83P INHIBITION OF CARRAGEENAN-INDUCED INCREASES IN PAW HYPERALGESIA, OEDEMA AND PROSTAGLANDIN SYNTHESIS BY SELECTIVE INHIBITORS OF CYCLO-OXYGENASE-2

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Selective inhibitors of cyclo-oxygenase 2 (Cox-2) have been reported to inhibit carrageenan-induced development of paw oedema and/or hyperalgesia (Boyce et al, 1994; Masferrer et al, 1994). However, no effects on paw prostaglandin synthesis were reported. In the present study we have compared the effects of diclofenac (non-selective Cox inhibitor) with the recently described Cox-2 inhibitors, DuP697 and SC58125 (Aslanian et al, 1994) on carrageenan-induced development of paw hyperalgesia, oedema and prostaglandin E_2 (PGE_2) levels. Compounds were dissolved in either distilled water (diclofenac) or 1% DMSO and 75% PEG-400 in distilled water (DuP697, SC58125) and administered orally at 0.1-10.0 mg kg^{-1}, 30min before an intraplantar injection (100µl) of 2% (w/v) carrageenan into the right hind paw of male , Glaxo bred, random hooded rats (120-150g) which had been fasted overnight. Three hours later, paw hyperalgesia (algesymeter) and oedema (plethysmometer) were measured. Subsequently, paws were snap frozen in liquid N_2, pulverised and PGE_2 extracted into ethanol and measured by enzyme immunoassay. Inhibition of either hyperalgesia, oedema or PGE_2 is expressed as a percentage of the maximum response which is defined as the difference between the right and left paws of rats treated with carrageenan alone. Results are either presented as geometric mean ED_{50} values (with 95% confidence limits) or as mean ± s.e.mean for percent inhibition. In all cases n=12. Results are shown in Table 1.

Table 1. Comparison of inhibition of hyperalgesia and PGE_2

Compound	ED_{50} mg kg^{-1} po	
	Hyperalgesia	PGE_2
Diclofenac	1.0 (0.4-2.2)	0.7 (0.2-2.6)
DuP697	0.7 (0.2-2.1)	1.2 (0.3-3.9)
SC58125	0.8 (0.4-1.7)	0.6 (0.1-2.3)

All compounds produced a dose dependent inhibition of hyperalgesia and PGE_2 synthesis with a maximum inhibition at 3 or 10 mg kg^{-1} of 100%. Inhibition of oedema was similarly dose dependent, however maximum inhibition values were 54.1 ±4.4% (diclofenac), 38.9±5.1% (DuP697) and 54.1±3.8% (SC58125) respectively at 3 or 10 mg kg^{-1}.

These data suggest that carrageenan-induced elevation of PGE_2 via the activity of Cox-2 appears to be associated with the development of hyperalgesia and oedema in this model. In addition, our results with DuP697 and SC58125 suggest that Cox-2 is the sole determinant of hyperalgesia but is only partially responsible for the development of oedema.

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84P THE MECHANISM OF 6-HYDROXYDOPAMINE (6-OHDA)-INDUCED PLASMA EXTRAVASATION IN RAT SKIN

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6-OHDA activates sympathetic terminals and is used to study the role of sympathetic nerves in inflammation. High concentrations (>50mM) produce plasma extravasation in rat joints (Coderre *et al.*, 1989) however 3mM 6-OHDA has also been shown to release sensory neuropeptides in rat trachea (Sulakviledze *et al* 1994). We have investigated the mechanism of 6-OHDA-induced plasma extravasation in skin. Plasma extravasation induced by i.d. injection of test agents (100µl) was measured by accumulation (over 30min) of ^{125}I-albumin (1.5µCi i.v.) in shaved dorsal skin of anaesthetised (pentobarbitone, 50mgkg^{-1}) male Wistar rats (200-350g). The effect of chemical sympathectomy (6-OHDA, i.p., 50mgkg^{-1} on days 1&2, 100mgkg^{-1} on days 6&7) on the response to subsequent i.d. injection of 6-OHDA and other mediators of increased vascular permeability (on day 8) was also measured. 6-OHDA (1-10 µmoles/site, vehicle 1% ascorbate in Tyrode) produced dose dependent plasma extravasation which was inhibited by mepyramine and methysergide but not by an NK_1 antagonist, SR140333, or by indomethacin. Compound 48/80 and GR73632 (a selective NK_1 agonist) plus CGRP were used as controls. Interestingly the response to 6-OHDA and other agents was increased by sympathectomy. (Table 1). We conclude that 6-OHDA probably produces oedema in rat skin by degranulating mast cells rather than activating sensory or sympathetic nerves. Thus, at least at high concentrations, 6-OHDA should not be assumed to selectively or exclusively act on sympathetic nerves. In sympathectomised animals loss of vasoconstrictor tone, (or loss of reflex sympathetic vasoconstriction following i.d. injection) may account for the observed potentiation of the responses.

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Support of the ARC is gratefully acknowledged.

Table 1. Modulation of 6-OHDA induced plasma extravasation. Results are expressed as µl plasma/site, mean ±s.e.mean, (n)=no. of rats.*p<0.05 treated vs control, ANOVA, followed by Tukey's multiple comparison test

Test agent	untreated (4)	mep (2.8nmol/site) + methys (1.9nmol/site)		SR140333 (1nmol/site)		indomethacin (5mgkg^{-1}, i.p., -30min)		sympathectomy	
		control (3)	treated (4)	control (3)	treated (3)	control (4)	treated (4)	vehicle (6)	6-OHDA (5)
6-OHDA									
vehicle	11.2±1.8					11.6±2.9	17.8±2.9	7.9±0.9	19.8±4.5
1µmol	32.3±7.5					31.8±7.6	16.0±1.8	28.4±4.5	79.4±8.0*
3µmol	38.0±13.2	39.6±10.7	13.0±1.2*			51.0±7.9	32.5±3.7	52.4±6.9	107.3±9.9*
10µmol	55.1±10.7	74.6±12.5	25.4±5.2*	56.5±11.4	75.0±15.2	75.1±15.2	52.5±9.2	97.2±14.9	170.5±18.7*
48/80									
1µg		61.9±7.1	13.1±1.0*	100.2±8.9	70.8±8.1	107.0±18.9	95.2±10.6	121.3±10.9	174.6±10.9*
GR+CGRP									
100pmol + 10pmol		72.6±22.5	40.1±10.6	88.4±9.5	30.2±4.0*	56.8±11.9	53.2±9.7	98.8±9.6	149.9±8.5*

85P STEROID SULPHATASE REGULATES CELLULAR INFILTRATION, BUT NOT OEDEMA, IN CONTACT SENSITISATION IN MICE

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Immune responses can be regulated by the opposing influences of glucocorticoids (immunosuppresive) and other steroid hormones such as dehydroepiandrosterone (DHEA) (immunostimulatory). DHEA is sulphated to its inactive circulating form, DHEAS, requiring conversion back to DHEA for biological activity. Inhibition of steroid sulphatase activity in mice inhibits the ear swelling seen in contact sensitisation (CS) *in vivo*, and reverses the augmentation seen with DHEAS (Suitters *et al.*,1995). In this study we investigated whether the effects of steroids, DHEAS and dexamethasone (DEX) (Sigma) and a steroid sulphatase inhibitor 3-estrone-sulphamate, CT2251 (Howarth *et al.*, 1994.) in a CS model were mediated via changes in cellular infiltrate or oedema formation. Male Balb/c mice (Harlan UK Ltd) 16-20g, were shaved on the right flank on day -1. On day 0 mice were topically sensitised with 50µl of either 2.5% oxazalone in 4:1 acetone:olive oil, or vehicle only. On day 5 mice were challenged on their right dorsal ear surface with 25µl of 0.25% oxazalone. Ear measurements were taken prior to and 24 hours post challenge. Animals (n=10/group) were treated with DHEAS (5mg/kg) ± CT2251 (10mg/kg) or DEX (5mg/kg) in olive oil subcutaneously on days 0 & 4. Ears were removed 24 hours post challenge for immunocytochemical analysis. Cryostat sections (6µm) were stained for T cells (anti-CD3) and macrophages (anti-Mac-1) (Serotech). Numbers of positive cells were counted blind in 10 random fields per tissue section. In this experiment, augmentation of the CS response was seen with DHEAS (97.5%±15.86) (mean±SEM), which was reversed by CT2251 to show an inhibition of 20.4%±10.4 ($p<0.05$ ANOVA). CT2251 alone reduced the response by 22.4%±8.9. DEX inhibited the response by 50%±8.1 ($p<0.05$). Correlating with the changes in ear thickness in the treated animals, DHEAS increased the number of macrophages (107.01± 28.77%) and T cells (65.27±10.54%) compared to vehicle control ($p<0.05$). This was prevented when DHEAS was given in combination with CT2251 (10mg/kg) ($p<0.05$). CT2251 alone reduced the number of T cells (41.58±12.77%) compared to vehicle ($p<0.05$), with a lesser effect on macrophages (17.4%±19.03). However it should be noted, in this experiment CT2251 alone did not inhibit the ear swelling (see above) as much as seen in previous experiments (Suitters *et al.*, 1995). DEX caused a significant decrease in both T cells (68.29%±11.51) and macrophages (91.58±2.85%). In a separate experiment, ears (n=10/group) were removed from animals treated with DHEAS ± CT2251 or DEX (as above). Ear weight measurements were made prior to and after drying in an oven overnight to estimate tissue water content. In this experiment DHEAS augmented the ear swelling response (64.2%±10.1) compared to vehicle control ($p<0.05$) which was inhibited when DHEAS (5mg/kg) in combination with CT2251 (10mg/kg) (35.6%±12.4) ($p<0.05$). CT2251 alone caused an inhibition of the CS response (15.6%±10.8) compared to vehicle control. The CS response alone increased water content by 15% which was unaffected by treatment with DHEAS ± CT2251 or DEX. This study demonstrates that CS swelling is more cell mediated than oedematous, and that inhibition of steroid sulphatase reduces the numbers of infiltrating cells into the site of an ongoing immune response with no effect on oedema formation.

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86P LEUKAEMIA INHIBITORY FACTOR (LIF) INDUCES MECHANICAL ALLODYNIA BUT NOT THERMAL HYPERALGESIA IN THE RAT

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Leukemia inhibitory factor (LIF) is responsible for axotomy-induced alterations in neurotransmitter phenotype in the sympathetic nervous system *in vivo* and *in vitro*[1] and will induce neuropeptide expression in sensory neurones *in vitro*[2]. Following peripheral nerve injury, high expression and robust retrograde transport of LIF is observed in injured sensory fibres[3]. We have investigated the possibility that LIF is involved in the behavioural hypersensitivity following peripheral nerve injury. The effects of exogenous LIF administration have been compared with those of a closely related cytokine, ciliary neurotrophic factor (CNTF) and the neurotrophin, nerve growth factor (NGF).

Behavioural assessments were made of mechanical and thermal thresholds for the hindlimb nociceptive flexion withdrawal reflex in young rat pups (postnatal day 12-14). Mechanical thresholds were measured using calibrated von Frey filaments. Thermal thresholds were measured as the latency of hindlimb flexion from a radiant heat beam focused upon the plantar surface of the foot. All substances were delivered in PBS containing 50µg BSA per 1µ g cytokine, either in a 10µl subcutaneous injection to the left hindpaw (5µl: dorsal surface, 5µl: plantar surface) or administered systemically (100µl i.p).

Subcutaneous LIF injection to the hindpaw induced a dose-dependent decrease in the von Frey threshold required to evoke ipsilateral hindlimb flexion. A significant difference was present 2.0 hours following LIF injection and was maintained at the same level for 7 hours. The mean threshold for hindlimb flexion fell from 5.77±0.24g in naive animals to 3.91±0.17g (100ng LIF) and 2.28±0.14g (1.0µg LIF) 2.0 hours following injection ($p<0.01$ *t-test*) and to 2.24±0.27g (1.0µg LIF) 7.0 hours following injection. Mechanical thresholds at these time points were within the innocuous range for naive animals. A significant difference was still present 24 hours following the highest dose of LIF when compared to naive (4.56±0.88 vs 6.52±0.29g, 1.0µg LIF, $p<0.05$, *t-test*). Vehicle injection (50µg BSA in PBS) was without significant effect. Systemic LIF injection (1.0µg i.p) also induced a significant reduction in von Frey threshold for hindpaw withdrawal (2.54±0.25 vs 6.07±0.33g 4 hours after LIF, $p<0.05$). Hindpaw sensitivity to a noxious thermal stimulus was unaffected by systemic LIF (12.1±1.12s latency before injection vs 12.2±1.06s, 4 hours following LIF). The sensitising effect of LIF to innocuous mechanical stimuli was fully reversed by simultaneous administration of an equimolar concentration of monoclonal anti LIF (αLIF). αLIF had no effect upon hindpaw withdrawal thresholds when injected alone nor influenced the mechanical hypersensitivity produced by a subcutaneous injection of NGF (1.0µg) (3.52±0.49g 7 hours following NGF vs 8.08±1.66g naive)

Subcutaneous CNTF injection (1.0µg) did not alter the mean von Frey threshold for hindpaw withdrawal at any time point following injection.

These experiments indicate that exogenously applied LIF induces a prolonged hypersensitivity of a nociceptive flexion withdrawal reflex in a specific and dose-related manner. LIF expression following peripheral nerve injury may therefore contribute towards the associated behavioural hypersensitivity.

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87P INTERLEUKIN-1β POTENTIATES FACIAL SKIN VASODILATATION EVOKED BY CAPSAICIN BUT NOT BY ELECTRICAL STIMULATION OF THE TRIGEMINAL GANGLION IN ANAESTHETISED RATS

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Intraplantar injection of interleukin-1β (IL-1β) enhances the cutaneous hyperaemic response to intraplantar injection of capsaicin in rat skin (Herbert & Holzer, 1994). In this study, the action of IL-1β on neurogenic vasodilator responses to local injection of capsaicin or electrical stimulation of the trigeminal ganglion in the rat facial skin was investigated. Male Wistar rats (180-220g) were anaesthetised (sodium pentobarbitone 60 mg kg^{-1}) and changes in facial skin blood flow were measured by laser Doppler flowmetry (Moor MDF3D). Test agents were injected intradermally (i.d., 10 µl volume) in the facial skin directly under the laser Doppler probe. Rats were pretreated with i.d. IL-1β (50 pg) or saline 40 min prior to i.d. capsaicin (0.33 to 10 nmol), capsaicin vehicle, Tyrode or CGRP (10 pmol). In anaesthetised and paralysed rats electrical stimulation of the trigeminal ganglion (2 - 5 Hz, 10 V, 1 ms for 30 s) led to an increase in facial skin blood flow (Escott *et al*, 1995). Two responses to electrical stimulations were obtained and IL-1β (50 pg) or saline (10 µl) were injected i.d. into the facial skin 40 min prior to the second stimulation. Results are expressed as the increase in blood flow (arbitrary units of flux, AU) from basal blood flow levels, mean ± s.e.mean. Comparisons between treatments were made using Student's *t* test or ANOVA followed by Tukey's modified *t* tests and statistical significance was accepted when $p < 0.05$.

Table 1 Effect of IL-1β on the vasodilator responses to i) i.d. capsaicin or CGRP or ii) trigeminal stimulation (n = 4 - 8 rats, *p < 0.05, **p < 0.01)

Treatment	Saline (-40 min)	IL-1β (-40 min)
i)	Increase in blood flow (flux, AU)	
Vehicle	0.3 ± 2.6	-2.2 ± 2.5
Capsaicin (nmol)		
0.33	34.5 ± 4.9	51.5 ± 4.6*
1	36.0 ± 7.3	86.4 ± 16.2*
3.3	62.0 ± 2.5	97.5 ± 6.6**
10	89.5 ± 8.4	141.8 ± 19.4*
Tyrode	4.3 ± 4.2	8.0 ± 4.0
CGRP (10 pmol)	75.0 ± 4.0	65.5 ± 3.6
ii)	Increase in response to second electrical stimulation (flux, AU)	
2 Hz, 10 V, 1ms	32.0 ± 5.4	36.3 ± 3.8
5 Hz, 10 V, 1ms	66.3 ± 9.7	60.5 ± 5.6

Intradermal capsaicin (0.33 - 10 nmol) caused a dose-related increase in facial skin blood flow, which was significantly enhanced by IL-1β (50 pg, 40 min) pretreatment (table 1). In contrast, IL-1β pretreatment had no significant effect on facial skin vasodilatation evoked by intradermal CGRP or by trigeminal ganglion stimulation (table 1). In conclusion, IL-1β selectively potentiates the increase in blood flow evoked by capsaicin but not by electrical stimulation of the sensory trigeminal ganglion.

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Escott, K.J. *et al.* (1995) *Brain Res.* 669, 93 - 99.
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88P THE ANTI-INFLAMMATORY AND BEHAVIOURAL EFFECTS OF PHOSPHODIESTERASE INHIBITORS IN THE CONSCIOUS RAT

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The anti-inflammatory properties of phosphodiesterase (PDE) inhibitors suggest that they have therapeutic potential in the treatment of airways inflammation. However, the clinical usefulness of these compounds may be limited by their associated side-effects (Nicholson & Shahid, 1994). This present study investigates the anti-inflammatory and behavioural effects of a range of PDE inhibitors in the rat by comparing their ability to attenuate lipopolysaccharide (LPS)-induced neutrophilia in the lung, and to affect locomotor activity (LMA). Animals were dosed orally with either PDE inhibitor or vehicle. LMA was assessed in mobility recording boxes (Dubocovich *et al.*, 1995) in the 5-20 minutes after dosing. Thirty minutes after dosing, lung neutrophilia was induced by exposing the same rats to an aerosol of LPS (0.1 mg ml^{-1}) for 10 minutes. Four hours later, the rats were killed with an overdose of sodium pentobarbitone (200 mg kg^{-1}), and the lungs lavaged with 2 x 5ml of heparinised (10 u ml^{-1}) phosphate buffered saline. The degree of neutrophilia was assessed by differential cell counts, using light microscopy of cytospin preparations stained with May-Giemsa, and total cell counts, using a Coulter JT haemotology analyser, to calculate the number of neutrophils in the lavage fluid from each animal. Rolipram (0.1-10 mg kg^{-1} p.o.) and IBMX (1-30 mg kg^{-1} p.o.) both caused a dose-related inhibition of the LPS-induced neutrophilia while zardaverine (0.1-10 mg kg^{-1} p.o.) and zaprinast (0.1-10 mg kg^{-1} p.o.) had no effect. However, all these compounds inhibited LMA in the rat (Table 1.). As a result, the therapeutic indices calculated (Table 1.) from these experiments were low. In conclusion, we have shown that anti-inflammatory and the behavioural effects of PDE inhibitors can be assessed in a single animal and a therapeutic index estimated. This model could be useful in attempts to identify novel PDE inhibitors with anti-inflammatory properties but reduced side-effect liability.

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Table 1. Effects of PDE inhibitors on LPS-induced neutrophilia and LMA (for all compounds $n \geq 4$ at each dose).

PDE Inhibitor (selectivity)	Doses mg kg^{-1} p.o.	Inhibition of neutrophilia mean ED_{50NEU}, mg kg^{-1} (range)	Inhibition of LMA mean ED_{50LMA}, mg kg^{-1} (range)	Therapeutic index (ED_{50LMA}/ED_{50NEU})
Zardaverine (type III/IV)	0.1, 1 and 10	>10	3.8 (2.1-5.0)	<0.38
Rolipram (type IV)	0.1, 1 and 10	5.1 (2.1-7.2)	0.28 (0.11-0.42)	0.05
Zaprinast (type V)	0.1, 1 and 10	>10	4.6 (1.2->10)	<0.46
IBMX (non-selective)	1, 10 and 30	11.4 (2.9-19.1)	19.6 (12.5-26.7)	1.72

89P EFFECTS OF Ro 20-1724, A SELECTIVE INHIBITOR OF PHOSPHODIESTERASE IV, ON A LONG LASTING BRONCHOCONSTRICTION AND EOSINOPHILIA IN CHRONICALLY ANTIGEN CHALLENGED GUINEA-PIGS

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Both an early (EAR) and a late asthmatic response (LAR) have been demonstrated in this laboratory in conscious sensitised guinea-pigs following inhalation of antigen (Lewis & Broadley 1995). Ishida et al (1989) have shown that chronic exposure to antigen produces a long-lasting bronchial hyperreactivity and eosinophilia in anaesthetised, sensitised guinea-pigs. We have therefore examined the effects of chronic ovalbumin (OvA) exposure of conscious, sensitised guinea-pigs on airway function and eosinophil accumulation.

Male Dunkin-Hartley guinea-pigs (200-250g) were sensitised to OvA (10μg/ml, in an alum suspension). 14 days later a series of 8 twice weekly exposures to nebulised OvA (0.5%, for 10 min.) was commenced. Mepyramine (30 mg/kg ip) was given 30 min. before challenge to protect against fatal anaphylaxis, the test group also receiving the PDE IV inhibitor, Ro 20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) (3mg/kg ip). Conscious, whole animal plethysmography (Griffiths-Johnson et al. 1988) incorporating a computerised acquisition system, was used to monitor changes in specific airway conductance (sGaw) over a 72 h. period for the 1st and 8th OvA challenges. 72 h. after the 8th challenge, all animals were overdosed with pentobarbitone (60mg/kg ip) and broncho-alveolar lavage (BAL) performed.

Following the 1st OvA challenge, all animals showed both an EAR and a LAR. In the control and Ro 20-1724 treated groups, the early fall in conductance (EAR) peaked at 67.2 $^{+}/_{-}$ 8.0% and 48.3 $^{+}/_{-}$10.7% respectively (n=5 for both, $p>0.05$), which recovered to baseline over the next 5 h. All animals showed a later fall in conductance (LAR) between 17 and 24 h. post challenge, peaking at 29.1 $^{+}/_{-}$5.4% and 17.1 $^{+}/_{-}$5.5%, for control and treated groups respectively ($p>0.05$). By 48 and 72 h, all animals were normal. On the 8th challenge to OvA, both control and treated groups initially showed an EAR of 53.7 $^{+}/_{-}$ 7.5% and 55.7 $^{+}/_{-}$7.5% respectively. The control group, however, failed to recover to baseline values over the next 5 h, and then showed a LAR with a further constriction to 59.2 $^{+}/_{-}$3.5%. Values for sGaw were still 44.9 $^{+}/_{-}$8.3% and 34.5 $^{+}/_{-}$5.4% of the baseline at 48 and 72 h. respectively. In the Ro 20-1724 treated group, sGaw recovered from the EAR within 5 h, and the LAR was significantly attenuated (38.3 $^{+}/_{-}$5.9%, $p<0.05$). By 48 and 72 h, sGaw had returned to baseline. Eosinophils and macrophages were observed in BAL fluid in both groups, the eosinophilia being slightly but significantly attenuated from 46.2 $^{+}/_{-}$2.7% to 37.4 $^{+}/_{-}$2.8% ($p<0.05$) by the Ro 20-1724 treatment.

In conclusion, Ro 20-1724 given prior to each OvA challenge in sensitised guinea-pigs had no significant effect on the responses to the 1st exposure, however it attenuated the long-lasting bronchoconstriction, the enhanced LAR and the eosinophilia associated with the chronic challenges. These effects are presumably the result of inhibition of PDE IV.

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90P INHIBITION OF NITRIC OXIDE SYNTHESIS IS AS EFFECTIVE AS DEXAMETHASONE IN TREATMENT OF ADJUVANT ARTHRITIS

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Nitric oxide (NO) was recently identified as a pro-inflammatory mediator in inflammatory joint diseases (Stefanovic-Racic *et al.*, 1993). In a previous study we found a more than 3-fold increase of urinary nitrate excretion, reflecting total body NO synthesis, in adjuvant arthritic rats compared with healthy control rats (Stichtenoth *et al.*, 1994). To compare the therapeutic effects of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) and dexamethasone in adjuvant arthritis, male Wistar rats were treated from day 20 to day 24 after induction of adjuvant arthritis with either 2 doses of 20 mg L-NAME p.o./kg body weight/day or 0.5 mg dexamethasone/kg body weight p.o./day ($n = 12$, each group). 12 healthy and 12 untreated adjuvant arthritic rats of the same strain and age served as control. At day 20 (before treatment) and day 24 after induction of adjuvant arthritis or at comparable days in the healthy control group, we determined body weight, paw volume and nitrate excretion in 24 h urine. Food and water intake were measured throughout the study period.

Adjuvant arthritis was induced by a suspension of killed Mycobacterium butyricum in mineral oil, injected in the right hind paw under light ether anaesthesia. The volume of the left hind paw was measured by plethsmography. Urinary nitrate was determined by gas chromatography/tandem mass spectrometry using ^{15}N-NO_3 as stable isotope internal standard and corrected by urinary creatinine (Gutzki *et al.*, 1992). Test substances were dissolved in water and administered by gavage.

Before treatment, paw volume (mean 2.4 ± s.d. 0.4 ml) and urinary nitrate excretion (446 ± 110 μmol/mmol creatinine) of arthritic rats were significantly ($p < 0.001$, 2-tailed unpaired t test) higher as compared to healthy controls (1.1 ± 0.2 ml and 141 ± 27 μmol/mmol creatinine, resp.); in contrast, the body weight was lower in arthritic rats (206 ± 20 g versus 259 ± 6 g, $p < 0.001$, 2-tailed unpaired t test). Paw volume and urinary nitrate excretion of arthritic rats were decreased significantly ($p < 0.001$, 2-tailed paired t test) by treatment with L-NAME (mean 1.8 ± s.d. 0.4 ml and 224 ± 45 μmol/mmol creatinine, resp.) as well as by dexamethasone (1.7 ± 0.3 ml and 188 ± 45 μmol/mmol creatinine, resp.). In untreated adjuvant arthritic rats no significant changes of these parameters could be observed. The body weights remained unchanged in all groups during the study period. The effects of L-NAME were slightly, but not significantly lower than those of dexamethasone. Analysis of alimentary nitrate intake exclude exogenous nitrate as reason for differences in urinary nitrate excretion between our study groups.

Our data support the importance of NO as pro-inflammatory mediator in arthritis and suggest that inhibition of NO synthesis might be as effective as dexamethasone for treatment of the acute signs of joint inflammation.

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91P THE ANTI-INFLAMMATORY AND EMETOGENIC PROPERTIES OF PHOSPHODIESTERASE (PDE) INHIBITORS IN CONSCIOUS FERRETS

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PDE inhibitors may possess anti-inflammatory activity and hence be useful in the treatment of respiratory diseases such as asthma (Nicholson & Shahid, 1994). The anti-inflammatory activity of rolipram, zardaverine and IBMX (3-isobutyl-1-methylxanthine) has been investigated using lipopolysaccharide (LPS)-induced neutrophilia in ferret lungs. Simultaneously, the emetogenic properties of these PDE inhibitors were evaluated. Neutrophilia was induced by exposing the ferrets to an aerosol of LPS for 10min, and after a specified time period (1-48h) the lungs were lavaged. The degree of neutrophilia in the bronchioalveolar lavage fluid was assessed by carrying out both total cell counts, and differential cell counts, allowing the calculation of total numbers of neutrophils. LPS (1-1000μg ml^{-1}) produced dose-related neutrophilia, from basal levels (mean±SEM) of 1.83±0.11 (n=7) to 9.35, 12.61 (n=2) million cells with 1000μg ml^{-1}. The maximal increase in neutrophils was achieved after 6-8h, returning to near basal levels after 48h. In all further studies, an LPS dose of 100μg ml^{-1}, and a time course of 6h were used. Ferrets were dosed orally 30min prior to LPS exposure with either a PDE inhibitor or vehicle control (20% cremophor in water). Rolipram (1, 10mg kg^{-1} p.o.), zardaverine (10mg kg^{-1} p.o.) and IBMX (10mg kg^{-1} p.o.) all produced inhibition of LPS-induced neutrophilia (Table 1.), whereas dexamethasone (10mg kg^{-1} i.p.) was used as a positive control and caused 77.1% reduction. Emetogenic activity was variable, IBMX caused marked emesis in both animals tested, rolipram caused moderate emesis in some animals, whereas zardaverine was without emetogenic activity.

These experiments show that exposing ferrets to inhaled LPS induces an acute neutrophilia, which may be attenuated by PDE inhibitors. In addition, emetogenic properties could be evaluated simultaneously, suggesting this model may be useful in the identification of novel PDE inhibitors with better side-effect profiles.

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Table 1. Effects of PDE inhibitors on LPS-induced neutrophilia in the lung and their emetogenic properties in the ferret.(mean±SEM)

Compound	Dose (mg kg^{-1} p.o.)	n	Neutrophils (million cells)	% inhibition of neutrophilia	Emetogenic responders	Mean no. of retches + vomits
Vehicle	-	9	16.73 ± 3.92	-	0/9	0
Rolipram	1	4	7.20 ± 2.78	57.0	1/4	8.0 ± 8.0
	10	4	4.36 ± 1.47	73.9	1/4	2.3 ± 2.3
Zardaverine	10	4	8.27 ± 0.97	50.6	0/4	0
IBMX	10	2	5.14 , 3.76	73.4	2/2	159 , 89

92P MESENTERIC STRETCH AS A MODEL OF VISCERAL NOCICEPTION

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An experimental model to study visceral mechanonociception in anaesthetized rats has been developed and characterized. This model allows to study the effects of stretching the mesentery, a stimulus that is well known from the clinical setting to induce strong autonomic reactions in patients.

Under phenobarbitone anaesthesia a carotid artery was cannulated for measuring mean arterial pressure (MAP) and heart rate (HR). The stomach was cannulated via the pylorus for monitoring intragastric pressure (IGP) at a filling volume of 5 ml. A thread was placed loosely around a jejunal artery and vein just proximal to their branching to the gut in order to stretch the vessels with defined weights. The effects of pulling on the jejunal vessels was compared with the effect of clamping the same vessels right before their branching ('distal clamp') or at their leaving the mesenteric vessels ('proximal clamp') or with the effect of dropping 100 μl 0.6% acetic acid onto the jejunal vessels ('acid').

Basal MAP was between 85 and 111 mm Hg, basal HR between 322 and 355 bpm, and basal IGP amounted to 711 to 885 Pa. Each noxious stimulus led to a prompt and transient change in MAP and HR, which mostly consisted of a short fall followed by a rise. The stomach reacted to such stimuli only with a lowering of the IGP. Graded stretch of the jejunal vessels with weights from 2 to 30 g led to changes of all three parameters. The magnitude of the responses correlated with the weight applied, the correlation for MAP and HR being best when the total absolute deviations from baseline rather than the isolated falls or rises were taken as response parameter (MAP: $r_s = 0.77$, HR: $r_s = 0.71$, IGP: $r_s = -0.86$; each $P < 0.001$ for $n = 6 - 9$). Whereas only one rat responded to a pull with 2 g, the changes in MAP at 30 g were -12 (25 - 0)/+6 (0 - 18) mm Hg (median with range, $n = 9$), changes in HR were -7 (-14 - 0)/+0 (0 - 4) bpm ($n = 6$), and IGP fell by 174 (-263 - 75) Pa ($n = 6$). A weight of 20 g was subsequently used as the standard stimulus.

Phentolamine (1 mg kg^{-1} i.v.) or propranolol (0.3 mg kg^{-1} i.v.) each significantly reduced basal MAP. Only propranolol reduced basal HR and increased basal IGP. Administration of either phentolamine or propranolol alone had no influence on the reflex changes of MAP, HR, or IGP in response to the various noxious stimuli. Only the combination of both reduced the changes in MAP after a pull with 20 g or after acid from 24 (10 - 38) to 9 (0 - 22) mm Hg and from 29 (20 - 39) to 10 (0 - 30) mm Hg (each $n = 10$), respectively, and had no significant effect on the responses to distal or proximal clamping. This combination of α- and β-blocker significantly reduced the changes in HR to all four stimuli and the changes in IGP in response to acid.

