650P

OPIATE INFLUENCES ON DRUG-INDUCED YAWNING IN THE RAT

H.H.G. Berendsen and Alma J. Gower, Department of CNS Pharmacology, Scientific Development Group, Organon, Oss, The Netherlands.

Yawning can be elicited in laboratory animals by various pharmacological agents including apomorphine and physostigmine (Yamada and Furukawa, 1980; Holmgren et al, 1980). The response has been shown to be influenced by androgens: castration will reduce drug-induced yawning and pretreatment with testosterone will restore it (Berendsen and Nickolson, 1980; Holmgren et al, 1980). Since several studies, e.g. Cicero et al (1980), indicate an interaction between androgens and opiates, we tested whether an opiate link was involved in the effect of testosterone on yawning.

All experiments were carried out between 8.30-12.30, using naive male Wistar rats weighing 250-350 g. The effects of naloxone HCl, morphine HCl, haloperidol and atropine SO_4, injected subcutaneously (s.c.) 20 min. before testing, were determined on apomorphine HCl (40 and 80 µg/kg s.c.) or physostigmine salicylate (100 µg/kg s.c.) induced yawning in normal animals. Yawning responses were observed in perspex observation cages (7.5 x 18 x 30 cm) as described previously (Berendsen and Nickolson, 1980) over a 20 min. period starting immediately after apomorphine or 10 min. after physostigmine. The effects of single doses of naloxone, morphine, haloperidol and atropine were also determined on the dihydrotestosterone propionate-(DHTP) mediated increases in apomorphine-induced yawning in chronically castrated (at least 6 weeks) rats. DHTP, 125 µg/rat/day or arachis oil was injected s.c. once daily for 3 consecutive days. 24 h. later test drugs were injected s.c. 20 min. prior to apomorphine (80 µg/kg) and yawning scored as stated above.

Naloxone (1-10 mg/kg) partially antagonised apomorphine or physostigmine-induced yawning. In castrated rats, naloxone, 10 mg/kg inhibited the DHTP mediated increases but had no effect on the low level residual apomorphine yawning. In normal rats, 0.32 and 1 mg/kg morphine had no effect on apomorphine yawning but 3.2 mg/kg caused marked inhibition; morphine (0.32-3.2 mg/kg) produced dose related inhibition of physostigmine yawning. In castrated rats, morphine, 1 mg/kg, inhibited the residual apomorphine response but had no significant effect on the DHTP-mediated increases. Haloperidol (0.01-0.1 mg/kg) reduced apomorphine yawning in normal rats. Haloperidol 0.01 mg/kg significantly increased physostigmine yawning but higher doses, 0.03 and 0.1 mg/kg had no significant effect. Atropine reduced both apomorphine and physostigmine yawning. In castrated rats, haloperidol 0.1 mg/kg and atropine 10 mg/kg inhibited both residual apomorphine yawning and the DHTP-mediated increases.

The finding that naloxone could not completely block yawning in normal rats, but blocked the DHTP effects suggests that this drug inhibits drug-induced yawning by antagonising the androgenic influences. However, in these experiments, there was no evidence that morphine treatment will enhance yawning. The blocking by haloperidol and atropine of yawning in normal rats and in castrated rats treated with DHTP or arachis oil, supports the hypothesis that DHTP acts as a permissive agent on yawning (Berendsen and Nickolson, 1980). The effects of these drugs also confirm previous findings (Yamada and Furukawa, 1980) of an interaction between dopaminergic and cholinergic effects on yawning.

Berendsen, H.H.G. and Nickolson, V.J. (1980). Behav. and Neural. Biol. 33, 123-128
Cicero, T.J. et al. (1980). Brain Res. 202, 151-164
Holmgren, B. et al. (1980). Acta Neurobiol. Exp. 40, 515-519
Yamada, K. and Furukawa, T. (1980). Psychopharmac. 67, 39-43

FURTHER STUDIES ON THE BIOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF THE ENANTIOMERS OF MIANSERIN

A.M. Johnson, D.R. Nelson, Jane M. Nicholass, P.E. Richards, and D.R. Thomas, Beecham Pharmaceuticals Research Division, Harlow, Essex, CM19 5AD.

