RELATIONSHIP BETWEEN DIALYSATE SEROTONIN AND RAPHE UNIT ACTIVITY IN FREELY MOVING CATS

L.O. Wilkinson*, K.F. Martin[1], S.B. Auerbach[2], C.A. Marsden[1], & B.L. Jacobs, Neuroscience Program, Princeton University, Princeton, NJ, USA 08544, [1]Department of Physiology & Pharmacology, Medical School, Nottingham, UK NG7 2UH, and[2]Department of Biological Sciences, Rutgers University, Piscataway NJ USA 08855.

A large body of evidence suggests a specific role for serotonin (5-HT) in various behaviors and physiological psocesses. However, 5-HT neuronal activity remains unchanged under a number of behavioral and physiological conditions (see Fornal & Jacobs, 1988 for review). This suggests that 5-HT may be released partially independent of neuronal firing. These experiments begin to examine how changes in the neuronal activity of 5-HT cells correlate with extracellular 5-HT levels in the terminal fields of these neurons.

Extracellular 5-HT was measured in the corpus striatum of the awake, freely moving cat using the in vivo dialysis technique of Hernandez *et al.* (1986). A concentric probe with a permeable tip was lowered through an implanted guide cannula at least 12 hours before an experiment. Ringers solution was pumped through the probe at 2 µl/min, collected every 30 mins, and injected into an HPLC with EC detection. During sleep/wake experiments (using a BAS 400 detector), limit of detection was 3 pg 5-HT on column, and 10 µM of the specific 5-HT reuptake inhibitor fluoxetine was added to the Ringers solution in order to increase the recovery of 5-HT above the limit of detection. In several 8-OH-DPAT experiments using a Princeton Applied Research EGG detector, the limit of detection was 500 fg 5-HT, and no reuptake inhibitor was used. In all 8-OH-DPAT experiments, two different applied potentials were monitored in parallel in order to verify the authenticity of the 5-HT peak. All experiments used an ODS 3µm column, and a 0.15 M monochloroacetate buffer (pH 3.3) containing 55 mM octane sulfonic acid and 2% acetonitrile.

Since 5-HT neuronal activity is highest during active waking behavior (Fornal & Jacobs, 1988), we examined extracellular 5-HT when the animal was maintained in an active waking state, and compared this to 5-HT collected when the animal was undisturbed. The cat was placed in an isolation chamber which allowed remote withdrawal of samples, and sleep state was polygraphically monitored. During undisturbed periods, cats maintained a quiet waking state with occasional periods of slow wave sleep. Extracellular 5-HT was significantly increased during active waking behavior [$F_{(2,8)} = 7.241$, $P < 0.05$] to 126.1 $\pm$ 10% of the previous sample obtained during a somnolent state ($\bar{X} \pm$ s.e. mean, n=5). Following the active waking period, extracellular 5-HT was 90.4% $\pm$ 12% of baseline, as the cat again became somnolent.

In order to examine extracellular 5-HT during long periods of suppressed 5-HT neuronal activity, the 5-HT autoreceptor agonist 8-OH-DPAT was administered. 8-OH-DPAT produced a 100% suppression of unit activity, lasting for approximately 1 hour following 50 µg/kg s.c. and 4 hours following administration of 250 µg/kg s.c. There was a significant decrease in extracellular 5-HT following each dose, though the magnitude of the decrease was not significantly dose dependent [$F_{(12,60)} = 0.57$, $P = 0.86$]. The minimal dialysate 5-HT levels (50% $\pm$ 4% following 50 µg/kg (n=3), and 41% $\pm$ 11% following 250 µg/kg (n=4)) were observed 2 hours following drug administration, though 8-OH-DPAT produced complete suppression of 5-HT unit activity within 15 mins following administration of either dose. Thus, decreases in extracellular 5-HT are less dynamic than those observed in unit activity. These data further suggest that approximately 60% of extracellular 5-HT measured by dialysis is a consequence of neuronal activity.

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IPSAPIRONE AND GEPIRONE INHIBIT 8-OH-DPAT-INDUCED ROTATION IN RATS WITH UNILATERAL 5-HT LESIONS

L.I. Backus, T.Sharp, S.R. Bramwell and D.G. Grahame-Smith, MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE.

Rats with unilateral 5,7-dihydroxytryptamine (5,7-DHT) lesions of central 5-HT pathways rotate contralaterally when injected with 5-HT agonists (Jacobs et al, 1977; Blackburn et al, 1980). Recent work by Blackburn et al (1984) demonstrated that various 5-HT agonists selective for the 5-HT_1 receptor subtype induce rotation in this model of postsynaptic 5-HT function. In the present study, we attempted to establish the 5-HT turning model, in part to evaluate the actions of the 5-HT_{1A} ligands gepirone and ipsapirone which have an unclear action at postsynaptic 5-HT_{1A} receptors (Traber and Glaser, 1987).

Male adult rats (250-275 g) were pretreated with desmethylimipramine (25 mg/kg) 45 min prior to lesioning. Under 1.8% halothane anesthesia, 5,7-DHT (4 µg in 4 µl 0.1% ascorbic acid solution) was stereotaxically injected into the right medial forebrain bundle (MFB). After 2 weeks recovery, animals were tested for a rotational response to the 5-HT_{1A} agonist 8-OH-DPAT (1 mg/kg s.c.). Only those rats that consistently turned contralaterally (>20 turns/20 min) were selected for further use. In these rats, 5-HT levels in the right striatum were reduced by at least 50%. For drug trials, the number of complete turns in either direction was counted over 5 min intervals for 30 min after s.c. drug injection. At least 72 h was allowed between drug trials and animals were used for no more than 6 trials.

In the unilaterally 5-HT lesioned rats, 8-OH-DPAT produced dose-dependent (0.01-2 mg/kg) contralateral rotation. The putative 5-HT_{1B} agonists, RU 24969 (1,5 mg/kg) and mCPP (5,10 mg/kg), the proposed 5-HT_2 agonist DOI (0.1, 1 mg/kg) and the 5-HT_{1A} ligands, gepirone (1,5,10 mg/kg) and ipsapirone (5 mg/kg) did not cause rotation. However, pretreatment (30 min) with ipsapirone (7.5 mg/kg) and gepirone (10 mg/kg) significantly inhibited rotation induced by 8-OH-DPAT (Table 1).

Table 1. Effect of 5-HT_{1A} ligands on 8-OH-DPAT-induced rotation in 5-HT lesioned rats

TREATMENT (mg/kg s.c.)	mean contralateral turns/30 min ± s.e. mean (n)
8-OH-DPAT (1.0)+saline	104 ± 12 (17)
8-OH-DPAT (1.0)+ipsapirone (2.5)	116 ± 22 (6)
8-OH-DPAT (1.0)+ipsapirone (7.5)	37 ± 16*(8)
8-OH-DPAT (1.0)+gepirone (10.0)	33 ± 18*(8)

*$p<0.001$ vs. paired controls

In summary, rats with unilateral 5,7-DHT lesions of the MFB rotate contralaterally to 8-OH-DPAT but not to RU 24969, mCPP or DOI. Furthermore, in this model the 5-HT_{1A} ligands ipsapirone and gepirone antagonize the response to 8-OH-DPAT. Blackburn et al (1984) previously observed RU 24969-induced contralateral rotation in rats with 5,7-DHT lesions of the dorsal raphe nucleus. The present method, therefore, might be more selective for drugs acting on the 5-HT_{1A} receptor.

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8-OH-DPAT CAN DECREASE 5-HT NEURONAL FIRING AND RELEASE BUT NOT METABOLISM

Jeni C. Garratt, C.A. Marsden & F. Crespi, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

A low dose of 8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OHDPAT), a specific $5HT_{1A}$ agonist, depresses the firing rate of 5HT neurones in the dorsal raphe nucleus (DRN) (Fallon et al., 1983) and infusion of the drug into the DRN decreases the hippocampal release of 5HT in vivo (Hutson et al., 1988). Following systemic administration of a relatively high dose of 8-OHDPAT (0.32 mg/kg s.c.) both 5HT release and metabolism are decreased in the frontal cortex (FC) (Routledge & Marsden, 1985). The present study investigates the relationship between 5HT neuronal firing, release and metabolism following a low dose of 8-OHDPAT (0.01 mg/kg i.v.).

5HT neuronal firing was monitored electrophysiologically using a glass electrode implanted into the DRN of the anaesthetised rat (n=7). 5HT neurones were identified initially by their characteristic slow firing pattern. These neurones were inhibited by 8-OHDPAT (see below) and this effect was antagonised by spiroxatrine (0.1 mg/kg i.v.) but not by ritanserin (0.5 mg/kg i.v.). Neurones showing different firing patterns were not inhibited by 8-OHDPAT. In parallel extracellular 5HT release and metabolism were monitored voltammetrically in the FC using conventional 12 μm diam. carbon fibre electrodes (CFE) for 5HIAA and 30 μm diam. Nafion coated CFE to monitor 5HT (Crespi et al., 1988). Both electrodes were implanted into the same animal; one in the right and the other in the left FC (n=7). Following administration of 8-OHDPAT (0.01 mg/kg i.v.) there was a rapid decrease in both 5HT neuronal firing (-60% of 0.9% saline injected controls) and extracellular 5HT (-50% of controls) but there was no significant change in extracellular 5HIAA. 5HT neuronal firing and release returned to pre-injection control values within 15-20 min and 25-30 min respectively. When the rats were given a second identical injection of 8-OHDPAT both 5HT neuronal firing and release were reduced again but for a shorter time and on this occasion 5HT release only decreased by 30%. Control rats (n=4) given 0.9% saline (100 μl i.v.) showed no significant change in 5HT neuronal firing, release or metabolism.

The results support the view that 5HT neuronal activity and release can be regulated by the somatodendritic $5HT_{1A}$ autoreceptor in vivo (Hutson et al., 1988) but it appears that alterations in 5HT release are not necessarily reflected by changes in 5HT metabolism.

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IS THE HYPERPHAGIC RESPONSE TO 8-OH-DPAT A MODEL FOR $5HT_{1A}$ RECEPTOR RESPONSIVENESS?

A.J. Sleight, R.J. Smith, C.A.Marsden & M.G. Palfreyman[+], Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [+]Merrell-Dow Research Institute, Cincinnati, Ohio 445215-6300, U.S.A.

In freely feeding rats 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) produces hyperphagia (Dourish et al., 1986); an effect that may be a model for $5HT_{1A}$ receptor responsiveness since it is produced by other $5HT_{1A}$ agonists (Gilbert & Dourish, 1987). Chronic treatment with E-β-fluoromethylene-M-tyrosine (MDL 72394) down-regulates the binding parameters of the $5HT_{1A}$ binding site in rat frontal cortex and the $5HT_{1A}$ mediated inhibition of forskolin-stimulated adenylate cyclase in rat hippocampus (Sleight et al., 1988). Consequently, if the hyperphagic feeding response is useful for investigating $5HT_{1A}$ receptor function it should be possible to demonstrate decreased responsiveness following chronic treatment with MDL 72394.

Male Wistar rats were housed singly and given free access to food and water. Food measurements were made between 0900 and 1300 h. Either saline or 8-OH-DPAT (0.32 mg/kg s.c.) was given at 0850 and 30 mins prior to that either saline, methysergide (1 mg/kg i.p.), methiothepin (1 mg/kg i.p.), metergoline (2 mg/kg i.p.) or idazoxan (2 mg/kg i.p.) were given. Other groups of freely feeding rats were given either saline or MDL 72394 (0.25 mg/kg p.o.) daily for 14 days. 24 h after the final dose the rats were given either saline (n=6) or 8-OH-DPAT (0.32 mg/kg s.c., n=6).

In saline pretreated rats, 8-OH-DPAT produced significant hyperphagia (food intake: saline treated rats=0.3±0.2 g/4 h; rats treated with 8-OH-DPAT=2.4±0.3 g/4 h). Pretreatment with the 5HT antagonist methiothepin and the α_2-adrenoceptor antagonist idazoxan significantly attenuated the hyperphagia produced by 8-OH-DPAT while methysergide and metergoline had no effect. However, pretreatment with either methysergide, metergoline or idazoxan significantly increased food intake themselves 4 h after an injection of saline. Chronic treatment with MDL 72394 reduced food intake itself but 8-OH-DPAT still produced a marked hyperphagia with the increase in food intake caused by 8-OH-DPAT in rats pretreated chronically with MDL 72394 being similar to that in control (chronic saline) rats (0.7±0.3 g in MDL 72394 treated rats compared to 0.9±0.4 g in saline treated rats $P>0.05$).

The results suggest that the hyperphagic response to 8-OH-DPAT is mediated by $5HT_{1A}$ receptors with the possible involvement of α_2 adrenoceptors. The antagonist produced hyperphagia may be a result of postsynaptic receptor blockade which would have a similar effect to 8-OH-DPAT if the latter produced hyperphagia by stimulation of somatodendritic autoreceptors (Hutson et al., 1986). Down-regulation of the hyperphagic response was not observed following chronic treatment with MDL 72394 suggesting that hyperphagia is not a simple model for $5HT_{1A}$ receptor responsiveness, probably because of compensatory changes in other systems involved in the control of food intake.