Atropine (1 mg kg^{-1} i.v.) slightly reduced basal MAP, but did not influence the reflex responses to the noxious stimuli. If given on top of the combination of α- plus β-blockers it further led to a significant reduction in the gastric response to stretch and proximal clamping.

It is concluded that the cardiovascular and gastric responses of anaesthetized rats to a pull on the mesentery correlate with the strength of the stimulus. Adrenergic α- or β-receptors or muscarinic receptors seem to mediate the reaction of the end organ in the reflex but have no role in the afferent or central processing.

93P EFFECTS OF OPIOID AGONISTS ON SYRIAN HAMSTER SUPRACHIASMATIC NUCLEI (SCN) NEURONES *IN VITRO*

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The hypothalamic SCN function as the dominant circadian pacemaker controlling daily behavioural, physiological and hormonal processes in mammals. Photic entrainment of the SCN biological clock by environmental lighting cycles is mediated via the retinohypothalamic tract and indirectly via the retino-geniculo-hypothalamic pathway, originating from the thalamic intergeniculate leaflet. The putative transmitters of the geniculo-hypothalamic projection to the SCN include neuropeptide Y and enkephalin (Morin *et al.*, 1992). Within the SCN, high levels of μ-, δ- and κ–opioid receptors have been demonstrated using *in vitro* autoradiography (Desjardins *et al.*, 1990). Further, *in situ* hybridization studies reveal high levels of κ-receptor mRNA expression in the SCN (Mansour *et al.*, 1994). The present study addresses the electrophysiological effects of opioid receptor agonists on SCN neurones.

Extracellular recordings were made from single SCN neurones in fifteen male Syrian hamster hypothalamic slices using an *in vitro* submerged preparation (Mason *et al.*, 1987). The effects of superfused (1.5 ml min^{-1}) morphine (1-100 μM), leucine-enkephalin (leu-Enk; 1-100 μM) or dynorphin 1-13 or 1-17 (1-10 μM) contained in artificial cerebrospinal fluid were assessed on basal or NMDA-evoked firing rates of SCN neurones during 5-20 min applications. On basal firing rates, leu-Enk elicited effects (>20% change from baseline) on 2 out of 8 neurones tested (one weak increase and one weak decrease); no effects were observed with morphine (n= 16/18 neurones) apart from one weak increase and one weak decrease. Similarly, no effects on NMDA-evoked responses were elicited by leu-Enk (n=5/5), morphine (n=7/7) or the dynorphins (n=5/5). However, a sustained "withdrawal" excitation was observed following high concentrations (>10 μM) of leu-Enk (n= 6/8) or morphine (n=5/11) 1-10 min after rapid washout of the drug or during co-application of naloxone (100 μM; n= 4/6).

The unresponsiveness of SCN neurones to opioid agonists suggest that the opioids do not alter SCN activity at a postsynaptic site. The possibility that opioid receptors may be located presynaptically to modulate the visual input to the SCN, is currently being investigated.

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94P CHARACTERISATION OF CYCLIC AMP RESPONSES IN A LOCUS COERULEUS-LIKE CELL LINE

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The cell bodies of ascending noradrenergic neurons in the brain are located predominantly in the locus coeruleus (L.C.). In this communication the characterisation of cyclic AMP responses in a L.C.-like cell line (CATH.a) is described. The line is derived from a brainstem tumour of a transgenic mouse in which the transgene consisted of a 773 base pair fragment of the tyrosine hydroxylase promotor fused to the SV-40 T antigen (Suri *et al.*, 1993). The cells synthesise noradrenaline but not adrenaline and express an immunological profile typical of neurons.

Cyclic AMP (cAMP) responses were assessed by measuring the accumulation of [^{3}H]-cAMP in subconfluent monolayers of CATH.a cells prelabelled with [^{3}H]-adenine. Cells were incubated with agonists in the presence of the phosphodiesterase inhibitor rolipram (10μM) for 10 mins at 37°C. [^{3}H]-cAMP was separated from other [^{3}H]-labelled products by alumina chromatography using the single column method described by Johnson *et al.*, (1994). The data are expressed as % conversion (means ± s.e.mean) of [^{3}H]-adenine and [^{3}H]-nucleotides to [^{3}H]-cAMP. Adenylyl cyclase activity was measured in cell membranes by monitoring the conversion of ^{32}P-ATP to ^{32}P-cAMP and data represent percentages of control responses.

In intact cells the basal conversion was 0.48 ± 0.04% (n = 5). Vasointestinal polypeptide (VIP) produced a maximal increase to 8.92 ± 1.24% with an EC_{50} of 240 ± 40nM (n = 3). Forskolin (10μM) increased conversion to 6.39 ± 0.38% (n = 5). The receptor agonists isoprenaline (1μM), 5'-N-ethylcarboxamido- adenosine (10μM), noradrenaline (300μM), histamine (1mM) and L-glutamate (1mM) had no significant effect ($p>0.05$, n = 3, Student's 't' test). The response to forskolin was inhibited by the α_2-adrenoceptor agonist UK14304 with an IC_{50} of 6.76 ± 0.11nM. This inhibition was reversed by yohimbine (Ki = 9.61nM). In contrast, the forskolin responses were unaffected by morphine (100μM), somatostatin (33μM), L-glutamate (1mM), carbachol (100μM), cyclopentyl adenosine (100nM) and 8-hydroxy-2-(dipropylamino)tetralin.

In cell membranes, cyclase activity was enhanced 9-fold by VIP (EC_{50} = 100nM, n = 2). In the presence of forskolin (5μM) conversion was 912 ± 54% of basal (n = 3). Corticotrophin releasing factor enhanced conversion to a maximum of 537 ± 63% of basal with an EC_{50} of 73 ± 10nM (n = 3). The responses to forskolin were inhibited by neuropeptide Y (IC_{50} = 110nM, n = 2) and D-ala-D-leu-enkephalin (IC_{50} = 270nM, n = 2).

The results indicate that the CATH.a cell line displays some of the functional properties expected of noradrenergic neurons and it could prove to be a useful *in vitro* model system for functional investigations of noradrenergic networks.

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95P SUBSTANCE P FRAGMENTS (1-4) AND (8-11) MODULATE ENDOGENOUS DOPAMINE OUTFLOW IN STRIATAL SLICES

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Substance P (SP) can be cleaved by various enzymes and increasing evidence suggests that the resulting fragments are biologically active. Thus, we have shown that SP(1-7) and SP(5-11) modulate locally endogenous dopamine (DA) outflow in rat striatum (Khan *et al.*, 1995a). However, the effects of the complementary fragments SP(1-4) and SP(8-11) have not been explored yet. This study investigated the effect of SP(1-4) and SP(8-11) on spontaneous outflow and potassium (K^+)- induced overflow of endogenous striatal DA.

The experiments were carried out using a method described previously (Khan et al., 1995b). Striatal slices (400-500 μm) obtained from adult male Wistar rats (150-200 g), 2 per tissue holder, were incubated sequentially for 5 min periods in tubes containing 1 ml buffer in the absence (basal outflow) or presence of peptide (2 x 5 min) and / or K^+25 mM (1 min). DA was quantified by HPLC-ECD. Changes in DA outflow were expressed as a percent of basal outflow. This was determined by extrapolating the basal outflow measured in the first 10 min, for the duration of the effect of the drugs.

Basal DA outflow (± s.e.mean, n=72), was 5.39 ± 0.27 pmol/ml/mg protein. One way ANOVA showed that SP(1-4) and SP(8-11) at 10^{-10} and 10^{-9} M significantly increased DA outflow ($F(6,65)= 7.17$ and 6.19, $P<0.05$ respectively)(Table 1). Two way ANOVA showed that the effects of K^+ 25 mM and SP(1-4) and SP(8-11) (10^{-9}M) on DA outflow were additive ($F(1,32)= 0.24$ and 0.705, n.s. respectively) (Table 2).

This study shows that SP(1-4) and SP(8-11) can locally modulate DA outflow in rat striatum. Therefore, the cleavage of SP may lead to fragments which are as efficient as the parent peptide in modulating DA outflow,(Khan *et al.*,1995a) and thus prolong its action.

	DA outflow (% basal)					
Peptide	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6} M
SP(1-4)	124±16	151±11*	162±16*	105±3	77±13	99±12
SP(8-11)	129±27	162±15*	181±17*	113±14	77±6	93±14

Table 1. Effects of SP(1-4) and SP(8-11) on DA outflow from striatal slices. Values expressed as mean ± s.e.mean of 6 determinations. *P<0.05, Bonferroni's test versus respective non-treated controls.

Treatment	**DA outflow (% basal)**
K^+ 25 mM	177.2 ± 12.4*
SP(1-4) 10^{-9}M	164.7 ± 21.7*
SP(8-11) 10^{-9}M	171.7 ± 15.5*
K^+ 25 mM + SP(1-4) 10^{-9}M	257.2 ± 31.4*
K^+ 25 mM + SP(8-11) 10^{-9}M	270.6 ± 27.9*

Table 2. Effect of SP(1-4) and SP(8-11) on K^+ induced DA outflow in striatal slices. Values expressed as mean ± s.e.mean of 6 determinations. * P<0.05, Bonferroni's test versus respective non-treated controls.

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96P SUBSTANCE P AND ITS FRAGMENTS (1-7) AND (5-11) MODULATE STRIATAL DOPAMINE OUTFLOW VIA A CHOLINERGIC LINK MEDIATED BY MUSCARINIC RECEPTORS

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We have shown that substance P (SP) and its fragments SP(1-7) and SP(5-11) modulate dopamine (DA) outflow in rat striatal slices (Khan *et al.*, 1995a). Our results led us to suggest the involvement of NK1 receptors in the observed effects. NK1 receptors are present almost exclusively on striatal cholinergic neurones (Gerfen *et al.*,1991), therefore the modulation of DA outflow by SP and its fragments may involve release of endogenous acetylcholine, which in turn modulates the outflow of the catecholamine. The present study was devoted to the exploration of the cholinergic link and the identification of the type of cholinergic receptors involved in the modulation of DA outflow.

Using a method previously described (Khan *et al.*, 1995b), striatal slices (400-500 μm) , obtained from adult male Wistar rats (200-220 g) were sequentially incubated in oxygenated Krebs-Ringer solution at 34°C containing the agents to be tested. 2 slices were transferred between incubation tubes containing 1 ml solution, at 5 min intervals. Basal outflow was monitored for 10 min, followed by 10 min exposure to SP, SP(1-7) and SP(5-11) (1 nM), in the presence or absence of a muscarinic antagonist (atropine, 1 μM) or a nicotinic antagonist (dihydroerythroidine, 0.5 μM). DA was quantified by HPLC-ECD. Changes in DA outflow were expressed as percent of basal, representing the area under the curve.

Mean basal outflow (±s.e.mean, n=66) was 8.4 ± 0.4 pmol/ml/mg protein. Two way ANOVA showed that SP, SP(1-7) and SP(5-11) induced a significant increase in DA outflow, which was completely reversed in the presence of atropine ($F(1,52)= 10.57$, 16.2, 24.4, all $P<0.05$ respectively). In contrast, dihydroerythroidine failed to antagonise peptide induced overflow ($F(1,72)= 0.69$, 1.312, 2.083, ns. for SP, SP(1-7) and SP(5-11)).

Both antagonists were devoid of intrinsic effects on DA outflow. Our results show that the local modulation of striatal DA outflow by SP involves a cholinergic link, in accordance with data reported in vivo (Anderson *et al.*, 1993). Furthermore, the effects of SP(1-7) and SP(5-11) appear to involve a similar mechanism. The endogenous acetylcholine released activates muscarinic receptors, leading to increased DA outflow. The nature of this cholinergic modulation, i.e. presynaptic or indirect, via additional links, remains to be established.

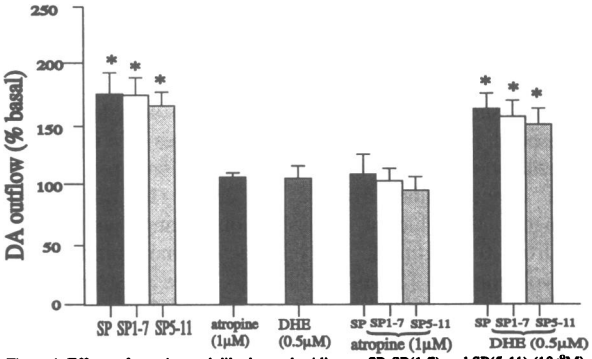

Figure 1. Effects of atropine and dihydroerythroidine on SP, SP(1-7) and SP(5-11) (10^{-9}M) induced endogenous DA outflow. Means ± s.e.mean, n= 4-18. *P<0.05, Bonferroni's test versus respective non-treated controls.

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97P STRIATAL DOPAMINE RELEASE IN AN *IN VITRO* MODEL OF CEREBRAL ISCHAEMIA: 1. MODULATION OF ENERGY SUPPLY AND DEMAND

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Release of neurotransmitters, including dopamine (DA), is thought to play a central role in neuronal death during acute cerebral ischaemia [Obrenovitch & Richards, 1995]. In the present study we investigated the effects of changes in energy demands and supply on DA release in an *in vitro* model of cerebral ischaemia.

Rat striatal slices were superfused (400ml/h) with an artificial cerebrospinal fluid (ACSF) at 34°C, unless otherwise stated. Episodes of 'ischaemia' were mimicked by removal of O_2 and reduction in glucose concentration from 4 to 2mM. The ensuing DA release was monitored by fast cyclic voltammetry [Stamford, 1990]. Three measures were recorded; (a) the time to onset of DA release (T_{on}), (b) the rate of DA release as expressed as time from onset to peak (T_{pk}) and (c) the maximum DA release (DA_{max}). Each brain slice had a paired control from the same rat. Statistical comparisons were made by paired t-test.

TABLE 1	T_{on} (s)	T_{pk} (s)	DA_{max} (μM)
Control (34°C)	164±11	21±7	88±13
Hypothermia (24°C)	608±72**	234±78*	40±11*
Control (34°C)	150±8	17±2	65±12
Hyperthermia (38°C)	130±5	19±3	55±10

** P<0.05, ** P < 0.01, vs paired controls. Means ±s.e.m., n=6/7*

Table 1 (above) shows the effect of temperature upon DA release in ischaemia.Table 2 (below) shows the effect of pre-incubation with creatine (25mM) on DA release in ischaemia.

TABLE 2	T_{on} (s)	T_{pk} (s)	DA_{max} (μM)
Control	164±11	32±20	125±16
Creatine (30 min)	183±10	34±10	130±39
Control	158±11	18±2	85±24
Creatine (180 min)	239±33**	77±29*	77±21

** P<0.05, ** P < 0.01 vs paired controls. Means ± s.e.m., n=5/7.*

Profound hypothermia (24°C) is known to reduce the severity of ischemic injury [see Obrenovitch & Richards, 1995]. These data show that it also delays and reduces ischaemia-induced DA release relative to controls (34°C), although mild hyperthermia (38°) had no significant effect. Preincubation with creatine increases tissue creatine phosphate levels, thus allowing more ATP to be regenerated from ADP during ischaemia [Whittingham 1986]. Our data shows that preincubation with 25mM creatine for 3 hours but not 30 minutes also delays DA release without affecting its magnitude. We conclude that DA release during *in vitro* 'ischaemia' is a useful index of ischemic injury and may allow the screening of neuroprotecive drugs.

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98P STRIATAL DOPAMINE RELEASE IN AN *IN VITRO* MODEL OF CEREBRAL ISCHAEMIA: 2. EFFECT OF GLUCOSE SUPPLY DURING HYPOXIA

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Neurotransmitter release during acute cerebral ischaemia is thought to play a central role in neuronal death. Reduction of dopamine (DA) release *in vivo* has been shown to reduce neuronal cell damage [Buisson et al, 1992] following ischaemia. In the present study we investigated the effects of changes in glucose concentration on DA release in an *in vitro* model of cerebral ischaemia.

Rat striatal slices were superfused (400ml/h) with an artificial cerebrospinal fluid (ACSF) at 34°C. Episodes of "ischaemia" were usually mimicked by removal of O_2 and reduction in glucose concentration from 4 to 2mM. In the present experiments, the glucose concentration during hypoxia was varied from 0 to 10mM. DA release was monitored by fast cyclic voltammetry [Stamford, 1990]. Three measures were recorded; (a) the time to onset of DA release (T_{on}), (b) the rate of DA release as expressed as time from onset to peak (T_{pk}) and (c) the maximum DA release (DA_{max}).

Glucose (mM)	T_{on} (s)	T_{pk}(s)	DA_{max}(μM)
0	164±25	55±22	72±6
2	174±12	20±7	78±12
4	190±14	18±4	86±7
10	1238±522[1]	143±20[2]	38±12[3]

Means ± s.e.m., n=4-6. [1] P<0.05 vs 0, 2 and 4mM glucose. [2] P < 0.01 vs 0mM and P < 0.001 vs 2 & 4mM glucose.[3] P<0.05 vs 4mM glucose (ANOVA with Tukey-Kramer multiple comparisons test).

Table 1 (above) shows that elevation of glucose concentration to 10 mM during hypoxia substantially delayed, slowed and reduced DA release. Table 2 (below) shows that mannitol had no significant effect.

	T_{on} (s)	T_{pk}(s)	DA_{max}(μM)
Control	155±9	23±14	104±23
Mannitol (10mM)	138±16	50±16	86±25

Means ±s.e.m., n=4.

There is some discrepancy between *in vivo* and *in vitro* data on the role of glucose in hypoxia. In hippocampal slices, glucose concentrations above 2 mM protect against loss of field potentials [Dong et al, 1988]. However, *in vivo*, hyperglycaemia (19mM) normally exacerbates ischaemic brain damage, measured histologically [Kofke et al, 1994]. This may be, in part, due to greater lactacidosis *in vivo*. The current data offer further functional evidence that hyperglycaemia is protective *in vitro*. This effect is not due to elevated extracellular osmolarity since mannitol (10mM) had no effect.

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Current concepts on the cause of Parkinson's disease (PD) suggests that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-like toxic substances may be responsible for nigral cell death. Isoquinoline derivatives structurally related to MPTP are possible endogenous neurotoxins. We and others have shown that these compounds are potent mitochondrial inhibitors and are moderate substrates for the dopamine re-uptake system (McNaught *et al.*,1994;1995a,b,c), but their dopaminergic toxicity is unclear. Therefore, we have examined the effects of exposing PC12 cells in culture to increasing concentrations (up-to 1000 µM) of 4 isoquinoline derivatives and MPP^+ (Fig. 1) for 3 days by techniques described elsewhere (Hartley *et al.*, 1994). Cytotoxicity was determined from the release of lactate dehydrogenase (LDH).

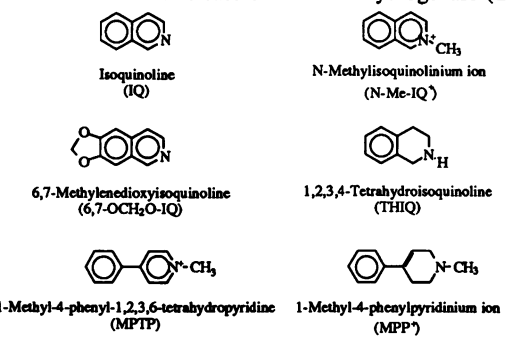

Fig. 1. Structures of the isoquinoline derivatives, MPP^+ and MPTP.

All compounds showed concentration-dependent toxicity towards PC12 cells, but with different potencies (Fig. 2). None of the isoquinoline derivatives were more toxic than MPP^+ which produced 50% LDH release at 375 µM.

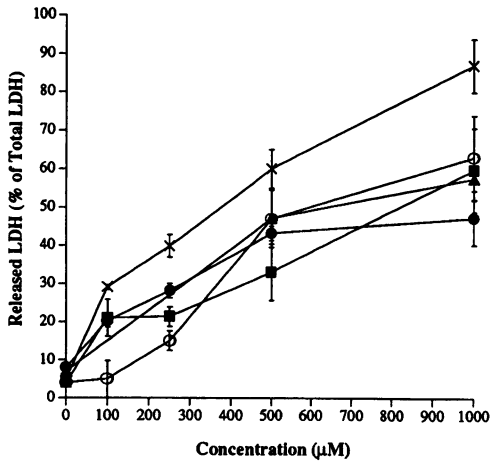

Fig. 2. Effects of isoquinoline derivatives and MPP^+ on PC12 cells. Results are presented as mean ± SD for duplicate experiments.

The concentrations of IQ, 6,7-OCH_2O-IQ and THIQ producing 50% LDH release were 640, 600 and 820 µM, respectively. N-Me-IQ^+ failed to produce 50% LDH release, but at the maximum concentration tested (1000 µM), this reached 47%. The cytotoxicity of these compounds correlates well (r = 0.9) with their substrate affinity for the dopamine re-uptake system (McNaught *et al.*, 1995c) and supports the hypothesis that isoquinoline derivatives may be responsible for degeneration of the dopaminergic nigrostriatal pathway in PD.

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100P ACTIVATION OF RAT LOCUS COERULEUS NEURONES BY HALOTHANE: A REGIONAL EVALUATION OF TERMINAL NORADRENALINE OVERFLOW AND FOS PROTEIN IMMUNOREACTIVITY

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Few *in vivo* dialysis studies have investigated regional effects of general anaesthetic agents on brain monoamine turnover. Therefore, in the present studies we have examined the effects of halothane (HA) on extracellular levels of noradrenaline (NA) in the rat hippocampus and frontal cortex. In parallel studies, expression of FOS protein in the locus coeruleus was measured as a marker of neuronal activation.

Concentric dialysis probes (4 mm Hospal) were vertically implanted in the hippocampus (AP-5.8; L5.0; V-7.0 *cf* bregma) and frontal cortex (AP+3.0; L3.2; V-4.0 *cf* bregma) of male SD rats (250-280 g) and, 24 h later, perfused with phosphate-buffered medium (pH 7.4) at 1.0 $\mu l.min^{-1}$. Basal monoamine levels were determined over 4x20 min samples before rats were injected with saline (2 $ml.kg^{-1}$) and, 5 mins later, anaesthetised with HA/O_2 (2%) for 80 min. Body temperature was maintained at 37°C. Thereafter, rats were returned to their home-cage for an 80 min recovery period. Dialysate levels of NA, DOPAC and 5-HIAA were measured by HPLC-ECD. Statistical analyses were carried out on raw data using one-way ANOVA for repeated measures. The cellular localisation of FOS protein was measured essentially as described by Meddle & Follet (1995). The primary antibody used was rabbit anti-FOS protein (X-FOS-3) at 1:10,000 dilution for 48 h at 4°C. Differences in FOS cell counts in comparative 50 µm sections of the locus coeruleus were assessed using a Mann-Whitney U test.

HA significantly elevated NA efflux in cortical dialysates ($F_{8,53}$=4.48; P<0.001) but failed to modify hippocampal NA efflux (see Table 1). Cortical NA efflux was unaffected by saline administration ($F_{8,35}$=0.6; P=0.7). The rise in cortical NA efflux after HA was accompanied by a 3-fold rise in DOPAC efflux ($F_{8,53}$=17.1 P<0.001) and a significant decline in 5-HIAA efflux ($F_{8,53}$=21.3 P<0.001). In addition, HA significantly enhanced the cellular expression of FOS protein in the locus coeruleus from 11±2 cells (n=4) in control animals to 78±5 cells (n=4) in HA-treated animals (P=0.03).

Table 1. HA-induced changes in dialysate NA levels ($fmol.20min^{-1}$ ± s.e.mean)

	basal (0 min)	halothane (80 min)	recovery (160 min)
frontal cortex (n=6)	19.8±2.7	28.4±2.6	18.8±3.8
hippocampus (n=5)	15.6±1.0	13.5±1.1	13.5±1.6

The results show that HA depolarises neurones in the locus coeruleus and augments NA overflow in the frontal cortex but not in the hippocampus. It is concluded therefore that HA selectively activates coeruleo-cortical noradrenergic neurons and/or local mechanisms play a crucial role in the regulation of terminal NA release, in agreement with a previous report (van Veldhuizen *et al.*, 1994).

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We acknowledge the generous gift of anti-FOS protein from Dr. D. Hancock (I.C.R.F.). This study was funded by UCB Pharma.

101P BRAIN CORTICOSTEROID RECEPTORS: A COMPARISON OF METHODS FOR THEIR ASSESSMENT IN THE RAT CORTEX

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Most studies employing ^{3}H-dexamethasone to measure corticosteroid receptors in rat brain have used either Sephadex LH20 column chromatography or dextran coated charcoal for the separation of bound from free ligand (Przegalinski & Budziszewska, 1993; Reul *et al* 1993). Although these methods provide a reasonable separation and give good specific to non-specific binding ratios, they are both time consuming and are subject to high inter-assay variability. The aim of this study was to compare column chromatography with vacuum filtration through glass fibre filters for the separation of bound from free ^{3}H-dexamethasone to rat cortical corticosteroid receptors.

Male Sprague Dawley rats (200-250g) were stunned and killed by decapitation. Following dissection, the cortex was homogenized in 10 or 20 volumes (w/v) of TEDGM buffer (10mM Tris, 1mM EDTA, 35mM sodium molybdate, 10% glycerol and 1mM dithiothreitol, pH 7.4) and centrifuged at 105,000g for 60mins at 0-4°C to yield the cytosolic fraction (supernatant) containing corticosteroid receptors. Aliquots (100μl) of this fraction were incubated at 0-4°C for 20-24 hours with ^{3}H-dexamethasone (3nM for characterisation and competition experiments and 0.6-20nM for saturation experiments) in a final assay volume of 250μl. Specific binding was defined either by 5μM or 500-fold excess hydrocortisone. Hydrocortisone (10^{-12} - 10^{-3}M) was used in competition studies. Bound and free ^{3}H-dexamethasone were separated either by eluting 100μl aliquots of the incubation mixture through the columns with a 600μl buffer wash or by filtering the entire mixture through filters (pre-soaked in 0.3% polyethylenimine for 4 hours) with a 4x4 ml buffer wash. Binding parameters were determined by non-linear regression analysis to a one-site model.

The specific binding (in dpm and as % of total binding) of ^{3}H-dexamethasone (3nM) obtained using GF/B (3745±424, 87%), and GF/F (4623±689, 88%) glass fibre filters was not significantly different from that obtained using column chromatography (4030±727, 78%). Binding obtained with GF/C filters (2691±366, 85) was significantly lower than that obtained using GF/B/F and columns ($p<0.05$, ANOVA). Values are means±SEM, n=3.

The results from competition and saturation experiments using column chromatography and filtration through GF/F filters are shown in Table 1.

Table 1. Binding parameters of ^{3}H-dexamethasone from parallel saturation and competition experiments.

	Filtration	Columns
Bmax (fmol/mg prot)	158±9 (*6*)	149±12 (*6*)
Kd (nM)	1.1±.0.3 (*6*)	0.96±0.15 (*6*)
Competition IC_{50}nM	14.8±2.5 (*4*)	9.2±0.6 (*4*)

Values are means±SEM from (*n*) experiments.

Our study has shown that the binding of ^{3}H-dexamethasone to corticosteroid receptors in rat cortex assessed by filtration is comparable to that obtained with column chromatography. In view of the high specific to non-specific binding ratio and the ease of processing larger numbers of samples, filtration could prove to be the method of choice in studies of corticosteroid receptors.

We would like to thank NESCOT for supporting this work.

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102P IS CEREBELLAR GRANULE CELL NECROSIS IN THE RAT FOLLOWING L-2-CHLOROPROPIONIC ACID DUE TO GLUTATHIONE DEPLETION?

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Neutralised L-2-chloropropionic acid (L-CPA) when dosed orally at 750 mg/kg to male AP rats (approx. 180-200g body weight) causes cerebellar granule cell necrosis between 36-48 h after dosing. We have been unable to detect any morphometric, behavioural or biochemical changes following L-CPA administration up until 36h following dosing except for a decrease in both cerebellar and liver glutathione (GSH) concentrations (Anderson, 1985), that may be related to the development of the neurotoxicity. Maximal reductions in cerebellar GSH concentrations occur 24h after L-CPA dosing (*P<0.01; Student's t-test). (t= 0h, GSH = 1.8 µmol/g wet weight, t =24h GSH = 0.2 µmol/g wet weight, data shown as mean of n = 5-9 experiments). Reductions in liver GSH concentrations occured more rapidly than in cerebellum with maximal reductions being acheived 4h after dosing (control = 6.0 µmol/g wet weight, L-CPA = 1.3 µmol/g wet weight) and remaining significantly lower than controls up to 12 h after L-CPA administration before slowly returning to control values over the following 24 h (n = 3/time point). The reduction in liver GSH concentration is due to the conjugation of L-CPA with GSH as has been demonstrated using liver cytosol preparations *in vitro* (Km= 145 µM; Vmax = 1.26 nmol/min/mg protein). However no conjugation of L-CPA to GSH has been detected with cerebellar preparations *in vitro* and therefore the slow reductions in cerebellar GSH concentrations may be due to a reduction in GSH synthesis following L-CPA administration.

One hypothesis for L-CPA-induced depletion of GSH in the cerebellum may be related to inhibition of amino acid transport into neuronal cells, in particular the transport of cysteine which is a component of GSH. We propose that cerebellar granule cell necrosis may be due to oxidative stress as a result of the lower antioxidant GSH concentrations. [^{14}C]Cystine (10 µM) was incubated with cerebellar slices (0.5 mm), (obtained from rats killed by fluothane overdose) in oxygenated Krebs-Ringer buffer at 37 °C for between 15 and 60 min. The effect of glutamate, L-CPA and the L-CPA conjugates with GSH and cystine were investigated. L-CPA-cysteine (0.1 mM) significantly reduced [^{14}C]cystine uptake into cerebellar slices by 50% over 1 h (P< 0.01 as compared to control). L-CPA up to 1 mM was without effect. L-glutamate at 1 mM was able to reduce [^{14}C]cystine uptake by approximately 50% over 1 h (P< 0.01 as compared to controls). The DL-CPA/GSH conjugate was also able to reduce [^{14}C]cystine uptake (control = 5.97 ± 0.29; 0.1 mM = 4.90 ± 0.47; 1 mM =2.38 ± 0.45; nmol/100mg tissue wet weight/h; mean ± S.D., n = 4). In conclusion the ability of the L-CPA-GSH metabolite, L-CPA-cysteine, to reduce [^{14}C]cystine transport into cerebellar slices may be relevant to the reductions in cerebellar GSH concentratons observed following L-CPA administration *in vivo*. The reduced antioxidant capacity of cerebellar granule cells as a result of L-CPA-induced reductions in GSH concentrations may make them more susceptible to cytotoxicity.

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103P A METHOD FOR MEASURING EXTRACELLULAR GLUTAMATE IN THE NUCLEUS ACCUMBENS OF THE RAT *IN VIVO*

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The nucleus accumbens (NAc) has a key role in psychomotor function and the mechanism of action of many classes of psychotropic drugs, including antidepressants (Willner & Scheel-Krüger, 1991). Glutamatergic pathways to NAc provide a major source of afferent information to this region. However, surprisingly little work has focussed on psychotropic drug effects on glutamate release in NAc. In the present study we have developed and validated an HPLC-based assay for the detection of glutamate, and used it in combination with microdialysis to measure glutamate in NAc of the awake rat.

