharge, and induction of the kinin $B_1$-mediated increase in neural discharge also appear to involve prostanoids.

Birrell, G.J., McQueen, D.S., Iggo, A. *et al.* (1993) *Neuroscience*, 54, 537-544.
Kelly, D.C., Asghar, A.U.R., McQueen, D.S. *et al.* (1996) *Br. J. Pharmacol.*, 117, 90P.
Schepelmann K., Messlinger, K., Schaible, H-G., *et al.* (1992) *Neuroscience*, 50, 237 - 247.

Table 1: Effects of indomethacin on IL-1β sensitisation of kinin mediated activation of C-fibres. *P<0.05 paired Student t-test; NS P>0.05 compared to untreated.

| | UNTREATED (n=5) *peak increase (i.p.s.)* | IL-1β + Indomethacin 1 $mg.kg^{-1}$ (n=5) *peak increase (i.p.s.)* | IL-1β (n=4) from Kelly et al, 1996 *peak increase (i.p.s.)* |
|---|---|---|---|
| Bk 2.8 nmol | 1.6 ± 0.7 | 3.9 ± 2.0 NS | 11.8 ± 5.7* |
| Bk 9.4 nmol | 4.8 ± 2.3 | 3.9 ± 1.7 NS | 12.8 ± 4.0* |
| Bk 28.3 nmol | 5.4 ± 2.0 | 3.3 ± 0.5 NS | 12.2 ± 2.7* |
| $desArg^9$-Bk 104 nmol | 0.4 ± 0.07 | 0.3 ± 0.2 NS | 1.6 ± 0.1* |

## 358P HEPARIN INHIBITS AGONIST-INDUCED HOMOTYPIC AGGREGATION OF HUMAN AND RABBIT POLYMORPHONUCLEAR LEUCOCYTES *IN VITRO*

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Downregulation of polymorphonuclear leucocyte (PMN) activation and adherence in inflammatory disease could prevent the excessive transendothelial migration and degranulation of these cells, thereby attenuating tissue damage effected by the release of cytotoxic enzymes and mediators. Heparin has been shown to exert anti-inflammatory effects in addition to its traditional role as an anti-coagulant (*e.g.* Matzner *et al.* 1984; Sasaki *et al.* 1993; Ley *et al.* 1991). Here, we have studied the homotypic aggregation of human and rabbit PMN's *in vitro* as a marker of PMN activation, and have investigated the effects of heparin upon this phenomenon.

Venous blood was taken from healthy human volunteers (n = 8), and arterial blood obtained from NZW rabbits (n = 8). PMNs (>95% neutrophils), were separated by density-dependent centrifugation, and resultant cell pellets resuspended in cell-free plasma (5 x $10^6$ cells $ml^{-1}$). 250 µl samples were placed in a Payton aggregometer under constant stirring at 37°C, in the presence of 5 $\mu g ml^{-1}$ cytochalasin B in the case of the human cells (Bazzoni *et al.* 1993). The chemotactic peptide f-met leu-phe (fMLP, $10^{-9}$M - $10^{-5}$M) or platelet activating factor (PAF, $10^{-9}$M - $10^{-5}$M) was added, and the change in light transmission recorded over a three minute period against cell-free plasma as a blank. Aggregation was expressed as the % change in light transmission. In some experiments, unfractionated heparin (50 - 10,000U$ml^{-1}$), was added prior to $10^{-6}$M fMLP or PAF. Results are presented as mean ± s.e. mean, and were analysed using ANCOVA or a modified t-test, as appropriate.

Concentration-response relationships were found for both cell types using both agonists, although fMLP ($10^{-7}$M - $10^{-5}$M) was found to elicit a significantly ($P<0.05$) greater peak aggregatory effect upon human PMNs (21 ± 2%) than upon rabbit PMNs (13 ± 2%). Conversely, PAF ($10^{-8}$M - $10^{-5}$M) was found to have a significantly ($P<0.05$) greater peak aggregatory effect upon rabbit PMNs (17 ± 2%) than upon human PMNs (10 ± 2%). This may be due to differential expression of receptors or transduction systems, though further studies will be required in order to confirm this.

Heparin in the range 100 - 10,000U$ml^{-1}$ was found to inhibit the aggregation of both human and rabbit PMN's in response to either fMLP{36 ± 7% to 76 ± 10% (human, n = 8; $P< 0.05$); 47 ± 14% to 89 ± 8% (rabbit, n = 5; $P<0.05$)}, or PAF {33 ± 12% to 94 ± 13% (human, n = 6; $P<0.05$); 46 ± 15% to 84 ± 10% (rabbit, n = 5; $P<0.05$)}. This result suggests that a direct effect upon PMN activation may be contributory to the anti-inflammatory properties of heparin.

Bazzoni, G., Nunez, A.M., Mascellani, G. *et al.* (1993) *J.Lab. Clin. Med.* 121, 268-275.
Ley, K., Cerrito, M., & Arfors, K-E. (1991) *Am. J. Physiol.* 260, H1667-H1673.
Matzner, Y., Marx, G., Drexler, R., & Eldor, A. (1984) *Thromb. Haemostas.* 52, 134-137.
Sasaki, M., Herd, C.M., & Page, C.P.(1993) *Br. J. Pharmacol.* 110, 107-112.

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## 359P VERY EARLY CHANGES IN NEUROPEPTIDES AND THEIR ENCODING mRNAs IN THE FREUND'S COMPLETE ADJUVANT-INDUCED RAT MODEL OF NEUROGENIC INFLAMMATION

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Injection of adjuvant around a joint causes neurogenic inflammation and subsequent development of monoarthritis. A role for sensory neuropeptides in the aetiology and maintenance of this process has been suggested. Eight hours after injection of Freund's Complete Adjuvant (FCA) around the left tibio-tarsal joint of rats, there is a marked induction of β preprotachykinin-A (βPPT-A) mRNA, encoding the sensory neuropeptides substance P (SP) and neurokinin A, and α-calcitonin gene related peptide (α-CGRP) mRNA, in innervating dorsal root ganglia (DRG; L4-6) (Donaldson *et al* 1992). We have now investigated the changes in mRNA and the encoded peptides, SP and CGRP over a very early time course

FCA (0.15 mg) was injected subdermally around the left tibio-tarsal joint of rats (male, Wistar, 200-250g, temporarily anaesthesised with 4% halothane). L5 DRG ipsilateral to the injected limb were removed 0.5,1,2,4,and 8 hours after injection (rats killed by $CO_2$ inhalation). Levels of βPPT-A and α-CGRP mRNA were quantified by *in-situ* hybridisation in individual, small (diameter <20µm) DRG neurons. Peptides were extracted with acetic acid and measured by radioimmunoassay. Levels of mRNAs and peptide in experimental animals were expressed as a percentage of levels found in untreated animals, and shown in table 1.

| Time(h) | βPPT-A | SP | α-CGRP | CGRP |
|---|---|---|---|---|
| 0.5 | 216±20* | 143±13* | 181±22* | 146±14* |
| 1 | 244±48* | 158±7* | 194±14* | 148±10* |
| 2 | 171±8* | N/A | 184±17* | 119±9 |
| 4 | 129±4* | 123±32 | 140±7* | 102±5 |
| 8 | 137±4* | 55±12* | 142±1* | 105±4 |

Table 1 Levels, expressed as % untreated, of βPPT-A and α-CGRP mRNAs and the encoded peptides Substance P and CGRP in L5 DRG ipsilateral to FCA injection (mean±s.e.mean, n=3-5, $p<0.05$ ANOVA & Dunnett's post hoc test) N/A = not available

A probe generated against intron E of βPPT-A, was used to measure newly transcribed intronic mRNA. Quantitative in-situ hybridisation showed that levels of RNA with sequences containing intron E significantly increased to 157±13% control levels 0.5 after FCA injection (n=4, $p<0.05$ ANOVA & Dunnett's test)

Thus a marked and rapid increase is seen β-PPTA and α-CGRP mRNA lasting up to 8h after injection of FCA, which is least partly due to increased transcription. This is initially paralled by an increase in the levels of the encoded neuropeptides. These data implicates the induction of SP and CGRP in the very early biochemical and pathological changes seen in neurogenic inflammation.