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FOOD INTAKE, BEHAVIOUR AND THERMOREGULATORY EFFECTS OF 8-OH-DPAT IN PREWEANLING RATS

Helen C. Jackson & I. Kitchen, Department of Biochemistry, Division of Pharmacology and Toxicology, University of Surrey, Guildford, Surrey, GU2 5XH, U.K.

8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) is a centrally-active 5-HT receptor agonist which has been shown to have high affinity for 5-HT_{1A} recognition sites in receptor binding studies (Middlemiss & Fozard, 1983). In adult rats, 8-OH-DPAT induces the 5-HT behavioural syndrome (Tricklebank et al., 1984) and produces hypothermia (Hjorth, 1985 ; Hutson et al., 1987) and there is some evidence that these responses may be mediated by post-synaptic 5-HT_{1A} receptors. In comparison, 8-OH-DPAT induces hyperphagia in non-deprived rats probably via activation of 5-HT_{1A} somatodendritic autoreceptors (Dourish et al., 1986). The pharmacological profile of this compound in neonatal rats has not been previously reported, hence, we have examined the behavioural responses to 8-OH-DPAT in 5-, 10- and 15-day old rat pups, and additionally, determined its effect on body temperature and food intake in preweanling rats.

The behaviours of individually-housed Wistar rat pups (male and female) were scored for one hour using a time-sampling procedure as previously described (Jackson & Kitchen, 1987). Rectal temperatures were recorded (to the nearest 0.1^{o}C) immediately before, and 30 min following, drug administration. Food intake was measured as the change in body weight of 15-day old pups given 2 h access to a wet mash diet. 8-OH-DPAT (Research Biochemicals Inc.) was dissolved in 0.9% NaCl and injected i.p. in a dose volume of 0.1ml/20g body weight using a blind protocol. Treatment group means (n = 8) were statistically compared using analysis of variance and the Mann-Whitney U test or Dunnett's test as appropriate.

8-OH-DPAT (1, 10mg/kg) induced hyperlocomotion and head-weaving in 5-, 10- and 15-day old rat pups. Other aspects of the 5-HT syndrome including reciprocal forepaw treading and hind-limb stretching (with signs of straub tail, tremor and flattened body posture) were only expressed by the oldest age group. 8-OH-DPAT also induced hypothermia in the 15-day old pups (1 and 10mg/kg doses of this compound produced 2.6 and 2.9^{o}C decreases in body temperature respectively). A low dose of 8-OH-DPAT (0.01mg/kg) produced a significant increase in body weight at this age which correlated with observed increases in food intake. In contrast, a higher dose of the 5-HT agonist (1mg/kg) significantly reduced body weight in the 15-day old animals over the 2 h period.

The results of this study suggest that both pre- and post-synaptic 5-HT_{1A} receptors are present in 15-day old rat pups. Younger animals did not display the full-blown 5-HT syndrome to 8-OH-DPAT, but exhibit a behaviour akin to reciprocal forepaw treading during normal suckling behaviour (Drewett et al., 1974) and hence are capable of performing this aspect of the motor syndrome from an early age.

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EVIDENCE FOR THE IMPORTANCE OF DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNELS IN ETHANOL TOLERANCE AND DEPENDENCE

S. J. Dolin[1], T. Patch[1], M. A. Whittington[2], R. J. Siarey[2] & H. J. Little[2], [1]Clinical Pharmacology Unit, Addenbrookes' Hospital, Hills Road, Cambridge, CB2 22Q and [2]Department of Pharmacology, The Medical School, University Walk, Bristol, BS8 1TD.

We have recently obtained evidence that dihydropyridine-sensitive binding sites may play a role in ethanol dependence. Chronic administration of ethanol increases both the B_{max} of dihydropyridine-sensitive binding sites and the actions of the dihydropyridines (Dolin *et al.*, 1987) and acute administration of calcium channel antagonists prevents the ethanol withdrawal syndrome (Littleton & Little, 1987; Little *et al.*, 1986), suggesting a possible causal relationship between the binding changes and the adaptations to chronic ethanol intake. An important aspect of this work was the discovery that the dihydropyridine calcium channel antagonist, nitrendipine, prevented the development of tolerance to ethanol, when it was given *concurrently* with ethanol in chronic treatment, (Little & Dolin, 1988). The ethanol withdrawal syndrome was also prevented when nitrendipine was given during the chronic ethanol treatment (Whittington & Little, 1988). In both these studies the nitrendipine treatment was stopped 24h before the tests, so that effective concentrations were not present in the brain during the tests. We now report that concurrent administration of nitrendipine prevents the increase in dihydropyridine binding sites caused by chronic ethanol intake.

In the first method of ethanol treatment (the same as that used in the tolerance studies, Little & Dolin, 1987), male Sprague-Dawley rats (150-170g) were injected with ethanol, 2 g/kg i.p., once daily for ten days, with concurrent injections of nitrendipine 50 mg/kg, or its vehicle (Tween 80, 0.5%). Separate groups received nitrendipine or tween injections without ethanol. At 24h after the last injections, the rats were killed by cervical dislocation and the cerebral cortices dissected out and used for binding studies. In the second method (that used in the withdrawal studies, Whittington & Little, 1988), male mice, C57 strain (25-30g) were given ethanol, 24% v/v, as sole fluid, for twelve weeks. For the last two weeks, injections, i.p., of nitrendipine, 50 mg/kg, or vehicle, were given every 12h, until 24h before withdrawal of ethanol. The animals were killed 5h after withdrawal (the time of peak behavioural signs) and the whole brains removed for binding studies. Binding was measured with [3H]-nimodipine, by the method of Glossman & Ferry,1985. Nonspecific binding was defined with 1μM nitrendipine. The results were subjected to Scatchard analysis.

In both experiments the B_{max} values were significantly increased (see table, *P<0.05, Student's t-test) by chronic ethanol alone, but not by ethanol plus nitrendipine. In the cerebral cortex, ethanol plus nitrendipine treatment significantly lowered the B_{max}, although nitrendipine alone did not alter this value. There were no differences in Kd values.

B_{max} values, fmol mg-1 (n)	Rat cerebral cortex	Mouse whole brain
Controls:	152 ± 3 (6)	232 ± 10 (11)
Ethanol alone:	196 ± 11 * (6)	277 ± 50 * (9)
Ethanol + nitrendipine:	131 ± 7 * (6)	249 ± 23 (7)
Nitrendipine:	142 ± 8 (6)	

Nitrendipine, given chronically during ethanol administration, prevented the development of ethanol tolerance and the withdrawal syndrome. These results show that the same treatment also prevented the increase in dihydropyridine binding sites. This suggests that these sites may be causally involved in the adaptive changes which occur during chronic ethanol intake.

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ANTAGONISM BY XANTHINE AMINE CONGENERS OF 2-CHLOROADENOSINE-INDUCED CALCIUM UPTAKE INHIBITION IN RAT BRAIN SYNAPTOSOMES

M.L.Gonçalves, Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781-Oeiras, Portugal.

It has been described that adenosine (Ribeiro et al., 1979) and adenosine agonists (Wu et al., 1982) decreases uptake of ^{45}Ca by rat brain synaptosomes stimulated by potassium. Xanthines have been used to study the antagonist profile of the adenosine receptor that mediates adenosine binding to brain membranes and adenosine analogues induced stimulation of adenylate cyclase and/or cyclic AMP formation (Lohse et al., 1987; Daly et al., 1987).

The present work was undertaken to investigate the antagonism of 2-chloroadenosine-induced calcium uptake inhibition by xanthine amine congeners, namely: 1,3-dipropyl-8-cyclopentylxanthine (DPCPX); 1,3-dipropyl-8-(p-$HOCOCH_2$-OC_6H_4)-xanthine (XCC); 1,3-dipropyl-8--(p-$H_2N(CH_2)_2$-$NHCOCH_2$-OC_6H_4)-xanthine (XAC).

The experiments were carried out in synaptosomes from rat brain homogenates incubated at 37°C in a medium (pH 7.4) containing (mM): NaCl 132; KCl 5; $MgCl_2$ 1.3; NaH_2PO_4 1.2: $CaCl_2$ 1.2; Glucose 10. Calcium uptake (^{45}Ca) was induced by potassium depolarization during 30 seconds. The uptake was terminated by adding EGTA solution followed by filtration. The radioactivity associated with the synaptosomes was determined in a scintillation spectrometer.

In the presence of 2-chloroadenosine (1nM-100μM) a decrease of calcium uptake in rat brain synaptosomes was observed.The inhibitory effect of 2-chloroadenosine was concentration-dependent and the IC_{50} value calculated by linear regression analysis was 19nM.The xanthine amine congeners DPCPX, XAC and XCC in concentrations that did not modify calcium uptake (50nM-100nM) antagonized the inhibitory action of 2-chloroadenosine. The effect of XAC was dose-dependent and in a concentration of 100nM shifted to the right the concentration--response curve for the inhibitory action of 2-chloroadenosine. Comparing the antagonistic effect of DPCPX, XAC and XCC in the same concentration of 100nM on 2-chloroadenosine-1μM-induced inhibition of calcium uptake it was observed that DPCPX was the most potent.

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AN INVESTIGATION OF THE NEUROCHEMICAL MECHANISM OF CAFFEINE-INDUCED ANXIETY IN THE RAT

Helen A. Baldwin & Sandra E. File, Department of Pharmacology, School of Pharmacy, London University, London WC1N 1AX.

Caffeine increases anxiety in man (Uhde et al, 1984) and has been demonstrated to have anxiogenic-like effects in rats (File & Hyde, 1979; Pellow et al, 1985). There is evidence that caffeine acts at several different sites in the CNS including adenosine and BDZ receptors (Gould et al, 1984) and noradrenergic neurones (Corrodi et al, 1972). The purpose of these experiments was to determine the mechanism by which caffeine increases anxiety.

Pairs of male hooded Lister rats (180-300g) were tested in the social interaction test of anxiety (File & Hyde, 1979) after the administration of caffeine (40 mg/kg) alone or in combination with various compounds. Both members of a pair received the same drug treatment. Rats were tested in a dimly-lit, familiar arena in which anxiogenic compounds normally reduce the time spent in active social interaction. In order to investigate the role of adenosine receptors, caffeine was given in combination with 2-chloroadenosine (2-CA, 0.1 & 1 mg/kg). To investigate the role of BDZ receptors, chlordiazepoxide (CDP, 5 mg/kg), a BDZ antagonist, flumazenil (RO 15-1788 1 & 10 mg/kg) and a triazoloBDZ (U-43,465, 32 mg/kg) were used. Finally, DL-propranolol (5 & 10 mg/kg) was used to study the role of B-receptors in the effects of caffeine.

In agreement with previous studies, caffeine (40 mg/kg) significantly decreased the time spent in social interaction indicating an anxiogenic effect. 2-CA and flumazenil had no effect on social interaction alone and did not reverse the effect of caffeine. CDP (5 mg/kg) significantly increased the time spent in social interaction alone and significantly reversed the decrease in social interaction produced by caffeine. U-43,465 had no effect alone but significantly reversed the effect of caffeine. The higher dose of propranolol (10 mg/kg) significantly increased social interaction compared with controls, whilst 5 mg/kg of propranolol had no effect. The lower dose of propranolol (5 mg/kg) significantly reversed the effect of caffeine.

The results with 2-CA and flumazenil suggest that the anxiogenic effects of caffeine are unlikely to be due to its effects at adenosine or BDZ receptors. The reversal of caffeine's effects by CDP was probably merely a cancellation of 2 opposite effects. As well as acting at the BDZ receptor, U-43,465 has some noradrenergic receptor activity: therefore the reversal of caffeine's effects by U-43,465 and propranolol may reflect a noradrenergic site of action for caffeine.

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A COMPARISON OF THE EFFECTS OF THREE TESTS ON ANXIETY ON PLASMA CORTICOSTERONE CONCENTRATIONS IN THE RAT

Helen A. Baldwin, Peter F. Curle & Sandra E. File, Department of Pharmacology, School of Pharmacy, London WC1N 1AX.

Over the last few years, several behavioural tests have been developed which are designed to measure anxiety in the rat. Most of these tests proved successful at predicting the anxiolytic properties of the benzodiazepines (BDZs). However, with the recent advent of various new putative anxiolytic drugs, it has become clear that the tests are not all equally sensitive to different types of drugs. Whilst all of the tests produce stress, it is possible that they are not equally stressful. This could explain the differential effects of various drugs in these tests. In this study plasma corticosterone concentrations were used provide a measure of the stress produced by 3 different tests of anxiety.