Glutamate was measured using a novel HPLC assay adapted from one used previously for the detection of GABA (Smith & Sharp, 1994a, 1994b). Glutamate, either in standard solution or perfusate samples, was derivatized by reaction with OPA/sulphite reagent (44 mg OPA, 1 ml ethanol, 1 ml 1 M sodium sulphite, 18 ml 0.4 M boric acid buffer, pH 10.4) at room temperature for 25 min. Derivatized glutamate was separated on a reversed-phase HPLC column (Rainin Dynamax 25 cm, C_{18}, 5 µm) using a mobile phase comprised of; 0.1 M NaH_2PO_4, 0.5 mM EDTA, 10 % (v/v) methanol at pH 5.0. Detection was achieved using a glassy carbon electrode (+0.85 V *vs.* Ag/AgCl). Initial experiments determined the effect on detector response of injected amounts of glutamate (0 to 20 pmol), derivatization reaction time (0 to 240 min), working electrode potential (+0.4 to +1.0 V) and pH of mobile phase (pH 3.5 to 6.5).

For microdialysis experiments, a single cannula probe was stereotaxically implanted into NAc of halothane anaesthetized male Sprague-Dawley rats weighing 270-300 g (Smith & Sharp, 1994b). After a 24 h recovery period, awake, freely-moving rats were connected to a perfusion system and the probe was perfused (at 1 µl/min) with artificial cerebrospinal fluid (CSF); 1.2 mM NaH_2PO_4, 0.27 mM Na_2HPO_4, 140 mM NaCl, 3.0 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 7.2 mM glucose. Perfusate samples were collected every 25 min. Experiments were performed to determine the basal level of extracellular glutamate in NAc and its response to perfusion with artificial CSF containing a glutamate uptake blocker, high potassium, tetrodotoxin (TTX) and calcium-free medium.

OPA-derivatized glutamate eluted at about 12 min and there was a linear relationship between amount injected and detector response. The limit of detection of glutamate (twice baseline noise) was approx. 0.1 pmol. Derivatization of glutamate was complete by 30 min with the derivative formed slowly degrading over the following hours. The hydrodynamic voltammogram for derivatized glutamate had a half-wave potential of about +0.60 V *vs.* Ag/AgCl. Results indicated that the glutamate derivative was susceptible to on-column hydrolysis at low mobile phase pH.

Basal levels of glutamate in perfusate samples from the NAc of the awake rat were constant over several hours (about 5 pmol/20 µl sample). Addition of either 50 mM KCl or 0.5 mM L-trans-pyrollidine-2,4-dicarboxylic acid (a glutamate uptake blocker) to the perfusion medium for 25 min increased glutamate by 92±25 % (mean ± s.e.mean, n=4) and 326±30 % (n=3), respectively. Glutamate levels were not significantly altered (n=3 in each case) by perfusion with calcium-free medium or medium containing TTX (1 µM).

In summary, we report a method for the measurement of extracellular glutamate in NAc of the awake rat. Results indicate that glutamate is increased by a depolarising stimulus but not reduced by calcium omission or addition of TTX. Future studies of psychotropic drug effects on glutamate in NAc should take into account the possibility that basal extracellular glutamate in this region may not be derived from a neuronal source.

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104P METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED EFFECTS ON RESPONSES OF SPINAL NEURONES TO IONOTROPIC AMINO ACID AGONISTS AND SYNAPTIC EXCITATION

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Metabotropic glutamate receptor (mGluR) agonists can increase background activity and potentiate the responses of rat spinal neurones to ionotropic agonists *in vivo* (Baker *et al.*, 1993; Jones & Headley, 1995). The potentiation of NMDA, AMPA and kainate responses produced by both (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD, a non-selective mGluR agonist) and (RS)-3,5-dihydroxyphenylglycine ((RS)-3,5-DHPG, selective for group I, PI-linked, mGluRs) was secondary to an enhanced excitability since there was no effect when NMDA/AMPA cycling was superimposed on background discharge held constant with kainate ("excitability controlled"). We have now tested these mGluR agonists and the group III (presynaptic, cAMP-linked) mGluR agonist L-2-amino-4-phosphonobutyric acid (L-AP4) on responses to electrophoretically administered GABA and on synaptic responses to peripheral noxious pinch.

Experiments were performed on α-chloralose anaesthetized, spinalized male Wistar rats (300-400g). Action potentials of single spinal neurones were recorded extracellularly and EAA agonists were ejected microelectrophoretically: AMPA (10mM in 200mM NaCl, pH 7.8-8.0), kainate (5/200mM, pH 7.4-8.0), GABA (100/100mM, pH 4.0), (1S,3R)-ACPD (25/150mM, pH 7.9-8.1), (RS)-3,5-DHPG (50/150mM, pH 4.3) and L-AP4 (50/150mM, pH 8.0). Ionotropic agonists were ejected in regular cycles, in some cases alongside a pinch (1.0-3.0N over 20mm^2) applied to the ipsilateral hindpaw. mGluR agonists were tested with or without excitability control (see above). Their effects on evoked responses are expressed as % of the last three pre-drug control responses ± s.e.m.

During excitability controlled tests, (1S,3R)-ACPD did not significantly potentiate NMDA, AMPA or GABA-evoked responses, which remained at 105 ± 2% (n=17), 95 ± 2% (n=18) and 96 ± 9% (n=7) control respectively (*cf* results of Baker *et al.*, 1993 on GABA). (RS)-3,5-DHPG similarly had no significant effect under these conditions, the responses to the three ionotropic agonists remaining at 106 ± 4% (n=6), 94 ± 4% (n=6) and 105 ± 16% (n=4) control. These results suggest that a membrane depolarisation, rather than an increase in membrane resistance, was involved in the increase in cell excitability and that this effect was mediated by group I mGluR.

In tests on synaptic responses, (RS)-3,5-DHPG significantly potentiated pinch responses to 143 ± 5%, at the same time increasing AMPA-evoked responses to 193 ± 10% (n=6, $p<0.05$, Wilcoxon) as well as any spontaneous activity. In contrast, preliminary data with L-AP4 show that responses to pinch were reduced to 46 ± 4% on 3 of 5 units tested, whereas responses to AMPA remained at 89 ± 6% control.

These results suggest that group I mGluR activation in the spinal cord has postsynaptic actions and that the enhancement of synaptic responses is secondary to an increase in cell excitability, whereas group III mGluR agonists can directly and selectively affect excitatory synaptic responses, implying a presynaptic site of action.

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105P BOTH INTRINSIC MEMBRANE PROPERTIES AND SYNAPTIC TRANSMISSION ARE MODULATED BY ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS IN SPINAL CORD VENTRAL HORN NEURONES

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Activation of metabotropic glutamate receptors (mGluRs) in the hippocampus has a dual effect on intrinsic membrane properties and neurotransmission (Watkins & Collingridge, 1994). In this study we investigated the effects of the mGluR agonists 1SR,3RS-ACPD (*t*-ACPD), (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) and 2S,1'S,2'S-2-(2'-carboxy-cyclopropyl)glycine (L-CCG-1) on primary afferent synaptic transmission and 1S,3R-ACPD and L-CCG-1 on neuronal excitability in ventral horn (VH) neurones in the rat hemisected spinal cord *in vitro*.

Urethane-anaesthetised rats (10-14 days) were cooled prior to a laminectomy and removal of the spinal cord (King *et al.*, 1992). Hemisected cords were perfused with gassed modified ACSF and intracellular techniques were used to record from VH neurones. Dorsal root (DR)-evoked EPSPs were elicited using suction electrodes and supramaximal stimulation (200 μA, 200 μs). For analysis of neuronal excitability, action potentials were evoked by applying long duration direct depolarizing current pulses (0.1-0.4 nA, 350 ms) and the rheobase (threshold) current determined for each neurone.

All three mGluR agonists produced dose-related reductions of the DR-EPSP mean ± s.e.m. amplitude even when the membrane potential was clamped at its pre-drug resting level. *t*-ACPD superfused at 10 μM (n=6), 25 μM (n=4), 50 (n=4) and 100 μM (n=4) produced reductions of 13.4 ± 3.4 % ($p < 0.02$), 29.3 ± 3.5 % ($p < 0.005$), 42.3 ± 3.3 % ($p < 0.005$) and 55.8 ± 6.0 % ($p < 0.005$) respectively. 1S,3R-ACPD at 10 μM (n=6), 25 μM (n=7) and 50 μM (n=4) attenuated the mean ± s.e.m. DR-EPSP amplitude by 19.2 ± 4.5 % ($p < 0.01$), 45.0 ± 3.8 % ($p < 0.001$) and 64.0 ± 8.9 % ($p < 0.02$) respectively. Similarly, L-CCG-1 at 2 μM (n=4), 5 μM (n=5) and 25 μM (n=8) depressed the DR-EPSP by 13.2 ± 2.8 % ($p < 0.01$), 32.5 ± 6.8 % ($p < 0.05$) and 59.2 ± 7.4 % ($p < 0.001$) respectively.

1S,3R-ACPD and L-CCG-1 produced an apparent increase in VH neurone excitability manifest as an increase in the number of spikes in response to rheobase current and a decrease in the mean ± s.e.m. peak amplitude of the spike AHP. 1S,3R-ACPD (10-20 μM) increased the average numbers of spikes from 2 ± 0.3 to 5 ± 1.4 ($p < 0.02$) (n=15) while L-CCG-1 (10-20 μM) increased the average numbers of spikes from 1 ± 0.3 to 3 ± 0.6 ($p < 0.02$) (n=7). 1S,3R-ACPD over two concentrations (10 and 20 μM) attenuated the amplitude of the AHP by 20.5 ± 3.9 % ($p < 0.01$) and 30.0 ± 8.3 % ($p < 0.05$) respectively. L-CCG-1 at 10 μM and 20 μM reduced the AHP amplitude by 24.5 ± 5.2 % ($p < 0.01$) and 28.0 ± 0.96 % ($p < 0.02$) respectively.

In the ventral spinal cord, as in other CNS areas, activation of mGluRs can modulate intrinsic membrane properties and synaptic transmission. Both effects will impact on information processing in spinal circuits.

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106P EFFECTS OF METABOTROPIC GLUTAMATE RECEPTORS ON PAIRED PULSE DEPRESSION IN THE RAT DENTATE GYRUS *IN VITRO*

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Paired pulse depression (PPD) of the second synaptic response to paired stimuli in the medial perforant pathway of the dentate gyrus has been well characterised across a broad range of interstimulus intervals (ISIs) both *in vivo* and *in vitro* (Bekenstein *et al.*, 1993; Harris & Cotman, 1985). There is now evidence that metabotropic glutamate receptors (mGluRs) modulate perforant path responses, via both pre- and post-synaptic mechanisms in the hippocampus (Kahle & Cotman, 1993). In the present study we have investigated the effect of a number of mGluR agonists and antagonists on PPD in the medial perforant pathway of the rat dentate gyrus *in vitro*.

Transverse hippocampal slices were prepared from male Wistar rats (50-150g) by standard methods. Briefly slices were equilibrated for at least 1 hour in a perfusion chamber at 31-33°C, pH 7.3, in oxygenated artificial cerebrospinal fluid (composition in mM: NaCl, 120; KCl, 2.5; $MgSO_4$, 2.0; $CaCl_2$, 2.0; $NaHCO_3$, 26; NaH_2PO_4, 1.25; D-Glucose, 10). A monopolar glass stimulating electrode was placed in the middle one-third of the molecular layer of the dentate gyrus to evoke field excitatory post-synaptic potentials (epsps) in the proximal medial perforant pathway every 20s. Paired pulses were applied at ISIs of 10, 20, 50, 100, 200 and 800ms. Recordings were analysed off-line using the Strathclyde electrophysiology software (Dr J Dempster). All drugs were bath applied onto the slice for at least 30min, except 1s,3r-1-amino cyclopentane-1,3-dicarboxylic acid (ACPD; 5min). All values are given as the mean±sem.

In control slices the second synaptic response was consistently smaller than the first at all ISIs tested (e.g. by 11.4±1.6% and 18.7±1.4% at 50 and 100ms ISI respectively; n=16). The mGluR agonist, ACPD (10μM) reduced the amplitude of the first synaptic response (S_1) by 41.3±1.4%. ACPD also reduced PPD at all ISIs less than 800ms (e.g. by 8.0±1.7% at 50ms ISI; $p<0.01$; n=4; Students t test). Application of the mGluR antagonist, α-methyl carboxy-phenylglycine (MCPG; 100μM) had no effect on its own but blocked the transient depression of S_1 by ACPD (n=3). MCPG (100μM) inhibited the ACPD-induced reduction in PPD at all ISIs tested (n=3).

The mGluR agonist, L-2-amino-4-phosphonobutyric acid (L-AP4; 20μM) also decreased S_1 amplitude by 27.9±3.5% (n=4) and reduced PPD at ISIs less than 800ms (e.g. by 5.1±1.5% at 50ms ISI; $p<0.01$; n=4). The putative presynaptic mGluR antagonist α-methyl-L-AP4 (MAP4) at 20μM enhaced PPD at ISIs less than 100ms on its own (e.g by 2.8±1.0% at 50ms ISI; $p<0.05$; n=3). However MAP4 at 20μM and 100μM did not block the LAP4-induced reduction in PPD. (e.g at 50ms ISI, PPD was reduced by 5.1±1.5% in 20μM L-AP4 compared to 9.0±1.6% in both LAP4 and MAP4; n=3).

These results further demonstrate the involvement of mGluRs in the modulation of PPD in the medial perforant pathway. They may also indicate an important role for presynaptic mGluRs in this form of plasticity. However, the effect of MAP4 in our experiments requires further investigation.

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107P THE ANTICONVULSANT FPL 12495 LIMITS SUSTAINED REPETITIVE FIRING OF RAT CA1 HIPPOCAMPAL NEURONES *IN VITRO*

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FPL12495 (1,2–diphenyl–2–propylamine) is thought to interact with the ion channel site of the *N*–methyl–D–aspartate (NMDA) receptor complex as it is a noncompetitive antagonist of [^{3}H]–MK801 binding (Palmer *et al.*, 1992). Like the established anticonvulsants phenytoin, carbamazepine and valproate, FPL12495 is effective against maximal electroconvulsive shock (Palmer *et al.*, 1992). As each of the former compounds has been shown to limit high–frequency firing (MacDonald, 1989) the aim of the present study was to determine if the novel anticonvulsant FPL12495 acts similarly.

Blind whole–cell recordings were made from CA1 neurones in submerged slices (400 μm) of adult rat hippocampus, perfused with ACSF (22–23 °C). Neurones had a resting membrane potential (RMP) of -64.2 ± 0.7 mV (n=18; mean ± S.E.M.) and input resistance (IR) of 144.5 ± 10.9 MΩ (n=18). Trains of action potentials (APs) were evoked by injection of depolarising current pulses (20–400 pA, 500 ms) from the RMP. NMDA and FPL12495 were perfused for 1 and 15 min, respectively. FPL12495 (4–400 μM) caused a hyperpolarisation (1–6 mV, n=8), depolarisation (2–4 mV, n=2) or no change of the RMP (n=8). The IR in the presence of FPL12495 (400 μM) was increased to 208.0 ± 31.2 MΩ (n=5; p≤0.05). FPL12495 (40 μM) reduced the depolarisation evoked by NMDA (10 μM) from 9.4 ± 0.5 mV to 6.1 ± 0.2 mV (n=3, p≤0.05). FPL12495 caused a concentration-dependent reduction in the number of APs elicited by a given depolarising pulse (EC_{50} 80 μM). FPL12495 also caused a concentration–dependent reduction of the AP amplitude and rate of rise and an increase in AP duration (see Table 1).

The present study suggests that the novel anticonvulsant FPL12495 may have a dual action on central neurones. In addition to a reduction of NMDA–evoked excitations, FPL12495 limits sustained repetitive firing and reduces the rate of rise of APs. These latter effects are consistent with a partial block of voltage–dependent sodium channels.

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Palmer, G.C., Murray, R.J., Wilson, T.C.M. *et al.*, (1992). *Epilepsy Res.* 12, 9–20.

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Table 1. The effects of FPL12495 on the amplitude, duration and rate of rise of the first action potential in a train.

	n	Amplitude (mV)	Duration (ms)	Rate of rise (V s^{-1})	
Control	15	105.1 ± 2.9	4.1 ± 0.1	166.0 ± 12.6	* denotes statistical significance
FPL12495 (40 μM)	6	98.8 ± 2.2	5.4 ± 0.3*	136.3 ± 13.5	(p≤0.01; students paired t-test)
FPL12495 (400 μM)	5	33.5 ± 9.6*	13.2 ± 1.9*	27.8 ± 11.8*	

108P mGluRs NEGATIVELY COUPLED TO ADENYLYL CYCLASE ARE POTENTLY ANTAGONISED BY NOVEL PHOSPHONO- AND SULPHONO- BUT NOT TETRAZOLYL-SUBSTITUTED PHENYLGLYCINE DERIVATIVES

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In adult rat cortical slices metabotropic glutamate receptors (mGluRs) negatively coupled to adenylyl cyclase are potently and relatively selectively stimulated by L-2-amino-4-phosphonobutyric acid (L-AP4), and (2S,3S,4S)-α-(carboxycyclopropyl)-glycine (L-CCG-1) (Bedingfield *et al.*, 1995).

We have previously reported the antagonist effects of 3- and 4-carboxy substituted phenylglycine derivatives against the agonists L-AP4 and L-CCG-1 at these mGluRs (Kemp *et al.*, 1994, 1995). We now report the antagonist effects of three novel phenylglycine derived compounds: (RS)-α-methyl-4-phosphono-phenylglycine (MPPG), (RS)-α-methyl-4-sulphono-phenylglycine (MSPG) and (RS)-α-methyl-4-tetrazolyl-phenylglycine (MTPG).

Antagonism of the depression of monosynaptic excitation elicited by L-AP4 and (1S,3S)-1-aminocyclopentane-1,3-dicarboxylate in neonatal rat motoneurones by these compounds has been reported by Jane *et al.*, (1995).

Adenylyl cyclase present in adult rat cortical slice (0.2x0.2mm) was stimulated with 30μM forskolin in the presence of 10μM L-AP4 or 0.3μM L-CCG-1 (EC_{50} values for cyclic AMP inhibition in this system), adenosine deaminase, Ro 20-1724 and antagonists.

Accumulated cyclic AMP was assayed by displacement of [^{3}H] cyclic AMP from cyclic AMP binding protein. Data regarding the reversal of L-AP4 and L-CCG-1 agonist effects by the novel phenylglycine derivatives are presented as IC_{50} values in table 1.

MPPG is the most potent antagonist of cortical mGluR for both L-AP4 and L-CCG-1 found to date. It is approximately twice as potent against L-CCG-1 than L-AP4. MSPG is also a very potent antagonist but is unselective. MTPG whilst having low potency *versus* both agonists (in contrast to effects on mGluRs of neonatal rat spinal cord motoneurones (Jane *et al.*, 1995)), has good selectivity for L-AP4.

Bedingfield J.S. *et al.*, (1995) Br. J. Pharmacol., (in press).
Jane D.E. *et al.*, (1995) Neuropharmacology, (in press).
Kemp M.C. *et al.*, (1994) Br. J. Pharmacol., 113, 147P.
Kemp M.C. *et al.*, (1995) Br. J. Pharmacol., 114, 325P.

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

		L-AP4	L-CCG-1
(RS) α methyl-4-phosphonophenylglycine	MPPG	156.2 ± 28.8nM	69.5 ± 0.5nM
(RS) α methyl-4-sulphonophenylglycine	MSPG	169.6 ± 75.9nM	162.9 ± 40.7nM
(RS) α methyl-4-tetrazolylphenylglycine	MTPG	59.4 ± 7.3μM	607.3 ± 135.1μM

109P CHARACTERISATION OF METABOTROPIC GLUTAMATE RECEPTOR COUPLED PHOSPHOINOSITIDE HYDROLYSIS IN CULTURED RAT CEREBELLAR GRANULE CELLS

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To date, eight mGluRs have been cloned in the rat and have been subdivided into three groups. Group I mGluRs (mGluR1 & 5) are coupled to phosphoinositide (PI) hydrolysis and are potently activated by L-quisqualate, whereas both Group II (mGluR2 & 3) and Group III (mGluR4, 6, 7 & 8) mGluRs are negatively coupled to adenylyl cyclase (Pin & Duvoisin, 1995). Previous studies have demonstrated the ability of several excitatory amino acids (EAA's) to stimulate PI hydrolysis in cultured cerebellar granule cells (Nicoletti *et al.*, 1986; Chavis *et al.*, 1994). The aim of the present study was to investigate the ability of several phenylglycine derivatives to influence PI hydrolysis in a neurone reported to contain high levels of mGluR1 mRNA and negligible amounts of mGluR5 mRNA (Santi *et al.*, 1994), the cerebellar granule cell.

Cultures of rat cerebellar granule cells were prepared as previously described and seeded onto 12-well plates containing medium supplemented with fetal calf serum (10% v/v) and L-glutamine (4mM) (Toms & Roberts, 1995). PI hydrolysis was determined by pre-labelling the cells with [^{3}H]-*myo*-inositol for 24hrs as described by Nicoletti *et al.* (1986). Briefly, cells (4-6 days old) were washed, challenged with agonists (and antagonists if appropriate) for 30min in the presence of 10mM LiCl and [^{3}H]-inositol monophosphate separated by ion exchange chromatography. All experiments were carried out in the presence of 50μM D-2-amino-5-phosphonopentanoic acid, 30μM 6-cyano-7-nitroquinoxaline-2,3-dione and 1μM tetrodotoxin.

Three EAA analogues were tested for their ability to stimulate PI hydrolysis, in order of potency (EC_{50}): L-quisqualate (2$\pm$1μM) »L-glutamate (50$\pm$3μM) > (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (102$\pm$11μM). Furthermore, L-quisqualate (2μM)-stimulated PI hydrolysis was inhibited in a dose-dependent manner by, in order of potency (IC_{50}): (S)-4-carboxy-3-hydroxyphenylglycine (41$\pm$7μM) ≥(S)-4-carboxyphenylglycine (51$\pm$2μM)>(+)-α-methyl-4-carboxyphenylglycine (243$\pm$62μM).

These data demonstrate that Group I mGluR pharmacology in cultured rat cerebellar granule cells is similar to that reported for mouse cerebellar granule cells (Chavis *et al.*, 1994) and mammalian cell line mGluR1 pharmacology (Hayashi *et al.*, 1994).

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Toms, N.J. & Roberts, P.J. (1995). *Br. J. Pharmacol.* **114**, 277P.

110P EFFECT OF INCREASING CHAIN LENGTH OF PHENYLGLYCINE DERIVATIVES ON ACTIVITY AT METABOTROPIC GLUTAMATE RECEPTORS LINKED TO PHOSPHOINOSITIDE HYDROLYSIS

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Of the eight metabotropic glutamate receptors (mGluRs) cloned to date, group 1 (1 and 5) mGluRs couple to phosphoinositide (PI) hydrolysis. As yet the most active antagonist at PI linked mGluRs in the rat neonatal cortex is the known non-selective mGluR antagonist (+)-α-methyl-4-carboxyphenylglycine ((+)MCPG) (Jane *et al.*, 1993). Replacement of the methyl group with an ethyl group has been shown not to affect potency (Bedingfield *et al.*, 1995). It was therefore of interest to investigate other homologues of 4-carboxyphenylglycine. Here we report the effect of increasing the chain length on the activity at mGluRs linked to PI hydrolysis.

PI hydrolysis was measured as previously described (Porter *et al.*, 1992). Briefly, cross-chopped (300μm x 300μm) slices from the cerebral cortices of 6-8 day old rats were loaded with D-[^{3}H]-*myo*-inositol prior to incubation (in the presence of LiCl) with the antagonist. Following the addition of the agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) and a further incubation, the reaction was terminated with $CHCl_3/CH_3OH$ and centrifugation. [^{3}H]-inositol-monophosphate was separated from the aqueous phase by ion exchange chromatography and counted by liquid scintillation spectrometry.

All experiments were performed three times in triplicate and the K_B values determined as a measure of the activity of each compound, these values are summarised in Table 1. From comparison of the compounds tested here with (+)MCPG and (RS)-α-ethyl-4-carboxyphenylglycine (K_B = 0.18 and 0.36mM respectively) (Bedingfield *et al.*,1995) it is evident that they are of a similar order of magnitude except for (RS)αProp4CPG which was found to be of lower potency.

The activity of these phenylglycine derivatives at other mGluRs has yet to be determined. Their activity in this system most likely reflects activity at mGluR5 since *in situ* hybridisation studies suggest that this subtype is the most abundant PI linked mGluR in the cortex (Abe *et al.*, 1992). Should these compounds prove to be selective for PI-linked mGluRs they may be useful pharmacological tools in view of their similar potency to (+)MCPG, which is still the most active known antagonist at group 1 mGluRs.

Supported by the MRC.

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Jane D.E. *et al.*, (1993) Neuropharmacology, 32, 725-727.
Porter R.H. *et al.*, (1992) Br. J. Pharmacol., 106, 509-510.

Table 1. K_B (mM) ± s.e.m values for test compounds at mGluRs linked to PI hydrolysis (n=3)

(RS)-α-propyl-4-carboxy phenylglycine	((RS)αProp4CPG)	1.13 ± 0.2
(RS)-α-butyl-4-carboxy phenylglycine	((RS)αBut4CPG)	0.33 ± 0.16
(RS)-α-pentyl-4-carboxy phenylglycine	((RS)αPent4CPG)	0.37 ± 0.04
(RS)-α-phenyl-4-carboxy phenylglycine	((RS)αPhe4CPG)	0.37 ± 0.05

111P VERATRINE-STIMULATED RELEASE OF PRE-LOADED [^{3}H]GLUTAMATE FROM GUINEA-PIG CEREBROCORTICAL SYNAPTOSOMES IS INHIBITED BY METABOTROPIC GLUTAMATE RECEPTOR AGONISTS

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It is assumed that (mGluR) agonists that depress neurotransmission by a presynaptic action do so by reducing release of excitatory amino acids. Such an action at presynaptic glutamate receptors has been reported for L-2-amino-4-phosphonobutyrate (APB) (Abdul-Ghani, 1985; Gannon *et al.*, 1989). Subsequently, mGluR agonists which act selectively on particular mGluR subtypes have been synthesised. Whilst none of the agonists are specific for a particular mGluR their rank order of potency facilitates the fuller characterisation of the mGluR subtypes which are acting as inhibitory autoreceptors. We have investigated the action of the mGluR agonists APB, L-*cis*-1-aminocyclopentane-1,3-dicarboxylic acid (cACPD), *o*-phosphoserine (oPS), (2S,3S,4S)-α-(carboxycyclopropyl)-glycine (L-CCG-I), *trans*-(1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (tACPD), on the release of pre-loaded [^{3}H]glutamate by veratrine from purified synaptosomal fractions.

Synaptosomes were prepared from the cerebral cortex of 500g male guinea pigs by sucrose density centrifugation.. The synaptosomes were resuspended in oxygenated HEPES-buffered Krebs saline and exposed for 10 min to 5 μCi/ml of [^{3}H]glutamate at 37°C. After washing in Krebs at room temperature the loaded synaptosome suspensions were decanted into aliquots of 200 μg in 200 μl Krebs. Following a 20 min equilibration period mGluR agonists were added. After a 1 min incubation with the mGluR agonist, veratrine was added to give a final concentration of 75 μM. Following a 5 min incubation at 37°C the incubations were spun at 1000 g for 1 min. The pelleted synaptosomes were separated from their supernatants and both were measured in a β-counter. The release was expressed as the amount of radioactivity in the supernatant over the total amount in the synaptosomes at the beginning of the incubation. Inhibition of release by a given mGluR agonist was expressed as the percentage difference in release in the presence and the absence of the mGluR agonist. The mGluR agonists were tested at concentrations from 10 nM to 100 μM.

All agonists tested were capable of inhibiting veratrine-stimulated release of pre-loaded [^{3}H]-glutamate from guinea pig cerebrocortical synaptosomes. The calculated maximal inhibitions were 24.08 ± 0.95%. IC_{50} values ± s.e.m. are given in Table 1.

Table 1 Inhibition of veratrine stimulated [^{3}H]glutamate release by mGluR agonists

	IC_{50} (μM)
APB	3.79 ± 0.78
cACPD	4.52 ± 0.78
oPS	5.33 ± 0.98
tACPD	5.97 ± 1.28
L-CCG-I	6.61 ± 1.35
CHPG	9.89 ± 2.54

When incubated with varying concentrations of the mGluR agonists the release of pre-loaded [^{3}H]glutamate from guinea-pig cerebrocortical synaptosomes was inhibited in a concentration dependent manner. All the mGluR agonists showed a maximal inhibition of glutamate release of about 20-25%. The rank order of potency of the agonists for the release inhibiting mGluRs as measured by the calculated IC_{50} values was APB ≥ cACPD ≥ oPS ≥ tACPD ≥ L-CCG-I > CHPG.

These results suggest that in the guinea-pig cerebral cortex glutamate release from nerve terminals is inhibited by presynaptic metabotropic autoreceptors of both type III and II.

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112P HIGH EXTRACELLULAR GLYCINE DOES NOT POTENTIATE DEPOLARIZATIONS EVOKED BY APPLICATION OF N-METHYL-D-ASPARTATE TO THE RAT STRIATUM *IN VIVO*

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N-methyl-D-aspartate (NMDA) receptor ionophore complexes have a distinct positive allosteric regulatory site for glycine, and the occupation of this site by glycine is required for NMDA/glutamate receptor activation (Thomson, 1989). Submicromolar concentrations of glycine markedly potentiate NMDA responses in cultured neurons and other *in vitro* preparations, and at higher concentrations glycine may also act as an agonist at the NMDA recognition site. These findings led to the hypothesis that elevated extracellular glycine may contribute to ischaemia-induced NMDA/glutamate receptor activation and, therefore, to excitotoxicity (Globus *et al.* 1991). The purpose of this study was to examine, *in vivo*, the effect of high extracellular glycine on NMDA-evoked responses.

Male Sprague-Dawley rats (250-350 g) were anaesthetized throughout with halothane in N_2O:O_2 (1:1). Microdialysis probes incorporating an electrode were implanted in the striatum and perfused at 1 μl/min with artificial CSF (Obrenovitch *et al.*, 1994). Changes in the extracellular field (d.c.) potential were recorded with the microdialysis electrode and a reference placed beneath the scalp. In the first series of experiments, increasing concentrations of glycine (from 1 μM to 100 mM) were perfused sequentially through the probe for 3 min, each glycine application being followed by 17 minutes recovery. In a second series, depolarising stimuli were evoked by perfusion of 200 μM NMDA for 2 min through the probe, either alone, or with co-application of glycine (1 to 100 mM). Application of up to 1 mM glycine had no effect on the local d.c. potential. Above 10 mM, glycine application produced concentration-dependent depolarization, but the amplitude of these responses remained very small (0.33 ± 0.08 and 0.52 ± 0.05 mV for 30 and 100 mM glycine, respectively; n = 10; mean ± s.e.mean) (Figure 1). Similarly, co-application of up to 1 mM glycine did not potentiate the responses to 200 μM NMDA. Only 30 mM and 100 mM glycine increased significantly NMDA-induced depolarizations (110.2 ± 7.8 and 111.4 ± 9.2 % of control, respectively; mean ± s.e.mean; n=10, $P < 0.05$ by Students *t* test).

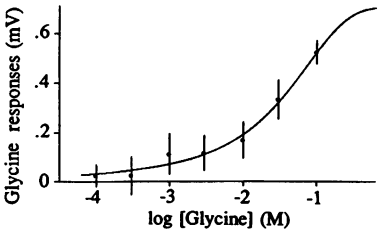

Figure 1. Effects of high extracellular glycine on local d.c. potential. Values are mean ± s.e.mean, n = 10.