Mianserin, an effective antidepressant, is a racemic mixture of the S(+) and R(-) enantiomers (Van Rij and Feil, 1973). Schoemaker et al (1981) recently reported that the S(+) enantiomer, like racemic mianserin, potentiated K^+ evoked release of [^{3}H]-noradrenaline from rat brain cortex slices at 10^{-9} to 10^{-5}M whereas the R(-) enantiomer was inactive at 10^{-6}M. We now report that the S(+) enantiomer and racemic mianserin at 2 x 10^{-7}M also significantly inhibit p-aminoclonidine-induced inhibition of the contractions of the isolated guinea-pig ileum due to transmural stimulation, whereas the R(-) enantiomer is inactive at this concentration (dose ratios 10, 5.4 and 1.1 respectively). This stereoselectivity also extends to in vivo tests as the S(+) enantiomer is more active than the R(-) enantiomer at inhibiting clonidine-induced suppression of the pinna reflex in mice (ED_{50} 12.6 compared to >30mg/kg p.o.). Similarly, clonidine-induced hypoactivity in rats is significantly inhibited (31%) by the S(+) but not by the R(-) enantiomer when administered at 25mg/kg i.p.

Interestingly, the ability of the enantiomers to inhibit [^{3}H]-clonidine binding to rat cortex membranes in vitro is similar (Ki's 85 and 108nM for the S(+) and R(-) enantiomers, respectively), thus supporting evidence that [^{3}H]-clonidine does not predominantly bind to presynaptic α_2-adrenoceptor sites. In contrast, evidence of stereoselectivity at α_1-adrenoceptors has been obtained as the S(+) enantiomer is 10 times more potent than the R(-) enantiomer at inhibiting [^{3}H]-prazosin binding to rat cortex membranes in vitro (Ki's 320 and 3200nM, respectively).

Pinder and van Delft (1983) reported that the S(+) enantiomer was 4.5 times as potent as the R(-) enantiomer at inhibiting [^{3}H]-mianserin binding to rat cortex membranes (IC_{50}'s 4 and 18nM, respectively). We now report that in the presence of excess mepyramine to block binding to histamine (H_1) sites and hence to restrict the binding of [^{3}H]-mianserin to 5HT$_2$ sites, the S(+) enantiomer is 10 times more potent than the R(-) enantiomer (Ki's 2.9 and 30nM, respectively). As would be predicted from our binding data, the S(+) enantiomer is more potent than the R(-) enantiomer at inhibiting 5-methoxy-N,N-dimethyltryptamine-induced head twitches in mice, ED_{50}'s 1.7 and >10mg/kg p.o., respectively. Interestingly, the difference in potency between the enantiomers at inhibiting the 5-hydroxytryptophan-induced body shakes in rats is considerably less, 74% and 63% inhibition at 0.3 mg/kg p.o., S(+) and R(-) enantiomers, respectively, suggesting that the two procedures differ in their basic mechanisms.

The similar potency of the two enantiomers in inhibiting [^{3}H]-mepyramine binding to rat cortex membranes, in vitro, (Pinder and van Delft, 1983) contrasts with the small but significant difference observed in their ability to inhibit the binding of [^{3}H]-mepyramine in mouse whole brain, in vivo (ED_{50}'s 0.7 and 2 mg/kg p.o. for the S(+) and R(-) enantiomers, respectively).

In conclusion, these results confirm and extend the results of Pinder and van Delft (1983) that the enantiomers of mianserin exhibit stereoselectivity for 5HT and NA receptors, the S(+) enantiomer being more potent than the R(-) form, whereas the difference on histamine receptors is less marked.

Pinder, R.M. and van Delft, A.M.L. (1983) Br. J. Clin. Pharmacol. 15, 269S-276S.
Schoemaker, H. et al (1981) Psychopharmacol., 74, 137-142.
van Rij, C, and Feil, D. (1973) Tetrahedron, 29, 1891-1893.

We thank Dr. R.M. Pinder, Organon, Oss, for the enantiomers and Frances Jewitt, Jane Patterson, and Debbie Shreeve for excellent assistance.