Donaldson L.F *et al* (1992) *Molecular Brain Research*, 16 143-9

## 360P PERMEABILISATION REVERSES THE INHIBITORY EFFECT OF SIALIC ACID DEPLETION ON THE RESPONSE OF MAST CELLS TO AMPHIPHILIC PEPTIDES

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Secretion of inflammatory mediators such as histamine by rat peritponeal mast cells has been extencively used as a model to study exocytosis (Aridor *et al.*, 1993). This response can be triggered by a family of cationic-amphiphilic molecules such as substance P (SP), compound 48/80, and mastoparan (MP) (Mousli *et al.*, 1990). Mast cell activation by these molecules involves a pertussis toxin-sensitive G proteins (Mousli *et al.*, 1989). Pretreatment of rat peritoneal mast cells with neuraminidase (Ndase), an enzyme which cleaves sialic acid from oligosaccharides of the cell surface, led to inhibition of histamine release induced by basic compounds (Coleman *et al.*, 1986). The preliminary binding of triggers to sialic acid residues is thus proposed as a requirement to the entry of triggers into the cell.

Purified (95-98%) rat peritoneal mast cells ($3x10^4$/ tube) were preincubated with neuraminidase (0.3 U/ml) for 1 h at 37°C. The cells were washed and were left intact or permeabilised by exposure to steptolysin O (SLO) (15 U/ml) for 5 min at 37°C. The cells were then stimulated either with SP or MP for 10 min. The reaction was stopped by addition of ice-cold buffer and histamine measured.

Results from table 1 showed that pretreatment of intact mast cells with neuraminidase resulted in an 80% inhibition of histamine release induced by SP or MP. However, when mast cells were permeabilised with streptolysin O this inhibitory effect of neuraminidase was abolished. This demonstrate that a preliminary binding of cationic triggers to the cell surface oligosaccharides having sialic acid residues is required for their later interaction with intracellular G proteins. Furthermore, the results showed that permeabilisation reverses the inhibitory effect of neuraminidase on histamine release induced by SP and MP.

In the other hand, [p-Glu$^5$, D-Trp$^{7,9,10}$]-$SP_{5-11}$, an antagonist of G proteins (Mukai *et al.*, 1992), inhibited histamine release induced by MP from permeabilised mast cells.

Our results suggest that, in neuraminidase-pretreated mast cells, peptides cannot bind to the cell surface and therefore do not penetrate the cell to stimulate G proteins. The present findings support the notion that MP and SP stimulate histamine release from neuraminidase-pretreated mast cells by interacting with their intracellular target (G proteins) accessible only in streptolysin-permeabilised mast cells which have their plasma membrane permeable to large molecules.

Table 1 : Effect of SLO-permeabilisation on histamine secretion induced by MP or SP from Ndase-pretreated mast cells

| | Histamine secretion (%) | | |
|---|---|---|---|
| | Intact Cells | + Ndase | + Ndase + SLO |
| SP (10 µM) | 45 ± 4.2 | 9 ± 3.6 | 41 ± 4.8 |
| SP (30 µM) | 58 ± 5.1 | 11 ± 4.5 | 51 ± 5.3 |
| MP (10 µM) | 51 ± 3.1 | 8 ± 4.5 | 47 ± 5.3 |
| MP (30 µM) | 65 ± 4.9 | 14 ± 5.4 | 55 ± 4.8 |

References :

Aridor, M. *et al.* (1993) *Science* 262: 1569-1572.
Coleman, J. W. *et al.* (1986) *Peptides* 7: 171-176.
Mousli, M. *et al.* (1989) *J. Pharmacol. Exp. Ther.* 250: 329-335.
Mousli, M. *et al.* (1990) *Trends Pharmacol. Sci.* 11: 358-362.
Mukai, H. *et al.* (1992) *J. Biol. Chem.* 267: 16237-16243.

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## 361P INVESTIGATING THE REGULATION OF *IN VITRO* NEONATAL RAT FACIAL MOTONEURONE EXCITABILITY BY THE HYPERPOLARIZATION-ACTIVATED CURRENT, $I_H$, WITH ZD-7288

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The hyperpolarization-activated current, $I_H$, is prominently expressed in facial motoneurones (FM's) and its modulation by 5-hydroxytryptamine (5-HT) contributes to the regulation of membrane excitability by this neurotransmitter (Larkman & Kelly, 1992). External $Cs^+$ ions block $I_H$, however, this ion is not selective for this channel and blocks inwardly rectifying potassium ($K^+$) channels. Recent reports suggest that a novel bradycardic agent, ZD-7288, (4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride), selectively blocks $i_f$, the cardiac cell equivalent of neuronal $I_H$ (BoSmith,R.E *et al.*, 1993). Here we examine the effects of ZD-7288 on $I_H$ and the modulation of neonatal rat FM excitability by 5-HT. Whole-cell recordings were obtained from FM's maintained at ~23°C in brainstem slices prepared from 4 to 14 day old rats. FM's were routinely voltage-clamped close to -70 mV in artificial cerebrospinal fluid (ACSF) containing 12mM $K^+$ to enhance the amplitude of $I_H$. In some experiments external $Ca^{2+}$ was removed or $Mn^{2+}$(2mM) included to prevent $Ca^{2+}$-dependent events.

Bath-application of ZD-7288 (1-10µM) reduced the amplitude of $I_H$ activated by hyperpolarizing voltage step commands (-40 mV, 1.5 sec, n = 15). Block of $I_H$ was time- and concentration-dependent. With 1µM ZD-7288, $I_H$ was completely abolished within 20 minutes. No recovery was observed over the course of whole-cell recording (up to 90 minutes). An outward current (45 ± 15 pA, mean ± SEM) and decrease in conductance (14 ± 2.5%) was associated with the block of $I_H$ by ZD-7288 (n = 8). Subtraction of current responses evoked by hyperpolarizing voltage commands in the absence of ZD-7288 from those evoked in its presence provided difference currents displaying the time and voltage dependence characteristic of $I_H$. The ZD-7288-sensitive current had a reversal potential of -35 ± 4 mV (n = 5) close to that previously reported for $I_H$ (Takahashi & Berger, 1990). The action of ZD-7288 was selective for $I_H$. Activation of a transient and sustained outward currents by depolarizing voltage steps was unaffected by ZD-7288 (n = 5).

5-HT evokes an inward current associated with an increase in conductance in FM's voltage clamped at -70mV in high $K^+$ ACSF. Sensitivity to block by external $Cs^+$ and an enhancement of $I_H$ evoked by negative voltage steps suggests this current mediates the response. After ZD-7288 had abolished $I_H$, the 5-HT-induced inward current was blocked and replaced by a 5-HT-induced outward current (n = 6). The outward current had a linear current-voltage relationship and reversed at -55.3 ± 1.9 mV, close to the predicted $K^+$ equilibrium potential.