These data do not support the view that increased extracellular concentrations of glycine, such as those observed in ischaemia (Globus *et al.* 1991), are potentially excitatory/excitotoxic. Nevertheless, as occupation of the glycine site coupled to the NMDA receptor is required for NMDA activation, this site remains an attractive target for potential neuroprotective agents.

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Obrenovitch, T.P., Urenjak, J. & Zilkha, E. (1994) *Br. J. Pharmacol.* 113, 1295-1302.

113P CHARACTERISATION OF STABLY EXPRESSED RECOMBINANT HUMAN N-METHYL-D-ASPARTATE RECEPTORS USING A $^{45}Ca^{2+}$-INFLUX ASSAY

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The N-methyl-D-aspartate (NMDA) receptor is comprised of at least one NR1 subunit and at least one of a family of NR2 subunits (Monyer *et al.*, 1992). cDNA encoding the human NR1a subunit was stably co-transfected with either human NR2A or NR2B cDNAs into L(tk-) cells under the control of a dexamethasone inducible promoter. Cells were plated into 24-well plates at a density of 1 x 10^5 cells / well and induced in the presence of 0.5 mM ketamine. 72 h after induction, cells were extensively washed (3 x 1 ml) with warm Ca^{2+}- and Mg^{2+}-free Locke's buffer and preincubated with test compounds for 5 min, prior to addition of 0.5 µCi $^{45}CaCl_2$/ well (final $[Ca^{2+}]$ of 2.3 mM). After 2 min incubation at 37 °C, the $^{45}Ca^{2+}$ was rapidly aspirated and the cells washed (3 x 1 ml) with ice-cold 2.3 mM Ca^{2+}-Locke's buffer. Cells were solubilised with 1 % SDS and samples taken for liquid scintillation counting using a β-counter. $^{45}Ca^{2+}$-influx was maximal in the presence of 100 µM L-glutamate and 100 µM glycine (NR1a/NR2A: 3038 ± 1149 dpm (mean ± s.e.mean; n=4); NR1a/NR2B: 3235 ± 826 dpm), addition of 100 µM dizocilpine (MK-801) determined background levels (NR1a/NR2A: 302 ± 67 dpm; NR1a/NR2B: 278 ± 36 dpm).

Inhibition curves were constructed for NMDA receptor antagonists inhibiting $^{45}Ca^{2+}$-influx in the presence of 100 µM L-glutamate and 100 µM glycine (Table 1).

Table 1. Inhibition of glutamate- and glycine-stimulated $^{45}Ca^{2+}$-influx by NMDA receptor antagonists.

	NR1a/NR2A	NR1a/NR2B
Dizocilpine	0.191 (0.125, 0.291)	0.125 (0.098, 0.159)
Phencyclidine	2.14 (1.68, 2.74)	1.07 (0.652, 1.75)
Ketamine	10.1 (4.22, 24.0)	4.88 (3.35, 7.11)
Ifenprodil	298, 329*	0.491 (0.395, 0.612)

Data shown are mean K_d values (geometric mean (-s.e. mean, +s.e.mean) (µM) of ≥ three experiments. *n=2.

No significant differences were observed between affinity values obtained with the two cell lines, for the ion channel ligands dizocilpine, phencyclidine and ketamine (Table 1). The sub-type selective polyamine site antagonist ifenprodil (Williams, 1993) had a 600-fold higher affinity for NR1a/NR2B receptors (Table 1). These results show that the pharmacology of stably expressed NMDA receptors can be investigated using $^{45}Ca^{2+}$-influx, and that subtype-selective compounds such as ifenprodil will distinguish between the two cell lines.

Monyer, H., Sprengel, R., Schoepfer, R., et al. (1992) Science 256, pp 1217-1221.
Williams (1993) Mol. Pharmacol. 44, pp 851-859.

114P INTRACORTICAL INFUSION OF NMDA RECEPTOR ANTAGONISTS: NEUROCHEMICAL EFFECTS

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In contrast to the effects of non competitive NMDA receptor antagonists, systemic administration of antagonists for either the glutamate agonist or glycine coagonist recognition sites do not activate mesocorticolimbic dopamine (DA) metabolism and consequently do not induce locomotor activity(Bristow *et al.*,1994). However, recent studies have indicated that infusion of the competitive NMDA receptor antagonist 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphonate (CPP) directly into the medial prefrontal cortex (mPFCx) increased cortical DA metabolism (Hata *et al.*,1990). Therefore, in the present study we have compared the effects of antagonists for the glycine and glutamate sites of the NMDA receptor on DA and 5-HT metabolism in various brain regions following their infusion into the mPFCx.

Male Sprague Dawley rats (250-300g) were implanted under isoflurane anaesthesia with a unilateral guide cannula (23G) into the mPFCx (coordinates according to Paxinos and Watson (1982): A, 3.7 mm from bregma; L, 1.0 mm from midline; H, 2.0 mm below dura) and secured with dental acrylic. 48 h later an injection unit(30G), (1 mm longer than the guide) was inserted into the mPFCx and rats infused with 0.5 µL of either vehicle or drug solution at a rate of 0.5 µL /min.

Animals were killed at 0.5, 2 or 4 hours after infusion, brains removed,dissected ipsilaterally into regions, and stored at -80°C until required for analysis of DA, 5-HT, and their metabolites by HPLC. The ratio (DOPAC)+(HVA)/(DA) and (5HIAA)/(5HT) provided an index of DA and 5HT metabolism respectively.
CPP (1.5 µg/ 0.5 µl) significantly increased DA metabolism in mPFCx and nucleus accumbens (n. Acc.) and striatum 0.5 h after infusion. By 2 and 4 h after infusion DA metabolism was significantly increased only in mPFCx and n. Acc. In contrast, the glycine site antagonist 5,7-dichlorokynurenic acid (5,7-DCKA) (1.5 µg/0.5 µl) did not significantly affect DA metabolism in mPFCx or n. Acc. and significantly decreased that in striatum at 2 h (Table 1). 5-HT metabolism was not affected by either CPP or 5,7 DCKA in mPFCx, n. Acc. or striatum at any time examined (data not shown). These results further illustrate that antagonists for different modulatory sites on the NMDA receptor differentially affect mesocorticolimbic DA function.

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Hata, N. *et. al.*, (1990) Neurosci. Lett., 120, 101-104.
Paxinos, G. and Watson, C. (1982) The Rat Brain in Stereotaxic Coordinates, Sydney: Academic Press

Table 1.	(DOPAC) + (HVA) / (DOPAMINE) (mean ± s.e.mean)								
	0.5 hours			2 hours			4 hours		
Treatment (dose)	mPFCx	n.Acc.	Striatum	mPFCx	n.Acc.	Striatum	mPFCx	n.Acc.	Striatum
Vehicle	0.82±0.05	0.32±0.01	0.23±0.01	1.5±0.20	0.21±0.01	0.20±0.01	1.5±0.1	0.23±0.01	0.20±0.01
CPP (1.5µg)	1.52±0.23*	0.36±0.01*	0.28±0.01*	2.8±0.10*	0.31±0.02*	0.22±0.02	3.2±0.16*	0.30±0.01*	0.27±0.01
5,7-DCKA (1.5µg)	0.74±0.07	0.34±0.01	0.22±0.01	1.5±0.10	0.21±0.01	0.15±0.01*	1.8±0.12	0.23±0.01	0.20±0.01

* $p < 0.05$ compared with vehicle controls by Tukeys test following one way ANOVA. n = 6/10 per group.

115P HYPERACTIVITY INDUCED BY COMPETITIVE BUT NOT GLYCINE/NMDA RECEPTOR ANTAGONISTS FOLLOWING INJECTION INTO RAT MEDIAL PREFRONTAL CORTEX

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Whilst non-competitive NMDA receptor antagonists (e.g. MK-801) induce a distinct hyperactivity response in rodents, increased locomotor activity is usually not observed following systemic injection of competitive or glycine/NMDA receptor antagonists (Bristow et al., 1993, 1994). Recent studies, however, have shown that increased locomotion is observed following direct microinjection of the competitive NMDA receptor antagonist (±)-CPP (3-(±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid) into the rat medial prefrontal cortex (mPFCx; O'Neill and Liebman, 1987). Thus in the present study, we have examined the behavioural effects of mPFCx injection of (±)-CPP and CGS 19755 (cis-4-(phosphonomethyl)piperidine-2-carboxylic acid) and compared them with those of the glycine/NMDA receptor antagonist 5,7-dichlorokynurenic acid (5,7-DCKA) in the rat.

Male Sprague Dawley rats (270 - 320g) were anaesthetised with isoflurane and bilaterally implanted with 12 mm, 23 gauge guide cannulae for microinjection into the mPFCx (A/P 3.7 mm, L ± 1 mm from bregma; V - 2 mm from dura (Paxinos and Watson, 1982)). Animals were allowed at least 3 days recovery and tested on one occasion only. Rats were habituated to individual activity cages equipped with 5 infrared beams, 1 positioned at each end of the base of the cage to record cage crossings i.e. consecutive beam breaks and 3 positioned across the top of the cage to record rearing. One hour later they were removed and drug or appropriate vehicle infused at a rate of 0.5 µl/min via a 13 mm, 31 gauge injection unit which remained in place for a further 60 s. Photocell beam breaks were then monitored for 4 h and results expressed as the mean ± s.e. mean total number of cage crosses and rears recorded. Data was analysed by analysis of variance followed by Dunnett's t test comparing all animals to vehicle-infused rats (*P<0.05). Doses are expressed as µg administered in 0.5 µl into each cannula (n = 7 - 10 rats/group).

Drug	Dose	Crosses	Rears
(±)-CPP	0	17 ± 3	15 ± 4
	0.15	69 ± 17*	104 ± 29*
	0.5	360 ± 94*	251 ± 71*
	1.5	2093 ± 323*	1197 ± 263*
CGS 19755	0	14 ± 4	11 ± 2
	0.05	42 ± 11*	37 ± 7*
	0.15	195 ± 55*	185 ± 46*
	0.5	1233 ± 287*	426 ± 106*
5,7-DCKA	0	19 ± 4	13 ± 3
	0.5	17 ± 4	10 ± 3
	2.5	24 ± 4	10 ± 4

In contrast to the marked increase in locomotor activity and rearing induced following intra-mPFCx injection of (±)-CPP and CGS 19755, the glycine/NMDA receptor antagonist 5,7-DCKA failed to alter rat activity at the doses tested. These results provide further evidence of the contrasting behavioural profiles of different classes of NMDA receptor antagonist in rodents.

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116P INVESTIGATION OF FOCAL ISCHAEMIA IN THE RAT: BEHAVIOURAL CONSEQUENCES AND NEUROPROTECTION

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Occlusion of the middle cerebral artery (MCAO) is one of the most common forms of stroke in man. The artery perfuses both neocortex and striatum. This study assessed the relative contribution of cortical and sub-cortical damage to the associated behavioural deficit, in the rat, and the effect of the potent neuroprotectant MK801.

The behavioural paradigm used to assess skilled forepaw reaching was the staircase test (Marston *et al.*). The apparatus was designed so that only the left paw could be used to recover pellets from the left stair and *vice versa*. Performance was quantified as the number of pellets recovered either contra- or ipsi-lateral to the lesion. The Lister hooded rats (male, 285-300g) were trained to perform the task to a preoperative criterion. They were then randomly allocated to six experimental groups. Under stereotaxic guidance and halothane/nitrous oxide anaesthesia rats received either an endothelin-1 induced MCAO (60pmol in 3.0 µl infusion), a quinolinic acid lesion of the striatum (2 x 100pmol in 0.75µl infusions) or appropriate control operation. Each group was then further divided, with either *i.v.* MK801 (0.3mg/kg) or vehicle being administered one minute post-lesion. Each group contained six rats. Animals were allowed to recover for four days before behavioural testing was re-commenced. Following a further 18 test sessions animals were sacrificed and the brains processed for histological assessment. Analysis (3-way ANOVA, Tukey *post hoc*) revealed that MCAO induced a profound bilateral impairment in paw reaching ($p<0.001$), while the striatal lesioned rats were contralaterally biased ($p<0.01$). Treatment with MK801 attenuated both impairments, but in both cases left a residual contralateral bias ($p<0.01$). Histological analysis confirmed that MCAO induced damage to both cortex and striatum, which MK801 significantly reduced ($p<0.01$). The quinolinate lesion was restricted to the striatum, which was also attenuated by MK801 ($p<0.01$). The data illustrate that MK801 mediated neuroprotection can be quantified behaviourally. Further, the data confirm a major role for cortical processing during the performance of a skilled reaching task.

Table 1.	Histological Damage		Paw Reaching	
Treatment	Cortical	Striatal	Contra.	Ipsi.
Control	2.6 ± 0.2	0	96 ± 1.5	98 ± 1.1
MCAO	39 ± 2.0*	64 ± 5.3 *	27 ± 5.8 *	36 ± 6.2 *
Striatal	3.5 ± 0.3	79 ± 6.9 *	44 ± 2.0 *	83 ± 8.3
MCAO + MK	13 ± 3.5†	51 ± 5.5 †	64 ± 5.2 †	93 ± 5.4 †
Striatal + MK	3.1 ± 0.2	37 ± 3.2 †	60 ± 6.8 †	101 ± 0.6

Histological damage expressed as percent contralateral structure ± 1 s.e.m.. Behavioural data as percent of pre-operative baseline ± 1 s.e.m. * significant compared with Control ($p<0.01$), † significant compared with lesion alone ($p<0.01$).

Marston H.M. *et al.*, (1995) Neuroreport, **6**, 7, 1067-1071

117P EFFECTS OF NMDA RECEPTOR ANTAGONISTS ON ACETYLCHOLINE EFFLUX *IN VIVO*

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NMDA receptors are involved in the regulation of CNS cholinergic function and a recent study (Hasegawa et al., 1993) showed that systemic administration of the non-competitive NMDA receptor antagonist dizocilpine (MK-801) enhanced cortical acetylcholine (Ach) release. In the present study we have examined the effects of antagonists for the glutamate agonist, glycine co-agonist and ion channel sites of the NMDA receptor on hippocampal and striatal Ach efflux *in vivo*.

Male Sprague Dawley rats (250-300g) were implanted, under isoflurane anaesthesia, with a concentric dialysis probe either in the hippocampus (A -4.8mm; L 5.0mm; V -8.0mm) or striatum (A 0.2mm; L 3.0mm; V -7.5mm, coordinates from bregma and dura according to Paxinos and Watson, 1982). After overnight recovery, the probe was perfused with physiological salt solution (125mM NaCl, 2.5mM KCl, 1.2mM $MgCl_2$, 1.3mM $CaCl_2$) containing 2μM neostigmine at a flow rate of 2μl/min. Samples were collected at 20 min intervals and analysed by HPLC with a post-column enzyme reactor and electrochemical detection (Hutson et al., 1990). In hippocampus, Ach efflux was increased by MK-801 (0.25 and 0.5mg/kg i.p.) to a maximum of 179 ± 19% and 368 ± 34% of basal and by phencyclidine (PCP) (5 and 10mg/kg i.p.) to a maximum of 314 ± 51% and 451 ± 29% of basal respectively. In contrast, the glycine site antagonist R-(+)-HA-966 (30 and 60mg/kg i.p.) and the competitive glutamate receptor antagonists CGS19755 (5mg/kg i.p.) and 3-[(±)-2-carboxy piperazin-4-yl]-propyl-1-phosphonate (CPP) (10mg/kg i.p.) did not affect Ach efflux. In striatum, extracellular Ach concentration was increased by vehicle injection but not further affected by MK-801 (0.5mg/kg i.p.), PCP (10mg/kg i.p.) or HA-966 (30mg/kg i.p.) (Table 1). The marked increase of hippocampal but not striatal Ach efflux by MK-801 and PCP, combined with the lack of effect of glycine or glutamate site antagonists on Ach efflux, parallels the effects of these compounds on mesolimbic and striatal dopamine metabolism (Bristow et al., 1993). Therefore, it is conceivable that the increase of hippocampal Ach efflux by MK-801 and PCP is secondary to their effects on mesolimbic dopamine metabolism.

Bristow, L.J. et al. (1993) Br. J. Pharmacol. 108, 1156-1163.
Hasegawa, M. et al. (1993) Neurosci. Lett. 150, 53-56.
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Table 1	Maximal Ach efflux (% of basal, mean ± s. e. mean)								
	vehicle	MK-801		PCP		R-(+)-HA-966		CGS19755	CPP
dose (mg/kg)		0.25	0.5	5	10	30	60	5	10
hippocampus	129 ± 3	179 ± 19*	368 ± 34*	314 ± 51*	451 ± 29*	118 ± 18	125 ±15	149 ± 19	142 ± 22
striatum	163 ± 25	-	119 ± 12	-	159 ± 15	146 ±13	-	-	-

* $p < 0.05$ compared with vehicle controls by Students t test following ANOVA, n = 4-11

118P THE INCREASE OF HIPPOCAMPAL ACETYLCHOLINE EFFLUX BY MK801 IS MEDIATED VIA DOPAMINE D_1 BUT NOT D_2 RECEPTORS

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Hippocampal acetylcholine (ACh) efflux is increased by the non-competitive NMDA receptor antagonist dizocilpine (MK801) at doses which also increase mesocorticolimbic dopamine (DA) metabolism (Hogg & Hutson, this meeting). DA neurones arising from the ventral tegmental area are known to innervate, via GABA interneurones, cholinergic cells in the medial septum which project to the hippocampus. Therefore, in the present study, we have determined if the increase in hippocampal ACh efflux induced by MK801 is mediated indirectly by activation of mesolimbic DA pathways.

Male Sprague-Dawley rats (250-200 g, B&K) were anaesthetised using isoflurane and implanted with a 5 mm concentric dialysis probe (Hospal membrane) in the hippocampus (Paxinos & Watson, 1982: anterior -4.8 mm from bregma, lateral ±4.8 mm, depth 8.0 mm from dura, incisor bar - 5.0 mm). After overnight recovery, the probe was perfused with artificial CSF (125 mM NaCl, 2.5 mM KCl, 1.18 mM $MgCl_2$, 1.26 mM $CaCl_2$) containing 2 μM neostigmine, at a flow rate of 2 μl/min. Twenty minute samples were collected and analysed by HPLC with a post-column enzyme reactor and electrochemical detection (Hutson et al., 1990). Rats were injected subcutaneously with either vehicle (saline, 1 ml/kg), the D1 receptor antagonist SCH23390 (0.03 mg/kg and 0.3 mg/kg) or the D2 receptor antagonist haloperidol (0.1 mg/kg) 20 mins before either saline (1 ml/kg, i.p.) or MK801 (0.5 mg/kg, i.p.). Mean area under curve (A.U.C.) ± s.e.mean was calculated for the 120 min preceding either vehicle or MK801 injection (pre-injection) and for the 320 min following either vehicle or MK801 injection (post-injection). Data were subjected to two-way analysis of variance followed by Tukeys test.

Basal ACh efflux in rat hippocampus was 1.2 ± 0.07 pmol/20μl/min (mean ± s.e.mean., n=64). MK801 (0.5 mg/kg, i.p.) increased hippocampal ACh approximately 400% above basal levels at 2 hours after injection. Pre-treatment of rats with SCH23390 (0.3 mg/kg, s.c.), but not 0.03 mg/kg, i.p. (data not shown), attenuated ACh efflux in response to MK801. In contrast haloperidol (0.1 mg/kg, s.c.) pretreatment did not affect the increase of ACh following MK801. Neither SCH23390 (0.3 mg/kg) or haloperidol (0.1 mg/kg) significantly affected hippocampal ACh efflux per. se.(Table 1).
Results indicate that MK801-induced ACh efflux in the rat hippocampus may be regulated by endogenous dopamine acting at D1 but not D2 receptors.

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Table 1. Effects of SCH23390 and haloperidol on MK801-induced hippocampal acetylcholine efflux

	A.U.C. (pmol/ 20 μl/ min)					
	Vehicle + Vehicle	Vehicle + MK801	SCH23390 + Vehicle	SCH23390 + MK801	Haloperidol + Vehicle	Haloperidol + MK801
pre-injection	0.07±0.01	0.07±0.01	0.07±0.01	0.06±0.01	0.08±0.01	0.06±0.01
post-injection	0.07±0.01*	0.15±0.01	0.09±0.01*	0.07±0.01*	0.08±0.01*	0.16±0.03

Values are means ± s.e.mean. n=7-9 per group. * $p < 0.05$ compared with vehicle/MK801 group by ANOVA and Tukeys test.

119P EVALUATION OF GLYCINE/NMDA RECEPTOR ANTAGONISTS IN AN ANIMAL MODEL OF DEPRESSION

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The non-competitive NMDA receptor antagonist, dizocilpine, produces an antidepressant-like increase in mobility in the mouse Porsolt test (Trullas & Skolnick, 1990). However, the side effects of this compound have restricted its use in man. Recently, several drugs have been described which can antagonise strychnine-insensitive glycine sites on the NMDA receptor complex. They provide a subtle way of controlling NMDA receptor function; potentially without the side effects observed with NMDA antagonists. This study compares the effects of dizocilpine in the mouse Porsolt test with those of the glycine site partial agonist/antagonist R(+)-HA-966 (R-(+)-3-amino-1-hydroxy-2-pyrrolidinone) and the glycine site antagonists, L-701,252 (7-chloro-3-(cyclopropylcarbonyl)-4-hydroxy-2(1H)-quinoline; Rowley et al., 1993) and 5,7-DCKA (dichlorokynurenic acid).

Male CD1 mice (20-30g) were injected i.p. with test drug and 30 min later placed in water for the 6 min test. Mobility (arbitrary units) was measured using a Doppler recording system (Buckett et al., 1982). A separate study examined whether the effects of dizocilpine in the Porsolt test were due to behavioural activation. Mice were injected with dizocilpine and 30 min later placed individually into novel activity boxes. Locomotor activity was measured over 6 min using automated photocell counters.

Dizocilpine at doses of 0.1 and 0.3 mg/kg produced similar increases in mobility in the Porsolt test to the antidepressant amitriptyline (mean mobility counts±s.e.mean: vehicle i.p. 340±31; amitriptyline 15 mg/kg i.p. 599±39; $P<0.05$; n=10). Locomotor activity was significantly ($P<0.05$) increased by dizocilpine 0.3 mg/kg, but not a by lower dose of 0.1 mg/kg (mean activity counts±s.e.mean (n=7-8) : vehicle 765±42; 0.1 mg/kg 1047±124; 0.3 mg/kg 1519±87). Thus the increase in mobility induced by dizocilpine is unlikely to be explained by its stimulant effects. These results confirm previous reports of the antidepressant-like effects of dizocilpine in the mouse Porsolt test (Trullas & Skolnick, 1990). In contrast to dizocilpine, R(+)-HA-966 (0.3-10 mg/kg), L-701,252 (0.3-10 mg/kg) and 5,7-DCKA (1-100 mg/kg) were inactive in the Porsolt test at doses shown by others to produce anticonvulsant and anxiolytic-like effects in rats and mice (Dunn et al., 1992; Corbett & Dunn, 1993; Rowley et al., 1993). These preliminary findings in an animal model predictive of antidepressant activity suggest that glycine site antagonists do not have potential as antidepressants.

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Rowley, M. et al. (1993) *J. Med. Chem.* 36, 3386-3396.
Trullas, R. & Skolnick, P. (1990) *Eur. J. Pharmac.* 185, 1-10.

Table 1 Effect of dizocilpine and glycine site antagonists in the mouse Porsolt test

Dose	Dizocilpine	Dose	R(+)-HA966	L-701,252	Dose	5,7-DCKA
Vehicle	334±31	Vehicle	340±31	428±33	Vehicle	428±33
0.01 mg/kg	328±26	0.3 mg/kg	327±60	423±53	1 mg/kg	353±139
0.03 mg/kg	388±35	1 mg/kg	315±47	416±35	10 mg/kg	393±72
0.1 mg/kg	540±41 *	3 mg/kg	342±43	386±69	30 mg/kg	401±41
0.3 mg/kg	625±19 *	10 mg/kg	277±47	401±37	100 mg/kg	320±41

Results are expressed as mean mobility counts ± s.e.mean; n=10-20 mice; *$P<0.01$ vs vehicle (ANOVA and Dunnett's test).

120P LOCALIZATION OF SODIUM CHANNEL-ENCODING mRNAs IN THE RAT CENTRAL NERVOUS SYSTEM

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Voltage-sensitive sodium channels in the rat brain are composed of three subunits (α, β1 and β2; Catterall, 1992), for which molecular cloning techniques have revealed at least three subtypes of the α subunit, designated as I, II and III. We have employed *in situ* hybridisation histochemistry to extend a previous report (Furuyama *et al.*, 1993) detailing the macroscopic distribution of sodium channel-encoding mRNA subtypes in the rat brain by examining their microscopic localization at the cellular level.

Subtype-specific, 50-oligomeric deoxyribonucleotide probes, complementary to nucleotide bases 1646-1695, 6091-6140 and 6541-6590 (subtype I); 1613-1662, 6019-6068 and 6501-6550 (subtype II); 1303-1352, 2011-2060 and 6060-6109 (subtype III), were synthesised. Computer-assisted GenBank screening revealed a lack of significant homologies of these probes with other mammalian cDNAs, thus minimising cross-hybridisation. Cocktails of oligoprobes were radiolabelled with α-[^{35}S]dATP using terminal deoxynucleotidyl transferase and incubated for 20 hours at 43°C with post-fixed cryostat-cut sections following the methodology of Pratt *et al.* (1993). After hybridisation, sections were washed under suitable stringency conditions and the cellular microscopic resolution of specific transcript localisation was achieved by dipping in Hypercoat™ LM-1 emulsion, exposing for 4-6 weeks followed by counter-staining with cresyl violet to reveal the cell populations associated with transcript signals.

Only a weak general expression of type III mRNA was apparent in the adult rat brain although more concentrated levels were evident in the inferior olivary nucleus, nucleus of the solitary tract, locus coeruleus, medial habenula and also granule cells of the dentate gyrus. In contrast, expression of type II mRNA predominated in the cerebral cortex, the granule cell layer of the cerebellum, pyramidal cells of the hippocampus and dentate gyrus granule cells. Comparatively lower levels were found in the caudate putamen and thalamus. Type I mRNA expression was generally less pronounced than that of type II, although a number of discrete regions exhibited particularly high transcript levels. These were notably, motor neurones of the spinal cord, the spinal trigeminal nucleus, pontine nucleus, deep cerebellar nuclei, thalamic nuclei and layer III-IV of the somatosensory cortex.

Sodium channel encoding mRNAs are differentially located throughout the brain with a clear rostral-caudal expression gradient existing for type II vs. type I mRNA. The anatomical substrates associated with type I mRNA may implicate the specific involvement of type I sodium channels with sensory spinal neurotransmission and also corticothalamic processing.

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121P THE EFFECTS OF CHRONIC (21-DAY) LAMOTRIGINE TREATMENT ON BASAL AND VERATRIDINE-EVOKED GLUTAMATE RELEASE IN THE RAT VENTRAL HIPPOCAMPUS *IN VIVO*

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Glutamate is the major excitatory neurotransmitter in the CNS (Meldrum, 1994). An excess in glutamatergic neuronal tone has been implicated in a number of neurological disorders including epilepsy (Meldrum & Garthwaite, 1990). Thus substantial interest has focussed on finding novel mechanisms for decreasing glutamatergic neurotransmission. One such approach is to reduce neuronal membrane excitability. Lamotrigine (LTG) is a novel anticonvulsant which acts as a voltage- and use-dependent inhibitor of Type IIa sodium channels (Xie *et al*, 1994). Previous studies have shown LTG to inhibit veratridine-evoked amino acid release acutely (Ahmad *et al*, 1995).

We have now evaluated the effects of chronic LTG administration, at different time points during a 21day treatment period, on extracellular glutamate release in the ventral hippocampus using *in vivo* microdialysis.

Male Wistar rats (250-350g) were injected twice daily (8.00h & 20.00h) with 10mgkg^{-1} LTG (isethionate salt) or control (saline) i.p.. On days 1,3,6,13 and 20 of treatment, groups of animals were implanted with concentric dialysis probes in the ventral hippocampus under chloral hydrate anaesthesia (400mgkg^{-1}). On the following days, all animals received their appropriate treatment at 8.00h. At 13.00h microdialysis was commenced. Probes were perfused with artificial CSF at 0.5µlmin^{-1}. Following the collection of 2x60min basal samples, 50µM veratridine was infused via the probes for 30min. Then a final 3x60min post-veratridine samples were collected. Dialysates were analysed for glutamate content by HPLC with flourometric detection.

The results indicate that LTG failed to modify basal glutamate release at any time-point investigated (Day 2: 32.59 ± 3.61(C), 38.25 ± 6.76 (LTG); Day 4: 69.57±12.40(C),65.83±3.97 (LTG); Day 7: 31.72 ± 3.19(C), 26 .17± 6.64(LTG); Day 14: 33.79 ± 4.44(C), 37.73 ± 5.56(LTG); Day 21: 32.80 ± 1.20(C), 31.70 ± 7.0(LTG),pmol/15µlsample,mean±s.e.mean;n=5-6animals/group). However, veratridine-evoked glutamate release was dramatically and significantly reduced in the LTG-treated animals, and this effect was sustained for the duration of the study (Day 2: 50.38 ± 0.38(C), *2.05 ± 0.45(LTG); Day 4: 79.92 ± 4.17 (C), *3.21 ± 0.44 (LTG); Day 7: 43.95 ± 2.58 (C), *2.52 ± 1.46 (LTG); Day 14: 42.28 ± 11.76(C), *1.05 ± 0.45 (LTG); Day 21: 41.97 ± 9.87(C), *3.64 ± 0.61 (LTG), *$p<0.05$, Mann-Whitney U-test, LTG vs control.

These *in vivo* data suggest that LTG is effective in stabilizing excitable (glutamatergic) neurotransmission, thus conferring protection in epilepsy without impairing normal physiological neurotransmission.

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SA is a BBSRC CASE student with The Wellcome Foundation.

122P CHANGES IN EXTRACELLULAR LEVELS OF NEUROTRANSMITTER AMINO ACIDS IN THE KINDLED AMYGDALA *IN VIVO*

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We have previously reported changes in extracellular amino acid levels in the amygdaloid complex *in vivo* following local K^+ or veratridine stimulation (Kaura *et al.*, 1994). We now report the effects of electrical kindling, an animal model of epileptogenesis, on basal and electrically stimulated amino acid levels in the ipsilateral and contralateral amygdaloid nuclei in conscious unrestrained rats.

Male Sprague-Dawley rats (290-300g) were implanted bilaterally with a guide-cannula and guide cannula/bipolar electrode unit aimed at the basolateral amygdalas using stereotaxic surgery. Following full kindling, animals were left to recover for a week before insertion of dialysis monoprobes. Details of the surgery, kindling protocol and dialysis procedure are described elsewhere (Young *et al.*, 1988; Croucher *et al.*, 1992). The amino acid content of perfusates, collected every 10 min, was analysed using HPLC. The results are summarised in Table 1.