ANALYSIS OF CORTICAL EEG SYNCHRONIZATION INDUCED BY CLONIDINE IN THE CONSCIOUS RABBIT

B. Gadie, P.S. McCarthy & I.F. Tulloch, Department of Pharmacology, Reckitt and Colman, Dansom Lane, Hull, HU8 7DS.

Clonidine induces EEG synchronisation in a number of animal species, an effect which is associated with pronounced behavioural sedation. This action of clonidine on EEG activity is thought to be mediated by activation of central α_2-adrenoceptors (Florio et al, 1975; Dettmar et al, 1983). In this study we have attempted to characterise the cortical EEG synchronisation produced by clonidine in the conscious rabbit, using a computerised analysis method to measure EEG amplitude and frequency. The central action of clonidine was also examined after treatment with the selective α_2-adrenoceptor antagonist idazoxan (RX 781094) (Doxey et al, 1983).

In conscious rabbits (male NZW, 2.5 - 3 kg) EEG activity was recorded differentially between platinum ball electrodes implanted chronically over the frontal and parietal regions of the cerebral cortex. EEG signals were amplified (bandwidth 0.3 - 35 Hz) and routed via a Microlink A/D converter (Biodata) to a 32K CBM microcomputer. EEG voltage was digitized at 500 Hz during 10 s epochs and analyzed (Biodata Background EEG Analysis System) in terms of Hjorth parameters (Hjorth, 1970) to give direct on-line values of r.m.s. voltage (μV) and mean frequency (Hz). EEG activity was also monitored.

Clonidine (12.5 - 50 μg/kg,i.v.) caused an increase in cortical EEG amplitude and decrease in EEG frequency; the duration of synchronization was dose-related. During EEG synchronization the rabbits were clearly sedated. EEG changes seen after 50 μg/kg clonidine are shown in Table 1. At this dose the time to onset of EEG synchronization was 0.7 ± 0.2 min and the effect lasted 30.5 ± 2.6 min.

Table 1. Effect of clonidine on rabbit cortical EEG

Treatment	EEG amplitude (μV)	EEG frequency (Hz)
Saline (0.2ml/kg)	60.7 ± 3.0	13.9 ± 0.4
Clonidine (50μg/kg)	139.3 ± 7.2	7.5 ± 0.5

Values are mean EEG amplitude and frequency recorded in the 10 min period after clonidine or saline injection (n=10 rabbits).

Pretreatment (10 min) with idazoxan (0.1-1.0 mg/kg,i.v.) caused a dose-related antagonism of the EEG synchronization and sedation induced by clonidine (50 μg/kg). After the highest idazoxan dose the EEG amplitude and frequency recorded after clonidine were not significantly different from values obtained in untreated awake rabbits. Injection of idazoxan (1.0 mg/kg) caused an immediate but transient fine muscle tremor, lasting 1-2 min; no other consistent drug-related behavioural or EEG effects were observed. This dose exerted detectable antagonism of clonidine after pretreatment periods of up to 2h. In summary, these results indicate that clonidine induces EEG synchronisation and sedation in the rabbit by activation of central α_2-adrenoceptors.

Dettmar, P.W. et al (1983) Neuropharmacology (In press).
Doxey J.C. et al (1983) Br. J. Pharmac. 78, 489-505.
Florio, V. et al (1975) Neuropharmacology, 14, 707-714.
Hjorth, B. (1970) EEG Clin. Neurophysiol., 29, 306-310.

A NON-INVASIVE BEHAVIOURAL ASSESSMENT FOR ANXIOLYTIC DRUGS IN RATS

J.T. Rick, M. Simkins, J. Sutherland & P.V. Taberner[1], Department of Psychology, University of Sheffield, Sheffield S10 2TN and [1]Department of Pharmacology, University of Bristol Medical School, Bristol BS8 1TD.

Current methods for the behavioural assessment of anxiolytic drugs tend to utilize procedures which involve either aversive stimuli or time consuming observations of animal behaviour (File, 1980). At present there is no ideal animal model of anxiety with the advantages of behavioural relevance and automated measurement. However, the measurement of exploratory behaviour of weanling rats has recently been shown to differentiate between anxiolytics, sedative-hypnotics and stimulants (Salt & Taberner, 1983). In these experiments, the drugs were added to the mother's powdered diet; the weanlings receiving the drug via the mother's milk. The exploratory activity of the weanlings in an arena not accessible to the mother was quantified by ultrasonic detection (Morris & Taberner, 1980).