Male hooded Lister rats (200-230g) were housed individually for 5 days before testing. For the social interaction test (File, 1980), pairs of rats were placed into a low-lit, unfamiliar (LU) arena for 7.5 mins. The elevated plus-maze test (Pellow et al, 1985) was in a brightly-lit room with an observer sitting 1m from the maze. The rats were allowed free access to all arms for 5 mins. For the punished-drinking test (modified from Vogel et al, 1971), rats were water-deprived for 24h and then placed into the apparatus for 6 mins. After a 1 min unpunished period, rats received one 0.18 or 0.4mA footshock per 20 licks. Thirty mins after testing, rats were anaesthetised with ether and blood was removed by cardiac puncture. Plasma corticosterone concentrations were measured using ^{125}I-corticosterone radioimmunoassay (RIA) (Radioassay Systems Laboratories, Inc.)

Test condition	plasma corticosterone (ug/dl)
home-cage	1.97 ± 0.31
social interaction - LU	7.16 ± 1.68 *
home-cage	4.60 ± 1.44
elevated plus-maze	9.88 ± 1.92 *
punished-drinking - 0.18mA	20.74 ± 4.14 **
" " - 0.4mA	29.14 ± 3.10 **

Significantly different from home-cage: *p<0.05; **p<0.01.

Whilst social interaction and elevated plus-maze were equally stressful, the punished-drinking test produced significantly greater increases in plasma corticosterone levels. These results may explain differences between the effects of drugs in tests using novelty and conflict tests but cannot explain differences between the social interaction and elevated plus-maze tests.

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THE BENZODIAZEPINE ANTAGONIST FLUMAZENIL HAS LONGLASTING EFFECTS ON ALCOHOLIC RATS

R.Armstrong, P.Bennett-Richards & A.W.Caan (introduced by M.A.Simmonds), Department of Pharmacology, The School of Pharmacy, Brunswick Square, London WC1N 1AX.

Flumazenil (Ro15-1788) is in use to manage benzodiazepine overdose (O'Sullivan & Wade, 1987) and has been found to reduce withdrawal anxiety in rats tolerant to benzodiazepines (Baldwin & File, 1987). In the course of testing effects of chronic alcohol on behaviour, we studied the effect of flumazenil on withdrawal anxiety in male, hooded Lister rats previously given ethanol in a liquid diet over seven weeks. The rats had reached intake levels averaging 16g/kg/day of alcohol. Anxiety was assessed with the Elevated Plus-Maze test, 6-8 hours after withdrawal of alcohol, 15 minutes after i.p. doses of 4mg/kg flumazenil or vehicle given to equal numbers of alcoholic or control (alcohol-naive) animals (total n=32). Flumazenil had no significant effect on anxiety levels in controls or withdrawn alcoholics. No longlasting effects on the behaviour of controls was seen. However, over the next 101 days, ex-alcoholics which had received the drug acutely, showed three persistent differences compared to ex-alcoholics given vehicle: they failed to gain weight at the same rate, they showed a different pattern of behaviour across trials of a Swimming Maze test involving a series of detours, and they learned to gain more rewards in a Trail task ("Rat Automat": Caan, 1988) by sampling all holes more frequently, rather than by visiting selectively those holes which were baited.

For example, whereas ex-alcoholics given vehicle on day 1 gained an average of 215g over 101 days, ex-alcoholics given flumazenil gained only 168g ($P<0.005$, Rank sum test). In successive variants of the Swimming Maze differing only in illumination, rats tend to generalize across this change with faster average speeds following the same route on the second trial, but ex-alcoholics given flumazenil took a (96%) longer time on the second trial ($P<0.05$, Matched-pairs signed-ranks test). During the eight training sessions of the Trail task, ex-alcoholics given vehicle acquired an increased rate of reward without any significant increase in the total number of holes visited per session, whereas ex-alcoholics given flumazenil showed a 103% increase in the total number of holes visited ($P<0.005$, Matched-pairs signed-ranks test).

In conclusion, alcoholics may be vulnerable to longlasting side effects of flumazenil, which are not apparent in rats never previously exposed to alcohol.

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CAPTOPRIL IMPROVES BASIC PERFORMANCE AND ANTAGONISES SCOPOLAMINE IMPAIRMENT IN A MOUSE HABITUATION TEST

B. Costall, Z.P. Horovitz[1], M.E. Kelly, R.J. Naylor & D.M. Tomkins, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP and [1]The Squibb Institute for Medical Research, Princeton, New Jersey, USA.

Captopril inhibits angiotension-converting enzyme (ACE) in body tissues and within the brain. Angiotensin II and angiotensin peptides are reported to disrupt passive avoidance behaviour in rats and retention performance in rodents (Koller et al, 1979; Melo and Graeff, 1975; Morgan and Routtenberg, 1977). Sudilovsky et al. (1984) have also shown that captopril delays the extinction of a conditioned avoidance response in the rat. This may indicate that ACE is involved in cognitive processes and in the present study we investigate the actions of captopril in a mouse habituation test.

Male albino (BKW) mice (25-30g) were maintained on a 12 hr light and dark cycle with lights off at 8.00 am. Mice taken at 8.30 am from the dark holding room to the dark test room were placed into the centre of a brightly illuminated white area of a black and white test box (see Costall et al., 1986). Mice were normally averse to the white section and the latency of movement from the white to the black section, and the number of exploratory rears, line crossings and time spent in the two sections was assessed over a 5 min period.

Habituation to the test box occurred over a 5 to 6 day period, mice (n = 5) moving increasingly quickly from the white to the black area (12 to 14s reducing to 4 to 6s), with an approximate 30% increase in time spent in the black area associated with increased rearing and line crossings, these being reduced in the white section. On days 2-6 following twice daily treatment with captopril (0.05 mg/kg i.p.) mice moved more rapidly than vehicle treated controls into the black section, the latency being reduced by at least 50% ($P<0.001$). Similarly, mice receiving captopril spent more time in the black section with a reduced incidence of rearings and line crossings in the white section ($P<0.001$).

The acute administration of a single dose of scopolamine (0.25 mg/kg i.p.) to mice on the 6th day of vehicle treatment disrupted the learning pattern, animals delaying movement into the back section by 276% ($P<0.001$), with increased time spent and rearing and line crossings in the white area ($P<0.001$). Two days following the injection of scopolamine the habituation curve had returned to control values; the dose of scopolamine was selected as one causing minimal autonomic disturbance (0.25 mg/kg i.p. methyl scopolamine failed to influence behaviour). In mice that had received the 6 day treatment with captopril, scopolamine failed to modify the habituation response. Epicaptopril (0.1-10 mg/kg), an analogue of captopril, but with no effect to inhibit ACE, failed to modify the habituation response or the response to scopolamine.

The results indicate that captopril may modify the adaptive or cognitive processes of learning or memory in the mouse and we are presently extending such observations to tests performed in the rat and marmoset. The failure of epicaptopril to modify the habituation response indicates that the effects of captopril may be mediated via an inhibition of ACE and prompted a further study to determine the actions of other ACE inhibitors on habituation behaviour and the effects of such compounds, and angiotensin I and II, to modify cholinergic function (see Barnes et al. 1988).

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CENTRALLY INJECTED NORADRENALINE, ADRENALINE OR CIRAZOLINE DOES NOT INDUCE SPECIFIC α_1-ADRENOCEPTOR MEDIATED LOCOMOTOR ACTIVITY

D.J. Heal, Research Department, The Boots Company PLC, Nottingham NG2 3AA.

Intracerebroventricular (i.c.v.) injections of phenylephrine or methoxamine dose dependently induce excitation and locomotor activity in mice and these responses have been proposed as a model of central α_1-adrenoceptor function (Heal, 1984). In this study, the behavioural effects of these drugs have been compared with those of noradrenaline, adrenaline and cirazoline, a putative α_1-adrenoceptor agonist with good brain penetration (Cavero et al, 1982).

Adult male C57/Bl/6Ola mice (Olac, Bicester) weighing 25-30g were used throughout. Drugs for i.c.v. injection were given in 2µl saline using the stereotaxic apparatus described by Heal (1984). The mice given noradrenaline or adrenaline were pretreated with tranylcypromine (5 mg/kg i.p.) 30 min earlier. Cirazoline was injected by both the i.c.v. and subcutaneous (s.c.) routes. Antagonists were all injected i.p. Phenylephrine (10-100 µg i.c.v.) or methoxamine (10-50 µg i.c.v.) dose dependently induced intermittent, stiff-legged locomotor activity performed with hunched body posture, head held down and abdomen clear of the cage floor. These α_1-agonists also induced marked piloerection, slight head-bobbing, mild Straub tail and, at higher doses, jumping. Noradrenaline (5-25 µg i.c.v.) or adrenaline (5-25 µg i.c.v.) both dose-dependently induced locomotor activity and other behavioural changes (hyperventilation, head-bobbing, sniffing and piloerection). However, the behavioural syndromes induced by the two catecholamines were not identical and both were distinguishable from that of phenylephrine. Cirazoline either 1.25-12.5 µg (i.c.v.) or 0.5-10 mg/kg (s.c.) also dose-dependently induced locomotor activity and behavioural changes which more closely resembled those of phenylephrine. Prazosin (3 mg/kg) markedly inhibited ($P<0.01$) the activity induced by phenylephrine (25 µg), methoxamine (25 µg) or cirazoline (5 µg). However, the responses produced by noradrenaline (12.5 µg) or adrenaline (12.5 µg) were respectively enhanced and unaltered by prazosin (3 mg/kg). Idazoxan (0.1 mg/kg) or (±)-propranolol (2.5 mg/kg) inhibited ($P<0.01$) the activity induced by cirazoline (5 µg), but did not alter the responses to the other adrenergic agents. Haloperidol abolished ($P<0.05$) the activity induced by phenylephrine (25 µg) or cirazoline (5 µg) at 0.1 mg/kg, but required 0.5 mg/kg to inhibit ($P<0.05$) the responses to noradrenaline (12.5 µg) or adrenaline (12.5 µg).

Overall, the data show that activity produced by phenylephrine or methoxamine was almost certainly specifically initiated via α_1-adrenoceptors, as previously reported (Heal, 1984). Haloperidol inhibits this response by antagonism of dopamine receptors "down-stream" of the α_1-adrenoceptors (Heal, 1984). Cirazoline-induced activity probably resulted from non-selective activation of noradrenergic function, because it was reduced by α_1-, α_2- and β-antagonists. Noradrenaline- or adrenaline-induced activity was unaffected by adrenergic antagonists, but was inhibited by haloperidol suggesting mediation by non-specific stimulation of dopamine receptors. Similar conclusions have been drawn from studies in rats (Pijnenburg et al, 1975). In conclusion, although noradrenaline, adrenaline and cirazoline all induced behavioural excitation and locomotor activity after i.c.v. injection, these responses, unlike those of phenylephrine and methoxamine, were not specifically α_1-adrenoceptor mediated.

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A PROTOCOL FOR THE ASSESSMENT OF NEURONAL SENSITIVITY USING THE 'HIGH-LOW' CURRENT THRESHOLD AND I.T_{50} METHODS SIMULTANEOUSLY

R. Mason & Catherine M. Cox. Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

Neuronal sensitivity to iontophoresed drugs is frequently assessed by the I.T_{50} method (de Montigny & Aghajanian, 1978; Gallager et al, 1984), but this has been criticised and the use of an alternating 'high-low' current method for assessing a threshold response has been proposed (Bloom et al, 1981; Shultz et al, 1981). We describe a protocol which enables measurement of neuronal sensitivity by both the I.T_{50} and threshold current methods simultaneously.

Extracellular recordings were made with multi-barrel micropipettes from male rats anaesthetised with urethane (1.3 g/kg, i.p.). Effects of iontophoresed 5-HT (20mM; pH 4.0) or NA (20mM, pH 4.5) and GABA (20mM, pH 4.0) were observed on the spontaneous discharge of cerebellar Purkinje cells or ACh-evoked hippocampal pyramidal cell discharge. Agonist ejections (30 sec) were repeated at 3 min intervals under automated control using automatic current balancing and a retaining current (-10nA) between ejections. Ejection currents were alternated between a 'high' (20-100nA) and 'low' (0-60nA) current, and the low current was reduced progressively until a threshold response was observed (Schultz et al, 1981). I.T_{50} values were measured as the charge (i.e. the product of the ejecting current (I nA) and the time (T_{50} sec)) required to obtain a 50% reduction in firing rate; a low I.T_{50} value reflecting a high sensitivity (de Montigny & Aghajanian, 1978).

For twenty hippocampal cells studied, I.T_{50} value-ejecting current curves were plotted. I.T_{50} values computed at low ejection currents (producing inhibitory responses <60-70%) were found to increase proportionally with increasing ejecting current. At higher ejecting currents (producing responses of 80-100%) a plateau of constant I.T_{50} values were obtained. With this experience the following protocol was adopted to investigate antidepressant-induced changes in neuronal sensitivity to NA. The ejecting current was adjusted, for a given agonist, to produce at least an 80% reduction in firing rate (mean 90% $\pm$ 5%; corresponding with the plateau of the I.T_{50}-current curve). This current (I) was then used for the determination of I.T_{50} values and as the 'high' current for threshold measurements of sensitivity of cerebellar Purkinje cells following chronic dothiepin treatment (15mg/kg, 21-28 days oral). Both methods detected the development of subsensitivity to iontophoresed NA, indicated by an increased I.T_{50} value (control: 211 $\pm$ 29.4 nC, n=46 cells; dothiepin: 620 $\pm$ 35.9 nC, mean $\pm$ s.e.m.; $p<0.001$ nC = nanocoulombs) and an increased NA threshold current (control: 14 $\pm$ 4 nA, n=46; dothiepin: 53 $\pm$ 5 nA, n = 31, $p<0.001$). There was no change in neuronal sensitivity to GABA.