Treatment	Aspartate		Glutamate		GABA		Glycine	
group	Ipsi.	Contra.	Ipsi.	Contra.	Ipsi.	Contra.	Ipsi.	Contra.
Kindled (n=6)								
Basal	2.59±1.69	3.70±1.07	78.93±10.55	64.43±10.16	0.53±0.10#	0.83±0.12##	6.50±1.44#	29.23±15.20
Stimulated	7.36±1.72*	5.05±1.17*	143.79±24.43**	101.14±20.62*	0.79±0.21	1.79±0.42*	17.46±9.92	35.91±17.11
Sham op (n=4)								
Basal	7.59±0.76	7.08±1.08	27.52±2.52	36.55±3.60	1.62±0.62	1.87±0.58	27.25±14.96	40.64±12.48
Stimulated	13.14±4.94	10.32±2.74	28.16±2.68	50.40±11.56	1.92±0.95	2.02±0.65	49.04±31.56	64.92±16.37

Table 1. Dialysate amino acid concentrations (nM), measured in the stimulated ipsilateral (Ipsi.) and unstimulated contralateral (Contra.) sides from sham-operated and kindled rats. Values are mean ± s.e.mean. * $P < 0.05$, ** $P < 0.01$ stimulated compared with basal (matched pairs t-test). # $P < 0.05$, ## $P < 0.01$ kindled compared with control (Sham-op.).

These results demonstrate elevated basal extracellular glutamate levels with decreased GABA levels in kindled animals when compared to controls. Electrical kindling stimulation evoked less selective increases in amino acid levels from the amygdaloid nuclei in kindled, but not control animals. These observed perturbations in amino acids strongly indicate a direct involvement of glutamate as the primary excitatory neurotransmitter involved in mechanisms generating kindled seizures in the amygdaloid complex.

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Young A.M.J., Crowder J.M. and Bradford H.F. (1988) J.Neurochem. 47, 1399-1404.

123P INHIBITION OF M-TYPE POTASSIUM CURRENT $I_{K(M)}$ IN RAT SYMPATHETIC NEURONES BY LINOPIRDINE (DuP996)

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Linopirdine (DuP996), a putative cognition-enhancing compound, augments the stimulated release of transmitters from brain tissue and increases performance in several animal models of learning and memory (Zaczek & Saydoff, 1993). Aiken & Brown (1994) have recently reported that linopirdine inhibits the M-type K-current ($I_{K(M)}$) in hippocampal pyramidal neurones.

In the present experiments, we have assessed its inhibitory activity against $I_{K(M)}$ and two other species of voltage-gated K^+ current, the delayed rectifier current $I_{K(V)}$ and the transient current I_A, in dissociated superior cervical sympathetic ganglion cells. Cells from 17 day-old rats were cultured for 1-3 days, then rounded-up by replating on the day of experimentation. Currents were recorded under voltage-clamp using amphotericin-perforated ($I_{K(M)}$) or whole-cell ($I_{K(V)}$ and I_A) patch electrodes.

$I_{K(M)}$ was measured from the deactivation relaxations produced by 20 mV hyperpolarizing steps (Brown & Adams, 1980) or from the reduction in outward rectification observed using 7 s, 70 mV hyperpolarizing ramps, both from a holding potential of -30 mV. Linopirdine produced a rapid, complete and reversible inhibition of $I_{K(M)}$. IC_{50} values were 2.47±0.13 μM (relaxations) and 3.35±0.27 μM (ramps) (mean±s.e.mean, curve fit to pooled data from 4 cells). Voltage-dependence of the current and time-constants for the current relaxations were unchanged by linopirdine. Atropine (1μM), which completely abolished the inhibition of $I_{K(M)}$ by the muscarinic agonist oxotremorine-M (10μM), did not affect inhibition by 10 μM linopirdine.

Delay rectifier ($I_{K(V)}$) and transient (I_A) currents were recorded following depolarizing steps to +10 mV from holding potentials of -50 and -100 mV respectively. Linopirdine inhibited both currents with similar potency but at >20 times higher concentrations than those required to inhibit $I_{K(M)}$. Mean IC_{50} values (±s.e.mean, 5 cells) were: $I_{K(V)}$, 62.7±2.4 μM; I_A, 69.5±3.08 μM.

Thus, in agreement with Aiken & Brown (1994), linopirdine inhibits $I_{K(M)}$ in preference to other voltage-gated K^+ currents at concentrations (~10μM) previously reported to enhance evoked transmitter release.

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124P THE EFFECT OF DENERVATION ON THE EXPRESSION OF THE APAMIN ACCEPTOR AND THE 52 kDa MYOTONIC DYSTROPHY GENE PRODUCT IN RAT SKELETAL MUSCLE

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Denervated skeletal muscle has some marked similarities with the muscle of myotonic dystrophy (DM) patients (Grampp *et al.*, 1972; Schmid-Antomarchi *et al.*, 1985; Rüdel & Lehmann-Horn, 1985; Vergara *et al.*, 1993) including the expression of the apamin acceptor at higher than normal levels (Schmid-Antomarchi *et al.*, 1985; Renaud *et al.*, 1986). The apamin acceptor is thought to be an apamin-sensitive, small-conductance calcium-activated potassium channel (SK_{Ca}) (Blatz & Magelby, 1986). Application of apamin blocked the fibrillatory potentials of both denervated (Behrens & Vergara, 1992; Vergara *et al.*, 1993) and DM muscle (Behrens *et al.*, 1994). Therefore the similar pattern of electrical activity observed for both DM and denervated muscle is thought to be due to over-expression of the apamin acceptor (Schmid-Antomarchi *et al.*, 1985; Vergara *et al.*, 1993).

In ^{125}I-apamin binding assays, skeletal muscle membranes (0.1mg protein) were incubated (1h, 4°C) in 10mM KCl, 0.1% [w/v] bovine serum albumin, 25mM Tris, pH 8.4 at 4°C and incubation stopped by rapid filtration and washing (Wadsworth *et al.*, 1994). The maximum ^{125}I-apamin acceptor density (B_{max}) was observed to increase linearly with time from 1.7 ± 0.2fmol/mg in innervated muscle to 52.7 ± 3.6fmol/mg (s.e. mean, n=3) at 12 days post-denervation. Crosslinking experiments using ^{125}I-apamin revealed labelling of polypeptides of 33 and 29kDa which were shown to be distinct from the similar apamin binding polypeptides of rat brain. In crosslinking experiments, membranes (0.5mg/ml) were incubated (1h) in 10mM KCl, 1mM EDTA, 0.1% BSA, 100pM ^{125}I-apamin, 25mM Borax, pH 9.0 at 4°C. Membranes were pelleted (20,000 xg, 10min) and resuspended in the crosslinking buffer (minus BSA and ^{125}I-apamin) containing 5mM bis(sulfosuccinimidyl) suberate. Crosslinking was quenched by addition of 1M glycine to a final concentration of 100mM. Using antibodies raised against the predicted protein sequence of the DM gene we have identified a 52kDa polypeptide (52 DMPK) in skeletal muscle and other tissues (Brewster *et al.*, 1993). Levels of 52 DMPK expressed in skeletal muscle membranes were measured using immunoblots with reference to a standard curve and the level of 52 DMPK expression was observed to decrease with time to 81.4 ± 7.8% (s.e. mean, n=3) of control values at 12 days post-denervation.

We have demonstrated that the increase in apamin acceptor levels in denervated muscle is accompanied by a decrease in the level of expression of 52 DMPK. These changes mirror those seen for apamin acceptor (Renaud *et al.*, 1986) and 52 DMPK expression (Fu *et al.*, 1993) in myotonic dystrophy muscle. This study therefore confirms the potential of denervated muscle as a model system for DM.

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125P BEHAVIOURAL EFFECTS OF AHR-14042, A NOVEL AZETIDINE ANXIOLYTIC, IN RODENT MODELS OF ANXIETY

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AHR-14042 [3(4-chlorophenoxy)-N-(2-propenyl)-1-azetidinecarboxamide; AHR] is a novel azetidine molecule with anxiolytic and anticonvulsant properties (Bill *et al.*, 1991). Here we describe it's effects in punished and non-punished rodent models of anxiety.

In a rat Vogel conflict test, male SD rats (180-230g; n=10) were water-deprived overnight (20-22h) and then injected s.c. with AHR, lorazepam (LOR) or vehicle. Thirty minutes later they were placed into test chambers and allowed to complete 20 licks on a water spout before a 5 min test period in which a mild electric shock (0.1mA) was delivered after every 20 licks. In the elevated zero-maze (Shepherd *et al.*, 1994), rats (n=12) were injected p.o. with the test compound 60 min before being placed onto a closed quadrant of the maze for a 5 min test session. The following behaviours were scored: (a) time spent in the open (%TO), (b) head dips (HDIPS) and (c) stretched attend postures (SAP). The mouse light/dark box test was as described previously (Bill *et al.*, 1992). AHR or vehicle were administered p.o. to female Tuck mice (28-35g; n=8) 60 min before a 5 min test session.

The results are presented in Table 1. AHR (16 mg/kg s.c.) and the positive control, lorazepam, were active in the rat Vogel test, significantly increasing the number of shocks received. In the rat zero-maze, AHR (15-60 mg/kg p.o.) mimicked the activity of the positive control, chlordiazepoxide (CDP), significantly increasing %TO and HDIPS, and decreasing SAP. AHR (0.3-1.0 mg/kg p.o.) was also active in the light/dark box, significantly increasing light compartment rearing (LCR) and time spent in the light (%LCTIME) to levels similar to those induced by diazepam (DZP). The benzodiazepine antagonist, flumazenil (10 mg/kg s.c.), completely blocked the anxiolytic response to diazepam but had no effect on that to AHR (1.5 mg/kg) in the light/dark box. This finding is in agreement with the lack of effect of flumazenil on the ability of rats to discriminate AHR (Goudie *et al.*, 1995). A wide receptor binding profile of AHR-14042 at concentrations $\leq 10^{-5}$M did not reveal significant binding affinity for $GABA_A$, benzodiazepine or any other major CNS neurotransmitter receptor or ion channel site. However, drug discrimination studies with AHR suggest an interaction with $GABA_A$ receptor mechanisms (Goudie *et al.*, 1995). These data indicate that AHR-14042 exerts an anxiolytic action in animals via a mechanism that is yet to be determined, but which does not involve benzodiazepine receptors.

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Table 1. Rat Vogel conflict test

Rat elevated zero-maze					Mouse Light/Dark box		
Treatment (mg/kg s.c.)	No. of Shocks	Treatment (mg/kg p.o.)	HDIPS	SAP	%TO	Treatment (mg/kg p.o.)	LCR	%LC TIME
Veh	6.6 ± 1.8	Veh	3.4 ± 0.5	3.3 ± 0.8	17.5 ± 2.2	Veh	8.1 ± 1.5	30.7 ± 3.9
AHR(4)	9.5 ± 3.2	AHR(10)	5.7 ± 1.3	2.4 ± 0.8	24.5 ± 2.6	AHR(0.1)	8.1 ± 2.2	30.0 ± 3.0
AHR(8)	20.5 ± 7.9	AHR(15)	10.3 ± 2.0*	2.8 ± 0.8	28.4 ± 4.1*	AHR(0.3)	15.3 ± 1.5*	41.3 ± 2.1*
AHR(16)	27.8 ± 7.7*	AHR(30)	15.2 ± 1.8*	0.6 ± 0.2*	30.5 ± 3.7*	AHR(1.0)	18.9 ± 2.8*	44.2 ± 3.4*
		AHR(60)	16.7 ± 1.7*	0.6 ± 0.2*	32.0 ± 2.6*	AHR(3.0)	12.1 ± 3.1	36.3 ± 3.6
LOR(0.2)	39.5 ± 9.0*	CDP(5)	13.5 ± 1.8*	1.0 ± 0.3*	33.6 ± 2.7*	DZP(0.5)	18.6 ± 2.5*	45.0 ± 3.6*

Data presented are mean ± s.e.mean. *$P<0.05$ versus vehicle-control group (one-way ANOVA/Dunnett's test).

126P LACK OF WITHDRAWAL/DEPENDENCE LIABILITY FOLLOWING CHRONIC TREATMENT WITH THE NOVEL AZETIDINE ANXIOLYTIC AHR-14042 IN RODENTS

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AHR-14042 [3(4-chlorophenoxy)-N-(2-propenyl)-1-azetidinecarboxamide; AHR] is a novel azetidine anxiolytic (Bill *et al.*, 1991; 1995). Here we present evidence to suggest that, after chronic treatment, the compound may lack the dependence liability associated with repeated benzodiazepine administration.

Male SD rats (170-230g;n=10) were dosed p.o. twice daily for 14 days with AHR, lorazepam (LOR) or vehicle. Punished drinking in a Vogel conflict test (see Bill *et al.*, 1995) was determined 1h after the final treatment. Female Tuck mice (27-35g; n=10) were dosed p.o. twice daily for 14 days with AHR, diazepam (DZP) or vehicle. Light compartment rears (LCR) were measured in a light/dark box (Bill *et al.*, 1992) 1, 24 or 72h after the final treatments.

The results are presented in Table 1. Following repeated administration there was no evidence of tolerance to the anxiolytic effect of AHR (60 mg/kg) in the Vogel test, whereas the effect of lorazepam (1 mg/kg) was significantly attenuated. Similarly, in the light/dark box there was no significant change in the anxiolytic effect of AHR (1.0 mg/kg) after repeated treatment, and also there was no anxiogenic effect of withdrawal from repeated AHR administration. However, 72h after withdrawal from chronic diazepam (10 mg/kg) a significant anxiogenic response (decreased LCR) was observed. Mice withdrawn from chronic diazepam (10 mg/kg p.o.) also exhibited a significant ($P<0.01$; ANOVA/t-test) body weight loss (decrease =1.2±0.4g; n=33) over the first 4 days of withdrawal compared with mice withdrawn from vehicle (gain =0.7±0.3g; n=33). In contrast, mice treated chronically with AHR (1.0 or 10 mg/kg) gained weight at a rate comparable to the vehicle-treated controls throughout the period of repeated dosing and over a 8 day period after drug withdrawal. These data indicate that AHR-14042 may be a clinically effective novel anxiolytic which appears to lack the dependence/withdrawal liability associated with prolonged benzodiazepine treatment. In addition, since it is difficult to train rats to discriminate AHR (Goudie *et al.*, 1995) the compound may also have low abuse potential. Initial studies into it's potential mechanism(s) of action indicate that benzodiazepine receptors are not involved (Bill *et al.*, 1995). However, drug discrimination studies suggest a possible interaction with the $GABA_A$ system (Goudie *et al.*, 1995).

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Goudie, A.J., Leathley, M.J. & Dourish, C.T. (1995) This meeting.

Table 1. Rat Vogel conflict test

			Mouse Light/Dark box				
Chronic Treatment (mg/kg p.o.)	Final Treatment (mg/kg p.o.)	No. of Shocks	Chronic Treatment (mg/kg p.o.)	Final Treatment (mg/kg p.o.)	1h withdrawal LCR	Chronic Treatment (mg/kg p.o.)	72h withdrawal LCR
Veh	Veh	5.6 ± 1.5	Veh	Veh	16.2 ± 1.8	Veh	15.2 ± 1.3
Veh	AHR(60)	32.0 ± 9.5*	Veh	AHR(1.0)	29.0 ± 3.3*	AHR(1.0)	15.8 ± 2.6
AHR(60)	AHR(60)	28.3 ± 8.9*	AHR(1.0)	AHR(1.0)	25.3 ± 3.9*	AHR(10)	16.1 ± 1.7
Veh	LOR(1.0)	50.2 ± 11.0*	Veh	DZP(0.5)	25.1 ± 3.2*	DZP(0.5)	15.4 ± 2.3
LOR(1.0)	LOR(1.0)	28.9 ± 5.1*§	DZP(0.5)	DZP(0.5)	23.0 ± 3.1	DZP(10)	7.3 ± 1.8*

Data presented are mean ± s.e.mean. *$P<0.05$ v. vehicle-control, §$P<0.05$ v. veh/LOR (one-way ANOVA/Duncan's test).

127P COMPARATIVE DISCRIMINATIVE STIMULUS PROPERTIES OF CHLORDIAZEPOXIDE AND THE PUTATIVE ANXIOLYTIC AHR-14042 IN RATS

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AHR-14042 [3(4-chlorophenoxy)-N-(2-propenyl)-1-azetidiecarboxamide] is a novel putative anxiolytic whose anxiolytic actions in rodents are resistant to the benzodiazepine receptor antagonist flumazenil (Bill et al., 1991; 1995). To characterise the in vivo mode of action of AHR-14042 (AHR) we compared the discriminative stimulus properties of AHR and chlordiazepoxide (CDP). Female rats were trained to discriminate AHR (n = 21, 10 mg/kg) or CDP (n = 19, 10 mg/kg) in a quantal Fixed Ratio drug discrimination assay (Goudie et al., 1989). Drug or vehicle (10% Cremophore) injections (i.p.) were given 15 min. before operant sessions. CDP discrimination was acquired rapidly (within 30 training sessions), and showed a consistently high level of asymptotic accuracy (group level of ca. 90% correct/day). In contrast, AHR discrimination was acquired very slowly. After 100 sessions the group level of accuracy was only ca. 80% correct/day on average. Furthermore, unlike CDP, AHR discrimination was unstable over days. Even after 200 sessions, on some days the group level of accuracy was as low as 60% correct, although on other days the level was 100%. Thus AHR clearly has a low level of "discriminability".

In CDP trained rats, CDP produced dose-related generalisation (ED_{50}=2.7 mg/kg, maximal drug lever selection = 81%). AHR did not generalise to CDP between 1.25-20 mg/kg (generalisation was never > 10% at any dose). In AHR trained rats, AHR produced dose-related generalisation (ED_{50} = 6.5 mg/kg, maximal drug lever selection = 100%). Furthermore, CDP generalised to AHR (ED_{50} = 4.6 mg/kg, maximal drug lever selection = 71%). Thus cross-generalisation between AHR and CDP was asymmetrical.

In antagonist tests in AHR trained rats flumazenil (10 mg/kg) reduced the ability of CDP (10 mg/kg) to generalise from 81% to 30% [Chi^2 = 10.9, $p < 0.01$]. However, the same dose of flumazenil did not significantly alter the ability of AHR (10 mg/kg) to induce drug lever selection [79% flumazenil present, 67% flumazenil absent, $Chi^2 = 0.75$, $p > 0.3$].

These data show clear differences between AHR and CDP. The drugs differ in "discriminability". They do not induce identical discriminative stimuli, as AHR did not generalise to CDP. Furthermore, in AHR trained rats generalisation of CDP, but not AHR, was flumazenil-reversible. However, since CDP generalised to AHR the cues also show similarities. Further studies (Goudie et al., 1995) indicate that AHR probably acts on the $GABA_A$ system, which presumably accounts for the similarity between the drug stimuli.

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128P ANALYSIS OF THE MODE OF ACTION IN RATS OF THE PUTATIVE ANXIOLYTIC AHR-14042 IN AN *IN VIVO* DRUG DISCRIMINATION ASSAY

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AHR-14042 [3(4-chlorophenoxy)-N-(2-propenyl)-1-azetidiecarboxamide] is a novel putative anxiolytic. The benzodiazepine (BZ) receptor antagonist flumazenil does not reverse the anxiolytic (Bill et al., 1991; 1995) or discriminative (Goudie et al. 1995) properties of AHR-14042. We have assessed the mode of action *in vivo* of AHR-14042 in female rats (n=21) trained to discriminate AHR-14042 in a quantal operant Fixed Ratio assay at a dose of 10 mg/kg (i.p.). After animals had been trained to discriminate AHR-14042 in over 100 sessions, generalisation tests were carried out over an 18 month period. All drugs were administered i.p. and tested with at least 4 doses. The following drugs generalised to AHR-14042 in a dose-related fashion:- the BZ partial agonist bretazenil (ED_{50}=2.21 mg/kg, maximal drug level selection = 61%); allopregnanolone, a neurosteroid modulator of the $GABA_A$ receptor (ED_{50}=14 mg/kg, maximal drug lever selection = 67%); the anticonvulsant valproate (ED_{50}=280 mg/kg, maximal drug lever selection = 60%) and the barbiturate phenobarbitone (ED_{50}=24 mg/kg, maximal drug lever selection = 76%). The following drugs did not generalise to AHR-14042 (less than 30% drug lever selection was seen at any dose):- the CCK_B receptor antagonist L-365,260 (tested at 0.001-1 mg/kg); the 5-HT_{1A} receptor partial agonist buspirone (tested at 0.625-5 mg/kg); the BZ receptor subtype agonists alpidem (tested at 3.75-30 mg/kg) and abecarnil (tested at 0.062-0.5 mg/kg); the 5-HT_3 receptor antagonist ondansetron (tested at 0.001-1 mg/kg); the $GABA_A$ receptor agonist muscimol (tested at 0.125-1 mg/kg); the NMDA receptor antagonist CPP (tested at 0.3-10 mg/kg); the $GABA_B$ receptor agonist baclofen (tested at 0.5-4 mg/kg) and the angiotensin II receptor antagonist losartan (tested at 0.01-10 mg/kg). These data show that the AHR-14042 discriminative stimulus (cue) involves the $GABA_A$ system, since many drugs which generalised (valproate, phenobarbitone, bretazenil and allopregnanolone) modulate the $GABA_A$ receptor at various different sites, as does chlordiazepoxide, which generalises to AHR-14042 (Goudie et al., 1995). The negative findings with the direct $GABA_A$ agonist muscimol are apparently anomalous. However, other studies suggest that direct and indirect $GABA_A$ receptor agonists do not cross-generalise (Rauch & Stolerman 1987; Grech and Balster 1993), thus there are important differences between direct and indirect $GABA_A$ agonists in drug discrimination studies. The AHR-14042 cue clearly differs from that of many established and putative anxiolytics from various pharmacological classes, since generalisation was not seen with L-365,260, buspirone, alpidem, abecarnil, CPP, losartan and ondansetron. The data suggest that AHR-14042 activates the $GABA_A$ system in a novel manner, which as yet is undefined. Ligand binding studies (Bill et al., 1985) indicate that AHR-14042 does not act at either the BZ or the $GABA_A$ receptor site. Further characterisation of the mode of action of AHR-14042 awaits the development of agents with selective actions at $GABA_A$ receptor subtypes.

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129P THE EFFECT OF AHR-14042 ON MEMBRANE POTENTIAL OF THE RAT ISOLATED VAGUS NERVE *IN VITRO* AND ITS INTERACTIONS WITH VERATRIDINE

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AHR-14042 [3-(4-chlorophenoxy) azetidine-1-carboxamide); AHR] is a novel agent with anxiolytic and anticonvulsant properties in animal models (Bill *et al.*, 1991). The mechanism of action of the compound is unclear but it appears not to involve benzodiazepine receptors (Bill *et al.*, 1995). In this study an attempt to characterize a depolarizing effect of AHR in the rat isolated vagus nerve is described together with the effects of AHR on depolarizing responses to other agents.

Extracellular recording of depolarization of the desheathed cervical vagus nerve was carried out using a "grease-gap" technique (Rhodes *et al.*, 1992). Male, Sprague Dawley rats (180-250 g) were killed by a blow to the head and cervical dislocation. The vagus nerve was placed in a two chamber tissue bath, one chamber being perfused (3 ml min^{-1}) with Krebs' solution (2.5 mM $CaCl_2$, at 27°C, gassed with 5% CO_2 in O_2) to which drugs were added. The potential difference between the chambers was recorded. Contact time for agonists was 3 min with a 10-15 min washout between doses. The concentrations of agonists were: AHR, 0.1-10 mM; veratridine, 3-100 μM; dimethylphenylpiperazinium (DMPP) 3 μM-3 mM; 5-HT, 10 nM-30 μM and GABA, 3 μM- 0.3 mM. Two non-cumulative concentration-response curves (CRC's) to a single agonist were obtained in each tissue. After constructing the first CRC tissues were equilibrated for 50 min with antagonist. AHR was studied as an agonist and as an antagonist of depolarizations evoked by veratridine (Na^+ channel opener), 5-HT (5-HT_3 receptor/Na^+ channel), DMPP (nicotinic receptor/Na^+ channel) and GABA ($GABA_A$ receptor/ Cl^-channel).

High concentrations of AHR depolarized the vagus nerve. The mean maximum depolarizations (95% limits) with AHR, veratridine, DMPP, 5-HT and GABA respectively were 2.34 (1.98-2.69) mV (n=21), 10.8 (10.0-11.6) mV (n=18) 1.05 (0.74-1.36) mV (n=6), 653 (560-746) μV (n=10) and 440 (306.1-573.9) μV (n=6) respectively. Geometric mean EC_{50} values (95% limits) were 1.0 (0.95-1.10) mM, 23.2 (19.6-27.5) μM, 14.6 (12.2-17.4) μM, 0.53 (0.40-0.70) μM and 18.6 (15.8-21.9) μM for these agonists respectively. The capsaicin antagonists capsazepine (3-10 μM; Bevan *et al.*, 1992) and ruthenium red (0.1-1.0 μM) produced concentration-related reductions in the maximum depolarization evoked by AHR, the pIC_{50} being 5.4 (5.3-5.5; n=10) for capsazepine and 6.8 (6.6-7.0; n=12) for ruthenium red. Hexamethonium (100 μM, n=4), ondansetron (1.0 μM, n=4), bicuculline (100 μM, n=4) and tetrodotoxin (100 μM, n=4) had no effect on responses to AHR. At concentrations lower than those depolarizing the vagus nerve AHR (10-100 μM) reduced responses to veratridine in a concentration-related manner with a pIC_{50} value of 4.6 (4.5-4.8; n=12). Depolarization evoked by 5-HT or by the nicotinic agonist DMPP was also reduced in the presence of AHR with pIC_{50} values for AHR of 3.9 (3.82-3.96, n=8) and 4.8 (4.52-5.56, n=8) respectively. Depolarization evoked by GABA was not reduced by AHR (30 μM, n=4).

AHR is thus a weak antagonist of depolarization of the vagus nerve evoked by agonists which act via Na^+ conducting channels. In contrast no block of depolarization evoked by $GABA_A$ receptor stimulation (Cl^- dependent) was observed. At higher concentrations AHR has a capsaicin-like agonist action.

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130P ATTENUATION OF ULTRASOUND-INDUCED DEFENCE BEHAVIOUR BY DIAZEPAM TREATMENT

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Rat ultrasonic vocalisations have been recorded in a variety of behavioural situations, particularly those associated with aversive situations. Previous studies have shown that these calls, consisting of frequencies between 20-32 kHz, can communicate information about situations such as predator threat. Production of these calls is inhibited by benzodiazepines (Blanchard *et al.*,1993). The present study examines the locomotor response of rats to artificially generated 20 kHz ultrasound and the effect of diazepam on this behaviour.

Male Lister hooded rats (250-300g, n=7-8) were injected with diazepam (0.3 or 3.0 mg/kg i.p.) or vehicle (0.9% saline 1 ml/kg i.p.) and 10 min later were placed in a circular open field arena (75 cm diameter) containing a wall mounted piezo-electric speaker. After 2 min they were exposed to a 1 min 20 kHz square wave ultrasound tone (5, 10 or 15v speaker intensity, randomised) followed by a further 2 min without sound. This procedure was repeated for each intensity with a 1 min inter-procedure interval. Behaviours were recorded and analysed via a computer tracking system (Beckett *et al.*, 1995).

Exposure to ultrasound produced intensity related locomotor behaviour, similar in type to previously reported chemically or electrically induced defence (Beckett *et al.* 1995). The maximum speed, average speed and distance travelled by the animal were quantified across 20 s "time-bins". Treatment with 0.3 mg/kg diazepam had no effect on the maximum speed of the animal, whereas 3.0 mg/kg significantly attenuated the response (Figure 1). Neither dose of diazepam altered basal (pre-ultrasound) locomotor behaviour. Values obtained for average speed and distance travelled showed a similar profile.

These results indicate that exposure to single tone ultrasound at a frequency produced by rats in aversive situations can elicit

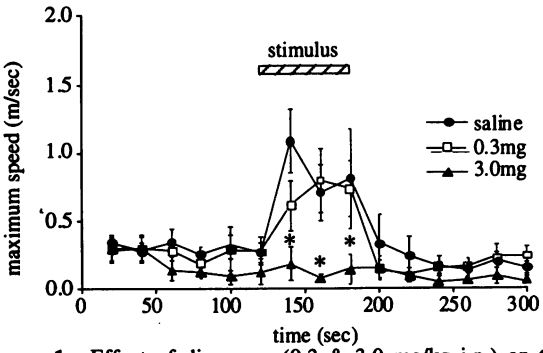

Figure 1. Effect of diazepam (0.3 & 3.0 mg/kg i.p.) on the maximum locomotor speed produced per 20 s time bin in response to 20 kHz ultrasound (10v speaker intensity) (mean ± s.e.mean, One way ANOVA with post-hoc Duncans NMR, n=7-8).

defence-like behaviour which is similar in type to that produced by stimulating brain regions (e.g., periaqueductal grey, amygdala, hypothalamus) important in the control of motivational aversion. This is supported by recent evidence that ultrasound exposure causes c-fos expression in such regions (Aspley *et al.*,1995). Furthermore, the behavioural response to ultrasound can be attenuated by diazepam in a similar manner to the aversive response produced by direct stimulation techniques (Graeff *et al.*, 1993).

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131P C-FOS IMMUNOREACTIVITY PRODUCED BY EXPOSURE TO 20 kHz ULTRASOUND IN RATS

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It is now well established that when rats are threatened by the presence of a predator or exposed to painful stimuli they vocalise with 20-32 kHz calls which communicate information about the threat (Blanchard *et al.*, 1991). Recent studies in this laboratory have shown that rats exposed to 20 kHz ultrasound display flight behaviour (Beckett *et al.*, 1995). This is identical to behaviour produced by electrical or chemical stimulation of brain regions associated with anxiety and defence. The present study examines the effect of ultrasound exposure on the central expression of the immediate early gene c-fos in the rat.

Male Lister hooded rats (250-300g, n=3-4) were placed in a circular open field testing arena (75 cm diameter). After 2 min they were exposed to a 1 min 20 kHz square wave ultrasound tone (Beckett *et al.*, 1995) followed by 2 min without sound. They were then returned to the home cage for 1 h. Control animals were either placed in the arena with no sound for 5 min or taken directly from the home cage. Rats were deeply anaesthetised (sodium pentabarbitone, 60 mg/kg i.p.) and trans-cardially perfused with saline then 4% (w/v) paraformaldehyde (PFA, pH 7.4, 60 ml each). Brains were removed and fixed for 24 h in PFA at 4 °C. Coronal sections (100 µm) were placed into immunobuffer (NaCl 12, KCl 0.005, Na_2HPO_4 900, Na_2HPO_4 1.5, merthiolate 0.1 mM and Triton X-100, 0.3% (w/v) used for subsequent solutions). Endogenous peroxidase activity was removed with H_2O_2 before sequential incubation in 2% (v/v) rabbit serum in immunobuffer (blocking buffer 24 h); sheep anti-c-fos immunoglobulin G (IgG, 1:2000, 72 h, in blocking buffer) and biotinylated rabbit anti-sheep IgG (1:200 in blocking buffer, 24 h). C-fos immunoreactivity was visualised using avidin/biotin and 3,3' diaminobenzidine and quantified by counting the number of positive cell bodies within brain regions using a 400x400 µm grid.

Exposure to ultrasound produced rapid locomotion followed by a period of freezing before normal exploratory behaviour was resumed. Animals which received ultrasound showed dense labelling in the dorsal periaqueductal grey matter, (PAG) basolateral amygdala, paraventricular thalamic nuclei and the dorsomedial hypothalamic nuclei (Figure.1), which was significantly greater than in either home cage or arena control animals.

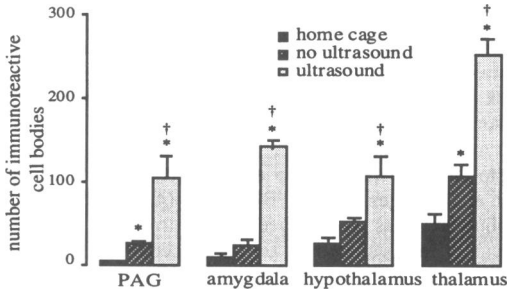

Figure 1 Number of c-fos immunoreactive cell bodies per 400x400 µm grid (mean ±s.e.mean *p<0.05 vs home cage, †p<0.05 vs no ultrasound, Mann Whitney U-test n=3-4).