We now report experiments which investigate the dose-dependent effects of a series of benzodiazepines in this model. Hooded Lister rats with litters of 6 pups were used. Mothers were placed on a powdered diet, with or without drug, when the pups were 11 days old and testing book place daily from day 16 to weaning at day 24. Each test lasted one hour. The breeding cage was connected to an enclosed arena by a short circular tunnel of 5cm diameter. The pups' entry and activity in the arena was monitored by ultrasonic detection and recorded using a Nascom II microprocessor. Drugs were added to the food as follows: chlordiazepoxide, 2.5 or 5mg/10g; nitrazepam, 0.5mg/10g; clobazepam, 1 or 2mg/10g.

Clobazepam and chordiazepoxide significantly increased exploratory activity in the arena in a dose-dependent manner whereas nitrazepam reduced activity from the control (drug-free) level. The average daily consumption of food by the mother was also measured under drug and control conditions. Food consumed was significantly increased by chlordiazepoxide and reduced by the higher dose of clobazepam. With both chlordiazepoxide and clobazepam the total daily activity of the litter showed a significant positive correlation (Table 1) with the amount of drug consumed over the previous 24h. Subsequent assays of plasma drug concentrations in mothers and weanlings were carried out. It is concluded that the exploratory behaviour of weanlings can be used to distinguish between the anxiolytic and sedative effects of the benzodiazepines in a dose-dependent manner.

Table 1. Exploratory activity of litters versus maternal drug consumption

	Number of daily pairings	Spearman's Rank Correlation, rho	Probability
Clobazam	22*	0.65	$p<0.005$
Librium	18*	0.58	$p<0.025$
Nitrazepam	9	0.21	n.s.

The activity of the litter, in a one-hour test, was directly proportional to the amount of clobazam or librium consumed by the mother over the 24h prior to the test period. This relationship was not observed with diazepam.

*Combined observations from two litters

This work was supported in part by an MRC project grant.

File, S.E. (1980) J. Neurosci. Methods, 2, 219
Morris, R.G. & Taberner, P.V. (1980) Br. J. Pharmac. 70, 189P
Salt, J.S. & Taberner, P.V. (1983) Progr. Neuropsychopharmacol. in press

INHIBITION BY β-ENDORPHIN AND D-ALA^2,D-LEU^5-ENKEPHALIN OF MEMBRANE-BOUND ADENYLATE CYCLASE IN THE RAT VAS DEFERENS

K.D. Bhoola & S. Pay, Department of Pharmacology, The Medical School, University of Bristol, Bristol BS8 1TD.

Opiate receptors are widely distributed and the question arises as to whether peripheral receptors, like central receptors, are coupled to adenylate cyclase. Recently the experimental conditions necessary to demonstrate inhibition of adenylate cyclase coupled to central receptors have been characterised using rat striatal membranes 'in vitro' (Law et al, 1981; Pay & Bhoola, 1983). The aim of the present study was to determine the effect of β-endorphin, D-ala^2-D-leu^5-enkephalin and morphine hydrochloride on the activity of adenylate cyclase in membrane fragments from the rat vas deferens.

Adult male rats were killed by cervical dislocation, the vasa deferentia removed, and homogenized (ultraturrax) for 15 seconds in ice cold buffer. The source of the adenylate cyclase was the 25,000g x 30 minute particulate fraction obtained after prior removal of the 800g x 10 minute pellet. Adenylate cyclase was measured according to the method of Albano et al (1973) and the concentration of cyclic AMP produced was measured by the binding protein method of Brown et al (1971).

In membrane fragments from rat vas deferens, β-endorphin (10^{-5}M) inhibited adenylate cyclase activity but only in the presence of GTP (10^{-8} - 10^{-5}M). The inhibition produced by β-endorphin and D-ala^2-D-leu^5-enkephalin (10^{-7} - 10^{-5}M) was dose dependent and reversed by the δ-opiate receptor antagonist ICI 154129 (10^{-8} - 10^{-5}M) (Shaw et al, 1982). Under the same conditions morphine hydrochloride (10^{-5}M) failed to inhibit adenylate cyclase activity.

Our results suggest that opiate receptor mediated inhibition of adenylate cyclase associated with membrane fragments prepared from the rat vas deferens, shows similar experimental requirements to those observed with rat brain striatal membranes. The ability of β-endorphin and D-ala^2-D-leu^5-enkephalin, and the failure of morphine hydrochloride to inhibit the adenylate cyclase of the rat vas deferens, supports the work of Lemaire et al (1978) who found β-endorphin and D-ala^2-D-leu^5-enkephalin more potent than morphine in inhibiting the electrically-evoked contraction of the isolated rat vas deferens. These results suggest that the predominant population of opiate receptors in this tissue are δ-receptors.