Using this protocol for the measurement of neuronal sensitivity we have been able to resolve antidepressant-induced changes in sensitivity (Buckett et al, 1985) and detect altered 5-HT sensitivity induced by continuous light exposure or altered photoperiod (Cox et al, 1986; Mason, 1986; Cox & Mason, 1987).

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THE EFFECTS OF SIBUTRAMINE ON ACETYLCHOLINE- AND CARBACHOL-EVOKED RESPONSES IN THE RAT HIPPOCAMPUS AND GUINEA-PIG ILEUM

M.T. Mahony, C.A. Evans, J.M. Hall, A.I. Lee, P.J.C. Sutcliffe & R. Mason. Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

Tricylic antidepressants have anti-muscarinic action both centrally and peripherally (Golds et al, 1980; Shein & Smith, 1978) which are responsible for some of their clinical side effects. Recently a putative antidepressant sibutramine (BTS 54 524) and its metabolites, all potent monoamine uptake inhibitors (Buckett et al, 1987; Luscombe et al, 1987), have been reported rapidly to down-regulate cortical β-adrenoceptors (Buckett et al, 1987). We report on a comparison of sibutramine with the antidepressants imipramine, dothiepin, iprindole and mianserin on cholinoceptive responses in the guinea-pig ileum and rat hippocampus.

Carbachol dose-response curves were obtained with the guinea-pig ileal preparation using a 2 min cycle in the absence and in the presence of the antidepressants at different concentrations, following a 30 min equilibration period and affinity constants (K_B, M^{-1}) computed (K_B atropine = 10^8). At low concentrations imipramine (K_B = 10^6), dothiepin (K_B = 10^6), iprindole (K_B = 10^5) and mianserin (K_B = 10^5) exhibited competitive anti-muscarinic activity, manifest (at dose-ratios <100) as a parallel displacement of the carbachol log dose-response curves to the right. At higher concentrations these antidepressants produced further rightward shifts and a decline in the maximal response. In contrast, sibutramine (BTS 54 524) and its secondary (BTS 54 354) and primary (BTS 54 505) amine metabolities exhibited non-competitive antagonism as revealed by an immediate reduction in the maximum response and slope of the dose-response curves downwards and to the right at concentrations exceeding 10^{-5} M. Sigmoid shaped stimulus voltage-response curves were obtained from electrically-evoked (atropine-sensitive) contractions of guinea-pig ileum. These were displaced downward and to the right following incubation with imipramine (10^{-7}M); comparable shifts were obtained with sibutramine at 10 to 50-fold higher concentrations.

Acetylcholine-evoked hippocampal neuronal discharge activity (using 30 s iontophoretic ejections of ACh (20mM) every 2-5 min) in the urethane anaesthetised rat were reversibly antagonised by concurrent ejection of atropine (2mM; 5-20nA over 5-10 min) in a dose dependent manner. Iontophoresis of sibutramine (2mM) or its metabolites (2mM) also attenuated the ACh-evoked discharge, but required higher ejection currents (sibutramine 40-100nA; metabolites 20-40nA) than atropine to achieve a similar degree of antagonism.

The present evidence indicates that sibutramine and its metabolites are less potent antagonists at muscarinic cholinoceptors in central and peripheral tissues in contrast to other antidepressants (Golds et al, 1980; Shein & Smith, 1978). This view is supported by binding studies which show that sibutramine and its metabolites are very poor (K_D >10^{-4} M) muscarinic ligands (Buckett et al, 1988).

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BEHAVIOURAL AND BIOCHEMICAL EVIDENCE FOR TRH MODULATION OF HISTAMINE H_1 RECEPTOR FUNCTION IN RAT BRAIN

L.J. Bristow & G.W. Bennett, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Microinjections of histamine into the rat nucleus accumbens induce an H_1 receptor mediated hyperactivity response which is markedly enhanced following chronic treatment with the TRH analogue CG3509 (Bristow & Bennett, 1988) thus suggesting TRH modulation of histamine H_1 receptor function. The present study has further investigated this proposal by examining the effect of chronic treatment with specific TRH antibodies on CG3509 and histamine induced behaviours. In addition, possible interactions of CG3509 with histamine H_1 receptors was examined by radioligand binding.

Male Wistar rats (300 g) were cannulated for microinjections into the nucleus accumbens and seven days later behavioural activity in response to intra-accumbens CG3509 and histamine were tested (Bristow & Bennett, 1988). Rats were initially tested for their responses to a) 0.9% saline (2 x 1 µl, day 1), b) histamine (10 µg/µl, 2 x 1 µl, day 3) and c) CG3509 (0.1 µg/µl, 2 x 0.5 µl, day 6) and then chronically treated with either IgG extracted TRH antiserum (diluted 1:20, 2 x 0.5 µl) or control serum (diluted 1:20, 2x0.5 µl) 5 bilateral intra-accumbens microinjections being given over 3 days (days 8-10). The behavioural responses to CG3509 and histamine were then retested (days 11, 12 respectively).

Rat cortex homogenates were prepared as described previously (Bristow & Bennett, 1987). To investigate the effect of CG3509 on ^{3}H mepyramine binding, 40 µl homogenates were incubated at 37°C for 30 minutes with 20 µl ^{3}H mepyramine (0.5-10nM), 400 µl buffer or 2 µM promethazine (to define non specific binding) and 40 µl CG3509 (5×10^{-5} M) or buffer. Incubations were terminated by addition of 5 mls ice cold 50 mM Na-K phosphate buffer and rapid filtering through Whatman GF/B filters. In addition, cortex homogenates were preincubated for 30 minutes with CG3509 (5×10^{-5} M) prior to addition of ^{3}H mepyramine.

Chronic treatment with TRH antibodies significantly enhanced CG3509 induced hyperactivity (counts from 30-90 mins ↑57%, $P<0.05$, n=6) suggesting the development of TRH receptor supersensitivity. In contrast, histamine induced hyperactivity responses measured from 60-120 mins were significantly reduced (↓72%, $P<0.05$, n=6). Chronic treatment with control serum did not alter CG3509 or histamine induced behaviour compared to pretreatment responses. Central administration of TRH antibodies did not affect the hypothalamic/pituitary axis since plasma free T_4 levels were not altered (TRH-AS group; $T_4 = 33.1 \pm 4.4$ pmol/l; control AS; $T_4 = 28.4 \pm 1.3$ pmol/l) and histological evidence suggested that the injected TRH antibodies do not diffuse away from the nucleus accumbens.

Preincubation of rat cortex homogenates with CG3509 (5×10^{-5} M) for 30 minutes significantly ($P<0.01$) increased B_{max} values determined by ^{3}H mepyramine binding ($B_{max} = 3.5 \pm 0.26$ pmol/g wet wt) compared to those measured following preincubation with buffer ($B_{max} = 2.3 \pm 0.06$ pmol/g wet wt) with no significant change in K_D (CG 3509, $K_D = 3.8 \pm 0.45$ nM; control $K_D = 2.8 \pm 0.24$ nM). In contrast, addition of CG3509 to the binding assay per se did not alter K_D or B_{max} values.

The present results are consistent with interactions between histamine and TRH and suggest that this may occur at the H_1 receptor level.

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A SINGLE INJECTION OF PENTYLENETETRAZOL OR PICROTOXIN MODIFIES NORADRENALINE STORES AND β-ADRENOCEPTORS IN MOUSE CEREBRAL CORTEX

D. Gettins, N. Goldsack[+], V. Ibegbuna & S.C. Stanford. Department of Pharmacology, University College & Middlesex School of Medicine, University College London, Gower Street, London WC1E 6BT

We have reported that a single injection of the benzodiazepine partial inverse agonist, FG7142, causes delayed (7 days) β-adrenoceptor upregulation in mouse cerebral cortex (Stanford *et al.*, 1986). This change is mediated by the $GABA_A$ receptor since it is blocked by co-administration of Ro 15-1788. We now report the effects on cortical β-adrenoceptors of pentylenetetrazol (PTZ) and picrotoxin: these compounds attenuate the actions of GABA through an action at the picrotoxin binding site of the $GABA_A$ receptor (Simmonds, 1982). In an attempt to distinguish between behavioural (anxiogenic) or convulsant effects of these compounds, we studied cortical noradrenaline levels and β-adrenoceptor binding after both convulsant and subconvulsant doses.

Male CD-1 mice (Charles River; 30-35g) were given a single i.p. injection (0.5ml/30g) of PTZ (25mg/kg or 60mg/kg), picrotoxin (1mg/kg or 4mg/kg) or saline, 7 days after injection animals were killed and the cortices removed and chopped. Samples of each tissue were taken for measurement of noradrenaline content by HPLC-ECD and radioligand binding to β-adrenoceptors (Stanford & Jefferys, 1985). Control and experimental tissues were treated in pairs at all times.

Table 1	Pentylenetetrazol			Picrotoxin		
	Saline	25mg/kg	60mg/kg	Saline	1mg/kg	4mg/kg
Bmax (pmol/gprot)	206±23 (11)	148±24* (11)	133±15* (10)	140±17 (6)	147±17 (6)	128±11 (6)
NA content (ng/g wwt)	158±10 (12)	144±10 (12)	129±10 (10)	102±9 (6)	134±18* (6)	115±14 (6)

Values show mean ± s.e.mean. * $P < 0.05$ (Wilcoxon's matched pair signed ranks test). Sample number in parentheses.

Table 1 shows that both a subconvulsant and convulsant dose of PTZ caused β-adrenoceptor downregulation 7 days after a single injection. Although picrotoxin had no effect on β-adrenoceptors at this time, a subconvulsant dose of this drug caused a significant increase in noradrenaline stores. None of the treatments cause β-adrenoceptor changes which resemble those seen after FG7142. These data suggest that drugs known primarily to attenuate the effects of GABA at the $GABA_A$ receptor have long-term but inconsistent effects on noradrenergic neurones; possible explanations will be discussed.

[+] MRC BSc Scholar. This work was funded by the MRC.

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THE TRH ANALOGUE, RX77368, HAS NO EFFECT ON OEDEMA FORMATION IN AN IN VIVO MODEL OF CEREBRAL ISCHAEMIA IN THE RAT

C. Hille, Celestine T. O'Shaughnessy and Joanne Shrewsbury-Gee, Department of Physiological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT.

Oedema has been implicated in the development of tissue infarction following ischaemia, and increased water content in brain tissue has been noted 1h after the induction of cerebral ischaemia in the cat (Schuier and Hossman, 1980). In a model of acute cerebral ischaemia, we have shown that the TRH analogue, RX77368, given 10 min after occlusion of the middle cerebral artery (MCA), reduces tissue infarction (O'Shaughnessy et al., 1987). In the present study, we have used a similar protocol to investigate possible effects of RX77368 on oedema formation.

Adult male Sprague-Dawley rats (200-250 g) were anaesthetized with halothane/ N_2O and O_2 and subjected to craniectomy and left MCA occlusion. In sham operated animals, a craniectomy alone was performed. RX77368 (50 µg/kg), or vehicle (10 µl), was administered intracerebroventricularly within 10 min of surgery. Rats were allowed to recover from anaesthetic and then killed at either 2h, 6h or 24h post-operatively to assess the development of oedema. Tissue was taken from the left cerebral cortex and its density determined on Percoll gradients calibrated with marker beads between 1.034 and 1.049 g/ml.

After 2h, no significant oedema was detected in the left cortex of sham operated rats (1.040±0.0012 g/ml, n=4) or those with MCA occlusion (1.0407±0.0037, n=4). Similar observations were made at 6h in sham operated (1.0435±0.0009, n=6) and MCA occluded (1.0405±0.0013, n=4) animals. However, after 24h, the density of tissue taken from the left cortex of MCA-occluded rats was significantly lower (1.0375±0.0013 g/ml, n=6) than that from sham operated controls (1.0416±0.0003, n=5; $P<0.02$ Students t-test). RX77368 administration had no effect on the density of cortical tissue from sham (1.0411±0.0003, n=6) or MCA-occluded (1.0373±0.0014, n=6) animals.

We conclude that RX77368 does not affect cortical oedema formation in this model of cerebral ischaemia.

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ADENOSINE ANALOGUES SUPPRESS K^+- AND KAINATE-INDUCED SYNAPTOSOMAL RELEASE OF ENDOGENOUS GLUTAMATE

J. Pan, L.F. Neville, B. Arvin and P.J. Roberts, Department of Physiology and Pharmacology, University of Southampton, Southampton SO9 3TU,

We recently reported that 2-chloroadenosine (CLAD) possesses potent neuro-protective properties when co-injected into rat striatum with the excitotoxin, kainate (Arvin et al., 1987) and provided some evidence that this action might be related to modulation of nerve terminal calcium entry (Arvin et al., 1988). In this study, we have investigated the K^+ and kainate-induced release of glutamate from cortical synaptosomes; calcium influx during depolarisation, and the effects of various adenosine analogues on these processes.