These results indicate that exposure to ultrasound at a frequency of 20 kHz (similar to that produced by rats in a naturally aversive situation) produces defence-like escape behaviour which is associated with c-fos expression in brain regions which participate in the modulation of aversive responses (Graeff *et al.*, 1993).

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132P AN *IN VIVO* MICRODIALYSIS STUDY IN THE FRONTAL CORTEX OF FREELY-MOVING RATS: EFFECTS OF FG 7142 OR YOHIMBINE ON SPONTANEOUS AND STRESS-INDUCED CHANGES IN NORADRENALINE EFFLUX

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The activity of central noradrenergic neurones is widely believed to be increased during anxiety and stress. The present experiments used *in vivo* microdialysis in freely-moving rats to compare changes in noradrenaline (NA) efflux caused by two compounds with established anxiogenic activity in humans: the benzodiazepine receptor partial inverse agonist, β-carboline-3-carboxylic acid methylamide (FG7142: Dorow et al., 1983) and the α_2-adrenoceptor antagonist, yohimbine (Charney et al., 1983). Since these compounds modify rats' behavioural response to novel stimuli (File & Pellow, 1985), their effects on the neurochemical response to the stress of a novel arena was also investigated.

Microdialysis probes were implanted vertically, under halothane anaesthesia, in the frontal cortex of male SD rats (260-310 g). Animals were left to recover from surgery in their home cage. The next day, probes were perfused with Ringer's solution (mM: NaCl 145, KCL 4, $CaCl_2$ 1.3; pH 6.6) at 1µl min^{-1}. Samples were collected at 20 min intervals and their NA content measured by HPLC-ECD. 1.5 h after the onset of perfusion, spontaneous ('basal') efflux of NA was assessed in 4 consecutive samples, after which animals were given an i.p. injection of FG7142 (10 mg kg^{-1}), yohimbine (2 mg kg^{-1}), or vehicle (FG7142: 10 µl of 'Tween 80' in 2 mls water: yohimbine: saline). Rats were then either returned to their home cage for 2 h, or returned to their home cage for 1 h before transfer to a novel arena for a further1 h. Statistics were performed on raw data, using split-plot ANOVA, comparing bins of data comprising 3 consecutive samples (Dalley & Stanford, 1995).

Basal efflux was 25.6 +/- 0.7 fmoles/20µl. Both injection of vehicle (Tween: peak effect 143 %, $F_{1,8}$=11.2, p<0.01; saline: 139 %, $F_{1,11}$ =6.33, p<0.05) and subsequent transfer of rats to a novel arena (Tween: 169 %, $F_{1,8}$ =9.7, p<0.05; saline: 152 %, $F_{1,11}$=7.51, p<0.05) caused a significant increase in NA efflux. FG7142 also increased NA efflux *cf* basals (158 %, $F_{1,11}$ =5.9; p<0.05) but this was no greater than after vehicle-injection. FG7142 had no effect on the increase in NA efflux induced by novel arena exposure. In contrast, yohimbine caused a significant increase in NA efflux *cf* basals (242 %, $F_{1,11}$=16.1; p<0.01) or *cf* vehicle-injection ($F_{1,12}$=15.4; p<0.01). Subsequent transfer of rats to a novel arena also increased NA efflux in yohimbine-injected rats *cf* basals (282 %, F1,11=20.7, p<0.01) and *cf* vehicle-injected controls ($F_{1,12}$ =15.87, p<0.01). However, this increase was not significantly different from that induced by yohimbine treatment alone.

In conclusion, NA efflux in rat frontal cortex was increased after both i.p. injection of vehicle and subsequent transfer to a novel arena. Administration of yohimbine, but not FG7142, also increased NA efflux. With neither compound was there a significant interaction with the stress of exposure to a novel arena. These results suggest that, in rat frontal cortex, anxiogenic agents have inconsistent effects on NA efflux. Furthermore stress does not modify the effects of these compounds on levels of extracellular NA.

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133P BU224, A HIGH AFFINITY SELECTIVE I_2 IMIDAZOLINE SITE LIGAND: A BEHAVIOURAL STUDY IN MODELS OF SPATIAL LEARNING AND MEMORY

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Imidazoline binding sites exist as two principle sub-types I_1 and I_2 (Ernsberger, 1992). The I_1 subtype appears to be involved in central cardiovascular control (Ernsberger *et al.*, 1990), but as yet no definitive role(s) for the I_2 subtype have emerged due, in part, to the lack of available selective ligands for these sites. However, there is some evidence that I_2 sites may be involved in cognition since the mixed α_2 antagonist / I_2 site ligand, idazoxan has been reported to facilitate memory retrieval in a complex maze task in rats (Sara & Devauges, 1989). BU224 (2-(4,5-dihydroimidaz-2-yl)-quinoline-HCl) has recently been shown to exhibit high affinity and selectivity for I_2 sites over α_2 adrenoceptors in mammalian brain (Hudson *et al.*, 1994) and thus provides an opportunity to explore possible cognitive functions for these sites. The aim of this study was to examine the effects of BU224 on locomotor activity (LMA) and on 2 tasks, the Morris water maze and delayed nonmatch to position (DNMTP) in normal young rats.

Male Hooded-Lister rats (220-250g) were injected with BU224 (0.3, 1, 3 and 10mgkg^{-1} i.p.; n=10 per group) or saline (1mlkg^{-1} i.p.; n=10) and their LMA measured in an unfamiliar cage 20min later over a 30min period using infrared beam activity meters. BU224 (1 or 3mgkg^{-1}; n=10 per group) or saline (1mlkg^{-1}; n=10) was given i.p. 30min prior to the training trials on the Morris water maze on each of the 4 training days. The Morris water maze protocol required rats to learn the location of a fixed 10cm platform located 2cm below the surface of the water in a 200cm circular pool over 60s. On day 4 each rat also received a probe trial in which it was allowed to swim for 60s with the platform removed. A separate group of animals was maintained at 90% of their free-feeding weight and trained on a DNMTP task, in a rodent operant chamber. Trained rats (n=18) received either BU224 (1 or 3mgkg^{-1}), tetrahydroaminoacridine (THA; 1mgkg^{-1}) or saline (1mlkg^{-1}) i.p. 30min prior to task in a balanced cross over design, with two drug free days between consecutive treatments.

BU224 (0.3, 1 and 3 mgkg^{-1}) had no effect on LMA, whilst rats given 10mgkg^{-1} showed a 39.1 ± 8.7%* decline in LMA 25min after dosing. Similarly 10mgkg^{-1} BU224 decreased total LMA at 30min by 34.3 ± 9.4%*, (*$p<0.05$, repeated measures (r.m.) ANOVA). BU224 (1 or 3mgkg^{-1}) alone was without effect on aquisition of the hidden platform Morris water maze task ($p>0.05$, r.m. ANOVA) and in the probe trials the doses indicated above had no effect on the distance swam in 60s ($p>0.05$, one-way ANOVA). A similar pattern of results was also found in the DNMTP task. Neither BU224 nor THA produced improvements or impairments in DNMTP performance in young rats when given alone ($p>0.05$, r.m. ANOVA).

These *in vivo* data suggest that BU224 neither facilitates nor impairs cognition in two separate models of spatial learning and memory in young rats when given alone. THA also failed to do so in the DNMTP model although it should be noted that these compounds have been tested in the absence of any cognitive impairment, thus further experiments in older or lesioned rats should be of interest. Moreover, whether BU224 is an I_2 site agonist or antagonist is unknown.

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LAL is a MRC CASE student with SmithKline Beecham.

134P A SELECTIVE NEURONAL NITRIC OXIDE SYNTHASE INHIBITOR IMPAIRS SPATIAL LEARNING IN THE RAT

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Nitric oxide (NO) is a transmitter which has been suggested to play a role synaptic plasticity and in memory formation. A novel selective NO synthase inhibitor, 7-nitro indazole (7-NI), has been shown in vivo to inhibit the neuronal isoform of NO synthase in a selective way while little affecting endothelial or macrocyte NOS (Babbedge *et al.*, 1993, Moore *et al.*, 1993). At a dose of 30 mg/kg *i.p.* 7-NI inhibited neuronal NOS in the hippocampus by 85 % while having no effect on blood pressure (Mackenzie *et al.* 1994).

The present study investigates if 7-NI has effects on learning of spatial tasks in a water maze and in an 8-arm radial maze (30mg/kg *i.p.*, Wistar rats, n=16). 3 arms of the radial maze were baited. Training was 6 trials per day for 4 days. After day 2, baited arms were changed to test reversal learning. Time needed by animals to retrieve food pellets was recorded (latency), as well as working memory errors (repeated entry of arm within one trial) and reference memory errors (entry on an unbaited arm). In the water maze, time required to find a hidden platform in a circular pool was measured as well as swim speed. 6 trials per day were given for 5 days to find the platform. Location of platform was changed after day 2.

In the water maze, latency was greater in the 7-NI group compared to the control group (2 way ANOVA, $p<0.0001$) while swim speed was not affected. When testing animals after training sessions by taking out the platform and analysing preferred location of rats in a 1 min swim, control animals swam more in the pool quadrant which contained the platform compared to the 7-NI group (Mean 26% ±2.5 S.E.M. spent in target quadrant by 7-NI group vs. 36% ±3.4 by control group, one-way ANOVA with a post-hoc Tukey t-test, ANOVA $p<0.0008$, t-test $p<0.04$). In the radial 8 arm maze, latency (2 way ANOVA, $p<0.0001$), working memory (2 way ANOVA, $p<0.001$) and reference memory errors (2 way ANOVA, $p<0.006$) were also higher in the 7-NI group. At the end of the second training day, no difference in performance between 7-NI and control groups was visible anymore. A change of baited arms caused a difference between groups in latency (2 way ANOVA, $p<0.001$) and WM errors (2 way ANOVA, $p<0.01$).

Learning of a visual discrimination task in the water maze using a visible platform was not affected. Analysis of behaviour in the Open Field showed that rats injected with 7-NI did not differ from vehicle injected rats.

We conclude from this that activity of the neuronal isoform of nitric oxide synthase is of importance in spatial learning.

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The presence of imidazoline binding sites in various tissues of several species is now well established. These sites exist in two principle types, the I_1- and I_2-imidazoline receptors (I_2-IR) (Ernsberger, 1992). The I_2-IR can be subdivided according to the affinity for amiloride in I_{2A}-IR displaying high affinity for the drug and I_{2B}-IR which bind amiloride poorly (Diamant *et al.*, 1992) (Table 1). The aim of this study was to further assess and extend the pharmacological differences between I_{2A}- and I_{2B}-IR and also to seek for molecular differences between these subtypes of I_2-IR. Radioligand binding studies were performed as described previously (Miralles *et al.*, 1993) with tissues from male New Zealand rabbits (3 kg) and Sprague-Dawley rats (250-300 g). *In vitro* competition curves of various drugs against [^{3}H]-idazoxan binding (in the presence of 1 μM adrenaline) to I_{2A}-IR in the rabbit cerebral cortex or I_{2B}-IR in the rat cerebral cortex were biphasic (Table 1). Among the drugs tested bromoxidine, moxonidine, L- and D-medetomidine and clorgyline were more potent on I_{2B}-IR than I_{2A}-IR (K_{iH} ratios 20 to 65) (Table 1). Preincubation (30 min at 25 °C) of cortical membranes with 1 μM clorgyline reduced by 60 % the B_{max} of [^{3}H]-idazoxan binding to I_{2B}-IR, but it did not affect the binding parameters of the radioligand saturation curves to I_{2A}-IR in the rabbit cerebral cortex. These results indicated that I_{2A}-IR and I_{2B}-IR differ in the affinity for some imidazolidine/imidazole drugs and in the ability to irreversibly bind clorgyline.

Immunological detection of IRs by immuno-(western) blot (Escribá *et al.*, 1994) revealed in rat cortical membranes a double band of 29-30 kDa and two less intense bands of 45 and 66 kDa. In rabbit cortical membranes the IR antibody detected proteins at 29-30 kDa, 57 kDa and 66 kDa. These results suggested that different IR proteins (45 vs 57 kDa) can account for the different pharmacological profiles of I_{2A}-IR and I_{2B}-IR.

Table 1

	Rabbit cerebral cortex I_{2A}-IR			Rat cerebral cortex I_{2B}-IR		
Drugs	K_{iH} (nM)	K_{iL} (μM)	R_H %	K_{iH} (nM)	K_{iL} (μM)	R_H %
Amiloride	52	247	84	-	3	-
Bromoxidine	3,157	67	72	93	4	70
Moxonidine	-	>1,000	-	-	5	-
L-Medetomidine	2,147	115	81	105	43	56
D-Medetomidine	1,087	79	80	55	36	75
Clorgyline	3,511	1,606	77	54	26	53

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136P CHRONIC TREATMENT WITH RESERPINE INCREASES THE DENSITY AND THE RATE OF APPEARANCE OF THE HIGH-AFFINITY FORM OF BRAIN α_2-ADRENOCEPTORS

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Recently, it has been reported that prolonged depletion of brain noradrenaline after treatment with reserpine induces in vivo functional supersensitivity of both pre- and postsynaptic α_2-adrenoceptors (α_2R) in the rat brain, and markedly increases the affinity of agonist radioligands ([^{3}H]clonidine or [^{3}H]UK 14304) for the brain α_2R with no apparent changes in receptor density (Giralt and García-Sevilla, 1989; Ugedo *et al.*, 1993).

The aim of the present study was to assess further whether reserpine-induced supersensitivity affect the turnover of α_2R in the cerebral cortex of male Sprague-Dawley rats (250 g), using radioligand binding methods as described previously (Ugedo *et al.*, 1993). First, saturation binding studies confirmed that chronic treatment with reserpine (0.25 mg/kg, s.c.; every 48 h for 14 days) led to marked reductions of the dissociation constants (K_d) for the agonists [^{3}H]clonidine (41%, n=3, P<0.001) and [^{3}H]UK 14304 (41%, n=5-10, P<0.001), but also increased the density of the α_2R agonist binding sites (26%, P<0.01 and 40%, P<0.05, respectively). Neither the affinity nor the density of the α_2R antagonist binding sites labeled by [^{3}H]RX 821002 were altered by reserpine treatment. The recovery of cortical [^{3}H]UK 14304 specific binding after almost complete irreversible inactivation (>95% at 6 h) of α_2R by the peptide coupling agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (1.6 mg/kg; i.p.) was then assessed in control (n=6) and reserpine-treated (n=3) rats (EEDQ was given at day 7 and treatment was continued until day 37). Recovery data were fitted to the equation $[Rt]= r/k(1-e^{-kt})$ (GraFit) (Mauger *et al.*, 1982), where [Rt] represents the receptor number (B_{max}) at a given discrete time t, r is the appearance rate constant and k is the disappearance rate constant of the receptor that allows to estimate the apparent half-life of the receptor ($t_{1/2}$= ln 2/k). The ratio r/k represents the density of α_2R at steady-state towards the system tends after irreversible inactivation of the receptors. Data are expressed as the best fit values± standard error calculated by the matrix inversion method (GraFit). The simultaneous analysis of recovery curves revealed that the turnover of α_2R in reserpine-treated rats (r=9.1±1.2 fmol/mg protein/day; k=0.115±0.020 days^{-1}; $t_{1/2}$=6.1±1.1 days; r/k=80±5 fmol/mg protein) was significantly different of that in control rats (r=5.8±0.7 fmol/mg protein/day; k=0.107±0.019 days^{-1}; $t_{1/2}$=6.4±1.2 days; r/k=54±4 fmol/mg protein) (F[2,96]=31.36, P<0.0001), and that the increased density of α_2R agonist binding sites in reserpine-treated rats (Bmax or r/k) was due to a higher appearance rate constant (Δr=57%, P<0.05) and not to a decreased disappearance rate constant. Therefore, the apparent half-life of the receptor was unchanged as a consequence of reserpine treatment.

The results indicated that reserpine-induced up-regulation and supersensitivity of brain α_2R are related to a greater expression of the high-affinity form of the receptor.

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137P THE EFFECTS OF ISOPRENALINE ON CYCLIC NUCLEOTIDE LEVELS IN THE GUINEA-PIG BRAIN

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The β-adrenoceptor-evoked stimulation of cyclic AMP levels is perhaps the best characterised of the G-protein-coupled receptor responses. We have previously suggested that elevated cyclic AMP levels in the guinea-pig cerebellum lead to enhanced cyclic GMP responses (Hérnandez *et al.*, 1993; 1994). In the present study, we have carried out an investigation of the cyclic AMP response to isoprenaline in the guinea-pig brain, together with an investigation of the potential for elevation of cyclic GMP levels in the guinea-pig cerebellum.

The accumulation of [^{3}H]-cyclic AMP or [^{3}H]-cyclic GMP was carried out at 37°C with guinea-pig (Dunkin-Hartley, either sex, 200 - 600g) brain slices pre-incubated with [^{3}H]-adenine or [^{3}H]-guanine, respectively, as previously described (Hernández *et al.*, 1993). Data were expressed as a percentage conversion from the total [^{3}H]-adenine or [^{3}H]-guanine nucleotides, from experiments carried out on at least 3 separate occasions. EC_{50} and K_i values were calculated as previously described (Hernández *et al.*, 1993).

Basal accumulation of [^{3}H]-cyclic AMP in tissue slices from the guinea-pig hippocampus, neostriatum, cerebral cortex and cerebellum were 0.08 ± 0.02, 0.12 ± 0.05, 0.27 ± 0.10 and 0.20 ± 0.04 % conversion, respectively. In slices from the hippocampus, neostriatum and cerebral cortex, isoprenaline failed to significantly stimulate [^{3}H]-cyclic AMP accumulation at concentrations up to 10 μM. However, in the cerebellum, isoprenaline evoked a concentration-dependent increase in [^{3}H]-cyclic AMP accumulation with an EC_{50} value of 297 ± 76 nM. The maximal increase in [^{3}H]-cyclic AMP accumulation evoked by isoprenaline was 1.79 ± 0.24 % conversion. The response to 1 μM isoprenaline was reversed in a concentration-dependent manner by the non-selective β-adrenoceptor antagonist propranolol, with an IC_{50} value of 19 ± 4 nM, allowing calculation of a K_i value of approximately 7 nM.

In the guinea-pig cerebellum, accumulations of [^{3}H]-cyclic GMP in the absence and presence of 1 mM sodium nitroprusside (SNP) were 0.40 ± 0.07 and 2.46 ± 0.30 % conversion, respectively. Isoprenaline evoked a concentration-dependent increase in SNP-elicited [^{3}H]-cyclic GMP accumulation with an EC_{50} value of 180 ± 41 nM with a maximal enhancement of 233 ± 11 % of the SNP response. The isoprenaline enhancement (1 μM, 227 ± 6 % SNP response) of the response to 10 μM SNP was prevented in the presence of 1 μM propranolol (116 ± 10 % SNP response).

These results indicate that isoprenaline evokes a cyclic AMP response in the guinea-pig cerebellum, but not in the hippocampus, neostriatum and cerebral cortex. Isoprenaline also evokes an increase in the SNP-evoked cyclic GMP response in the cerebellum. Both these responses appear to be mediated through β-adrenoceptors. The enhancement in cyclic GMP levels appears to be mediated through the stimulation of cyclic AMP levels, as we have previously observed for forskolin and adenosine receptor activation (Hernández *et al.*, 1993, 1994)

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138P LOCALISATION OF P_{2X}-PURINOCEPTOR TRANSCRIPTS IN THE RAT NERVOUS SYSTEM

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Evidence has been accumulating to suggest that adenosine 5'-triphosphate (ATP) is a neurotransmitter in both the central and peripheral nervous systems (see Chen *et al.*, 1995). Recently two P_{2X}-purinoceptor genes have been cloned from the rat vas deferens (Valera *et al.*, 1994) and PC12 cells (Brake *et al.*, 1994). Preliminary localisation studies in the nervous system indicated that the vas deferens P_{2X}-purinoceptor is not found in the brain although it is present in the spinal cord and the superior cervical ganglia (SCG), while the PC12 P_{2X}-purinoceptor is found in the brain, the spinal cord and the SCG. The aim of this study was to examine in more detail the localisation of the transcripts for the two recombinant P_{2X}-purinoceptors using specific antisense oligonucleotide probes.

Sequence-specific antisense oligonucleotide probes (45mers) were designed for the PC12 and the vas deferens P_{2X}-purinoceptors. The probes were 3' end-labelled with [^{35}S]dATPαS using TdT and purified by separation on a G50 Sephadex column. *In situ* hybridisation was carried out essentially as described by Senaris et al. (1994) on 12μm sections of Sprague-Dawley neonatal and adult male (200-250g) rat brain and adult nodose ganglia (NG) and SCG. Sections were exposed to Hyperfilm β-Max for 6 weeks at -80°C.

In Northern blot analysis the oligonucleotides directed against the vas deferens and the PC12 P_{2X}-purinoceptors were able to detect the mRNA of the expected size, 1.8kb and 2kb, respectively. In *in situ* hybridisation studies these oligonucleotides detected mRNA in the neonate and adult rat brain and in both the adult NG and SCG. In the neonatal brain the strongest labelling with the oligonucleotides directed against the vas deferens P_{2X}-purinoceptor was seen in the cerebellum. In addition, there was labelling in the striatum, hippocampus and cortex. The oligonucleotides directed against the PC12 P_{2X}-purinoceptor labelled mRNA in the olfactory tubercle, striatum, medial habenula, CA1-3 regions of the hippocampus, dentate gyrus, ventromedial hypothalamic nucleus, cortex and cerebellum. A qualitatively similar distribution was seen in the adult rat brain, but the overall level of expression was much lower. The PC12 P_{2X}-purinoceptor oligonucleotides showed very intense labelling of the NG but less in the SCG. However, there was a similar level of expression in the two ganglia with the vas deferens P_{2X}-purinoceptor oligonucleotides.

These findings have extended those of Brake et al. (1994) but differ from those of Valera and co-workers (1994) who did not find any evidence for mRNA encoding the vas deferens P_{2X}-purinoceptor in the rat brain. However, adult rat brain appears to contain low levels of mRNA for this receptor localised in discrete areas which may explain why this was not detectable in mRNA isolated from whole brain. Although these findings are consistent with many functional studies carried out in both the peripheral and central nervous systems (Chen *et al.*, 1995), discrepancies do exist, e.g. there is strong electrophysiological evidence for the presence of an α,β-methylene ATP-sensitive P_{2X}-purinoceptor in the medial habenula (Edwards et al., 1992). The presence of apparently only PC12 P_{2X}-purinoceptor (α,β-methylene ATP-insensitive) transcripts in this region suggests the existence of other, as yet unidentified, P_{2X}-purinoceptors.

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139P ACTIONS OF ADENOSINE RECEPTOR ANTAGONISTS ON THE RISE IN $[K^+]_e$ INDUCED BY COMBINED OXYGEN AND GLUCOSE DEPRIVATION IN THE RAT HIPPOCAMPUS *IN VITRO*

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We have previously reported that adenosine receptor antagonists reduce the rise in extracellular K^+ concentration ($[K^+]_e$) seen in the CA1 pyramidal cell layer of rat hippocampal slices as a result of oxygen deprivation (Croning *et al.*, 1994). In those experiments the increases in $[K^+]_e$ were modest - rising from 3 mM (the superfusate level) to 4-5 mM. In order to determine whether adenosine receptor antagonists would have a similar action when the insult was more severe we have established a model of combined oxygen and glucose deprivation where $[K^+]_e$ rises to ca. 20 mM in a biphasic manner as seen during anoxia *in vivo* (e.g. Hansen, 1977).

Transverse hippocampal slices were prepared from pentobarbitone-anaesthetised male Sprague-Dawley rats (70-110 g). Slices were submerged in a chamber and superfused with an aqueous solution at 30°C containing (mM): NaCl 125, KCl 2, KH_2PO_4 1, $MgSO_4$ 1, glucose 5, $NaHCO_3$ 25, $CaCl_2$ 2.5 gassed with 5% CO_2 in O_2 under control conditions. Oxygen and glucose deprivation was induced by gassing the aqueous solution with 5% CO_2 in N_2 and omitting glucose. $[K^+]_e$ was measured by ion-selective, valinomycin-based, double-barrelled electrodes (tip 5-8 µm). Recording electrodes were placed in the CA1 stratum pyramidale and near-maximal field potentials were evoked by a stimulating electrode placed in the CA3 stratum radiatum, the evoked potential being recorded via the reference barrel of the ion-selective electrode. Each slice was subjected to a single period of oxygen and glucose deprivation (10-25 min) with or without drug pretreatment (30 min). Data are presented as median (range, number). A significant effect was attributed to $P<0.05$ in the Mann-Whitney U-test.

Combined oxygen and glucose deprivation resulted in a biphasic increase in $[K^+]_e$. During phase 1 lasting 7.3 min (6.2 to 8.1, n=7) the $[K^+]_e$ rose gradually to 9.2 mM (6.9 to 11.7, n=7). Thereafter in phase 2 it rose sharply to 21.3 mM (14.9 to 73.4, n=7) over the next 1-3 min. Coincident with the phase 1 there was a reduction and an eventual abolition of the field potential. Treatment with theophylline (100 µM) or 8-cyclopentyl-1,3-dipropylxanthine (100 nM) significantly reduced the magnitude of the phase 1 $[K^+]_e$ increase: 5.6 mM (4.4 to 6.2, n=5) and 5.3 mM (4.2 to 6.4, n=4) respectively. They also significantly delayed the onset of phase 2 to 14.0 min (13.0 to 17.0, n=5) and 12.5 min (11.0 to 13.0, n=4) respectively, but the magnitude of the phase 2 increase in $[K^+]_e$ was not affected. As previously reported, both antagonists significantly delayed the depression of the evoked field potential caused by oxygen and glucose deprivation (Fowler, 1990).

These results suggest a facilitatory role for adenosine A_1 receptors in the increase in $[K^+]_e$ seen in the CA1 pyramidal cell layer as a result of oxygen+glucose deprivation.

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140P POTENTIATION OF PROPOFOL AND PENTOBARBITONE ANAESTHESIA BY CONVULSANT DRUGS

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High pressure appears to be the only consistent mechanism by which anaesthesia can be antagonised, although some central stimulant compounds have been shown to decrease anaesthesia (e.g. Dolin et al., 1988). We previously demonstrated that bicuculline antagonised anaesthesia due to ethanol but not that due to ketamine, whereas the excitatory amino acid N-methyl D,L aspartate (NMDA) had no effect on anaesthesia due to either agent (Watson et al., 1995). 4-aminopyridine (4-AP) antagonised both anaesthetics when given 5 min beforehand. When given 20 min before ethanol, it caused significant potentiation of anaesthesia. The present study examined the action of these convulsant drugs on the loss of righting reflex caused by either propofol or pentobarbitone. In addition the actions of thyrotropin releasing hormone (TRH) were examined as this compound has been reported to reduce the sleep time following administration of some anaesthetics.

Separate groups (n= at least 10) of male TO mice (25-35g) were used. Dose-response curves were constructed from the numbers which lost righting reflex after i.p. propofol (40 - 120 mg/kg) or pentobarbital (20 - 40 mg/kg i.p.) plus either 4-AP, NMDA, bicuculline or saline. The doses of the latter drugs were those which caused seizures in 50% of animals. At least 3 anaesthetic doses were used for each curve, measured 10 min after the anaesthetic injection to correspond with the peak anaesthetic effect. The doses of anaesthetic causing loss of righting reflex in 50 % of the animals (ED50 values with 95% fiducial limits) were calculated by the method of Lichfield and Wilcoxon (1949).

The results indicate the actions of both propofol and pentobarbitone were potentiated by NMDA and 4-AP. These results are opposite to the results obtained with ketamine and ethanol (Watson et al., 1995). In the case of pentobarbitone plus TRH the lines were not displaced in a parallel fashion; the ED50 was not altered, but the ED90 was significantly increased, (Saline - 60 (55.6-64.0) mg/kg, TRH - 105.5 (93.4-119.2) mg/kg) TRH therefore reduced the effects of higher doses of pentobarbitone, as reported when sleeping time was measured (Prange et al., 1973). Although pharmacokinetic effects cannot be ruled out until central drug concentrations are measured, the interaction of anaesthetics with convulsant drugs appears to operate via mechanisms which may be different for each anaesthetic agent.

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Watson, W.P. et al., (1995) Br. J. Pharmacol., in press.

Table 1: Doses of anaesthetic causing loss of righting reflex in 50 % of the animals (ED50 values with 95% fiducial limits)

* $P < 0.05$ c.f. control	Propofol				Pentobarbitone			
	Control		Drug treated		Control		Drug treated	
Bicuculline 2.9 mg/kg	93	(84-102)	86	(76-98)	33.8	(30.7-37.2)	30.4	(28.1-33.0) *
NMDA 300 mg/kg	97	(86-109)	53	(43-64) *	33.8	(30.7-37.2)	25.5	(21.1-30.9) *
4-AP 8 mg/kg	93	(84-102)	60	(47-76) *	32.6	(27.5-38.6)	27.0	(24.3-30.1) *
TRH 20 mg/kg	97	(86-109)	99	(91-108)	35.1	(33.0-37.3)	36.4	(32.2-41.1)

141P NO CHANGE IN EFFECTS OF AMPHETAMINE AND COCAINE AFTER REPEATED WITHDRAWAL FROM CHRONIC ETHANOL TREATMENT

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We have shown (this meeting) that chronic administration of ethanol can increase the locomotor stimulant effects of, and sensitisation to, amphetamine and cocaine. Repeated withdrawal from prolonged ethanol treatment is known to cause kindling (Pinel, 1980). We have investigated the effects of repeated withdrawal from chronic ethanol administration on the stimulation of locomotor activity by amphetamine and cocaine, and effects of repeated administration of these drugs.

Male TO mice (25-30g) were administered ethanol by liquid diet. All ethanol-treated mice first received control diet for 3 days, followed by 2 days 3.5% v/v ethanol in the diet, then 8 days 5% ethanol. This was then followed by either 17 cycles of 24h withdrawal plus 2 days 8% ethanol (schedule 1), or 9 cycles of 24h withdrawal plus 5 days 8% ethanol (schedule 2). Control groups were pair-fed control diet. Locomotor activity was measured for 10 min, in separate groups of mice, 20 min after administration of either amphetamine, 3 mg/kg, cocaine, 10 mg/kg or saline. Initial measurements were made either 24h (schedule 1) or 6 days (schedule 2) after the final withdrawal, followed by injections of amphetamine, 3 mg/kg, cocaine, 20 mg/kg or saline once daily for the next 15 days, with measurements of effects on locomotor activity on the fifteenth day.

After either treatment schedule, there were no significant differences (P>0.1) in the effects of amphetamine and cocaine on controls or ethanol treated mice, either on the first administration of the psychostimulants or after the repeated injections. Locomotor activity after saline was unaltered at either time (P>0.1,comparison between controls and ethanol treated). These results are in contrast to those obtained after 3 weeks continuous ethanol treatment (Manley and Little, this meeting), when increased effects of both psychostimulants were seen when testing was started either 24h or 6 days after cessation of ethanol treatment. Total ethanol consumption was greater in the intermittent treatments. It is possible that the repeated handling during withdrawal testing may have altered the effects of the ethanol treatment, or that the intervals without ethanol prevented the changes from occurring.

These results show that the pattern of ethanol intake determines whether or not changes are seen in the effects of psychostimulants. It appears that the prolonged kindling effect of repeated withdrawal from chronic ethanol administration may not occur with the psychostimulant drugs.