Sarah Pay is a SERC student.

Albano, J.D.M. et al (1973) Biochem. Soc. Trans. 1, 477-479
Brown, B. et al (1971) Biochem. J. 121, 561
Law, P.Y. et al (1981) J. Neurochem. 36, 1834
Lemaire, S. et al (1978) Br. J. Pharmacol. 64 (3), 327-9
Pay, S. & Bhoola, K.D. (1983) Adv. Cyc. Nuc. Res. in press
Shaw, J.S. et al (1982) Life Sci. 31, 1259-1262.

EFFECTS OF THE R- AND S-ENANTIOMERS OF SK & F 38393 ON (^{3}H)-PIFLUTIXOL AND (^{3}H)-SPIPERONE BINDING TO D_1 AND D_2 DOPAMINERGIC SITES

K.M. O'Boyle & J.L. Waddington, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2.

Evidence for the division of dopaminergic receptor sites into D_1 and D_2 subtypes derives from the profiles of drugs in inhibiting the stimulation of adenylate cyclase activity and in displacing ^{3}H-butyrophenone binding (Seeman, 1980). The introduction of ^{3}H-piflutixol as an antagonist ligand for the D_1 site (Hyttel, 1982) has facilitated such studies. However, selective agonists at the ^{3}H-piflutixol site have not yet been identified. SK & F 38393 (2,3,4,5,-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine) has been considered as a selective D_1 agonist (Setler et al, 1978; Waddington et al, 1982). We have studied the activities of its resolved *R*- and *S*-enantiomers in displacing the binding of ^{3}H-piflutixol, in comparison with that of ^{3}H-spiperone to the D_2 receptor.

Specific binding of 0.3nM ^{3}H-piflutixol to rat striatal membrane preparations was defined by 5 µm butaclamol and showed a typical D_1 profile; the rank order of displacement potencies was cis(Z)-flupenthixol > dopamine, with domperidone and metoclopramide displacing < 50% of specific binding at 10µM and 100µM respectively. Specific binding of 0.1nM ^{3}H-spiperone was defined by 1µM domperidone and showed a typical D_2 displacement profile of domperidone = cis(Z)-flupenthixol > metoclopramide > dopamine. 1µM domperidone was routinely included in ^{3}H-piflutixol assays to occlude residual binding to D_2 sites. The affinities of racemic SK & F and of its resolved enantiomers at these two sites are compared in the Table:

DRUG	IC_{50} (nM) ^{3}H-PIFLUTIXOL	^{3}H-SPIPERONE
R- SK & F 38393	810 ± 450	33,300 ± 7,280
S- SK & F 38393	>100,000	>50,000
racemic SK & F 38393	2,030 ± 230	30,000 ± 9,810

Means ± SEM (n = 3).

R- SK & F 38393 stereoselectively displaced ^{3}H-piflutixol binding, its *S*-antipode being >100 x less active. The enantiomers each showed little affinity for the D_2 site, which did not appear to show the same degree of stereospecificity. The relative selectivity of *R*- SK & F 38393 in displacing ^{3}H-piflutixol binding suggests it may be useful in further characterising the pharmacology of the D_1 site.

This work was supported by the Medical Research Council of Ireland and The Royal College of Surgeons in Ireland. We thank Smith Kline & French for 38393 enantiomers and Lundbeck for ^{3}H-piflutixol.

Hyttel, J. (1982). Adv. Biosci. 37, 147-152.
Seeman, P. (1980). Pharm. Rev. 32, 229-313.
Setler, P.E. et al (1978). Eur. J. Pharmacol. 50, 419-430.
Waddington, J.L. et al (1982). Adv. Biosci. 37, 143-146.

TRITIUM FILM AUTORADIOGRAPHY OF κ OPIATE RECEPTORS IN RAT BRAIN

S. Patel & P. Slater, Department of Physiology, Medical School, University of Manchester, Manchester M13 9PT

Binding studies using neuronal membranes have identified a unique κ-type opiate receptor in rat brain (Wood et al, 1981). We have used a quantitative autoradiographic technique to investigate the detailed distribution of κ binding sites in rat brain using [^{3}H]bremazocine as the ligand (Römer et al, 1980).