In conclusion, the selective block by ZD-7288 of $I_H$ in FM's confirms the involvement of this current in the modulation of excitability by 5-HT. Secondly ZD-7288 has revealed that the action of 5-HT on $I_H$ can mask a second effect on a resting $K^+$ conductance.

BoSmith,R.E. *et al.*, (1993) Br.J.Pharmacol. 110:343-349.
Larkman,P.M. & Kelly,J.S. (1992) J.Physiol. 456:473-490.
Takahashi,T. & Berger,A.J. (1990) J.Physiol. 423:63-76.

## 362P BINDING AND FUNCTIONAL RESPONSES ON HUMAN CELL LINES OF THE TACHYKININ $NK_1$ RECEPTOR ANTAGONIST MEN 11149: COMPARISON WITH FK888 AND CP 122,721

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Aim of this study was to compare binding and functional effects, on human cell lines, of three tachykinin $NK_1$ receptor antagonists: the retroinverse peptidomimetic MEN 11149 (Astolfi et al., 1997), the peptide FK888 (Fuji et al., 1992) and the non-peptide CP 122,721 (McLean et al., 1996). On human lymphoblastoma cells (IM9) Scatchard analysis with [$^3$H]substance P (SP) ($K_d$=0.16±0.02 nM and $B_{max}$=5092±325) revealed a non competitive inhibition by 3 nM MEN 11149 ($K_d$=0.28±0.04 nM and $B_{max}$=3000±270; P<0.001 for each parameter) and 0.1 nM CP 122,721 ($K_d$=1±0.18 nM and $B_{max}$= 3095±394; P<0.001 for each parameter) and a competitive antagonism by 10 nM FK888 ($K_d$=1.9±0.3 nM (P<0.001) and $B_{max}$= 5876±290)(n=3). In additional studies, IM9 cells were incubated for 60 min with MEN 11149 (3 nM), FK888 (3 nM), CP 122,721 (0.1 nM) or vehicle and then extensively washed for two hours in a drug free medium. Finally, a Scatchard analysis was performed for specific [$^3$H]SP binding. The reversible nature of FK888 antagonism was assessed since neither the $K_d$ nor the $B_{max}$ of [$^3$H]SP ($K_d$=0.1±0.01 nM and 0.15±0.02 nM; $B_{max}$=5248±102 and 5007±49, for vehicle and FK888, respectively; n=3) was changed. On the contrary, MEN 11149 and CP 122,721 behaved as irreversible or pseudoirreversible antagonists eliciting a decrease in the density of [$^3$H]SP binding sites ($B_{max}$=2333±77 and 2136±349 for MEN 11149 and CP 122,721, respectively; P<0.001, n=3). MEN 11149 significantly affected also the $K_d$ value (0.37±0.07 nM; P<0.001).

The effects of MEN 11149, FK888 and CP 122,721 on the functional consequences of human tachykinin $NK_1$ receptor activation were investigated in the astrocytoma cell line U373 MG. In [$^3$H]SP binding assays the $K_i$ values were 2.1±0.3 nM, 0.5±0.14 nM and 0.06±0.01 nM for MEN 11149, FK 888 and CP 122,721, respectively (n=3). All these antagonists inhibit functional responses triggered by SP (100 nM) i.e. the accumulation of inositol monophosphate ($IP_1$) (after 1 h of incubation) and the release of interleukin-6 (IL-6) (after 18 h of incubation). None of these compounds modified the basal level of $IP_1$ or IL-6 release. As shown in table 1, the rank order of potency was: CP 122,721>MEN 11149>FK888. Moreover, while the competitive antagonist FK 888 was able to antagonise both functional responses with comparable $IC_{50}$ values, the non competitive antagonists MEN 11149 and CP 122,721 inhibited at a greater extent SP-induced IL-6 release than $IP_1$ accumulation.

Table 1. $IC_{50}$ values (nM) of some $NK_1$ receptor antagonists against SP-induced $IP_1$ formation and IL-6 release (n=3).

| Antagonist | $IP_1$ | IL-6 |
|---|---|---|
| MEN 11149 | 77±18 | 4±1 |
| FK888 | 105±20 | 88±23 |
| CP 122,721 | 1.6±0.09 | 0.03±0.003 |

In conclusion, MEN 11149 binds with remarkable affinity and in a very tight manner to human tachykinin $NK_1$ receptors. Unlike FK888, MEN 11149 and CP 122,721 behaved as non-competitive pseudoirreversible/irreversible antagonists. A greater inhibition of long-lasting $NK_1$ driven cellular functional responses (like cytokine release) could be achieved with this type of antagonists.

Astolfi, M. et al. (1997) Br. J. Pharmacol. 120, 202P.
Fuji, T. et al. (1992) Br. J. Pharmacol. 107, 785-789.
McLean, S. et al. (1996) J. Pharm. Exp. Ther. 277, 900-908.

## 363P A NOVEL C-TERMINALLY DIRECTED ANTIBODY TO MEASURE BIOLOGICALLY ACTIVE NEUROTENSIN

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Neurotensin (NT) is a tridecapeptide present in the CNS. It is colocalised in dopamine (DA) neurones, modulates DA neurotransmission (Kitabgi, 1989) and has been implicated as a mediator of neuroleptic drug actions in the treatment of schizophrenia (Nemeroff, 1986). NT radioimmunoassay (RIA) (Moss *et al*, 1994) using an N-terminally directed antibody (NAb) binds both inactive NT N-terminal metabolites and intact NT and this has made it difficult to distinguish between basal and evoked NT release both *in vitro* (Holtom *et al*, 1997) and *in vivo* (Aspley *et al*, 1996).

The C-terminal sequence Cys,Cys-$NT_{8-13}$ was conjugated to keyhole limpet hemocyanin via m-maleimidobenzoyl-N-hydroxysuccinimide ester. An emulsion of conjugate/adjuvant was injected into sheep monthly for 7 months (250µl total conjugate sheep/ injection; s.c.). The final bleed produced a C-terminal antibody (CAb) titre in excess of 30% total radioactivity bound at 1:150,000 dilution. Comparison of NT standard curves was performed with both the NAb and CAb. In addition, concurrent RIAs were performed with CAb and other bioactive peptides (Table 1).

The intra- and inter-assay variation of the RIA standard curve with CAb was 5.8% (n=5) and 11.2% (n=4), respectively. The mean net displacement produced by the other bioactive peptides studied was no more than 15%. In particular, CAb does not bind to NT related peptides such as neuromedin-N, kinetensin or N-terminal fragments indicating selectivity for biologically active NT fragments. These results demonstrate that the novel CAb does not bind to bioactive peptides other than $NT_{8-13}$ or NT itself. If we compare this to data with NAb there is considerable cross-reactivity with N-terminal fragments. For example, $NT_{1-8}$ had 53.7%, $NT_{1-11}$ had 113.7% and $NT_{1-12}$ had 102.9% cross-reactivity with NAb (Moss, 1996). This necessitates the use of HPLC to separate biologically active NT from its N-terminal metabolites. These data characterise an antibody which will improve immunoreactive determination of NT

Table 1: Selectivity of CAb with respect to NT and metabolites.

| Ligand | Antibody | ED50 | Ligand | Antibody | ED50 |
|---|---|---|---|---|---|
| NT | NAb | 1.6 | Xenopsin | CAb | >102 |
| NT | CAb | 1.9 | Kinetensin | CAb | >85 |
| $NT_{8-13}$ | CAb | 8.53 | M-E | CAb | >174 |
| $NT_{1-6}$ | CAb | >128 | S-P | CAb | >74 |
| $NT_{1-8}$ | CAb | >97 | D-A | CAb | >47 |
| NMN | CAb | >134 | CCK | CAb | >25 |

ED50 = amount of ligand in fmoles/30µl required to displace 50% [$^{125}$I]NT binding (n=3-4 in all cases). NMN=neuromedin-N; M-E=met-enkephalin; S-P=substance-P; D-A=dynorphin-A; CCK=cholecystokinin.