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Table 1. Locomotor activity measurements, mean ± s.e.m., comparisons by Student's t-test. Con = controls; Eth. = chronic ethanol; A = amphetamine; C = cocaine; In = first administration; Rp = measurements after 15 daily injections; Sch 1,2 = schedule 1, 2

	Con/Sal In	Eth/Sal In	Con/A In	Eth/A In	Con/C In	Eth/C In	Con/A Rp	Eth/A Rp	Con/C Rp	Eth/C Rp
Sch 1	3514±746	2320±168	4848±267	4059±467*	3488±421	2835±153*	5139±466	5731±530	3648±571	3029±194
Sch 2	1847±153	2210±175	3660±241†	4052±357*	3750±193†	3666±237*	4162±533	3932±478	3099±377	3288±587

†P<0.05 cf.Con/Sal In; *P<0.05 cf. Eth/Sal In

142P AN INVESTIGATION OF THE POSSIBLE INFLUENCE OF STRESS AND ANAESTHESIA ON THE LEARNING AND MEMORY DEFICITS PRODUCED BY SIMULATED ELECTROCONVULSIVE THERAPY

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It has been reported that electroconvulsive therapy (ECT) produces significant memory loss in patients (Squire, 1977). In support of this observation, Lerer *et al* (1986) demonstrated that repeated electroconvulsive shocks (ECS) given to rats produced learning and memory deficits in the passive avoidance test. However, in this study, ECS was given without anaesthetic and this differs from the manner in which ECT is given to patients. In addition, there was no control for the possibility that the stress of ECS sensitised the rats such that their tolerance to the aversive illuminated chamber was markedly reduced; if this had occurred, it would have resulted in artifactual deficits being observed in the passive avoidance test. Using this model, we have now investigated the effects of ECS given under anaesthesia on learning and memory and have used convulsive footshock (FS) to control for the stress component of this procedure.

Learning and memory testing were conducted as described by Lerer *et al* (1986) using a two-compartment dark/light shuttle box (Camden model 451). Male CD rats (Charles River; 150 - 200 g) were anaesthetised with halothane before receiving either, once or five times over 10 days, an ECS (200V, 2s) via earclip electrodes, or a convulsive FS (200V, 2s) via both hindlimbs. Passive avoidance testing was performed 24h after acute or repeated shock treatment.

The results shown in Table 1 demonstrate that neither acute, nor repeated, administration of halothane anaesthesia or convulsive FS had a significant effect on learning and memory. By contrast, repeated but not acute ECS impaired both behavioural responses. The results, therefore, indicate that ECS produces cognitive deficits which are not stress-related. They are not modified by halothane anaesthesia or mimicked by spinal convulsions. Finally, the data also demonstrate that the cognitive side-effects of ECT can be effectively investigated using the light/dark shuttle box passive avoidance model.

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Table 1 Effects of acute and repeated ECS or FS on learning and memory

	Acute treatment		Repeated treatment	
Treatment	Learning(s)	Memory(s)	Learning(s)	Memory(s)
Untreated	600 (512-600)	504.5 (381-600)	600 (390-600)	576.5 (268-600)
Halothane	600 (423-600)	501 (281-600)	599 (412-600)	539.5 (319-600)
ECS	548 (315-600)	558 (261-600)	292 (53-487)**†††	232.5 (97-489)**††
FS	598 (472-600)	546.5 (346-600)	433 (261-600)	498 (268-600)

Median step through latency minimum and maximum in parentheses, n = 8; **P<0.01 versus Untreated controls, ††P<0.01, †††P<0.001 versus Halothane-treated group. Mann Whitney U test (two-tailed). s = seconds.

143P SIMULTANEOUS ASSESSMENT OF BEHAVIOUR AND *IN VIVO* ACETYLCHOLINE RELEASE FROM THE HIPPOCAMPUS OF RATS EXPOSED TO A NOVEL, AVERSIVE ENVIRONMENT

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Previous studies have reported that hippocampal acetylcholine (Ach) release is increased in response to chronic restraint stress (Imperato *et al.*, 1991) or during periods of increased locomotor activity (Toide, 1989). The present study monitors the level of Ach from the right hippocampus of rats undergoing simultaneous behavioural analysis in a novel, aversive environment.

Microdialysis probes were implanted into chronically indwelling cannulae located in the dorsal hippocampus (Ant. +2.0, Vert. -8.0 and Lat. ±4.8; Paxinos & Watson, 1982) of male Lister Hooded rats (375-500g) 16 hours prior to the experiment. Probes were perfused with artificial cerebrospinal fluid containing 2 μM neostigmine at a rate of 2.0 μl min^{-1}. After 100 min stabilisation, four 20 min basal level samples were determined. During treatment samples were taken (Table 1.) and immediately analysed for Ach content by HPLC-ECD (Mark *et al.*, 1992). At time=0 min rats were placed in the centre of the light part (Lux: light=420 or 1200; dark=10-20) of a light/dark box, (50x39x64 cm), doorway 17.5x8 cm, light 3/5, dark 2/5) facing away from the doorway. Rats remained in the test box for the duration of the experiment. Rat exploratory activity (EA) was monitored during each dialysis sampling period and assigned a value of 0-3 according to the level of EA (EA: 0=none (asleep), 1=periodic, 2=frequent, 3=continuous). In addition, the number of transitions between, time spent, number of exploratory rears and line crossings in both compartments was recorded in the first 10 min of box exposure.

The light/dark box exposure significantly increased Ach release, although not differentially, at both 420 and 1200 Lux (Table 1). The behaviour of rats (in the first 10 min in the box) which experienced 1200 Lux was significantly suppressed as indicated by decreased time spent (182±20 secs, 420 Lux to 112±26 secs, 1200 Lux) and number of rears (14.4±4.7, 420 Lux to 6.8±2.6, 1200 Lux) ($P<0.05$, 1 way ANOVA followed by Dunnett's t test) in the light section, compared to rats with 420 Lux in the light compartment. The results indicate an 'anxiogenic-like' profile in the more aversive environment of enhanced illumination (1200 Lux). A significant elevation of Ach levels occurred concomitant to an increase in EA (0.6±0.2, 420 Lux; 0.4±0.2, 1200 Lux at t=0 min to 3 at t=10-20 min), both variables showing habituation to the situation with time.

The results suggest that the increase in Ach after a novel yet aversive stimulus may be in response to stress, the emotional arousal of a new environment, regardless of it's nature and/or an increased locomotor activity from exploration of a novel environment.

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Table 1. Effect of light/dark box exposure at different light intensity on extracellular levels of Ach in the right hippocampus of the rat.

Time (min)	0	10	20	30	40	60	80
420 Lux light side	113±11	173±13*	208±12**	206±17**	193±16**	179±37*	150±40
1200 Lux light side	97±16	170±22**	201±13**	180±21**	156±27*	119±18	97±9

Data represent the mean±SEM (% of basal level), n=5.
Significant differences compared to the basal levels are indicated as *P<0.05 and **P<0.01 (ANOVA with repeated measures followed by Dunnett's t test). There was no significant difference in the response to test box exposure at the different light intensity (P>0.05).

144P EFFECTS OF METRIFONATE AND TACRINE IN THE SPATIAL MORRIS TASK AND MODIFIED IRWIN TEST: EVALUATION OF THE EFFICACY/SAFETY RATIO IN RATS

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Metrifonate (MTF) is a member of the so-called second generation cholinesterase inhibitors (ChEI) (Becker & Giacobini, 1988) and is currently under clinical evaluation as a potential therapeutic for the cognitive dysfunctions in Alzheimer's disease. In the present study we examined the possible therapeutic window for MTF by evaluating the dose-response relationship for efficacy as well as tolerability in rats. For comparison, we evaluated the effects of a first generation ChEI tacrine (THA).

The efficacy of MTF and THA was tested in a Morris task in 19-month-old male Wistar rats (MTF: n=10; THA: n=9). The rats were given daily injections with MTF (3, 10, and 30 mg/kg, p.o.) or THA (0.3, 1, and 3 mg/kg p.o.), 60 min prior to a session of 4 trials on 5 consecutive days. The time to escape on the submerged platform was taken as an index of spatial learning. The tolerability of both substances was evaluated in an observation test (modified Irwin test, MIT) in 19-month-old rats (all groups n=6). The doses used are shown in Table 1. In addition, the effects of repeated injections with a relatively high dose of MTF and THA (100 and 10 mg/kg, respectively) were evaluated. An MIT was performed after a fifth injection.

The results of the Morris task indicated that the MTF-treated rats acquired the task faster than the untreated rats (linear trend coefficient: $F(3,36)=3.67, P<0.05$). The rats treated with a dose of 30 mg/kg MTF seemed to perform not as good as the other two treatment groups. It was suggested that the most effective dose of MTF is within the range of 3-10 mg/kg p.o. THA treatment appeared not to improve performance of old rats in the Morris task (linear trend coefficient: $F(3,32)=1.09, P>0.10$). Therefore, no efficacy of THA could be determined in this test model. The results of the MIT are shown in Table 1.

Table 1: Effects of a single and repeated oral injections (5 in total) with different doses of MTF and THA in the MIT in old rats (rep.: repeated; 12: the symptom was 12 times observed during 8 time points within 2 h, maximal score was 48 (40 in case of THA rep.)).

	MTF				THA			
administration	acute			rep.	acute			rep.
dose (mg/kg)	10	30	100	100	1	3	10	10
salivation	4	14	34	24	19	26	41	36
diarrhoea	2	6	16	9	0	6	7	7
prone position	0	0	18	7	0	0	8	10
tremor	0	3	23	11	2	6	16	22
limb abduction	0	0	15	1	0	0	4	9
clonic seizures	0	0	8	1	0	0	0	0

The efficacy of MTF was found in a dose range of 3-10 mg/kg p.o. Severe cholinergic side effects were observed at a dose of 100 mg/kg. Repeated administration of MTF (100 mg/kg) led to a reduction of these side effects. No efficacy for THA could be determined in our model. The previously reported efficacy of THA appeared to be in a dose range of 1-3 mg/kg (i.p./s.c.) (Flood & Cherkin, 1988). We observed severe acute side effects at a dose of 10 mg/kg p.o. Repeated administration of THA (10 mg/kg p.o.) did not reduce the occurence of side effects (one rat died). We assume that the safety/efficacy ratio of MTF is more favourable than that of THA.

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145P THE EFFECT OF CHOLINESTERASE INHIBITION AND STIMULATION FREQUENCY ON JITTER OF ACTION POTENTIALS IN MOUSE SKELETAL MUSCLE

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Inhibition of acetylcholinesterase (AChE) by ecothiopate increases the variability of the latencies of muscle action potentials (APs) at 30Hz, i.e. increases jitter (Kelly *et al* 1990). In this study the role of frequency of activation on increased jitter was investigated.

Adult male albino mice were given ecothiopate 500nmol/kg + atropine 700nmol/kg by subcutaneous injection. Five days later phrenic nerve-diaphragm preparations were bathed in physiological saline. Trains of 30 APs were evoked by stimulation of the phrenic nerve and recorded intracellularly at 1 then 30Hz from the same muscle cells. Variation of the latency during trains of APs is represented as the mean consecutive difference of AP 11-30 (MCD). The general increase in the latency of the APs during the train is represented by the extra delay of the mean latency of the 14th, 15th and 16th APs compared with that of the 1st AP (Delay).

Functional AChE activity as described by Crofts *et al* (1995), i.e the asymmetric molecular forms assayed by the method of Ellman (1961), which was normally 0.50 ± 0.20 nmol ACh/min/mg tissue in untreated diaphragms was reduced to 0.05 ± 0.04 nmol/min/mg 3 hours after ecothiopate. Five days after treatment functional AChE was still reduced at 0.26 ± 0.14 nmol/min/mg.

In untreated muscle, the Delay at 30Hz was greater than at 1Hz, but the MCDs were not different. After ecothiopate there was no increase in MCD or Delay at 1Hz, but at 30Hz, the MCD and Delay were both increased.

Table 1 The effect of stimulation frequency on the MCD and Delay of APs in diaphragm muscle fibres. All values are expressed as mean ± s.d. with the number of fibres in parentheses. All data groups differ from each other except for those starred (Kolmogorov-Smirnov $p<0.01$).

Treatment	Delay (μs)	MCD (μs)
Untreated 1Hz	- 6 ± 24 (24)*	9.5 ± 4.7 (24)*
Untreated 30Hz	32 ± 21 (24)	11.4 ± 5.3 (24)*
Ecothiopate 1Hz	- 4 ± 12 (31)*	9.3 ± 5.1 (31)*
Ecothiopate 30Hz	53 ± 42 (31)	22.3 ± 20.0 (31)

It is concluded that, whilst increased jitter is a consequence of inhibition of AChE, it is manifest with stimulation at 10Hz and 30Hz (Kelly *et al* 1990) but not at 1Hz. Thus this effect of ecothiopate is not due to an on-going deficit in nerve and muscle and perhaps is use-dependent.

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146P FUNCTIONAL CHARACTERISATION OF MUSCARINIC RECEPTOR SUBTYPES IN GUINEA-PIG SUBMANDIBULAR SALIVARY GLAND

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Muscarinic receptor subtypes in salivary glands have not been extensively characterised despite the major role of the parasympathetic nervous system in the control of salivation. Functional studies in rat parotid and submandibular salivary gland show that the responses to cholinergic agonists in these tissues are mediated via M_3 receptors (Dehaye *et al.*, 1988; Laniyonu *et al.*, 1990; Kirkup & Moore, 1995). In the present study, functional muscarinic receptors in guinea-pig submandibular salivary gland have been characterised using subtype selective antagonists against both agonist and field-stimulated challenges in ^{86}Rb efflux studies.

Submandibular glands taken from female Dunkin Hartley guinea-pigs (300-400g) were finely minced and incubated in Krebs-Henseleit solution (37°C, gassed with 95%O_2/5%CO_2) containing $^{86}RbCl$ (10μCi/ml) for 90min. The tissue was then washed and aliquoted into individual chambers in a superfusion apparatus. Muscarinic antagonists were superfused in Krebs solution (37°C, gassed with 95%O_2/5%CO_2) for 20min prior to carbachol (10^{-5}M) or field stimulation (6Hz, 1msec pulse width, 5min train, 50mA) challenge. The potencies of muscarinic antagonists to inhibit the ensuing increase in the ^{86}Rb efflux rate coefficient (Durbin & Jenkinson, 1961) were expressed as the log of the concentration required to produce a 50% inhibition (pIC_{50}). The data are shown in Table 1.

The pIC_{50} values of the muscarinic antagonists used in this study and the rank order of potency relative to atropine, suggests that the muscarinic receptor subtype which mediates the ^{86}Rb efflux response to carbachol and neuronal stimulation in guinea-pig submandibular glands is the M_3 subtype. In the field stimulation studies, no evidence for pre-junctional modulation by other muscarinic receptor subtypes was found.

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Kirkup, A.J. & Moore, B.A. (1995). *Br. J. Pharmacol.* (Proceedings of Canterbury meeting).
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Table 1 pIC_{50} values for muscarinic antagonists on guinea-pig submandibular gland (mean ± s.e.mean, n = 4)

Challenge	Atropine	Pirenzepine (M1)	Methoctramine (M2)	4-DAMP (M3/M1)	Himbacine (M4/M2)
Carbachol	7.87 ± 0.14	5.89 ± 0.10	5.88 ± 0.19	7.23 ± 0.08	5.67 ± 0.06
Field Stimulation	8.65 ± 0.15	5.63 ± 0.33	5.47 ± 0.13	7.75 ± 0.17	6.65 ± 0.40

147P THE CARDIOPROTECTIVE EFFECTS OF CYCLIC GUANOSINE 3':5' MONOPHOSPHATE ON REPERFUSION-INDUCED VENTRICULAR ARRHYTHMIAS IN THE RAT ISOLATED HEART

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It has recently been shown that reperfusion-induced ventricular fibrillation (VF) is enhanced by N^G-nitro-L-arginine methyl ester. This pro-fibrillatory effect is L-arginine reversible and correlates with changes in coronary effluent nitric oxide content (NO) (Pabla & Curtis, 1994). The mechanism of signal transduction for NO is known to be the cGMP second messenger system. In the present study we have examined the effect of altering cardiac cGMP on susceptibility to reperfusion-induced VF.

Male Wistar rats (240-260g) were anaesthetised with pentobarbitone (60 mg kg^{-1}, i.p.) and given 250 iu sodium heparin (i.p.). Hearts were excised and perfused (Langendorff mode) with solution containing (in mM): NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4 and glucose 11.1, pH 7.4, 37°C. Hearts were randomised to control (vehicle: 0.1% NaOH), 20 μM methylene blue (MB; a guanylate cyclase inhibitor; Martin *et al*, 1985), 100 μM zaprinast (a cGMP-specific phosphodiesterase inhibitor; Souness *et al*, 1989), or MB plus zaprinast (n=10/group). Hearts were subjected to 60 min left ventricular regional ischaemia, followed by reperfusion. Ventricular arrhythmias were detected using a unipolar electrogram inserted into the ventricular apex. Additionally heart rate, coronary flow and recovery of flow were measured. Following 1 min of reperfusion hearts were rapidly frozen in liquid nitrogen for cGMP analysis by radioimmunoassay. Data were recorded and analysed by ANOVA plus Dunnett's test for mean ± s.e.mean data or Chi^2 for % incidence data.

MB significantly increased the incidence of reperfusion-induced VF from 10% in controls to 80% ($P < 0.05$). The pro-fibrillatory effect of MB was abolished by zaprinast co-perfusion ($P < 0.05$). Furthermore, co-perfusion with zaprinast led to a significant reduction in reperfusion-induced ventricular tachycardia incidence from 100% in control hearts to 50% ($P < 0.05$). Perfusion with zaprinast alone led to a 30% incidence of reperfusion-induced VF. The pro-fibrillatory effects of MB were associated with a significant reduction in cGMP content in the reperfused myocardium from 156 ± 15 fmol/mg in control hearts to 70 ± 7 fmol/mg ($P < 0.05$). The effects of MB on cGMP content was reversed by zaprinast co-perfusion to 293 ± 22 fmol/mg ($P < 0.05$). Perfusion with zaprinast alone led to an even greater rise in cGMP content to 393 ± 43 fmol/mg. In addition zaprinast significantly increased coronary flow 1 min prior to ischaemia by 40% compared to controls and recovery of flow during the first min of reperfusion by 52%. MB had no significant effect on either coronary flow or recovery of flow but reduced heart rate 1 min before ischaemia onset from 340 ± 14 beats/min to 251 ± 18 beats/min ($P < 0.05$). This bradycardic effect was overcome with zaprinast co-perfusion.

These results implicate cGMP as a potential anti-arrhythmogenic mediator and may explain the mechanism of action of NO as an anti-fibrillatory substance. Clearly further studies are required to confirm this possibility.

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148P DELAYED EFFECTS OF THE ENDOTOXIN DERIVATIVE, MONOPHOSPHORYL LIPID A, ON RESPONSES TO ISCHAEMIA-REPERFUSION IN RABBIT MYOCARDIUM

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Pretreatment with bacterial endotoxins has been shown to enhance myocardial resistance to ischaemia-reperfusion injury many hours later, possibly by augmenting myocardial cellular defences (Brown *et al* 1989, Song *et al* 1994). The endotoxin derivative monophosphoryl lipid A (MLA), at low doses, has been reported to reduce infarct size in the dog heart *in vivo* 24 h, but not 1 h, after treatment through an unknown mechanism (Yao *et al* 1993). In the present study, we have examined if low dose MLA treatment induces the cytoprotective stress protein hsp70i in rabbit myocardium and have studied the responses to ischaemia-reperfusion in (i) isolated buffer-perfused rabbit hearts and (ii)*in vivo* 24 h after MLA pretreatment.

MLA (35 mg/kg i.v.) or vehicle were administered to male New Zealand White rabbits (2.2-3.0 kg) 24 h before the ischaemia-reperfusion protocols. In the *in vitro* study, hearts were Langendorff-perfused with Krebs-Henseleit buffer at 37 °C. Global ischaemia was induced for 20 min followed by 120 min reperfusion. Recovery of left ventricular developed pressure (DP) during reperfusion was assessed and percentage infarction of the risk zone (I/R) was examined by tetrazolium staining. In the *in vivo* study, 30 min coronary artery occlusion was induced in pentobarbitone-anaesthetised, artificially ventilated rabbits, followed by 120 min reperfusion after which I/R was determined. In a separate series of animals, myocardial content of hsp70i 24 h after MLA or vehicle administration was determined by Western blot analysis (Marber *et al* 1994).

Myocardial hsp70i content was not elevated following MLA pretreatment. In the *in vitro* study, no protection against ischaemia-reperfusion injury was observed (Figure 1). However, *in vivo*, there was a marked reduction in myocardial susceptibility to infarction 24 h after MLA despite similar myocardial risk volumes (Figure 2).

FIGURE 1 *IN VITRO* STUDY

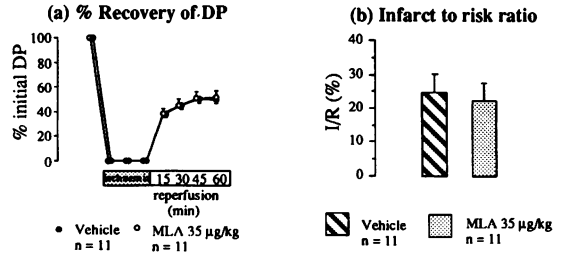
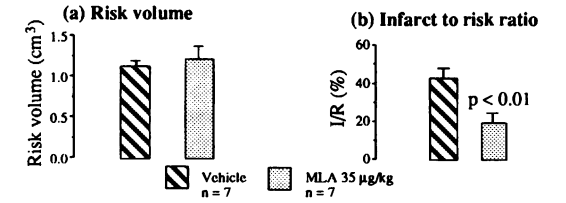


FIGURE 2 *IN VIVO* STUDY



Data are mean ± s.e.mean

We conclude that MLA at this dose confers protection only against ischaemia-reperfusion injury *in vivo* and that this protection is not related to enhanced hsp70i content. It seems possible that the delayed protection conferred by MLA is mediated by effects on humoral or blood-borne factors.

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149P RAPID DESENSITZATION OF THE INOTROPIC RESPONSE OF THE GUINEA-PIG LEFT ATRIUM CAUSED BY SHORT TERM EXPOSURE TO LOW CONCENTRATIONS OF ISOPRENALINE

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Studies on receptor desensitization have been mainly conducted on either cell membrane preparations (Lefkowitz *et al*, 1990) or, where whole tissue functional responses have been measured, have employed prolonged exposures to high agonist concentrations (Herepath and Broadley, 1990). In the present study we have examined receptor desensitization in the guinea-pig atrium using short exposure times and low concentrations of agonists.

Sections of left atrium from Dunkin-Hartley guinea-pigs of either sex (c.500g) were mounted between platinum electrodes under 1g tension in an insulated chamber and superfused with warmed (37°C) Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2 at a rate of 2 ml min^{-1}. The tissues were directly electrically stimulated (1Hz, 5 ms, voltage 20 - 100% x threshold) and the force of contraction was measured using an isometric transducer.

Isoprenaline (0.01 - 0.3 μM) superfused for 60 min at each concentration produced a concentration-related increase in peak force of contraction of the paced left atrium, (peak increase in force in mg at 0.01 μM = 205 ± 29, 0.03 μM = 261 ± 44, 0.1 μM = 457 ± 49, 0.3 μM = 519 ± 65, n=7), but the response was not maintained at each concentration, a significant decline from peak occurring in the first 30 min, $P < 0.05$. The decline in response was not due to exhaustion of energy sources or failure of the contractile machinery since the inotropic action of the Ca^{2+} channel opener, Bay K 8644, at 1 μM, produced a similar peak effect which was maintained over the same time frame (n = 7) (peak increase = 539 ± 32 mg at 60 min, after a further 30 min, force = 527 ± 34 mg). The desensitization to isoprenaline appeared to be homologous in nature since it did not occur with the inotropic response to the thromboxane receptor agonist U 46619 (0.1μM) (n = 6).

The desensitization appeared to be occurring primarily at the level of the receptor or its G protein interaction since the inotropic response to forskolin, which directly stimulates adenylate cyclase, showed a smaller ($P < 0.05$) decline over a 30 min period (1μM forskolin, 22 ± 3%, n=29, 0.1μM isoprenaline, 36 ± 4%, n=18). Even when the response to isoprenaline had declined to baseline and despite continued application of the β-agonist, U 46619 produced a strong inotropic response (246 ± 48 mg).

Thus rapid desensitisation of a functional response caused by activation of the β-adrenoceptor can be demonstrated in the guinea-pig atrium. Since it occurs rapidly it is most likely to be a consequence of uncoupling of the receptor from the Gs protein (Lefkowitz *et al*, 1990), but further experiments will be required to confirm this mechanism.

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150P THE EFFECT OF EICOSAPENTAENOIC ACID UPON CONTRACTURES OF ISOLATED AORTIC RINGS AND CALCIUM INFLUX INTO CULTURED AORTIC CELLS FROM THE RAT

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Eicosapentaenoic acid (EPA) causes vasorelaxation (Engler, 1992) and reduces blood pressure (Bønaa *et al.*, 1990) but its mechanism of action is unclear. In cardiac myocytes, it has been suggested that EPA modulates L-type calcium channels (Hallaq *et al.*, 1992). In this study, the vasorelaxant effect of EPA on rat (male, Wistar, 200-250g) aorta was compared to its action on calcium influx in cultured aortic cells (AOCs).

Isolated aortic rings (5-7mm), devoid of endothelium, were suspended in Krebs buffer containing propranolol (10^{-6}M), EDTA (10^{-5}M) and ascorbic acid ($5x10^{-5}$M) at 37°C, gassed with carbogen, under a resting tension of 2g. Isometric contractures were recorded. After 1h equilibration, contractures to KCl (60mM) were elicited, followed by non-cumulative concentration-response curves to KCl before and 30 min after EPA ($5x10^{-5}$M), nicardipine (NIC, 10^{-7}M) or ethanol (ETH, 1%; the EPA vehicle). AOCs were prepared by enzymatic dissociation using papain and collagenase and cultured in MEM D-valine medium, supplemented with 10% fœtal calf serum for 10-13 days. AOCs were incubated with ^{45}Ca (1μCi ml^{-1}) for 40 min in physiological saline at 37°C in the presence of 5, 10 or 60mM KCl (controls, CON). The addition of ETH (1%) or EPA ($5x10^{-5}$M) was compared. In some experiments, NIC (10^{-7}M) was added to the culture medium 30 min before experimentation.

KCl contractures were reduced by EPA. A rightward shift of the concentration-response curve was seen from the EC_{50} values; ETH 12.4±1.0mM (n=6), EPA 16.7±1.5mM (n=6) $P<0.001$, with no significant change in E_{max}; ETH 3.43±0.29g, EPA 2.81±0.46g. This effect of EPA was not dependent upon eicosanoid production. In contrast NIC (10^{-7}M) totally inhibited KCl contractures. In AOCs, KCl caused a concentration-dependent increase in ^{45}Ca influx which was reduced by NIC. EPA significantly reduced influx induced by 5mM KCl but enhanced influx induced by 60mM KCl (Table 1).

Table 1. The effect of EPA upon calcium influx into AOCs.

	Calcium influx (nmol μg^{-1} protein)			
	CON	NIC	ETH	EPA
KCl (mM)				
5	14.28±0.89†	* 4.07±0.38	15.12±1.47†	# 5.89±2.20†
10	19.41±1.59	*10.30±0.86	19.46±3.08	27.93±2.96
60	23.34±1.58	*14.59±0.76	25.82±3.55	#50.68±6.90

Values are mean±s.e.mean. * compared with control values (CON); # compared with ethanol (ETH) . $P<0.05$ using Students unpaired t-test (n=6, † n=4).

The results suggest that vasorelaxation induced by EPA is not simply associated with a decrease in calcium entry through voltage-operated calcium channels. Clarification of its mechanism of action remains for future study.

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151P CARDIAC AND TOXIC EFFECTS OF DIGITALIS GLYCOSIDES IN INTACT AND VAGOTOMISED ANAESTHETISED DOGS

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The responses to the therapeutic and toxic effects of cardiac glycosides appear to involve inhibition of myocardial Na^+,K^+-ATPase and indirect neuronal effects. The experiments were designed to evaluate the role of vagal activity on responses to six different cardiac glycosides (ouabain [O], digoxin [D], digoxigenin galactoside [DGa], digitoxigenin galactoside [DTGa], digoxigenin rhamnoside [DRh] and digitoxigenin glucoside [DTGu]).

All experiments were performed in mongrel dogs, anaesthetised with pentobarbitone (30 mg kg^{-1} i.v., then 3 mg kg^{-1} hr^{-1} i.v.). Heart rate and myocardial contractility were measured as previously described (Altman *et al.*, 1988) in control animals (no intervention) and in animals after bilateral vagotomy. Continuous i.v. infusion of one of the glycosides was administered to each animal until the onset of toxicity (continuous idioventricular rhythm, lasting at least 10 min). The rate of infusion of each compound was such that the cardiovascular responses could be observed for 90-120 min before the onset of toxicity. The effects of vagotomy on the inotropic and chronotropic responses were compared by repeated measures analysis of variance. The doses required to induce toxicity were compared using Student's t test. Significance was assumed where $P<0.05$.

In the control animals, all of the glycosides produced dose-dependent increases in myocardial contractility. The inotropic response to ouabain was significantly greater in vagotomised animals than in the control group, but there was no significant difference in inotropic responses to any of the other glycosides.

Digoxin had no significant effect on heart rate in the control animals but produced a dose-dependent decrease in heart rate in vagotomised animals. All of the other glycosides had a negative chronotropic effect in control animals. The effect of vagotomy on the responses to these drugs was variable. In the vagotomised animals, DGa did not alter heart rate, DGTa caused an increase in heart rate and the negative chronotropic effects of ouabain, DGu and DRh were not significantly different to those in control dogs.

The doses required to induce toxicity in control and vagotomised animals are shown in Table 1.

The results show that the glycosides differed significantly in their potential to interact with the parasympathetic branch of the autonomic nervous system. Since vagotomy did not affect the responses to all of the glycosides in the same manner, the changes observed are unlikely to be due to altered myocardial sensitivity as a consequence of the denervation procedure. Thus it is likely that indirect effects of the drugs are due to a real interaction with extracardiac receptors.

Altman, P.M., Einstein, R., Goodman, A.H. *et al.* (1988) *Arzneimettelforschung*, 38, 1115-1119.

Table 1. Dose (μg kg^{-1}) of glycosides (mean ± s.e.mean) required to induce toxicity

Drug	O	D	DGa	DTGa	DRh	DTGu
Control (n)	41.1±2.3 (6)	128.3±6.9 (6)	59.1±4.0 (6)	88.4±6.5 (8)	41.8±4.8 (8)	51.3±2.4 (6)
Vagotomy (n)	36.9±0.4 (6)	95.9±5.4* (6)	70.1±2.8 (6)	53.3±3.6* (7)	34.4±1.7 (6)	45.0±2.0 (6)

* $P< 0.05$

152P BINDING OF THE NUCLEOSIDE TRANSPORT INHIBITOR NITROBENZYLTHIOINOSINE TO RAT RENAL MEMBRANES: EFFECT OF ACUTE RENAL FAILURE

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We have reported changes in the binding characteristics of adenosine A_1 receptors in glycerol but not in $HgCl_2$-induced acute renal failure (ARF) in the rat (Gould *et al.* 1995a). Such changes may explain, in part, the selective increase in sensitivity to the renal vasoconstrictor actions of adenosine noted in glycerol but not in $HgCl_2$-induced ARF (Gould *et al.* 1995b). The aim of this work was to determine the binding characteristics of the nucleoside transporter in these two forms of ARF.