Experiments were performed using slide-mounted, cryostat-cut sections (15 μm) of brain from female Sprague Dawley rats. Each section was covered with 0.1 ml of 0.17 M Tris HCl buffer (pH 7.4) containing 1 nM [^{3}H]bremazocine and 100 nM [D-Ala^2, -Me Phe^4,Gly-ol^5]enkephalin and 100 nM [D-Ala^2,D-Leu^5]enkephalin to block any binding to μ and δ sites (Kosterlitz et al, 1981). After 30 min incubation at 2°C, the sections were washed twice in fresh buffer (3 min, 2°C), dipped briefly in ice-cold water and dried rapidly. The sections were exposed to tritium-sensitive film (LKB) at 4°C for 20 weeks. The autoradiographs were analysed using a fibre optic microdensitometer (Morten at al,1982). Non-specific binding was determined by co-incubating sections with 1 μM ethylketocyclazocine (EKC) and scraping the sections into vials for liquid scintillation counting.

Some guinea pig brain sections, treated exactly as above, were included in the study.

[^{3}H]Bremazocine bound to many areas of rat brain, with low binding to white matter. Nearly all the binding was displaced by 1 μM EKC - the mean specific binding defined in this way was 89% of the total.
The highest concentrations of κ-sites were in the nucleus accumbens, striatum, claustrum, fornix, medial habenula, superficial layer of superior colliculus and hippocampus. In the rostral striatum, there were dense patches of [^{3}H]bremazocine binding, similar to the binding of [^{3}H]naloxone (Kent et al,1982). Most of the hippocampal κ-sites were concentrated in the pyramidal cell layer and were entirely absent from the corresponding layer in the dentate gyrus.
There were moderate concentrations of κ-sites in some thalamic nuclei,the stria terminalis, periaqueductal grey and medial geniculate body.
The globus pallidus had a very low density of κ-sites.
The density and distribution of κ-sites in the cerebral cortex of rats and guinea pigs were compared. Rat, but not guinea pig, cortex had a high density of receptors in layer 1. There were low to moderate levels of κ-sites in layers 2-6 of rat cortex. By contrast, layers 5 and 6 of guinea pig cortex had a very high density of κ-sites, as described by Foote & Maurer (1982).

The findings show that there are considerable regional variations in the density of κ-sites in rat brain. The rat cerebral cortex has fewer κ-sites than the guinea pig cortex and also lacks the pronounced concentration of these receptors in the deep cortical layers.

S.P. is supported by S.E.R.C.
[^{3}H]Bremazocine was donated by Sandoz Ltd.

Foote,R.W. & Maurer,R. (1982) Eur.J.Pharmac. 85, 99
Kent,J.L. et al (1982) Dev.Brain Res. 2, 487
Kosterlitz,H. et al (1981) Br.J.Pharmac. 73, 939
Morton,B.E. et al (1982) J.Neurosci.Meth. 6, 113
Römer, D. et al (1980) Life Sci. 27, 971
Wood, P.L. et al (1981) Neuropharmac. 20, 1215

EFFECTS ON LIMB TONE OF NEUROTENSIN, TRH AND β-ENDORPHIN INJECTED INTO THE PERIAQUEDUCTAL GREY REGION OF RAT BRAIN

E.C. Griffiths, P. Slater & P.S. Widdowson, Department of Physiology, University of Manchester, Manchester M13 9PT

Neurotensin (NT), thyrotrophin-releasing hormone (TRH) and β-endorphin are present in the periaqueductal grey area (PAG) (Emson et al,1981, 1982; Finley et al,1981). Opiates cause limb rigidity when injected into the PAG (Jacquet & Lajtha,1974) whereas NT given intracerebrally was reported to produce muscle relaxation (Nemeroff et al, 1980). The interactive effects of NT, TRH and β-endorphin on limb tone have been investigated after injection into the PAG.

Male Sprague-Dawley rats (180-200g) were anaesthetized and fitted with a guide cannula for micro-injections in PAG (A 0.6, L 0.45, H + 0.5; König & Klippel, 1963). After 6 days, a limb tone apparatus (Dickinson & Slater,1982) was used to measure the force needed to partly flex each hind limb both before and after peptide injection in PAG. Synthetic peptides (Universal Biologicals,Cambridge) were injected in a total 1μℓ of sterile saline solution and injection sites were subsequently confirmed histologically.