Aspley, S., Holtom, P.E., Bennett, G.W., *et al* (1996) Proc. 7th Int. Conf. *In vivo* Methods, p193.
Holtom, P.E., Aspley, S., Needham, P.L. *et al* (1997) Brain Research Assoc. Abstracts **14**:P7.03.
Kitabgi, P. (1989) Neurochem. Int. **14**:111-119.
Moss, S. (1996) Thesis, University of Nottingham.
Moss, S., Bennett, G.W. and Marsden, C.A. (1994) Brain Research Assoc. Abstracts **11**:P7.
Nemeroff, C.B. (1986) Psychoneuroendocrinology, **11**:15-37.

## 364P $mGlu_{1\alpha}$ RECEPTOR UNDERGOES RAPID, PKC-INDEPENDENT DESENSITIZATION WHEN EXPRESSED IN CHO CELLS

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Previous studies, using neuronal cultures and brain slices, suggest that phospholipase C-coupled metabotropic glutamate (mGlu) receptors ($mGlu_{1\&5}$ receptors) desensitize in response to prolonged agonist exposure and are rapidly phosphorylated by PKC in an agonist dependent manner (Alaluf *et al.*, 1995; Thomsen *et al.*, 1993). The aim of this present study was to evaluate whether $mGlu_{1\alpha}$ receptors, stably expressed in CHO cells, undergo a rapid desensitization upon agonist application and to determine whether PKC plays a physiological role in $mGlu_{1\alpha}$ receptor desensitization.

CHO cells were cultured in DMEM supplemented with 10 % (v/v) fetal calf serum, 2 mM L-glutamine, 1 % (w/v) proline and 50 $Uml^{-1}$ penicillin / 50 $\mu gml^{-1}$ streptomycin / 0.25 $\mu gml^{-1}$ amphotericin B (Aramori and Nakanishi, 1992). For measurement of total [$^3$H]-inositol phosphate ([$^3$H]-$IP_x$) accumulation, cells were grown in 12 well plates and pre-incubated with [$^3$H]-*myo*-inositol (4 $\mu$Ci / 24 hours) prior to L-glutamate stimulation in the presence of 10 mM LiCl. [$^3$H]-$IP_x$ were isolated by anion exchange chromatography as previously described (Aronica *et al.*, 1993). Changes in $[Ca^{2+}]_i$ were measured by pre-loading the cells with Fura-2/AM (5 $\mu$M, 60 min, 37°C) and determining the ratiometric changes ($\lambda_{ex}$ = 340/380 nm) in Fura-2 fluorescence ($\lambda_{em}$ = 510 nm).

L-glutamate stimulated both [$^3$H]-$IP_x$ production and increased $[Ca^{2+}]_i$ in a concentration-dependent manner ($EC_{50}$ s = 10.6 ± 2.2 $\mu$M and 2.7 ± 0.1 $\mu$M, respectively (n = 4). L-glutamate failed to stimulate [$^3$H]-$IP_x$ accumulation in CHO cells transfected with vector DNA only (*i.e.* minus $mGlu_{1\alpha}$ cDNA). Repeated exposure (greater than 60 seconds) of 1 mM L-glutamate produced a desensitized $[Ca^{2+}]_i$ response (67.2 ± 0.9 % initial response (n = 6)) with mGlu1$\alpha$ receptor $[Ca^{2+}]_i$ responses recovering 15 minutes after agonist application. Furthermore, L-glutamate (1 mM) stimulated [$^3$H]-$IP_x$ production, displaying a temporally biphasic curve with a rapid rate of [$^3$H]-$IP_x$ formation over the first minute followed by a slower accumulation over the following 10 minutes. However, in our hands, pre-incubation with either the selective PKC inhibitor GF 109203X (10 $\mu$M, 40 minutes) or PMA (100 nM, 5 minutes) had no significant effect on 1 mM L-glutamate-stimulated [$^3$H]-$IP_x$ accumulation over 15 minutes (n = 3).

In summary, these data suggest that $mGlu_{1\alpha}$ receptors, when expressed in CHO cells, undergo a rapid and partial desensitization upon agonist stimulation which appears to be PKC independent.

S. Alaluf, E.R. Mulvihill and R.A.J. McIlhinny (1995) *FEBS Lett.* 367, 301-305

I. Aramori and S. Nakanishi (1992) *Neuron* 8, 757-765.

E. Aronica, D.F. Condorelli, F. Nicoletti, P. Dell'Albani, C. Amico and R. Balázs (1993) *J. Neurochem.* 60, 559-565.

C. Thomsen, E.R. Mulvihill, B. Haldeman, D.S. Pickering, D.R. Hampson and P.D. Suzdak (1993) *Brain Res.* 619, 22-28.

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## 365P NITRIC OXIDE-INDUCED ACTIVATION OF $BK_{Ca}$ CHANNELS IN RAT BASILAR MYOCYTES DOES NOT INVOLVE A DIRECT EFFECT ON THE CHANNEL

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Smooth muscle relaxation induced by the vasodilator molecule nitric oxide (NO) has been attributed in certain blood vessels and species to the opening of $K^+$ channels. Pharmacological analysis of the electrophysiological and mechanical response to NO has lead to the suggestion that the target channel for NO differs depending on the artery studied. In aorta, cerebral and pulmonary arteries it appears that large-conductance, calcium-activated $K^+$ channels ($BK_{Ca}$) are activated by NO (Robertson et al, 1993, Archer et al, 1994, Bolotina et al, 1994, Holland et al, 1996) while in mesenteric artery NO-induced hyperpolarisation may depend on $K_{ATP}$ channels (Murphy & Brayden, 1995).

We have previously shown that the NO donor molecule SIN-1 causes activation of $BK_{Ca}$ channels in rat basilar artery myocytes, (Holland et al, 1996). Here, we have extended our studies and have used authentic NO solutions to confirm the identity of the channels activated and also to examine the possible mechanisms underlying channel activation.

Smooth muscle cells were enzymatically isolated from rat basilar artery and currents recorded using conventional configurations of the patch clamp technique. Saturated solutions of NO were bath applied using a gas tight syringe and low gas permeability tubing. All experiments were conducted at room temperature.

NO solutions (~1mM) caused the slow development of an inward current in cells at -60 mV in 140/140mM $K^+$ solution. This current was not blocked by 5$\mu$M glibenclamide or 100nM apamin but was rapidly inhibited by charybdotoxin (ChTX) and iberiotoxin (IbTX) both 100nM. Voltage ramps (-50 to +50 mV) demonstrated that the NO-induced current was increased at all potentials and reversed at 0 mV. NO activated a single channel of unitary current amplitude -15.4 ± 0.4 pA, n=3 whose calculated single channel conductance was 257 pS. Similar results were obtained using 8-bromo cGMP suggesting that channel activation may be due to activation of cGMP-dependent protein kinase (G-kinase) by NO. Therefore, we examined the effects of NO or G-kinase (1$\alpha$ isoform) on isolated patches. NO solution failed to increase channel activity in any isolated patch studied (n=20). G-kinase (500 U/ml), in the presence of $Ca^{2+}$ (1$\mu$M), ATP (1mM) and cGMP (500$\mu$M) caused rapid activation of a large-conductance (250pS), $Ca^{2+}$-sensitive $K^+$ channel in all patches (n=12). Voltage ramps applied to these patches showed that the G-kinase cocktail activated the channel at all potentials and that the current reversed at 0 mV in symmetrical $K^+$. The removal of ATP from the cocktail caused a rapid reduction in channel activity towards control levels.