Purified guinea-pig synaptosomes were prepared on discontinuous ficoll/sucrose gradients. Following resuspension of pellets in Krebs bicarbonate medium and determination of the extracellular concentrations of glutamate, synaptosomes were then exposed to either 30 mM K^+ or 1 mM kainate, and the release of endogenous glutamate measured (Nicholls & Sihra, 1986). The effects of adenosine analogues were investigated by their inclusion in the incubation medium 5 min prior to addition of K^+ or kainate.

For the investigation of synaptosomal calcium influx, crude rat cortical synaptosomes were loaded with the fluorescent probe quin-2 ester, followed by exposure to two sequential 30 mM K^+ pulses, 5 min apart. Drug effects were examined by addition during the second depolarisation.

Both K^+ and 1 mM kainate induced a pronounced calcium-dependent release of glutamate (0.3 and 0.28 nmol/mg protein/min respectively). Exposure to CLAD (1nM - 1uM) markedly diminished the effect of both of these agents. Typical values with 100 nM CLAD were 0.15 and 0.16 nmol glutamate released/mg protein/min for K^+ and kainate respectively. Similar effects were observed wih the highly specific A1 receptor agonist, N^6-cyclopentyladenosine (CPA). The actions of CLAD and CPA were partially reversed by the selective A1 antagonist cyclopentyltheophylline (CPT).

Exposure of quin-2 loaded synaptosomes to two successive K^+ stimuli yielded S2/S1 ratios of 0.94. Inclusion of CLAD or CPA (10-1000 nM) 2 min prior to S2, resulted in a dose-dependent inhibition (max of 40% inhibition). This was fully reversed by 1 uM theophylline.

These results re-emphasise the likely importance of presynaptic glutamatergic mechanisms in kainate-induced toxicity, in view of the action of A1 agonists to prevent kainate toxicity and glutamate release, the latter possibly through an effect on calcium influx.

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PREVENTION OF NMDA RECEPTOR-MEDIATED NEURODEGENERATION IN THE RAT STRIATUM BY AN ANTAGONIST AT THE GLYCINE MODULATORY SITE

Robert Tridgett and Alan C. Foster, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR

Activation of the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors can result in degeneration of neurones within the central nervous system. Both competitive (Schwarcz et al, 1982) and non-competitive (Foster et al, 1987a) NMDA antagonists have been shown to prevent these neurodegenerative effects. NMDA receptor responses are potentiated by glycine acting at a modulatory site on the NMDA receptor which is distinct from the classical strychnine-sensitive glycine receptor (Johnson and Ascher, 1987). Recently, radioligand binding (Donald et al, this meeting) and electrophysiological (Kemp et al, this meeting) studies from this laboratory have revealed that 7-chloro-kynurenate (7-ClKYNA) is a selective antagonist at the glycine site associated with the NMDA receptor. In the present investigation we have evaluated the effectiveness of this compound in preventing neurodegeneration in the rat striatum caused by the endogenous NMDA receptor agonist quinolinate (QUIN).

Male Sprague-Dawley rats (250-300g), under equithesin anaesthesia, were given a unilateral intrastriatal injection of 200nmol QUIN (1µl, pH 7.4) followed 1 hr later by 1µl saline or 7-ClKYNA at the same stereotaxic coordinates. 7 days later the animals were killed and their striata removed for measurement of choline acetyltransferase (CAT) and glutamate decarboxylase (GAD) activities as described by Foster et al (1987a), to assess the loss of striatal neurons.

Table:

	Dose of 7-ClKYNA injected (nmol)			
	Control	10	20	50
CAT	60.8 ± 3.1 (29)	36.8 ± 5.5* (9)	31.3 ± 7.9* (10)	4.1 ± 1.6* (9)
GAD	65.7 ± 2.7 (29)	35.7 ± 3.3* (9)	35.5 ± 6.4* (9)	13.2 ± 5.8* (9)

7-ClKYNA was injected 1 hr after 200nmol quinolinate. Values are percent reduction of enzyme activity in the injected side v. the contralateral side (mean ± SEM of (n) animals) * $P \geq 0.01$

As shown in the table, when 7-ClKYNA was injected into a rat striatum which had received 200nmol QUIN 1 hr previously (a treatment time based on earlier results obtained with the non-competitive NMDA receptor antagonist, MK-801: Foster et al, 1987b) a dose-dependent protection was observed which was almost complete at the highest dose (50nmol). The specificity of the protection afforded by 7-ClKYNA was assessed by testing it against excitotoxins specific for other excitatory amino acid receptor subtypes. 7-ClKYNA was unable to prevent the neurodegeneration in the striatum induced by 5nmol kainate or 50nmol α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) when co-injected at a dose of 50nmol.

These results indicate that antagonists at the glycine site on the NMDA receptor may prevent neuronal degeneration caused by over-activation of this receptor. It is suggested that activation of the glycine site *in vivo* is important for the expression of NMDA receptor mediated neurodgenerative events.

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PHARMACOLOGICAL BLOCK OF EXCITATORY SYNAPTIC TRANSMISSION IN THE FROG OPTIC TECTUM IN VITRO

A. Nistri, Lucia Sivilotti & N. Bunce, Department of Pharmacology, St. Bartholomew's Hospital Medical College, London EC1M 6BQ.

While the precise identity of the optic nerve transmitter in mammals is not clear, acetylcholine (ACh) has long been regarded as the transmitter in lower vertebrates (Oswald & Freeman 1980). More recently, in the goldfish optic tectum, glutamate rather than ACh was proposed as the physiological transmitter acting via non-NMDA receptors (Langdon & Freeman, 1986). The availability of 6,7-dinitroquinoxaline-2,3-dione (DNQX) as a specific antagonist of non-NMDA receptors (Drejer & Honoré, 1988) prompted the present study, in which frog optic tectum preparations have been used. The relative contribution of cholinergic mechanisms to synaptic transmission in the optic tectum was examined by applying vesamicol, an agent known to deplete intravesicular stores of ACh (Marshall & Parsons, 1987). Previous work showed the relatively weak antagonism of tectal synaptic transmission by cholinergic blockers (Nistri & Berti, 1987).

Excitatory synaptic potentials elicited by electrical stimulation of the optic nerve were recorded from superficial layers of in vitro frog (Rana temporaria) optic tectum preparations maintained at 6-7°C as previously described (Nistri & Sivilotti, 1985). Drugs were applied by fast superfusion. Stock solutions of DNQX (10 mM) were prepared in dimethylsulphoxide; the latter was also added to control Ringer solutions at a concentration of 0.1% without affecting synaptic transmission.

Optic nerve stimulation evoked complex tectal field potentials with characteristic monosynaptic waveforms termed U_1 and U_2 (Chung et al. 1974). These responses were moderately reduced by 50 uM (-)vesamicol (69 $\pm$ 5% of controls; n = 4; $P < 0.001$); paired pulse stimulation of the optic nerve elicited about 25% potentiation of the second waveform and this phenomenon was little changed by 50 uM (-)vesamicol. DNQX (10 uM) halved the amplitude of the U_1 wave without significant effect on the U_2 component. The U_1 and U_2 waves were significantly depressed (84 $\pm$ 4 and 52 $\pm$ 3%; $P < 0.01$ and $P < 0.001$ respectively) by large concentrations of glutamate (2 mM), presumably because of excessive depolarization. During exposure to DNQX the action of glutamate on the U_1 wave was lost but remained essentially unchanged on the U_2 wave.

These results suggest that optic nerve mediated transmission is only moderately sensitive to vesamicol; conversely, the U_1 component of the excitatory potential was more strongly blocked by DNQX, indicating that non-NMDA type receptors for glutamate probably play a role in this response. The nature of the receptors mediating the U_2 wave is still to be established. It seems possible that in the frog the optic nerve (which comprises various classes of fibres) utilizes more than one transmitter substance to activate central neurones.

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CHARACTERIZATION OF [^{3}H]-GLYCINE BINDING TO A MODULATORY SITE WITHIN THE N-METHYL-D-ASPARTATE RECEPTOR COMPLEX FROM RAT BRAIN

A.E. Donald, R. Tridgett and A.C. Foster. Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR

A regulatory role for glycine within the N-methyl-D-aspartate (NMDA) receptor complex was recently suggested by Johnson and Ascher (1987). Low concentrations of glycine greatly potentiate the depolarising responses to NMDA agonists, an effect which is strychnine-insensitive and is thus distinct from the classical inhibitory effect of glycine. Autoradiographical experiments indicate that [3H]-glycine binding sites have an anatomical distribution in rat brain which parallels that of the NMDA receptor (Bristow et al, 1986). We have examined [3H]-glycine binding to rat brain membranes to investigate the pharmacological specificity of this putative regulatory site within the NMDA receptor complex.

P_2 membranes and synaptic plasma membranes (SPM) were prepared from rat cerebral cortex as described previously by Foster and Fagg (1987). The P_2 membranes were treated with 0.1% saponin and then washed once with 5mM Tris-acetate buffer (pH 7.0). The SPM were washed 4 times in the same buffer. For the binding assay, 100µg of protein were incubated at 4°C for 30 min with 50mM Tris-acetate buffer (pH 7.0) and 50nM [3H]-glycine in a final volume of 0.5 ml. Non-specific binding was determined by the inclusion of 1mM glycine, and bound radioactivity was separated by rapid centrifugation. The pellet was washed and then solubilized in 2% SDS before scintillation counting.

Saturation analyses of [^{3}H]-glycine binding using the P_2 and SPM membranes gave Bmax values of 11.67 $\pm$ 5.61 pmoles/mg protein and 7.23 $\pm$ 1.90 pmoles/mg protein, respectively (mean $\pm$ SEM), and Kd values of 724 (525, 1000)nM and 371 (302, 457)nM respectively (geometric mean (-SEM, +SEM); N=3). Glycine was the most potent inhibitor with an IC_{50} of 0.18 (0.16, 0.20) µM (N=4). Amongst other common amino acids, serine and alanine were the most potent. D-serine was more potent than L-serine, respective IC_{50}s being 0.57 (0.42, 0.77)µM and 30.3 (20.3, 45.1)µM (N=3). D-cycloserine was also active with an IC_{50} of 3.7 (1.1, 7.6)µM (N=3). Strychnine was inactive at 10µM. The known NMDA receptor antagonists kynurenic acid (KYNA) and 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) inhibited [3H]-glycine binding with IC_{50}s of 41 (27, 61)µM and 17.5 (15.2, 20.3)µM, respectively (N=3-4). Substitution of chlorine at the 7 position of KYNA (7ClKYNA) increased activity, giving an IC_{50} of 0.56 (0.38, 0.75)µM (n = 4). KYNA, 7ClKYNA and HA-966 all had IC_{50}s > 100µM against [3H]-strychnine binding to rat brain stem/ spinal cord membranes or radioligand binding to the recognition sites of the NMDA (L-[3H]-glutamate), quisqualate ([3H]-AMPA) and kainate ([3H]-kainate) receptors in rat cortex.

These results suggest that 7ClKYNA and HA-966 are relatively selective inhibitors of [3H]-glycine binding. Electrophysiological experiments indicate that the NMDA antagonist effects of 7ClKYNA and HA-966 can be reversed by the addition of either glycine or D-serine (Kemp et al, this meeting). Therefore these compounds appear to be antagonists at the glycine modulatory site within the NMDA receptor and should be useful tools to explore the function of this glycine regulatory site under both physiological and pathological conditions.

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ANTIPARKINSON-LIKE EFFECTS OF NEUROTENSIN: COMPARISON OF VARIOUS ROUTES OF ADMINISTRATION AND STRUCTURE ACTIVITY STUDY

R. Rivest and F.B. Jolicoeur, Department of Psychiatry, Faculty of Medicine, University of Sherbrooke, Sherbrooke, PQ, Canada JIH-5N4 (introduced by D. Regoli)

A dense population of neurotensin (NT) receptors is found in mammalian substantia nigra and, recently, a marked loss of NT receptors in this region was found in patient with Parkinson's disease (Uhl et al., 1984). Although the significance of this finding remains to be ascertained, it does suggest that NT may be implicated in the etiology and/or symptomatology of this neurological disease. Thus, we investigated the effects of NT on various Parkinson-like neurobehavioral deficits observed in the rat following 6-OHDA administration in the posterolateral hypothalamus (Rivest and Jolicoeur, 1986). Our results indicated that NT injection into the lateral ventricle (ILV) significantly attenuates the muscular rigidity and tremors of lesioned animals. The purpose of the present study was to further examine the effects of the peptide on muscular rigidity and tremors after administration in discrete areas of the CNS. In a second series of experiments, we have undertaken a structure activity study with two fragments, NT_{1-10} and NT_{8-13}, as well as two analogues, [D-Tyr^{11}]-NT and [Ala^{11}]-NT.