NT (0.5 - 10 μg) in PAG caused a dose-related reduction of limb tone. The maximum effect of the 1 μg dose was recorded 30 min after injection (Table 1). TRH (0.5 - 5 μg) in PAG had no effect on limb tone. β-endorphin (2.5 μg) in PAG caused a pronounced, naltrexone-reversible, limb rigidity, whereas naltrexone had no effect on NT-induced relaxation. Peptide interactions in PAG were also investigated:- TRH (1-5 μg) partly prevented the NT-induced relaxation but had no effect on β-endorphin-induced rigidity.

Table 1 Effects of peptides in PAG on hind limb tone

Injection	Mean limb tone (g) Initial	30 min
Saline	63 ± 3	61 ± 4
TRH (1 μg)	60 ± 3	55 ± 4
NT (1 μg)	70 ± 3	34 ± 4[a]
NT (1 μg) + naltrexone (5 μg)	62 ± 2	32 ± 2[a]
β-endorphin (2.5 μg)	52 ± 2	75 ± 2[a]
β-endorphin (2.5 μg) + naltrexone (5μg)	56 ± 2	55 ± 5
β-endorphin (2.5 μg) + TRH (1 μg)	51 ± 2	80 ± 2[a]
NT(1 μg) + TRH (1 μg)	66 ± 3	41 ± 2[b]
NT(1 μg) + TRH (5 μg)	68 ± 3	50 ± 3[b]

[a]$p<0.01$ vs.saline; [b]$p<0.05$ vs. NT alone; n= 6-8 ± S.E.M.

The findings demonstrate that the PAG is a sensitive site for controlling limb muscle tone in the rat. NT and β-endorphin have opposite effects and the partial reversal of NT-muscle relaxation by TRH provides a further example of specific interactions between the two peptides.

P.S.W. is supported by the S.E.R.C.

Dickinson, S.L. & Slater, P. (1982) Neuropharmac. 21, 787
Emson, P.C. et al (1981) Neuropeptides 2, 115
Emson, P.C. et al (1982) J.Neurochem. 38, 992
Finley, J.C.W. et al (1981) Neuroendocr. 33, 28
Jacquet, Y. & Lajtha, A. (1974) Science 185, 1055
König, J.F.R. & Klippel, R.A. (1963) The Rat Brain: A Stereotaxic Atlas.Publ. Williams & Wilkins, Baltimore
Nemeroff,C.B. et al (1980) Trends in Neurosci.3,212.

IS THE THALAMO-STRIATAL PATHWAY CHOLINERGIC?

S. Barrington-Ward, I.C. Kilpatrick[1], O.T. Phillipson[1] & C.J. Pycock,
Departments of Pharmacology and Anatomy[1], Medical School, University of Bristol,
Bristol BS8 1TD.

The transmitter chemistry of the pathways linking the thalamus with the caudate-putamen complex (CP) is controversial. Early reports that thalamo-CP fibres were non-cholinergic (e.g. McGeer et al, 1971), appear at variance with more recent suggestions that significant decreases of striatal choline acetyltransferase (ChAT) could be obtained following lesions of the parafascicular nucleus (PF) of the thalamus (e.g. Simke & Saelens, 1977). Recent anatomical studies have shown that the thalamic input to the rat CP is more extensive than was previously thought and arises also from neurones rostral to the PF in adjacent intralaminar cell groups (Veening et al, 1980). The present study re-examines the possible cholinergic nature of this projection in the rat by making lesions not only in the PF but also in the intralaminar thalamus and subsequently assaying ChAT activity in microdissected regions of CP.

Female Wistar rats (~200g) were anaesthetised with 1.5% halothane in O_2 and placed in a stereotaxic frame. Via burr holes in the skull, unilateral radio-frequency lesions (49°C, 1min) or sham lesions were placed at two sites in the PF and adjacent intralaminar thalamus (co-ords: Caudal to bregma, -2.7; L 1.1; V 6.0 and -3.4; L 1.0; V 5.6). At various survival times, rats were killed by cervical dislocation, brains rapidly removed and the entire CP sectioned coronally into 6 x 500μm blocks on a 'Vibroslice' following immersion in an ice-cold Krebs' solution. Under direct microscopic control, the CP sections were further divided into medial and lateral segments, taking care to avoid globus pallidus contamination. ChAT activity of each segment was assayed according to the method of Fonnum (1975). The diencephalon was retained for histological examination of the lesion sites.