This study provides evidence that NO-induced hyperpolarisation in rat basilar myocytes is likely to be secondary to activation of G-kinase probably followed by phosphorylation of either $BK_{Ca}$ channels or, as may be more likely, an associated protein.

Archer, SL, Huang, JMC, Hampl, V *et al* (1994) *Proc. Natl. Acad. Sci.*, **91**, 7583-7587.

Bolotina, VM, Najibi, S., Palacino, JJ *et al* (1994) *Nature*, **368**, 850-853.

Murphy, ME and Brayden JE (1995) *J. Physiol.*, **486**, 47-58.

Holland, M., Standen, NB & Boyle, JP (1996) *Br. J. Pharmacol.*, **118**, 106P.

Robertson, BE, Schubert, R, Hescheler, J., *et al* (1993) *Am. J. Physiol.*, **265**, C299-303.

## 366P ELECTROPHYSIOLOGICAL CHARACTERISATION OF THE NOVEL TETRODOTOXIN-SENSITIVE SODIUM CHANNEL, rPN4

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Neurones in rat dorsal root ganglia (DRG) express a variety of sodium channel subtypes (Caffrey *et al.*, 1992). Functionally, they can be divided into tetrodotoxin-sensitive ($TTX_S$) low-threshold channels, with rapid activation and inactivation kinetics, and TTX-resistant ($TTX_R$) high-threshold channels, with slow activation and inactivation kinetics (Kostyuk *et al.*, 1981; Elliott & Elliott, 1993; Ogata & Tatebayashi, 1993). A number of structurally novel sodium channels have now been cloned from DRG tissue *e.g.*, rPN3/SNS (Akopian *et al.*, 1996; Sangameswaran *et al.*, 1996), rPN1/hNE (Klugbauer *et al.*, 1995; Sangameswaran *et al.*, 1997) and rPN4/NaCh6 (Schaller *et al.*, 1995). In the present study, the novel sodium channels, rPN4 and its splice variant, rPN4a, were electrophysiologically characterised in the *Xenopus* oocyte expression system. The sensitivities of rPN4 to the sodium channel specific toxin, TTX, and the local anaesthetic, mexiletine, were also studied.

For expression and functional analysis of these channels, defolliculated stage V-VI *Xenopus* oocytes were injected with 0.1-0.2 ng (two-electrode voltage-clamp, TEVC) or 10 ng (macropatch) cRNA encoding rPN4 or rPN4a α-subunits. The oocytes were placed in sterile ND-96 solution (supplemented with gentamycin, theophylline and pyruvate) and kept in an incubator at 20°C.

In two oocytes, TEVC recordings, made 2-3 days after cRNA injection, showed that the channels encoded by rPN4 were potently inhibited ($IC_{50}$ values of 0.4 and 1.6 nM) in a concentration-dependent (0.1-10 nM) manner by TTX. In order to improve temporal resolution of the currents, the macropatch recording technique was used to study the activation, steady-state inactivation and recovery from inactivation (at -110 mV) of rPN4 and rPN4a sodium channels in devitellinised oocytes, 3-4 days after cRNA injection. The table summarises and compares the behaviours of rPN4 and rPN4a. Data are presented as mean±s.e.mean and the numbers in parentheses are the N-values. Statistical comparisons were made using the unpaired Student's t-test. The rate of recovery of rPN4a from inactivation was voltage-dependent since it was significantly (P=0.006) faster at -130 mV ($\tau_{rec}$=2.4±0.1 ms; N=3) than at -110 mV.

| parameter | rPN4 | rPN4a | P |
|---|---|---|---|
| max. conductance | 12.3±1.5 nS (7) | 34.7±9.2 nS (11) | 0.07 |
| activation $V_{half}$ | -37.7±1.2 mV (7) | -42.1±1.8 mV (11) | 0.09 |
| activation k | -8.4±0.4 mV (7) | -7.4±0.3 mV (11) | 0.05 |
| max. current | 0.8±0.2 nA (5) | 2.0±0.6 nA (7) | 0.11 |
| inactivation $V_{half}$ | -97.6±2.1 mV (5) | -103.6±2.0 mV (7) | 0.07 |
| inactivation k | 6.8±0.7 mV (5) | 8.3±0.5 mV (7) | 0.08 |
| time constant of recovery | 8.5±0.5 ms (4) | 5.7±0.3 ms (3) | 0.007 |

The affinities of mexiletine for the resting and inactivated states of rPN4 channels were estimated from the concentration-dependence (10-3000 μM) of the depression of $I_{max}$ and the shift in the midpoint of the steady-state inactivation curve (N=10). In this way, the affinity of mexiletine for resting rPN4 channels was 860±210 μM and for inactivated rPN4 channels it was 28±7 μM.

In conclusion, rPN4 is a rapidly activating and inactivating sodium channel which is sensitive to TTX and mexiletine.

Akopian *et al.*, 1996, *Nature*, **379**, 257-262.
Caffrey *et al.*, 1992, *Brain Res.*, **592**, 283-297.
Elliott & Elliott, 1993, *J. Physiol.*, **463**, 39-56.
Klugbauer *et al.*, 1995, *EMBO J.*, **14**, 1084-1090.
Kostyuk *et al.*, 1981, *Neurosci.*, **6**, 2423-2430.
Ogata & Tatebayashi, 1993, *J. Physiol.*, **466**, 9-37.
Sangameswaran *et al.*, 1996, *J. Biol. Chem.*, **271**, 5953-5956.
Sangameswaran *et al.*, 1997, *J. Biol. Chem.*, in press.
Schaller *et al.*, 1995, *J. Neurosci.*, **15**, 3231-3242.

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## 367P IN ADULT RATS, PRESSURE-NATRIURESIS IS BLUNTED BY A NEW PEPTIDIC AT2 RECEPTOR AGONIST

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Aims: We previously reported (Lo et *al.*, 1995) that in rats blocked with losartan, an AT1 receptor antagonist, PD 123319 (50 μg/kg/min), a specific AT2 receptor antagonist, enhanced the slope of the pressure-natriuresis curve. The present work was aimed to assess the effects of an AT2 receptor stimulation using TA (T2-(Ang II 4-8)2), a new AT2 receptor peptidic agonist (Grouzmann et *al.*, 1995).
Methods: In inactin anesthetized male adult Sprague Dawley rats, the endogenous angiotensin II synthesis was blocked by a converting enzyme inhibitor (quinapril, 10 mg/kg, i.v.). Using the *in vivo* technique of Roman & Cowley (1985), the influence of renal perfusion pressure (RPP) on renal blood flow (RBF), diuresis (UV) and natriuresis (UNaV) was studied at 3 RPP levels during the i.v. infusion of vehicle (Controls) or TA (10 μg/kg/min, as chosen after preliminary experiments). The experiments were repeated after AT2 receptor blockade by PD 123319 (50 μg/kg/min, i.v.).
Data in Fig 1 are mean ± SEM. Statistic analysis used a two way ANOVA.