Under anaesthesia, male hooded rats received bilateral injections of 26 µg 6-OHDA in the posterolateral hypothalamus (A.-0.8, L.2.0, V.8.0 in relation to bregma) and stereotaxically implanted with indwelling cannula in one of the following regions (n=8): striatum, globus pallidus, thalamic ventromedial nucleus, substantia nigra, in lateral ventricle (ILV), cisterna magna (IC) and in the subarachnoidal space of the spinal cord (IT). In brain regions, NT was administered bilaterally in a volume of 1 µl. The volume for ILV, IC and IT injections was 10 µl. Two days after 6-OHDA administration, animals were injected with the various peptides in doses ranging from 0.45 to 120 µg. The presence of tremors, observed when the hind quarter of animals was lifted by the tail, and the intensity of muscular rigidity, manifested by a prolonged grasping time when animals were suspended with their front paws holding a metal bar, were recorded at 15, 30, 45, 60, 90 and 120 minutes following injection. Results obtained were analysed by ANOVA analyses for repeated measures or by Fisher exact probability tests when non-parametric data was recorded.

Grasping time of lesioned animals was significantly attenuated starting with bilateral injections of 3.0 µg of NT in both substantia nigra and striatum and with 6.0 µg in the other regions examined. When injected in cerebro-spinal fluid cavities, significant decreases were found after 30 µg ILV, and following 3.75 µg for both IC and IT routes of administration. On the other hand, minimal effective doses for reducing tremors were 3.0 µg in the substantia nigra, 6.0 µg in the thalamus and globus pallidus, 30 µg in ILV and IC, and 7.5 µg after IT administration. These results indicate that 6-OHDA-induced muscle rigidity and tremors are differentially affected by regional administration of NT and that IT injection of the peptide is the most efficient route of administration for attenuating both symptoms.

Muscular rigidity and tremors were not altered by either NT_{1-10} or [Ala^{11}]-NT even with the highest dose, while 60 µg of NT_{8-13} decreased grasping time 15 min after ILV injection. However, the NT analogue [D-Tyr^{11}]-NT proved to be markedly more potent in its anti-Parkinson-like effects. Compared to NT, wich significantly decreased grasping time and tremors at 30.0 µg, the analogue produced similar effects at 1.8 and 0.9 µg respectively. Also we found a marked enhancement in both efficacy and duration of action with this analogue. Together, these results indicate that our previous observations on anti-Parkinson's actions of NT are not solely due to unspecific effects of peptide administration, are largely dependent on the carboxy terminal of NT, and that the tyrosine residue in position 11 plays a critical role in these actions of NT.

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HA-966 ANTAGONISES ENHANCEMENT OF RAT STRIATAL [^{3}H]-DOPAMINE RELEASE BY N-METHYL-DL-ASPARTATE

M. Crawford, P.J. Roberts & J.C. Watkins[1], Department of Physiology and Pharmacology, University of Southampton, Southampton SO9 3TU, and [1] Department of Pharmacology, University of Bristol, Bristol, BS8 1TD.

It was recently reported that glycine potentiates NMDA responses in cultured neurones, through a strychnine-insensitive mechanism (Johnson & Ascher, 1987). However, whether this represents a purely pharmacological effect, or is of physiological significance remains uncertain. The results of the present study support the latter possibility.

Rat striatal slices (0.4 mm thick) were prelabelled with [^{3}H] dopamine and superfused with Krebs bicarbonate medium (Snell & Johnson, 1986). The slices were depolarised twice (S1 and S2) with 20 mM K^+, with inclusion of test compounds during the second period of stimulation (4 min). Fractions of superfusate were collected, the released [^{3}H]dopamine determined and S2/S1 ratios calculated.

S2 Treatment	S2/S1	n
20 mM K^+	0.70 ± 0.04	5
20 mM K^+ + 1 mM NMDLA	1.31 ± 0.08	9
20 mM K^+ + 1 mM NMDLA + 1 mM gly	1.23 ± 0.04	4
20 mM K^+ + 1 mM NMDLA + 1 uM HA-966	0.93 ± 0.08	3*
20 mM K^+ + 1 mM NMDLA + 10 uM HA-966	0.89 ± 0.08	5**

*$P<0.05$ and **$P<0.01$ compared with K^+ + NMDLA (by t-test)

N-methyl-DL-aspartate (NMDLA) greatly enhanced [^{3}H]DA release, an effect that was significantly attenuated by the depressant drug 1-hydroxy-3-amino-pyrrolid-2-one (HA-966) (1 and 10 uM), which we have recently found to inhibit the binding of [^{3}H]glycine to strychnine-insensitive sites on rat brain synaptic membranes, whilst not affecting binding of glutamate to the NMDA site (unpublished).

This effect of HA-966 on the NMDLA-enhanced release of dopamine from striatal slices, suggests that glycine may exert a tonic facilitatory role; our observation that 1 mM glycine failed to influence the NMDLA response, suggests that facilitation may be occurring maximally at physiological glycine concentrations.

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A HIGHLY SENSITIVE METHOD FOR MEASURING ENDOGENOUS 3-METHOXY-TYRAMINE CONCENTRATION IN BRAIN BY HPLC-ECD

D.J. Heal, A.T.J. Frankland and W.R. Buckett, Research Department, The Boots Company PLC, Nottingham NG2 3AA

In brain, dopamine is mainly metabolised by oxidation and O-methylation to form 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The enzymes involved are respectively monoamine oxidase (MAO) and catechol-O-methyltransferase. Methylation can precede oxidation and 3-methoxytyramine (3-MT) is then the intermediate product. After administration of a MAO inhibitor, 3-MT becomes the sole product of dopamine metabolism. Davies and Heal (1986) have previously described a HPLC-electrochemical detection method which is suitable for measuring brain 3-MT concentrations under these circumstances. We have now developed a method using the ESA coulometric detector which is sensitive enough to measure basal 3-MT concentrations in brain.

Adult male CD rats (Charles River) weighing 140-180g were used. Drugs were dissolved in 0.9% NaCl solution and administered i.p. For measurement of 3-MT, brains were rapidly dissected and striatal and accumbens tissues were immediately frozen on "dry ice". These were homogenised in 10 vols w/v of 0.05M perchloric acid containing 0.4mM sodium metabisulphite and 0.13µM 3-methoxy-4-hydroxy-benzylamine (MHBA) as internal standard. Supernatants were prepared by centrifugation twice at 12,000g (6 and 5 min). Using a WISP 710B autoinjector, 50µl was injected onto the HPLC system which consisted of a Kratos Spectroflow 400 pump with membrane pulse damper connected to a 25 cm Spherisorb 1 ODS 5µ analytical column protected by a 3 cm Brownlee Aquapore RP 300 precolumn. 3-MT was detected using an ESA Coulochem detector with a 5011 analytical cell. Detector 1 was set at +0.2V and detector 2, used for recording, was set at +0.4V. The mobile phase was 10mM sodium dihydrogen phosphate-orthophosphoric acid buffer pH 2.7 containing 10% methanol, 1.2 mM 1-octanesulphonic acid and 4.5 mM di-n-butylamine pumped at 1 ml/min. MHBA and 3-MT chromatographed with retention times of 9.5 and 13.5 min, respectively. Basal 3-MT concentrations ± s.e. mean were striatum: 130 ± 3 and accumbens: 44 ± 2 ng/g wet-weight and this closely agrees with previously reported values (Westerink and Spaan, 1982; Davies and Heal, 1986).

Table 1

Drug		Striatal 3-MT (ng/g wet wt) Control	Treated
Tranylcypromine	(5 mg/kg)	145 ± 10	878 ± 65***
Pargyline	(50 mg/kg)	134 ± 4	800 ± 44***
Methamphetamine	(0.3 mg/kg)	168 ± 17	258 ± 18**
"	(1 mg/kg)	189 ± 12	311 ± 14***
"	(3 mg/kg)	127 ± 8	283 ± 8***
"	(10 mg/kg)	148 ± 10	427 ± 24***
Bupropion	(10 mg/kg)	115 ± 11	136 ± 10
Nomifensine	(10 mg/kg)	117 ± 8	123 ± 5

** P<0.01, *** P<0.001, n = 4-10 observations.

Striatal 3-MT concentration was measured 60 min after various drug treatments (Table 1). The MAO inhibitors, tranylcypromine and pargyline, markedly increased this, as previously reported by Westerink and Spaan (1982) and Davies and Heal (1986). Methamphetamine also dose-dependently elevated striatal 3-MT, but the dopamine reuptake inhibitors, bupropion and nomifensine were without effect. Hence, a highly sensitive HPLC technique is described which is capable of measuring brain basal 3-MT levels and permits the investigation of 3-MT as a possible index of central dopaminergic function.

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RAT STRIATAL AND MESOLIMBIC D1 RECEPTOR BINDING IS NOT ALTERED BY ANTIDEPRESSANT TREATMENTS INCLUDING ECS AND SIBUTRAMINE HCl

K. F. Martin, P. L. Needham, J. Atkinson, A. Cowan, D. J. Heal and W. R. Buckett
Research Department, The Boots Company PLC, Nottingham U.K.

It has been suggested by various authors that central dopamine function is involved in the mode of action of antidepressant drugs (see Willner 1983). Recently, Klimek and Nielsen (1987) reported that chronic treatment with antidepressant drugs decreased the number of dopamine D1 receptors in the rat striatum and limbic system. In this communication we report that neither acute nor chronic administration of antidepressant drugs, electroconvulsive shock (ECS) or sibutramine HCl affected the binding characteristics of [^{3}H]SCH 23390, a D1 receptor ligand.

Adult male CD rats (100-125g) were used. Drugs were given i.p. once daily for 1 or 10 days; sibutramine HCl (BTS 54 524, 3 mg/kg), desipramine (10 mg/kg) pargyline (10 mg/kg), amitryptyline (10 mg/kg) or water (1 ml/kg). Additionally, halothane anaesthetised rats received a single ECS (200V, 2s) or 5 ECS over 10 days. Controls were given halothane. Twenty-four hours after the final treatment the striata and limbic areas (tuberculum olfactorium and nuc. accumbens) were removed and stored at -80°C. Six point saturation binding analyses were performed on membranes from pairs of rats using [^{3}H]SCH 23390 (0.125-4.0 nM). Specific binding was determined by chlorpromazine (100 μM). Correlations for Scatchard plots were $r > 0.9$.

The data show (Table 1) that neither acute nor chronic antidepressant treatments, including ECS altered the number of D1 receptors in the striatum. Pargyline (10 days) decreased the number of D1 sites in the limbic areas. The only significant change in Kd value for [^{3}H]SCH 23390 binding occurred after 10 days DMI in the striatum.

Table 1

Treatment	1 day		10 day	
	Striatum	Limbic	Striatum	Limbic
Water	69.03 ± 3.85	42.81 ± 3.15	54.95 ± 2.97	33.22 ± 1.26
Sibutramine HCl	66.83 ± 4.67	42.25 ± 1.53	55.27 ± 1.09	33.99 ± 1.68
Desipramine	68.53 ± 9.23	47.78 ± 1.32	58.30 ± 3.18	34.60 ± 2.56
Pargyline	66.24 ± 9.26	47.78 ± 2.06	54.89 ± 1.85	28.05 ± 1.18
Amitryptyline	76.35 ± 5.82	43.19 ± 4.97	58.51 ± 4.62	32.87 ± 3.91
Halothane	54.60 ± 2.68	34.87 ± 3.32	56.27 ± 4.35	32.72 ± 1.50
ECS	53.57 ± 4.70	41.48 ± 2.23	62.30 ± 3.41	32.60 ± 2.06

Bmax [mean ± s.e. mean, n=5, fmol/mg wet wt. tissue]

Thus, the results presented in this communication suggest that changes in D1 receptor number or affinity are not a common feature of antidepressant treatments. They also show that the increased D1 receptor mediated behaviour following repeated ECS reported by Kingston et al (1988) is not reflected by changes in [^{3}H]SCH 23390 binding parameters.

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REPEATED COCAINE ADMINISTRATION DOES NOT INDUCE CHANGES IN D_1 RECEPTOR NUMBER OR AFFINITY

P. L. Needham, J. Atkinson and W. R. Buckett, Research Department, The Boots Company PLC, Nottingham NG2 3AA

Cocaine abuse has led to renewed interest in the neurochemical and pharmacological changes consequent to its repeated administration. Acutely, cocaine blocks neuronal dopamine reuptake, and adaptive changes occur chronically. For example, repeated cocaine administration to rats reduces striatal binding but increases binding in the nucleus accumbens of the D_2-dopamine receptor subtype (Goeders & Kuhar, 1987). However Dwoskin et al (1988) did not detect changes in striatal D_2-binding. D_2 receptors mediate the psychomotor actions of dopaminergic drugs. Dopamine receptors of the D_1 subtype (adenylate cyclase-linked) have been shown, by the use of the D_1-selective antagonist SCH 23390, to be involved in the modulation of some D_2-mediated behaviours (Braun et al, 1986). Therefore, in this study we have examined the effect of chronic cocaine administration on D_1-receptor binding parameters in limbic and striatal brain regions of the rat.