ARF was induced in male Wistar rats with either glycerol (10 ml kg^{-1} of 50% v/v glycerol in saline, i.m., subsequent to 24h withdrawal of drinking water) or $HgCl_2$ (2 mg kg^{-1} s.c.). Control animals were given equivalent volumes of saline. At various times, kidneys were removed, freeze clamped in liquid nitrogen and membranes prepared by standard centrifugation methods. Kidney cell membranes were diluted to 1 mg ml^{-1} protein and preincubated with adenosine deaminase (5 u ml^{-1}) for 30 minutes at 37°C; followed by 5 hours incubation at 4°C with concentrations of the nucleoside transport inhibitor [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBTI, 0.08-40nM) in 50 mM Tris buffer, pH 7.4.

A significant decrease in the number of binding sites (Bmax) for [^{3}H]-NBTI was noted in rats with glycerol-induced ARF (Table 1). This decrease was found as early as 30 min following glycerol injection and by 48h, Bmax was 52% of its value in control animals. Similarly, 48h following induction of $HgCl_2$-induced ARF, Bmax had declined to 32% of the control value. By contrast, binding affinity for [^{3}H]-NBTI increased in animals with $HgCl_2$-induced ARF as shown by a significant decrease in the apparent dissociation constant Kd. There were no significant differences in the values of Kd between rats with glycerol-induced ARF and their time-matched controls. However, in control animals for the glycerol-injected rats, there was a progressive decline in Kd such that 48h following saline injection, Kd was 30% of the value at 30 min.

Present findings suggest: (1) i.m. saline injection in dehydrated rats results in an increase in affinity for [^{3}H]-NBTI; (2) there is a reduction in the number of binding sites for [^{3}H]-NBTI during the development of both glycerol and $HgCl_2$-induced ARF and (3) changes in binding characteristics of the nucleoside transporter are unlikely to contribute to the enhanced sensitivity to the renal constrictor effect of adenosine noted in glycerol-induced ARF.

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Gould, J., Bowmer, C.J. & Yates, M.S. (1995b) *Nephron* (in press)

Table 1 Characteristics of renal [^{3}H]-NBTI binding sites in rats with acute renal failure.

Group	Kd (pM)	Bmax (fmol mg^{-1})	Group	Kd (pM)	Bmax (fmol mg^{-1})
Saline (i.m.) 0.5h	824 ± 52	978 ± 121	Glycerol (i.m.) 0.5h	581 ± 100	644 ± 37*
Saline (i.m.) 16h	452 ± 158#	891 ± 98	Glycerol (i.m.) 16h	513 ± 184	644 ± 77
Saline (i.m.) 48h	244 ± 54##	848 ± 76	Glycerol (i.m.) 48h	256 ± 23	442 ± 17**
Saline (s.c.) 48h	918 ± 119	1224 ± 38	$HgCl_2$ (s.c.) 48h	307 ± 32**	388 ± 50***

Estimate ± s.e. estimate (8 d.f.) from nonlinear least squares regression analysis of binding isotherms; * $P<0.05$, ** $P<0.01$, *** $P<0.001$ with respect to time-matched saline controls; # $P<0.05$, ## $P<0.01$ with respect to 0.5h saline (i.m.). Each value was obtained using protein derived from the kidneys of 12 rats.

153P INTERACTION BETWEEN NEUROPEPTIDE Y AND NIFEDIPINE IN THE REGULATION OF RAT RENAL FUNCTION

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Neuropeptide Y (NPY), a cotransmitter of the sympathetic nervous system, reduced renal blood flow and concomitantly enhanced diuresis and natriuresis but not kaliuresis in anesthetized rats (Bischoff et al. 1994). In many model systems the NPY-induced vasoconstriction depends on influx of extracellular Ca^{2+}, and thus is sensitive to dihydropyridine-type Ca^{2+} entry blockers (Michel & Rascher 1995). Therefore, we wished to dertermine whether NPY effects on renal blood flow, diuresis and natriuresis are also sensitive to Ca^{2+} entry blockade.

Two h after completion of instrumentation pentobarbital-anesthetized male Wistar rats (330-350 g) were given 3 μg/kg nifedipine intraperitoneally, and 1 h thereafter 1 μg/kg/min NPY was infused for an additional 2 h. Mean arterial pressure was measured via a catheter in the femoral artery connected to a Statham pressure transducer. Renal blood flow was determined by an electromagnetic flow probe (Skalar) placed on the renal artery. Urine samples were collected via a ureter catheter in 15 min intervals. Urinary electrolytes were determined by flame photometry, serum and urine creatinine with an autoanalyzer (Hitachi 707). Data are mean ± s.e.mean of 18-23 animals.

Nifedipine treatment did not significantly ($p > 0.05$ in t-test) effect mean arterial pressure (121±5 mm Hg), renal blood flow (7.9±0.4 ml/min) or endogenous creatinine clearance (1.3±0.7 ml/min) but reduced urine formation from 300±60 to 123±20 µl/15 min and Na^+ and K^+ excretion from 3.4±0.7 to 1.2±0.4 and from 2.0±0.1 to 1.4±0.2 µmol/min (all $p < 0.05$), respectively, as determined prior to the NPY administration.

NPY infusion had similar effects on mean arterial pressure (increase by ≈10 mmHg) and renal blood flow (peak reduction by ≈1.7 ml/min) in the absence and presence of nifedipine. NPY-induced enhancements of diuresis and natriuresis (cumulative excretion during 2 h infusion period 2199±627 µl and 1321±356 µmol, respectively) were significantly ($p < 0.05$ in two-way ANOVA) attenuated by nifedipine treatment (949±319 µl and 673±244 µmol). In contrast NPY increased kaliuresis more in nifedipine-treated than in control rats (cumulative excretion 253±54 vs. 93±79 µmol).

We conclude that under our experimental conditions nifedipine treatment did not affect systemic or renal hemodynamics or their alterations induced by NPY. However, nifedipine reduced basal and NPY-induced diuresis and natriuresis. Although nifedipine reduced basal kaliuresis, it revealed NPY-induced enhancements of kaliuresis which are not detectable under control conditions. Based on the kaliuresis data, we speculate that the Ca^{2+} entry blocker nifedipine unmasks a possible NPY effect on proximal tubular function.

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154P UPREGULATION OF Na/K PUMPS BY LOOP DIURETICS: NON-INVOLVEMENT OF Na/K/2Cl CO-TRANSPORT

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Loop diuretics increase Na/K pump numbers both *in vivo* and *in vitro*. Pump numbers in erythrocytes are increased in patients taking frusemide (Erdmann *et al.*, 1984), and this has been attributed to potassium depletion (since a low extracellular potassium concentration stimulates pump upregulation). Ethacrynic acid, which inhibits Na/K/2Cl co-transport (as do frusemide and bumetanide), increases Na/K pump numbers *in vitro* in HeLa cells and lymphocytes, and it has been suggested that this effect is secondary to a raised intracellular sodium concentration (Boardman *et al.*, 1974). However, we have previously shown that myoinositol reverses ethacrynic acid-induced upregulation, suggesting that phosphoinositide signalling may be involved (Murton & Aronson, 1995). We have therefore determined whether ethacrynic acid-induced upregulation of Na/K pump numbers *in vitro* is accompanied by a raised intracellular sodium concentration and whether loop diuretics other than ethacrynic acid upregulate pump numbers *in vitro* when the extracellular potassium concentration is normal.

We exposed Epstein-Barr virus-transformed lymphocytes for 72 h to ethacrynic acid, frusemide, and bumetanide in the presence of potassium 5 mM, and calculated the numbers of pumps from the B_{max} of [^{3}H]-ouabain binding (2.5–40 nM for 2 h; Kennedy *et al.*, 1990). We calculated intracellular sodium concentration from the sodium content (measured by flame emission spectrophotometry) and cell volume (measured by Coulter® Multisizer).

Ethacrynic acid increased Na/K pump numbers significantly, but frusemide and bumetanide did not (Table 1). The intracellular sodium concentration of cells exposed to ethacrynic acid did not change at 72 h (control 20.6 [s.e.mean 1.7] mmol/l; ethacrynic acid 18.4 [2.0]; n=9), nor at earlier times (3, 8, and 24 h).

We conclude that ethacrynic acid-induced upregulation of Na/K pump numbers, in the presence of a normal extracellular potassium concentration, is not secondary to inhibition of the Na/K/2Cl co-transport. *In vivo* upregulation in patients taking frusemide is probably due to potassium depletion.

We are grateful to the Medical Research Council for support.

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Table 1. Percentage increases in pump numbers due to ethacrynic acid, frusemide, and bumetanide

	Ethacrynic acid (20 µM; n=12)	Frusemide (20 µM; n=6)	Frusemide (100 µM; n=6)	Frusemide (1 mM; n=5)	Bumetanide (10 µM; n=9)
Control no. of pumps	286 620	299 160	299 160	290 160	360 600
s.e.mean	21 550	22 440	22 440	6 896	7 760
Pump no. after treatment	359 760	278 580	288 480	292 680	361 380
s.e.mean	24 080	21 580	15 211	2 361	7 640
Mean increase (%)	24	-7	-4	1	0
P (versus control; unpaired t-tests)*	<0.035	>0.5	>0.7	>0.7	>0.9

155P THE INFLUENCE OF CALCIUM CHANNEL BLOCKADE ON RENAL INTERSTITITAL HYDROSTATIC PRESSURE IN ANAESTHETISED WISTAR AND STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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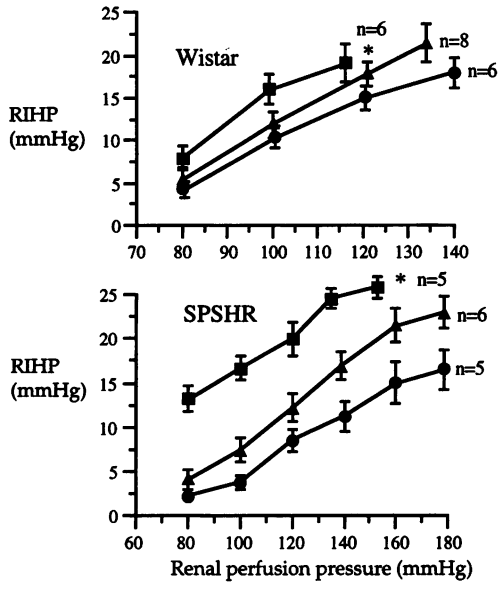
Previously we demonstrated that the calcium channel antagonist, nitrendipine, inhibited renal blood flow (RBF) autoregulation and steepened pressure-natriuresis curve in both Wistar (Huang et al, 1993) and stroke-prone spontaneously hypertensive rats (SPSHR) (Huang & Johns, 1995). This study aimed to determine whether a calcium channel blocker could influence renal interstitial hydrostatic pressure (RIHP) in response to changes in renal perfusion pressure (RPP).

Male rats, 280-320 g, were anaesthetised (pentobarbitone, 60 mg/kg ip). The left kidney was exposed via the abdomen, and a flow probe placed on its artery. For measuring RIHP, a capsule was implanted into the cortex of the kidney (Roman and Lianos, 1990). RPP was adjusted by aortic ligatures above and below the renal artery. Saline was infused at 6 ml/h iv and 1 h later aldosterone, hydrocortisone, norepinephrine and vasopressin (Huang & Johns, 1995) were added. After a further 30 min, RPP was raised to its highest level and then reduced in 20 mmHg steps and RBF and RIHP measurements were taken at each step. Two sets of rats were studied: Wistar (n=20) and SPSHR (n=16). There were three groups in each set: saline controls, rats given nitrendipine iv at 0.125 μg/kg/min or 0.25 μg/kg/min. Data are shown in the figure as mean ± s.e.mean. Comparisons between groups were undertaken using ANOVA and differences were taken as significant when $P < 0.05$.

Figure 1 shows that in both Wistar and SPSHR, RIHP was significantly raised by the high dose nitrendipine throughout the range of RPP measured. These results support view that following calcium channel blockade the attenuated renal myogenic response (Huang Davis & Johns, 1993; Huang & Johns, 1995) to changes in RPP allows greater hydrostatic pressure transmission into the interstitium and causes an increase in RIHP and hence sodium excretion.

Figure 1 Effect of RPP on RIHP



● Control ▲ Nitrendipine at 0.125 μg kg^{-1} min^{-1}
■ Nitrendipine at 0.25 μg kg^{-1} min^{-1} *: P<0.05 from control

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156P VASCULAR RESPONSES OF THE ISOLATED DUAL-PERFUSED RAT LIVER TO HEPATIC ARTERIAL INJECTIONS OF ENDOTHELIUM-DEPENDENT AND INDEPENDENT VASODILATORS

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Endothelium-dependent and independent vasodilators play an important role in regulation of hepatic blood flow, but the mechanisms by which this is achieved remain poorly understood. We have developed a new, *in vitro*, isolated dual-perfused rat liver preparation, which enables reproducible dose-response curves to hepatic arterial and portal venous injections of noradrenaline to be constructed. The present study was conducted to investigate whether dose-response curves could be constructed to hepatic arterial (HA) injections of the endothelium-dependent vasodilator acetylcholine (ACh) and direct, smooth muscle vasodilator sodium nitroprusside (SNP) in the same preparation.

6 male Wistar rats (250-300g) were anaesthetized with sodium pentobarbitone (3mg 100g^{-1} i.p.). The abdomen was opened through a midline incision. The common bile duct, common HA and portal vein (PV) were cannulated under an operating microscope. The livers were excised and perfused with Krebs-Bülbring buffer (pH 7.4, 37^{0}C) saturated with 95% O_2/5% CO_2 via the hepatic arterial and portal venous cannulae at constant flow rates of 0.32±0.01 (s.e. mean) and 0.98±0.03 ml min^{-1} g liver^{-1}, respectively. After a 10-15 equilibration period, the vascular tone was raised by the addition of methoxamine to the perfusate to a concentration of 7.5x10^{-6} M. Dose response curves were constructed from transient decreases in perfusion pressure following individual HA bolus injections (0.05ml) of ACh (5x10^{-13} - 5x10^{-11}moles) and SNP (5x10^{-11} - 5x10^{-9} moles).

The basal perfusion pressures of the HA and PV were 86.7±2.8 and 7.2±0.9 mmHg respectively following the period of equilibration. The HA pressure increased to 159.2±8.1 mmHg and remained stable after addition of methoxamine (7.5x10^{-6} M) to the perfusate. There was no significant change in the PV perfusion pressure after addition of methoxamine (7.2±0.9 vs 7.6±0.8 mmHg). Injections of ACh produced dose-related vasodilatation in the HA (-log mole ED_{50} = 12.18±0.08). There were no measurable pressure changes in the PV to HA injections of ACh. SNP produced dose-related vasodilatation in the HA (-log mole ED_{50} = 9.95±0.23), but again no measurable changes could be detected in the PV. The maximum decreases in perfusion pressure were 42.83±4.97 mmHg to HA injections of ACh and 29.00±2.39 mmHg to SNP.

Vascular responses to HA injections of both ACh and SNP were attained reliably and reproducibly during the perfusion period of 4 hours. The responses to ACh diminished following periods of prolonged perfusion, and studies are now in progress to determine whether this represents a deterioration in endothelial function and also to determine the stability of the preparation. There was no evidence of any transhepatic responses in this preparation (responses in the PV following HA injections), in contrast to the rabbit liver (Alexander *et al* 1992). This is unlikely to be due to the lack of increased PV tone following methoxamine infusion. These preliminary results demonstrate that the isolated dual-perfused rat liver model can be used to investigate endothelium-dependent and independent vasodilators for vascular pharmacological studies of the HA in the rat liver.

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157P THE ALDOSE REDUCTASE INHIBITOR SORBINIL HAS EFFECTS ON ISOLATED ATRIA FROM BOTH CONTROL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Aldose reductase inhibitors (ARI) can prevent or reverse certain diabetic complications and we have previously shown that diabetes-induced enhanced cardiac ß-adrenoceptor responsiveness can be prevented by treatment with the ARI ponalrestat (Austin and Chess-Williams, 1991). The aim of the present study was to investigate the effect of a structurally different ARI, sorbinil (Pfizer Inc.), on atria isolated from control and 14-day streptozotocin-induced diabetic rats.

Diabetes was induced by a single i.p. injection of streptozotocin (65mgkg^{-1}) dissolved in citrate buffer. Control rats received citrate buffer alone. Rats to be treated with sorbinil were then administered a daily oral dose of sorbinil (25mgkg^{-1}). Fourteen days later, left and right atria were isolated and mounted in gassed Krebs solution at 37°C. Right atria were left to beat spontaneously whilst left atria were paced at 1Hz. Concentration-response curves to isoprenaline and phenylephrine (in the presence of 1μM propranolol) were obtained. All experiments were performed in the presence of cocaine (10μM) and corticosterone (10μM).

Maximum increases in tension of left atria to isoprenaline were lower (P<0.01) in diabetics (0.28±0.06g) than in controls (0.77±0.10g). Sorbinil treatment did not prevent this diabetes-induced reduction (0.24±0.06g), but did reduce the maximum in controls (0.41±0.03g, P<0.01). Tension responses of left atria to phenylephrine were not altered by diabetes or sorbinil treatment. Resting spontaneous rate of right atria was lower (P<0.01) in diabetics (268.7±9.5 beats min^{-1}) than in controls (329±13.9 beats min^{-1}). Sorbinil treatment reduced this further to 241.2±7.9 beats min^{-1} in diabetics (P<0.05) and also reduced resting rate in controls (295.7±6.7 beats min^{-1}, P<0.01). Maximum rate increases to phenylephrine were greater in diabetic right atria (28.0±6.3 beats min^{-1}, P<0.05) than controls (7.5±10.5 beats min^{-1}). Sorbinil treatment further enhanced responses to phenylephrine in diabetics (56.4±10.1 beats min^{-1}, P<0.01) and also enhanced responses in controls (24.6±3.72 beats min^{-1}). Rate increases of right atria to isoprenaline were not altered by diabetes or sorbinil treatment.

In conclusion, the diabetes-induced changes in atrial reponsiveness in the rat were not prevented by sorbinil treatment. The effects of sorbinil suggest that it has actions in addition to its effects on the polyol pathway or that polyol pathway flux modulates atrial responsiveness.

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158P ENDOTHELIN-1 HAS A CARDIOPROTECTIVE EFFECT IN A RABBIT MODEL OF ACUTE MYOCARDIAL ISCHAEMIA AND REPERFUSION

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The plasma levels of endothelin-1 (ET-1) are enhanced in patients with acute myocardial infarction. It is currently perceived that endogenous ET-1 contributes to the extension of infarct size despite studies by Velasco *et al.* (1993) suggesting ET-1 has a cardioprotective effect. ET-1 may well have cardioprotective effects, as activation of ET_A receptors results in the opening of ATP-sensitive potassium channels. It is thought that the opening of these channels mediates the cardioprotective effects of "ischaemic preconditioning" (Parratt, 1994). Here we investigate whether pre-treatment with ET-1 reduces infarct size in a rabbit model of myocardial ischaemia and reperfusion.

New Zealand white rabbits were premedicated with Hypnorm (0.1 ml kg^{-1}, i.m.). General anaesthesia was then induced (30 ml kg^{-1}, i.v.) and maintained with sodium pentobarbitone. The animals were ventilated with room air. A left intercostal thoracotomy was performed and the first antero-lateral branch of the left coronary artery (LAL) was occluded for 60 min followed by 120 min of reperfusion. Mean arterial pressure (MAP) and heart rate (HR) were continuously recorded. Ten min prior to the onset of ischaemia, different groups of animals were treated with vehicle (saline + 0.1% bovine serum albumin) or ET-1 (0.03 or 0.3 nmol/kg). At the end of the experiment the LAL was reoccluded and Evans blue dye (2% w/v) was injected into the left ventricle (LV) to determine area at risk (AR). Infarct size (IF) was determined by incubation of AR with nitro-blue tetrazolium (NBT; 0.5 mg/ml for 20min).

The mean values for AR (% LV) were not significantly different between groups (AR was approximately 46% for all animal groups). ET-1 at 0.03 nmol/kg caused a reduction in IF (expressed as a % of AR) from 54±4% (control; n=9) to 43±3%* (*p<0.05, unpaired Students *t*-test; n=8). In contrast, ET-1 at 0.3 nmol/kg had no effect on infarct size (62±2%; n=4) when compared to control. The lower dose of ET-1 (0.03 nmol/kg) had no effect on MAP or HR, unlike the higher dose (0.3 nmol/kg) which produced a transient increase in MAP (of approximately 36±8 mmHg, n = 4) and pressure rate index (PRI) which lasted approx. 40 min. This hypertension was not associated with a reflex bradycardia.

Thus, ET-1 at a dose (0.03 nmol/kg) that does not produce an increase in MAP causes a reduction in myocardial infarct size in the anaesthetised rabbit when administered as a bolus into the left ventricle, 10 min before the onset of LAL occlusion. The mechanism of the potential cardioprotective effect of ET-1 is not known, but may well involve the opening of ATP-sensitive potassium channels. *This work is supported by a project grant from Schwarz Pharma AG, Monheim, Germany. EH is the recipient of a BHF studentship.*

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159P INHIBITORY EFFECTS OF THE CO-POLYMER SURFACTANT, PLURONIC F-68, ON SPONTANEOUS PLATELET AGGREGATION IN HUMAN WHOLE BLOOD

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The non-ionic, co-polymer surfactant, Pluronic F-68 (PF-68), can improve blood rheology and alter blood cell behaviour under pathological conditions (Carter *et al.*, 1992; Schaer *et al.*, 1994). This suggests possible therapeutic applications for PF-68 in the improvement of blood flow. However, a pre-requisite to such uses is assessment of the effects of the surfactant on blood cells and platelets. Therefore, the effects have been studied of PF-68 and its purified fraction on platelet aggregation in human whole blood *in vitro*.

A solution (10.0% w/v) of commercial grade (COMM) PF-68 (ICI, U.K.) was prepared in isotonic saline (0.9% w/v NaCl); purification was by passing 3 times through silica resin (Bentley *et al.*, 1989). Blood was obtained from healthy volunteers and 8.0 ml aliquots anticoagulated with 400 μg hirudin (Ciba-Geigy, U.K.; CGP39393). Aliquots (460 μl) of blood were placed in small plastic tubes containing a wire stirrer in a water bath (37°C) and, after 2 min, 20 μl of one of several two-fold dilutions of COMM PF-68 or its purified fraction were added to the blood samples. Samples were stirred at 1000 rpm and aliquots (15 μl) removed at 2 min intervals for 8 min and placed in 30 μl of a formaldehyde fixing solution. Platelet counts in fixed samples were measured using an automatic Ultra-Flo 100 Blood Platelet Counter. Platelet counts were expressed as percentage fall in the number of platelets compared with the time 0 value and expressed as percentage aggregation.

The median spontaneous platelet aggregation in normal whole blood was 18.4% [interquartile range (IQ): 10.5 - 24.2%; n = 15]. COMM PF-68 significantly ($P < 0.05$) reduced platelet aggregation at concentrations as low as 7.3 μM (median: 8.4%; IQ: 3.9 - 13.4%; n = 12) and almost eliminated aggregation at concentrations greater than 58 μM (median: 2.0%; IQ: 0.0 - 3.5%). Similar results were obtained with the purified PF-68 fraction (n = 3), with a median aggregation of 9.8% with 7.6 μM of surfactant ($P < 0.05$). These results show that PF-68 can inhibit spontaneous platelet aggregation in human whole blood *in vitro*. The beneficial effects of PF-68 in ischaemic injury (Schaer *et al.*, 1994) may be due, at least in part, to inhibitory effects on platelet aggregation in the microvasculature. Similarly, the beneficial effects of tissue perfusion with oxygen-carrying fluorocarbon emulsions containing PF-68 (e.g. Lowe, 1994) may also involve direct effects of the surfactant on platelets, although this remains to be clarified.

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160P THROMBOPOIETIN INDUCES THE PHOSPHORYLATION OF JAK2 IN HUMAN PLATELETS

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Thrombopoietin (TPO) is the main humoral factor that regulates the number of platelets in the blood. Recombinant TPO has been available since it was cloned last year and might be used as a therapeutic agent for treating thrombocytopenias. The signalling transduction mechanisms that TPO uses to exert its biological effects remain to be elucidated. The TPO receptor belongs to the cytokine receptor superfamily (Vigon et al., 1992), many members of which induce tyrosine phosphorylation of intracellular proteins through activation of the JAK (Janus kinase) family of tyrosine kinases (Ihle & Kerr, 1995). The presence of one member of the JAK family, JAK2 (Rodríguez-Liñares & Watson, 1994), and of the TPO receptor in human platelets (Methia et al., 1993) prompted us to study whether TPO phosphorylates JAK2 and whether TPO induces a functional response in these cells.

Antiphosphotyrosine blots of whole platelet lysates using the monoclonal antibody 4G10 showed that the pattern of tyrosine phosphorylation induced by TPO was different from that stimulated by thrombin, collagen and the platelet receptor for immune complexes, FcγRIIA, suggesting a different mechanism of transduction for this cytokine. Immunoprecipitation experiments using a specific JAK2 antibody demonstrated that TPO induced tyrosine phosphorylation of JAK2 in a concentration (50-1,000 units/ml) and time dependent manner. Phosphorylation of JAK2 is an early event, evident within the first 30 seconds of stimulation. TPO alone had no effect on [^{3}H]5-hydroxytryptamine (5-HT) secretion but when acting in combination with low concentrations of the above agonists induced a significant increase in [^{3}H]5-HT release (Table 1).

In conclusion, TPO induces a novel pattern of protein tyrosine phosphorylation, possibly mediated by activation of JAK2. The synergistic effect that TPO shows in stimulating secretion suggests that it might play a functional role when platelet activation occurs in thrombocytopenic states.

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Table 1. Thrombopoietin potentiates release of [^{3}H]5-HT

	-	TPO (400 units/ml)
Basal	0.0 ± 0.0	-0.5 ± 1.3
Collagen (10 μg/ml)	3.9 ± 1.7	10.1 ± 1.1**
Fcγ cross-linker	9.4 ± 2.4	14.2 ± 2.9*
Thrombin (0.02 u/ml)	3.3 ± 0.2	12.2 ± 0.2**

Results are shown as mean ± s.e.mean of three experiments and represent % release of total cellular [^{3}H]5-HT. *$P < 0.05$; **$P < 0.01$

161P CHIRAL REGIOISOMERS OF INS(1,4,5)P_3: STRUCTURE ACTIVITY PROFILE

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It is now well established that D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] releases Ca^{2+} from intracellular stores. It has previously been demonstrated that the naturally occurring tetrakisphosphate, Ins(1,3,4,6)P_4, also possesses Ca^{2+} mobilising activity (Gawler *et al.*, 1991). As a consequence of structure-activity studies performed on Ins(1,4,5)P_3 analogues, the importance of the vicinal 4,5-bisphosphate configuration in mediating intracellular Ca^{2+} mobilisation has been highlighted (Nahorski & Potter, 1989). Rational structure-based molecular design demonstrates that the vicinal 1,6-bisphosphate of Ins(1,3,4,6)P_4 can mimic the essential 4,5-bisphosphate of Ins(1,4,5)P_3. Moreover, since either the 4-phosphate or the 3-phosphate of Ins(1,3,4,6)P_4 can mimic the 1-phosphate of Ins(1,4,5)P_3 it is possible that Ins(1,3,4,6)P_4 is evoking Ca^{2+} release by a similar binding mechanism to Ins(1,4,5)P_3.

To establish this rationale both the D- and L-enantiomers of Ins(1,4,6)P_3 and the D- and L-enantiomers of Ins(1,3,4)P_3 (Riley *et al.*, 1994) have been synthesised. The Ca^{2+} mobilising potential of these compounds has been examined by evaluating their ability to release $^{45}Ca^{2+}$ from permeabilised platelets, performed as previously described (Mills *et al.*, 1993). The ability of these compounds to displace [^{3}H]Ins(1,4,5)P_3 from its binding site on the rat cerebellar membrane was also examined according to Challiss *et al.* (1994). The findings from this study (Table 1) demonstrate that both L-Ins(1,3,4)P_3 and D-Ins(1,4,6)P_3, but not D-Ins(1,3,4)P_3 or L-Ins(1,4,6)P_3, are able to release $^{45}Ca^{2+}$ from permeabilised platelets and also displace [^{3}H]Ins(1,4,5)P_3 from its binding site on rat cerebellar membrane. The observed $^{45}Ca^{2+}$ releasing activity of these two synthetic derivatives of the naturally occurring Ins(1,3,4,6)P_4 would appear to confirm the predicted structure-activity profile of these synthetic inositol phosphates.

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Table 1 Comparison of inositol phosphates on $^{45}Ca^{2+}$ release and displacement of [^{3}H]Ins(1,4,5)P_3 binding. Values are expressed as mean±S.E.M. (n=3-10)

Compound	$^{45}Ca^{2+}$ release (Conc. causing 40% release) [μM]	Displacement of [^{3}]HIns(1,4,5)P_3 binding IC_{50} [μM]
D-Ins(1,4,5)P_3	0.69±0.24	0.045±0.01
Ins(1,3,4,6)P_4	28.50±1.06	1.62±0.39
L-Ins(1,3,4)P_3	8.05±0.98	4.42±1.29
D-Ins(1,3,4)P_3	>100	>30
L-Ins(1,4,6)P_3	>100	>10
D-Ins(1,4,6)P_3	1.56±0.34	1.42±0.34

162P MEASUREMENT OF INTRACELLULAR CALCIUM IN CELL POPULATIONS LOADED WITH AEQUORIN

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We have loaded aequorin into populations of a human glioma cell line, U373MG using the gravity method of Borle et al (1986) and measured calcium dependent signals with a luminescence plate reader. Aequorin activity could be detected in U373MG cells up to 48 hours after loading. Activity was dependent on the aequorin concentration in the loading buffer (we tested between 10 and 100µg/ml) and on the plating density of the cells (300-60 000 cells/well). The cells responded with an increase in luminescence to a number of agents known to increase levels of intracellular calcium. These included substance P (SP), histamine, and ionomycin .

Substance P acts through NK1 receptors in U373MG cells (Lee et al 1989). In the aequorin assay, the EC_{50} for SP was 31 ± 4.6 nM, N=10 (all data are means ± SEM). Cells also responded to other NK1 agonists, SP methyl ester (SP-OMe) and [Sar^9, Met$(O_2)^{11}$]SP. There was little or no response to high concentrations of [ß-Ala^8]NKA, or [Phe(Me)^7]NKB, selective NK2 and NK3 agonists respectively. The signal induced by 30nM SP was completely blocked by the NK1 antagonists, CP99994 (IC_{50} = 3.0 ± 0.49 nM, N=6), FK888 (IC_{50} = 2.4 ± 1.1 nM, N=5) and SR 140333 (IC_{50} = 1.2 ± 0.35nM, N=5). The EC_{50} for histamine was 7.3 ± 1.4 µM, N=3. The histamine response has not been characterised any further.

We have also tried to load other cell types with aequorin using the gravity method, but have not yet succeeded in obtaining robust responses to agonists in any cell line except U373MG. Some cells, for example, WI-38 and IMR-90 took up aequorin and retained measureable activity for at least 18 hours, but gave very small or no responses to agonists. Other cells, for example CHO, LLCMK2, and AR42J also took up the aequorin but lost the activity relatively quickly, within 2 hours in some experiments.

Loading populations of U373MG cells with aequorin can provide a rapid and convenient assay for receptors that activate phospholipase C. The method may not be applicable to all cells.

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