Results:

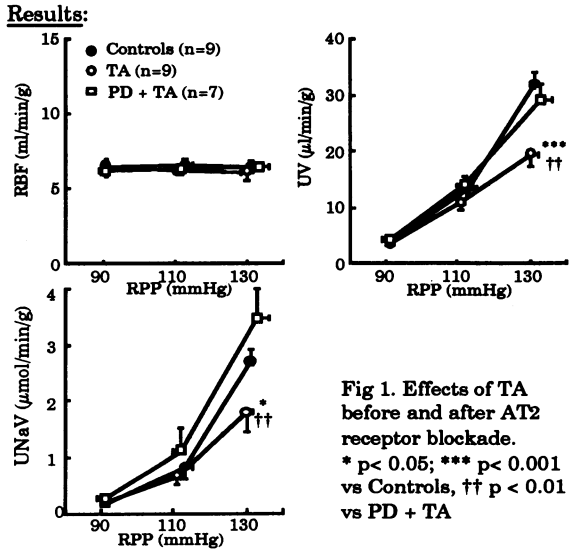


Fig 1. Effects of TA before and after AT2 receptor blockade. * p< 0.05; *** p< 0.001 vs Controls, †† p < 0.01 vs PD + TA

Conclusion: These results demonstrate that AT2 receptor stimulation with TA blunts the natriuretic response to acute increases in RPP. Since they are in close agreement with our observations using AT1 and AT2 receptor blockers they confirm that AT2 receptor stimulation limits the sodium excretion induced by acute increases in RPP.

References:

Grouzmann E. *et al.*, *Eur.J.Biochem.*1995,234:44; Lo M. *et al.*, *J.Clin.Invest.*1995,95:1394; Roman R.J. & A.W. Cowley Jr. *Am.J.Physiol.*1985,248:F190

## 368P ACTION OF LOVASTATIN ON CELL DEATH IN CULTURED SV-40 TRANSFORMED HUMAN VASCULAR SMOOTH MUSCLE CELLS

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Lovastatin reduces plasma cholesterol as a result of inhibition of mevalonate synthesis by HMG CoA reductase. Isoprenoids derived from mevalonate, farnesol pyrophosphate (FPP) and geranygeraniol pyrophosphate (GPP) are required for lipid modification (prenylation) of several proteins, including the ras superfamily (Maltese, 1990). Lovastatin may inhibit vascular smooth muscle cell proliferation by this mechanism (Munro et al., 1994). This study examined the effect of lovastatin on apoptosis in immortalised human vascular smooth muscle cells.

Cultured SM1 cells, a clonal smooth muscle cell line derived from human saphenous vein and immortalised with SV40 large T antigen, were used for all studies. Apoptosis was assessed using flow cytometry as previously described (Nicoletti et al.,1991). ~$5x10^5$ cells were plated onto 9cm Petri dishes coated with poly-l-lysine (0.01mg/ml) and exposed to drug or vehicle in serum-free NCTC-109 medium supplemented with 0.25% (w/v) bovine serum albumin. Apoptotic cells were identified as a hypodiploid population ($A_0$) and quantified as % total cell number. Additionally DNA fragmentation was confirmed in some experiments by agarose gel electrophoresis or immunocytochemistry using a terminal deoxynucleotide transferase-mediated dUTP-digoxigenin end labelling (TUNEL) reagent (Apop Tag $^{TM}$ Plus, Oncor Inc., MD, USA). Data are means±s.e. means of n observations.

Apoptotic cells were seen in the absence of any drug. Lovastatin (3-100μM; 0–48h) induced a time- and concentration-dependent increase in apoptosis. 24h incubation with lovastatin (10μM) increased apoptotic cells from 16±2% (n=7) to 25±3% (n=8; p=0.03 by Student's t-test). Apoptosis induced by 10μM lovastatin was recovered by co-incubation of cells with mevalonate (0.1mM; %recovery = 112±16%; n=4), GPP (10μM; %recovery = 83±22%; n=4) but not by FPP(10μM; %recovery = 23±17%; n=4), or cholesterol (10μg/ml; % recovery = 33±25%; n=3). None of these agents had any effect on apoptosis in the absence of lovastatin (n=2-4).

Inhibition of HMG CoA reductase by lovastatin causes increased apoptosis of SM1 cells. This action appears unrelated to depletion of cellular cholesterol but may be due to inhibition of prenylation, possibly by depletion of geranylgeraniol pyrophosphate.

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Maltese, W.A., (1990) *FASEB J.* **4,** 3319-3328.
Munro, E., Patel, M., Chan, P., *et al.* (1994) *Eur. J. Clin. Invest.* **24,** 766-772.
Nicolletti. I., Migiorati, G., Pagliacci, M.C. *et al.* (1991) *J. Immunol. Methods* **139,** 271-279.

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## 369P SIGNALLING PATHWAYS ACTIVATED BY ENDOTHELIN-1 IN VASCULAR SMOOTH MUSCLE FROM THE RAT PULMONARY ARTERY

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Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor in the pulmonary circulation. It can activate both $ET_A$ and $ET_B$ receptors, their ratio of expression being species dependent and varying throughout the pulmonary arterial tree. The detailed mechanism underlying ET-1 action at the level of pulmonary vascular smooth muscle has not yet been established. The aim of the present study was thus to investigate in the rat main pulmonary artery (RMPA): 1) the effect of ET-1 on both cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and membrane current, 2) ET-1 receptor subtypes implicated in this effect, 3) the role of these mechanisms in ET-1 -induced contractile response.
The effect of ET-1 on $[Ca^{2+}]_i$ and membrane current was studied in myocytes freshly isolated from RMPA dissected from pentobarbitone anaesthetised Wistar rats. Myocytes were loaded with 1μM indo-1acetoxymethyl ester for 30 min. $[Ca^{2+}]_i$ was estimated fluorimetrically from the 405:480 nm ratio using a specific calibration (Guibert *et al.*, 1997). Membrane currents were studied using the whole-cell patch-clamp technique. Isometric contraction was recorded in arterial rings using a computerised isolated organ bath system (Roux *et al.*, 1997). Short (30s) application of ET-1 (5-100 nM) induced 3-6 cyclic rises in $[Ca^{2+}]_i$ (oscillations) of decreasing amplitude, the first one reaching 724 ± 45 nM (mean ± SEM) from the resting value of 103 ± 3 nM (n = 45). ET-1-induced $[Ca^{2+}]_i$ response was abolished by BQ 123 (1μM) but not altered by BQ 788 (1μM), specific inhibitors of $ET_A$ and $ET_B$ receptors, respectively. In the presence of 100 μM $La^{3+}$ or in the absence of external $Ca^{2+}$ ions ET-1-induced $[Ca^{2+}]_i$ response was not modified, but disappeared after pre-treatment of myocytes with thapsigargin (1 μM) for 10 min. Addition of neomycin (0.1 μM, 10 min) or phorbol 12,13 dibutyrate (1μM) in $Ca^{2+}$ free solution progressively abolished the ET-1-induced $[Ca^{2+}]_i$ response. In myocytes clamped at -60 mV, ET-1 elicited an oscillatory inward current the pattern of which evoked that of oscillations in $[Ca^{2+}]_i$ (n = 15). The reversal potential of the current, estimated from a ramp pulse delivered at the first peak, was -2.3 mV (n = 4), close to chloride equilibrium potential ($E_{Cl}$ = -2.1 mV). Moreover, ET-1 induced current was blocked by 50 μM niflumic acid which failed to modify $[Ca^{2+}]_i$ oscillations. Finally, ET-1 (0.1-100 nM) induced concentration-dependent and slowly developing contractions. The amplitude of the maximal contraction was reduced by 35.7 ± 8 % (n = 6) and 34 ± 5 % (n = 6) in the presence of 50 μM niflumic acid and 1μM nifedipine, respectively; the two drugs having a similar (35 ± 6 %, n = 6) non additive effect.
In conclusion, our results show that ET-1 induces $[Ca^{2+}]_i$ oscillations in RMPA via the stimulation of $ET_A$ receptors coupled to phospholipase C activation. $[Ca^{2+}]_i$ oscillations are due to cyclic $Ca^{2+}$ release from internal store and secondary activate a chloride current which can depolarise the cell membrane leading to activation of voltage-gated $Ca^{2+}$ channels. The resulting $Ca^{2+}$ influx contributes to contraction.