Male CD rats (Charles River) weighing 150 to 180g were used in groups of 5. Cocaine HCl (10 mg/kg i.p.) was injected daily for 15 days; controls received saline. Either 20 mins or 48 hrs after the last dose, rats were killed and the brains rapidly dissected into striatal and limbic (tuberculum olfactorium and nucleus accumbens) regions. Tissue homogenates were prepared and incubated with [^{3}H]-SCH 23390 (0.125-4nM) at 37° for 30 min. D_1-receptor binding was determined by nine point saturation analysis (Billard et al, 1984). Specific binding was determined with chlorpromazine (100 μM). Correlations for Scatchard plots were $r > 0.94$.

The results showed no effect on receptor number (Bmax) or affinity (Kd) in either brain region from cocaine-treated rats compared with controls (Table 1).

Table 1 D_1-receptor number (Bmax) and affinity (Kd) in rats treated with cocaine daily for 15 days compared with controls.

15 day treatment	Limbic		Striatal	
	Bmax	Kd	Bmax	Kd
a. 20 mins after last dose				
Saline control	32.37 ± 3.53	0.29 ± 0.01	43.89 ± 2.94	0.32 ± 0.01
Cocaine (10 mg/kg i.p.)	42.25 ± 3.23	0.30 ± 0.02	43.98 ± 2.29	0.30 ± 0.01
b. 48 hrs after last dose				
Saline control	39.72 ± 2.65	0.27 ± 0.01	52.89 ± 4.44	0.31 ± 0.02
Cocaine (10 mg/kg i.p.)	40.98 ± 2.85	0.28 ± 0.02	45.19 ± 5.50	0.28 ± 0.02

Bmax [mean ± s.e. mean, n=5, fmol/mg wet wt. tissue]; Kd [nM]

Furthermore no changes in either Bmax or Kd were apparent after a single acute treatment with cocaine (10 mg/kg i.p.) Thus repeated cocaine treatment does not induce changes in D_1-receptor binding parameters, in contrast to changes in D_2-receptor binding reported elsewhere.

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A STUDY ON THE SOURCE OF DIHYDROXYPHENYLACETIC ACID (DOPAC) IN THE RAT STRIATUM

M.C.Garrett & P.Soares-da-Silva, Laboratório de Farmacologia, Faculdade de Medicina, 4200 Porto, Portugal

DOPAC is the major dopamine metabolite in the brain and is formed by degradation of the transmitter by monoamine oxidase. Altghough is has been accepted for years that recently released and uptaken dopamine is the main source of DOPAC, more recently it has been shown that blockade of neuronal uptake mechanisms do not affect DOPAC formation (Braestrup, 1977; Waldmeier et al., 1981); Westerink et al., 1987). Also, it has been demonstrated that the deamination of recently synthetized dopamine to DOPAC may have occurred before dopamine has been stored in nerve vesicles (Soares-da-Silva, 1987). The aim of the present work was to examine the effects of stimuli which promote the release of vesicular dopamine as well as the result of mobilization of cytoplasmic amine on the formation of DOPAC.

For that purposes we have used superfused rat striatum slices in conditions of COMT inhibition (tropolone 27 μM) and the overflow of endogenous dopamine and DOPAC were measured of high pressure liquid chromatography. Spontaneous overflow of dopamine and DOPAC was collected during three collection periods of 2 minutes each. During the fourth collection period the striatal slices were electrically stimulated (5 Hz, 3 ms, 24 mA) and in the four subsequent collection periods post-stimulation dopamine and DOPAC overflow was also determined. In another set of experiments striatal slices were superfused with a potassium-enriched (52 mM) medium for 10 minutes and dopamine and DOPAC overflow determined every 2 minutes; the spontaneous overflow was studied in parallel experiments. In order to mobilize cytoplasmic dopamine, striatal slices were superfused with decreasing concentrations of sodium (118 mM, 78 mM, 39 mM and 0 mM) and dopamine and DOPAC overflow measured every 4 min for 40 min.

Spontaneous DOPAC overflow was greater than dopamine spontaneous overflow and electrical stimulation produced a 10 fold increase in dopamine overflow without changing DOPAC overflow. Potassium-induced depolarization caused a 50 fold increase of dopamine overflow and DOPAC overflow was practically not affected. Decreasing sodium concentrations in the superfusion medium produced a concentration-dependent increase in dopamine overflow and the DOPAC/dopamine overflow ratio was significantly reduced.

These results suggest that most of the DOPAC found in the superfusate is formed from a cytoplasmic pool of dopamine and that recently released dopamine does not significantly account for the formation of DOPAC.

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[^{3}H]NIPECOTIC ACID BINDING TO GABA UPTAKE SITES IN THE AMYGDALA IN SCHIZOPHRENIA

C. Czudek and G.P. Reynolds, Department of Pathology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Neurodegenerative disease and neuronal loss in man is often associated with a loss of neurones containing γ-aminobutyric acid (GABA). This reflects the high proportion and ubiquitous nature of GABAergic neurones in the human brain. In the past, neurochemical studies of these neurones in post-mortem tissue have relied on the measurement of glutamic acid decarboxylase, the synthetic enzyme for GABA, and the transmitter itself. There are limitations associated with these parameters since the enzyme is sensitive to agonal state and GABA is involved in intermediary metabolism.

The introduction of a radioligand specific for GABA uptake sites, (3H)nipecotic acid, has provided a valuable alternative marker for GABAergic neurones (Lloyd and Vargas, 1982; Simpson et al., 1988). Having found that, in our hands, a filtration assay employing (3H)nipecotic acid seriously underestimates the amount of specific GABA-displaceable binding, we have developed a centrifugation assay for these binding sites. This is described here along with results from a preliminary investigation into possible neuronal deficits in schizophrenia.

Brain tissue was homogenised in 100 vol. 50 mM phosphate-HCl pH7.4 containing 400mM Na+ and was centrifuged at 48000g for 10 min. After two further washes to eliminate residual GABA, the pellet was resuspended in 100 vol. buffer and incubated with 100nM (3H)nipecotic acid at 4°C. Non-specific binding was defined by 20mM GABA (specific binding typically 80% of total). After 3h, the incubate was centrifuged at 12000g for 5 min. The supernatant was aspirated and the pellet superficially washed with 1ml buffer. Bound radioligand in the pellet (contained in the tip of a microcentrifuge tube) was determined by liquid scintillation counting. Scatchard analysis of binding to control post-mortem amygdala tissue employing 8 concentrations of ligand between 50 and 5000nM demonstrated binding to be saturable and consistent with a single site (Bmax 113±47 pmol/mg protein, K_D 1.82±0.18 μM; mean±s.d., n=3).

This method was applied to the investigation of GABA uptake sites in amygdalae dissected bilaterally from 17 cases of schizophrenia and 17 age-matched control subjects. The results are shown in table 1.

Table1 Binding of (3H)nipecotic acid to post-mortem amygdalae

	Left	Right
Controls	5317±2098	5124±2258
Schizophrenics	4933±1728	4658±1875

mean±s.d. in fmol/mg protein.

It is apparent that no significant loss of binding occurs in the schizophrenic group, indicating that the evidence consistent with an atrophy or disturbance of temporal lobe structures in the disease (e.g. Brown et al., 1986) is not reflected by a general deficit of GABAergic neurones in the amygdala. The lack of any asymmetric difference is notable in the light of our observation of increased dopamine in the left amygdala in schizophrenia (Reynolds, 1983); these results indicate that the change in dopamine is unrelated to any asymmetric loss of this indicator of GABAergic innervation of the amygdala.

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900P

EFFECTS OF DIMETHYLSULPHOXIDE ON CYCLIC AMP PRODUCTION AND INOSITOL PHOSPHOLIPID BREAKDOWN IN GUINEA-PIG BRAIN SLICES

D.A. Kendall, R.M. Straw and S.J. Hill, Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

Dimethyl sulphoxide (DMSO) is widely used in biomedical research as a relatively inert vehicle for water-insoluble compounds. In recent studies prompted by the use of adenosine receptor antagonists dissolved in DMSO we have observed some complex effects of the solvent which might influence its selection as a suitable vehicle for neurochemical procedures.

Inositol phospholipid hydrolysis was monitored by measurement of total ^{3}H-inositol phosphates (^{3}H-IP) accumulation in guinea-pig cerebro-cortical slices prelabelled with ^{3}H-inositol (Brown et al., 1984). ^{3}H-cyclic AMP formation was measured using the ^{3}H-adenine prelabelling method of Shimizu et al. (1969).

Despite having no effect alone, adenosine potentiated ^{3}H-IP accumulation in the presence of 10^{-3}M histamine by 2.3±0.1 fold (n=29). DMSO had no significant effects on basal ^{3}H-IP or histamine stimulated ^{3}H-IP formation, but it antagonized the potentiation due to adenosine analogues in a concentration dependent manner. 1.5% (v/v) DMSO reduced the maximum response to 2-Chloroadenosine (2-CA) by 62±7% (n=3) and at 6% (v/v) DMSO the potentiation was abolished.

DMSO had no significant effects on basal ^{3}H-cyclic AMP production but enhanced the maximum response to histamine by 2.3±0.2 fold (n=5) at a concentration of 6%, without changing the EC_{50} value of histamine. In contrast there was a concentration related reduction in the formation of 2-CA-stimulated cyclic AMP formation due to DMSO. The antagonism appeared to be competitive since the 2-CA dose response curve was shifted by 3.3±0.2 fold (n=3) to the right in the presence of 6% (v/v) DMSO. The cyclic AMP response to a combination of histamine and 2-CA, which is much greater than additive, was also antagonized by DMSO.

The DMSO induced potentiation of the histamine stimulated cyclic AMP response seemed to be mediated via an H_2 receptor since there was still a 2.3±0.2 fold (n=3) increase in the response to histamine in the presence of the H_1-antagonist mepyramine (10^{-6}M), due to 6% (v/v) DMSO.

In addition, 6% (v/v) DMSO potentiated the cyclic AMP response to 31mM KCl by 6.4±0.2 fold (n=3) whilst it reduced the response to the adenylate cyclase activator forskolin (10^{-4}M) by 73% (n=2).

These data do not support the contention that DMSO is biologically inert and we suggest that it be used with caution in neurochemical assays.

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EFFECTS OF PROTEIN KINASE C INHIBITORS ON POTENTIATION OF CYCLIC AMP FORMATION IN RAT BRAIN SLICES

Jean P. Robinson and D.A. Kendall, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Hormone and neurotransmitter stimulated cyclic AMP formation in the central nervous system can be modulated by indirectly-acting agents. Thus β-adrenoceptor mediated cAMP production is potentiated by α-adrenoceptor agonists which are inactive alone. Since it has been widely suggested that the mechanism of this potentiation relies upon activation of protein Kinase C (PKC), we have investgiated the effects of a number of putative inhibitors of PKC (Polymyxin B, H7 and staurosporine) on this amplified response.

Cyclic AMP formation was monitored by measuring the accumulation of ^{3}H-cyclic AMP in 350 μm cross-chopped slices from the cerebral cortices of male Sprague/Dawley rats prelabelled with ^{3}H-adenine as described by Shimizu et al. (1969).

β-adrenoceptor stimulated cyclic AMP was defined as that accumulating in the presence of a maximally effective concentration of isoprenaline (10^{-5}M) whilst the α-adrenoceptor potentiation was the difference between the maximum response to noradrenaline (NA 10^{-4}M) which was 840±119% and isoprenaline which was 304±18% of basal (n=3). Phorbol 12-myristate 13-acetate (PMA) (10^{-5}M) had no effect alone but potentiated the isoprenaline response (585±60% of basal (n=3)).

Polymyxin B at concentrations up to 10 μM had no effect on cyclic AMP stimulation due to isoprenaline in the presence or absence of NA or PMA. 100 μM Polymyxin B alone produced a four-fold stimulation of cyclic AMP.

1(5-isoquinoline sulfonyl)-2 methyl piperazine) (H7) had no effect on basal levels of cyclic AMP at concentrations up to 300 μM, but enhanced the response to noradrenaline from 678±41% to 864±46% (n=3) of basal. The α-adrenoceptor component appeared to be unaffected. 300 μM H7 also enhanced the response to isoprenaline plus PMA.

Staurosporine (3 μM) had no significant effect on basal cyclic AMP formation but that due to isoprenaline was increased to 1248±198% of basal (n=3). Isoprenaline plus PMA stimulated cyclic AMP was also increased in the presence of 3 μM staurosporine, but if the enhancement due to β-adrenoceptor stimulation alone was accounted for, it was evident that the PMA potentiation was abolished. There was no apparent inhibition of the α-adrenoceptor potentiation by staurosporine.

The results provide some support for the contention that phorbol ester potentiation of β-adrenoceptor stimulated cyclic AMP proceeds via PKC activation, but even staurosporine, the most active PKC inhibitor available (Watson et al., 1988) failed to reverse the α-adrenoceptor enhancement and a different biochemical mechanism may underlie this modulation.