Guibert, C., Marthan, R. & Savineau, J.P. (1997) *Cell Calcium* 21, 421-429
Roux, E., Guibert, C., Savineau, J.P. et al., (1997) *Br. J. Pharmacol.* 120, 1294-1301

## 370P $M_1$ MUSCARINIC RECEPTORS STIMULATE PHOSPHOINOSITIDE TURNOVER IN PRIMARY CULTURES OF RAT DISSOCIATED SUPERIOR CERVICAL GANGLION NEURONES

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Previous studies have demonstrated that muscarinic agonists can increase the production of inositol phosphates in intact rat superior cervical ganglion (SCG; reviewed by Caulfield, 1993). $M_1$ Muscarinic receptor activation also inhibits the M-type $K^+$ current ($I_{K(M)}$) in SCG neurones by activating Gq/11 (Caulfield et al., 1994) which in many systems has been shown to stimulate phospholipase Cβ (PLCβ), with resultant mobilisation of intracellular $Ca^{2+}$ (reviewed by Caulfield, 1993). However, the muscarinic receptor involved in PLC stimulation of SCG neurones has not been adequately characterised. Application of $Ca^{2+}$ to the intracellular surface of excised patches of SCG neurones results in M-channel inhibition, and it has been suggested that muscarinic $I_{K(M)}$ inhibition is mediated by intracellular $Ca^{2+}$ release (Selyanko & Brown, 1996). in the present experiments, we have tested whether the pharmacology of muscarinic activation of PLCβ in SCG cultures maintained in L-15 plus 10 % FCS indicates $M_1$ receptor involvement. To do this, we have measured PLC activity by monitoring accumulation of the intermediate in the resynthesis of phosphoinositides, CMP-PA (Bevilacqua et al., 1994). Primary cultures of SCG neurones were preincubated with $^3$H-cytidine and 10mM LiCl for one hour (37°C), then incubated with varying concentrations of oxotremorine-M (Oxo-M). $^3$H-CMP-PA was measured in lipid extracts. Oxo-M (10μM)-evoked increase in CMP-PA levels was maximal at 15 minutes and at this time point, effects were seen at Oxo-M concentrations ≥ 1 μM.

One hour pre-incubation with 100nM pirenzepine and 1 μM himbacine shifted the Oxo-M concentration-response curve rightwards by a factor of about 10-fold, corresponding to apparent $pK_B$ values of 8 and 7 (n = 4-6), respectively, consistent with the involvement of an $M_1$ receptor in muscarinic agonist stimulation of PLC. It is likely that the response measured is confined to SCG neurones, as glial accumulation of CMP-PA is negligible. Our demonstration that $M_1$ receptor stimulation leads to measurable CMP-PA accumulation indicates the possibility of localising PLCβ stimulation at the individual neurone level in SCG cultures (cf. Bevilacqua et al., 1994).

Bevilacqua, J.A., Downes, C.P. & Lowenstein, P.R. (1994) *Neuroscience* **60,** 945-958.
Caulfield, M.P. (1993) *Pharmacol.Rev.* **58,** 319-379.
Caulfield, M.P., Jones, S., Vallis, Y. *et al.* (1994) *J.Physiol.* **477,** 415-422.
Selyanko, A.A. & Brown, D.A. (1996) *Neuron* **16,** 151-162.

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## 371P TIME-DEPENDENT INHIBITION OF INTRACELLULAR CALCIUM MOBILISATION BY LOW CONCENTRATIONS OF THAPSIGARGIN IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

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Exposure for 1h to low concentrations (<10nM) of thapsigargin (TG) inhibits the replication of cultured human vascular smooth muscle cells (HVSMC; Shukla *et al.*, 1997). The mode of action of TG was not defined in this study. TG is a selective inhibitor of intracellular $Ca^{2+}$ sequestering ATPase which, because of continuous turnover of $Ca^{2+}$ through intracellular pools, not only increases cytosolic $Ca^{2+}$ ($[Ca^{2+}]_i$) but also reduces the $Ca^{2+}$ content of inositol 1,4,5-trisphosphate ($IP_3$)-mobilisable $Ca^{2+}$ pools (Putney and Bird, 1993). We therefore used dynamic video imaging of $[Ca^{2+}]_i$ in FURA-2 loaded HVSMC to determine the involvement of $Ca^{2+}$ pools in the inhibition of VSMC proliferation by TG.

HVSMCs were grown to confluence in DMEM (high glucose) containing 10% fetal calf serum and seeded on glass cover slips. Following culture for 48 h, cells were then incubated for 200 sec, 1h and 24 h with TG prior to loading with FURA-2. Mobilisation of intracellular $Ca^{2+}$ stores was stimulated by 1μM ionomycin in $Ca^{2+}$ free medium and the rise in $[Ca^{2+}]_i$ detected using $Ca^{2+}$ imaging techniques (McArdle *et al.*, 1993).

Continuous exposure of cells to low concentrations of TG (which failed to measurably increase in $[Ca^{2+}]_i$) reduced the ionomycin response in a time and dose - dependent manner (fig.1). After exposure of cells to 10 nM TG for 1h followed by washing and further incubation in culture medium for up to 72h there was a time-dependent recovery of the ionomycin response relative to zero time (24h: 47±12.5%; 48h: 64±7%; 72h: 75±3.6% [mean ± s.e.m., n = 4-6] ).

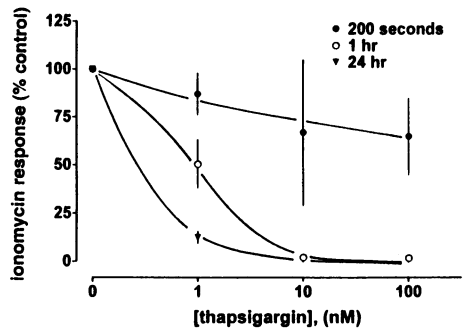


Fig. 1. Effect of different exposure times to TG on ionomycin stimulated $[Ca^{2+}]_i$ release in HVSMCs (mean ± s.e.m.; n = 4 - 6). (●) 200 sec, (○) 1h, (▼) 24 h.

These data demonstrate that exposure of HVSMCs to low concentrations of TG for 1h is sufficient to deplete $IP_3$-sensitive $Ca^{2+}$ pools. Since the concentrations of TG and exposure times are identical to those that inhibit replication in HVSMC (Shukla et al., 1997), it is proposed that $IP_3$-sensitive $Ca^{2+}$ pools play an important role in the progression of HVSMC replication. Furthermore, these data consolidate that short term (1h) *ex vivo* exposure of vein grafts to TG prior to implantation constitutes a potential strategy for treating vein graft failure.

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McArdle C.*et al.* (1992) *Mol. Cell Neurosci.* **13**: 124-132.
Putney, J.W. & Bird, G. (1993) *Endocr. Revs.* **14**: 610-6311.
Shukla, N.*et al.* (1997) *Br. J. Surg.* **84**: 325-330.

## 372P EFFECTS OF BACTERIAL LIPOPOLYSACCHARIDE TREATMENT ON NF-κB ACTIVITY IN CULTURED RAT AORTIC SMOOTH MUSCLE CELLS

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As found by some groups (e.g. Szabo *et al.*, 1993), we have shown that treatment of rat aortic smooth muscle cells (SMC) with bacterial lipopolysaccharide (LPS) induces expression of inducible nitric oxide synthase (iNOS). LPS-induced nitrite formation (an index of NO production) by SMC was blocked by putative inhibitors of NF-κB activation, including the protease inhibitor N-p-tosyl-L-phenylalanine chloromethylketone (TPCK) (Zhou *et al.*, 1996), an agent which inhibits nuclear NF-κB translocation and iNOS expression in mouse macrophages (Griscavage *et al.*, 1996). Here, we have investigated effects of LPS and TPCK upon NF-κB activation in SMC.

SMC obtained by collagenase digestion of rat aortae were maintained in culture as previously described, with use up to passage 16 (Zhou *et al.*, 1996). Cells were incubated with LPS (E. coli serotype 055:B5) for 1 h in Medium 199 (without foetal bovine serum and antibiotics), while corresponding control cells were incubated without LPS. In some cases, TPCK (20 μM) was added with LPS. Cells were then lysed and nuclear protein extracts prepared essentially as described by Brennan & O'Neill (1996).

NF-κB gel shift assays involved first incubating 8 μg protein extract for 20 min in 10 μl assay mixtures containing 35 fmol of a $^{32}$P-labelled oligomeric consensus binding sequence for NF-κB (from Promega) according to the manufacturer's protocol, followed by electrophoresis of the mixtures in 4% non-denaturing polyacrylamide gels and detection of radiolabelled bands by autoradiography.

Binding of the radiolabelled probe to protein extracts from control cells resulted in the detection of a single gel shifted band. This band was also found using cells treated with LPS (1, 10 or 100 μg/ml), but also with a more slowly migrating second band. Both bands involved specific binding of NF-κB, because they were absent when using the radiolabelled oligomer with a 100-fold excess of competing unlabelled NF-κB probe, whereas 100-fold excess of an unlabelled binding oligomer specific for AP-1 transcription factor did not prevent detection of the two bands. Only this LPS-induced band was abolished when using cells treated with 1 μg/ml LPS and 20 μM TPCK concomitantly.

Recent studies with interleukin-1β stimulated SMC have shown NF-κB activation (Hecker *et al.*, 1996) and its inhibition (as well as prevention of iNOS expression) by another protease inhibitor N-alpha-tosyl-L-lysine chloromethylketone (Schini-Kerth *et al.*, 1997). Here we show similar effects upon NF-κB with LPS as the stimulant and TPCK as the inhibitor. As found by these authors, we obtained evidence for a constitutive NF-κB band in control cells, and an additional band of slower electrophoretic mobility induced in LPS-treated cells which was inhibitable by TPCK.

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Brennan, P. & O'Neill, L.A.J. (1995) *Biochim. Biophys. Acta* **1260**, 167-175.
Griscavage, J.M., Wilk, S., & Ignarro, L.J. (1996) *Proc. Natl. Acad. Sci.* **93**, 3308-3312.
Hecker, M., Preiß, C., Schini-Kerth, V.B. *et al.* (1996) *FEBS Letts.* **380**, 224-228.
Schini-Kerth, V.B., Boese, M., Busse, R. *et al.* (1997) *Arterioscler. Thromb. Vasc. Biol.* **17**, 672-679.
Szabo, C., Mitchell, J.A., Gross, S.S. *et al.* (1993) *J. Pharmacol. exp. Therap.* **265**, 674-680.
Zhou, J., Struthers A.D., & Lyles, G.A. (1996) *Br. J. Pharmacol.* **119**, 313P.

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## 373P ENDOTHELIN-1 SYNTHESIS IS STIMULATED BY A TUMOR NECROSIS FACTOR α MUTANT SELECTIVE FOR THE p55 TNF RECEPTOR

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Tumor necrosis factor alpha (TNFα) is a strong inducer of endothelin-1 (ET-1) synthesis *in vitro* and *in vivo* (Corder *et al.*, 1995; Klemm *et al.*, 1995). The biological actions of TNFα are exerted via two distinct receptors of approximately 55 and 75 kDa (p55, CD120a; p75, CD120b) (Bazzoni & Beutler, 1996). In order to define which TNF receptor (TNFR) is involved in TNFα-induced ET-1 secretion, we studied ET-1 release from bovine aortic endothelial cells (BAEC), and from a human epithelial cell line, HEp-2, which expresses the p55 but not the p75 receptor (Brockhaus *et al.*, 1990). For selective stimulation, two human TNFα mutants which bind and activate specifically each type of TNFR, R32W-S86T (p55-selective) and D143N-A145R (p75-selective) were used (Loetscher *et al.*, 1993).

Confluent cultures of BAEC and HEp-2 were stimulated in serum-free medium with different concentrations of either wild-type human TNFα (wtTNFα) or the mutant TNFs. After 6h incubation, conditioned media were collected and ET-1 release was measured either by a specific immunoassay (Corder *et al.*, 1995) or by ELISA (R&D Systems).

Mean basal release of ET-1 was 139 ± 7 fmol/well/6h for BAEC (n=39) and 13.7 ± 1.4 fmol/well/6h for HEp-2 (n=13). The p55-selective mutant, R32W-S86T, was able to induce ET-1 release in both cell types (Table 1). However, with BAEC the maximum response was significantly less than wtTNFα, and combined stimulation with the two mutants had no greater effect than the p55-selective mutant alone. These results indicate that regulation of ET-1 synthesis in endothelial and epithelial cells is linked to stimulation of the p55 TNFR.

Bazzoni F. & Beutler B. (1996) *New Engl. J. Med.* 334, 1717-1725.
Brockhaus M. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87, 3127-3131.
Corder R. *et al.* (1995) *J. Cardiovasc. Pharmacol.* 26 (S3), S56-S58.
Klemm P. *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92, 2691-2695.
Loetscher H. *et al.* (1993) *J. Biol. Chem.* 268, 26350-26357.

Table 1. ET-1 release from BAEC and HEp-2 (values are % change from control basal release ± s.e.mean; n = 6-15; * = P<0.01 compared to wtTNFα in each cell line, ANOVA with Fisher's LSD test).

| | BAEC | | | HEp-2 | | |
|---|---|---|---|---|---|---|
| ng/ml | wtTNFα | R32W-S86T | D143N-A145R | wtTNFα | R32W-S86T | D143N-A145R |
| 1 | 34 ± 5 | 3.4 ± 1.8 * | 0.8 ± 1 * | 16 ± 6 | -2.7 ± 2 | |
| 10 | 57 ± 7 | 17 ± 2 * | 1.8 ± 2 * | 37 ± 10 | 37 ± 7 | 1.2 ± 4 * |
| 50 | 66 ± 8 | 25 ± 2 * | 2.7 ± 3 * | | | |
| 100 | | | | 57 ± 7 | 58 ± 9 | -3 ± 6 * |
| 200 | 64 ± 8 | 47 ± 5 * | 2.5 ± 3 * | | | |
| 500 | 82 ± 8 | 45 ± 3 * | 7.2 ± 2 * | 76 ± 8 | 67 ± 5 | -2.8 ± 3 * |