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[^{3}H]-FORSKOLIN BINDING IN THE VISUAL SYSTEM OF THE RAT AFTER UNILATERAL ORBITAL ENUCLEATION

Karen Horsburgh, Derek Chalmers and James McCulloch, Wellcome Surgical Institute, University of Glasgow, Garscube Estate, Bearsden Road, Glasgow G61 1QH.

Forskolin is a potent activator of adenylate cyclase. (^{3}H)-Forskolin has been used to map the anatomical distribution of adenylate cyclase in the CNS using quantitative autoradiography (Worley et al. 1986). Alterations of (^{3}H)-forskolin binding sites have been examined after lesioning of the visual pathway.

Male, black-hooded Long Evans rats (350-450g) were enucleated unilaterally (right eye) under 2% halothane anaesthesia. Receptor binding of (^{3}H)-forskolin to thaw-mounted brain sections was investigated at 1,5,10 and 20 days post-enucleation. After a pre-incubation at 20°C with two one-minute washes in 50 mm Tris-acetate buffer (pH 7.5) containing 10 mm $MgCl_2$; the sections were incubated in the buffer at 20°C for 20 min with 20 nM (^{3}H)-forskolin. Subsequently, they were washed twice for one min in buffer (4°C) and dipped in distilled H_2O. Non-specific binding was evaluated in adjacent tissue sections in the presence of 20 uM cold forskolin. In rats, 97% of the retinal efferent fibres are directed towards the contralateral hemisphere (Jeffery, 1984), thus allowing the ipsilateral (right) hemisphere to act as the reference against which changes in (^{3}H)-forskolin binding sites in the visually deprived (left) hemisphere could be contrasted.

Within the visual system of the un-operated control rats, the highest levels of (^{3}H)-forskolin binding were present in the visual cortex, layer IV (75 ± 4 pmol/g) and superior colliculus (80 ± 5 pmol/g) with a lower level in the dorsal lateral geniculate body (41 ± 3 pmol/g). The levels of (^{3}H)-forskolin binding in these regions in the ipsilateral hemisphere (non-visual deprived) did not deviate significantly from those in the control rats at any time after orbital enucleation (analysis of variance). Significantly lower levels of (^{3}H)-forskolin binding were present in the contralateral superior colliculus and lateral geniculate body at 5, 10 and 20 days post-enucleation, but remained unaltered in the visual cortex (Table 1). Thus, in the lateral geniculate body and superior colliculus approximately 20% of (^{3}H)-forskolin binding sites appear to be localised on retinal afferents to these regions. Chronic depression of cortical function is associated with minimal alterations in the number of (^{3}H)-forskolin binding sites.

TABLE 1. Interhemispheric differences in (^{3}H)-forskolin binding at varying times, post-enucleation.

	Ligand bound (ipsi-contra) (pmol/g tissue)				
Visual Areas:	Control (n=6)	1-Day (n=3)	5-Day (n=6)	10-Day (n=5)	20-Day (n=4)
Visual Cortex: Layer IV	-1 ± 1	3 ± 1	3 ± 1	0 ± 1	-1 ± 1
Superior Colliculus (Superficial Layer)	-3 ± 1	3 ± 1	-14 ± 2*	-14 ± 1*	-19 ± 2*
Dorsal Lateral Geniculate Body	-2 ± 1	-3 ± 1	-6 ± 2*	-8 ± 2*	-7 ± 1*
Non-Visual Areas:					
Auditory Cortex	1 ± 2	-1 ± 2	6 ± 4	-1 ± 2	2 ± 2
Inferior Colliculus	0 ± 1	-2 ± 2	1 ± 1	2 ± 1	-1 ± 1

*$p < 0.005$ significant difference from ipsilateral to contralateral.

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INTERACTION BETWEEN A TRH ANALOGUE (RX 77368), SUBSTANCE P AND PROCTOLIN ON RAT SPINAL CORD NORADRENALINE RELEASE IN VITRO

J.V. Johnson, K.C.F. Fone, C.A. Marsden and G.W. Bennett, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Immunohistochemical evidence shows that bulbospinal serotonergic neurones contain substance P, thyrotrophin-releasing hormone (TRH) and a proctolin-like peptide in addition to 5-hydroxytryptamine. Intrathecal TRH or TRH analogue injection produces wet-dog shake (WDS) behaviour in rats which is antagonised by pretreatment with the α_1-adrenoceptor antagonist prazosin suggesting that spinal noradrenergic neurones may be involved in this behaviour (Fone et al., 1987). Furthermore, previous in vitro studies show that the TRH analogue RX 77368 (pGlu-His-3,3-dimethyl-ProNH_2) augments potassium-induced noradrenaline release from rat ventral cord slices (Johnson et al., 1988). Since RX 77368-induced WDS are also antagonised by intrathecal proctolin pretreatment (Fone et al., 1988), the present study examined the effect of proctolin and substance P on RX 77368 and/or potassium-induced noradrenaline release from rat ventral cord slices.

The ventral thoracic spinal cord of rats were dissected, sliced, washed and incubated (Johnson et al., 1988) with RX 77368, proctolin or substance P and/or 15 mM potassium (20 µl). In separate experiments tissue was pre-incubated with substance P, proctolin or tetrodotoxin (TTX) for 5 min before adding RX 77368 and 15 mM potassium. Slices were then rapidly centrifuged (2000 g) and the noradrenaline content of the supernatant determined (Johnson et al., 1988). Results (n=6 in each group) were analysed using Student's t-test.

As previously noted RX 77368 (10^{-4}M) augmented the release of noradrenaline produced by addition of 15 mM K^+ to ventral cord slices (Johnson et al., 1988). The noradrenaline release evoked by RX 77368 ($P<0.001$) and potassium alone ($P<0.05$) were both reduced to basal levels (2.65±0.26 pmol/mg protein) by pre-incubation with TTX (10^{-7}M) suggesting that the TRH analogue may not be acting directly on noradrenergic nerve terminals although K^+-induced noradrenaline release was also abolished by TTX. Proctolin at 10^{-4}M, but not at 10^{-5}M, significantly decreased ($P<0.05$) K^+-induced noradrenaline release. Furthermore, pre-incubation of slices with proctolin (10^{-5}M) significantly attenuated ($P<0.05$) the RX 77368-induced release of noradrenaline, although this concentration of proctolin had no effect when it was added at the same time as RX 77368. In contrast, substance P (3×10^{-5}M and 10^{-4}M) had no significant effect on either the noradrenaline release evoked by 15 mM K^+ or (at 3×10^{-5} M) the augmentation of K^+-induced noradrenaline release evoked by RX 77368.

In conclusion, proctolin and RX 77368 have opposite effects on K^+-induced noradrenaline release from ventral cord slices while substance P is without effect at the doses tested. The ability of proctolin to attenuate RX 77368-induced noradrenaline release may contribute to the reduction in TRH analogue-induced WDS behaviour produced by intrathecal proctolin injection in conscious rats (Fone et al., 1988).

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κ RECEPTOR BINDING IN THE GUINEA-PIG CEREBELLUM: THE PRESENCE OF A DYNORPHIN-RESISTANT BINDING SITE THAT IS NOT μ OR δ

J.A.Carroll and J.S.Shaw, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Guinea-pig cerebellar membranes have been shown to express predominantly kappa opioid receptors (80-90%), with very low levels of mu and delta receptor contamination (Robson et al, 1984). Non-selective opioid ligands such as [^{3}H]bremazocine have therefore been used in this tissue, to selectively label the kappa receptor.

In this study guinea-pig cerebellum membranes were prepared in HEPES buffer at pH 7.4, according to the methods of Magnan et al. (1982). [^{3}H]bremazocine (0.2nM) and [^{3}H]EKC (0.5nM) binding assays were run for 40 min at 25°C in HEPES buffer (pH 7.4). Non-specific binding was defined using 10μM naloxone.

[^{3}H]bremazocine binding, under these conditions, was apparently selective for the kappa opioid receptor, with no evidence of any significant mu, delta or sigma receptor contamination. However, Hill coefficients of less than one ($P<0.05$) were obtained for all the agonists tested, with values of 0.639, 0.537 and 0.640 respectively for the kappa ligands U50488 and U69593 and the mu selective agonist [D-Ala^2,(Me)Phe^4,Gly(ol)^5]enkephalin (GLYOL). This finding is inconsistent with displacement from a single binding-site population. In addition, displacement curves to the kappa opioid peptides dynorphin A 1-13 and dynorphin A 1-17 were clearly biphasic, and suggested the presence of a "dynorphin resistant" component, comprising 15-20% of the specific binding.

The addition of 3μM [D-Ala^2,D-Leu^5]enkephalin (DADLE) to the assay, to suppress any remaining mu or delta receptor binding, produced some steepening of the U50488 displacement curve, but had no significant effect on the Hill coefficents for U69593 or the mu selective agonists GLYOL and morphine. There was no reduction in the "dynorphin resistant" component of specific binding.

Similar results were also obtained using the opioid agonist ligand [^{3}H]EKC, with Hill coefficients of 0.540 and 0.569 ($P<0.05$) for GLYOL and U69593 respectively. The "dynorphin resistant" component in this case comprised approximately 10% of the specific binding. There was no significant alteration in either the slopes, or the proportion of "dynorphin resistant" binding, in the presence of 10μM DADLE.

These results suggest that both [3H]bremazocine and [3H]EKC label a naloxone sensitive binding-site in guinea-pig cerebellum membranes, that cannot be defined as a high affinity mu, delta or kappa opioid site.

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INFLUENCE OF GR38032F ON THE BEHAVIOURAL CONSEQUENCES OF CEASING SUB-CHRONIC TREATMENT WITH DRUGS OF ABUSE

B. Costall, A.M. Domeney, B.J. Jones[1], M.E. Kelly, P.A. Gerrard, R.J. Naylor and M.B. Tyers[1], Department of Pharmacology, University of Bradford, Bradford, W. Yorks., BD7 1DP, and [1]Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DJ, U.K.

Mice (male, BKW, 25-30g) were treated for 7-14 days with diazepam (10mg/kg i.p., b.d.), nicotine (0.1mg/kg i.p., b.d.), cocaine (1.0mg/kg i.p., b.d.) or ethanol (8% w/v in drinking water) and were tested both during treatment (days 1, 3, 7 and 14) and after ceasing treatment (8, 24 and 96h) using a black:white test box (45 x 27 x 27cm, 2/3 white, brightly illuminated, 1/3 black, dimly illuminated). Naive mice were used on each test occasion. They were taken from dark home conditions and normally showed aversion to the brightly-lit environment.

During the treatment period, control, vehicle-treated mice moved into the black within 10-12s and exhibited most behaviour in the black (52-56% time spent in black, 51.6±5.4 rears/5min, 55.8±5.4 crossings/5min), corresponding reductions in behaviour occurring in the white section (24.2±3.1 rears/5min, 36.7±3.9 crossings/5min). For mice receiving diazepam (day 3, i.e. after tolerance to sedation), nicotine, cocaine or ethanol, behaviour in the black:white box redistributed in favour of the white (i.e. max. change [rears/5min] for diazepam was to 80.7±8.4, nicotine 79.6±8.1, cocaine 75.1±7.7, ethanol 74.3±7.6, all increases above control significant to $P<0.001$). The increased rearings in the white were associated with increased line crossings with corresponding decreases in behaviour in the black. Time spent in the black also decreased and latency to move to the black was delayed (to 17-38s, $P<0.01$-$P<0.001$ for all drugs).

Within 24h of ceasing treatment with diazepam, nicotine, cocaine or ethanol, the behaviour in the black and white sections redistributed in the opposite direction to that recorded during drug intake, with a marked preference for the black environment (latency to move to the black decreased to 2-5s, rearing in the black increased to 62.6±6.7 to 81.2±8.3/5min, line crossings increased to 79.7±8.1 to 89.4±9.0/5min, with time spent in black increasing to 61-70%, $P<0.001$ for all changes). This change in behaviour observed after ceasing treatment with the drugs of abuse could be prevented if mice were treated at the time of cessation of dosing with GR38032F (1-10µg/kg i.p. b.d.). Indeed, after treatment with all doses of GR38032F behaviour reverted to a preference for the white (max. changes: latency 33s, rearing in black 8.4±0.9/5min, line crossings in black 7.4±0.9/5min, % time 31, $P<0.001$ for all data).

In the marmoset using a human threat test (Costall et al., 1988), similar changes in behaviour were observed for nicotine (0.01mg/kg s.c. b.d. 14 days), cocaine (1.0mg/kg s.c. b.d. 14 days) and ethanol (1% w/v 7 days + 2% 35 days in blackcurrant juice). During treatment these drugs decreased the response to threat (assessed by characteristic posturing and time confronting the threat). The opposing behaviours which developed upon ceasing treatment with the drugs of abuse could be prevented by GR38032F (0.01mg/kg s.c.).

It is suggested that the behavioural consequences of ceasing sub-chronic treatment with diazepam, nicotine, cocaine and ethanol may be prevented by the selective 5-HT_3 receptor antagonist, GR38032F.

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