In confirmation of the results of Rea & Simon (1981), significantly higher ($p < 0.05$) ChAT activities were detected in lateral CP (111.5 ± 10.5 nmol ACh formed h^{-1} mg $protein^{-1}$) compared with medial CP (80.4 ± 5.6 nmol h^{-1} mg^{-1}), although no evidence for a rostro-caudal enzyme activity gradient was obtained.

Following either 7 or 14 days survival, no significant alterations in ChAT activity could be detected at any level of the CP or in any medial or lateral segments from 18 animals, despite histological confirmation of adequate thalamic lesions encompassing PF, centrolateral and part of the centromedian cell groups.

These results cannot support recent proposals that thalamo-striatal fibres are cholinergic. Indeed, preliminary evidence indicates that thalamic efferents to the rat motor cortex (from PF, n=6), prefrontal cortex (from mediodorsal nucleus, n=6) and nucleus accumbens (from periventricular nucleus, n=6) are also non-cholinergic. These data complement immunocytochemical studies in rats using monoclonal antibodies directed against ChAT, which failed to show immunoreactive perikarya anywhere in the thalamus (Sofroniew et al, 1982), and perhaps offer an axiom for the non-cholinergic nature of thalamic output neurones.

Supported by the Wellcome Trust

Fonnum, F. (1975) J. Neurochem. 24, 407-409
McGeer, P.L et al (1971) Brain Res. 35, 308-314
Rea, M.A. & Simon, J.R. (1981) Brain Res. 219, 317-326
Simke, J.P. & Saelens, J.K. (1977) Brain Res. 126, 487-495
Sofroniew et al (1982) Neurosci. Lett. 33, 7-12
Veening et al (1980) Neuroscience 5, 1253-1268

BEHAVIOURAL EFFECTS OF GABA-MIMETICS IN BARBITAL TREATED MICE

P.L. Gray & P.V. Taberner, Department of Pharmacology, University of Bristol, Bristol BS8 1TD.

It is well established that barbiturates augment GABA-mediated neuronal inhibition and recent observations of enhancement of [^{3}H]-GABA binding to brain membranes by barbiturates (Willow & Johnson, 1980; Olsen et al. 1981) suggest that this may occur at the level of the postsynaptic GABA receptor-ionophore complex. There is scant behavioural data on the augmentation of GABA effects by barbiturates: Sivam et al. (1982) used muscimol to assess the role of GABA in the acute and chronic effects of pentobarbital and found similar enhancement of sleep time in naive and barbiturate tolerant mice and an increase in [^{3}H]-muscimol binding after acute and chronic pentobarbital which was reversed on withdrawal. We have compared the behavioural effects of the GABA agonists muscimol and imidazoleacetic acid (ImAA) and the GABA metabolite γ-hydroxybutyrate (GHBA) following acute or chronic barbital treatment. We have also measured barbiturate enhancement of [^{3}H]-GABA binding in vitro in naive and dependent animals.

LACG mice were treated chronically with barbital mixed into their powdered diet for 4-5 weeks according to a schedule previously shown to induce tolerance and dependence, or acutely with a sub-anaesthetic dose of sodium barbital (NaB, 100mg/kg i.p.) 45 min prior to administration of the drug under test. Groups of six mice of either sex were given muscimol (1.5mg/kg i.p.) or ImAA (160mg/kg i.p.) and scored for immobility, ptosis, splayed limbs and sedation at 15 min intervals for 45 min. Mice given GHBA (200mg/kg i.p.) were scored for immobility, exophthalmos, splayed limbs, sedation and 'straub tail'. Results were analysed using the Mann-Whitney U-Test. For binding studies, crude membrane fractions of whole brains were prepared by the procedure of Greenlee et al. (1978) with some modifications. Na^+-independent [^{3}H]-GABA binding was measured in the presence of pentobarbital (10^{-5} - $2x10^{-3}$M) and 100mM KCl as described by Asano & Ogasawara (1982).

ImAA and muscimol had shorter latencies and significantly greater potency in barbiturate dependent mice compared to controls. In contrast, GHBA had little effect on dependent animals whereas naive animals became sedated with splayed limbs within 15 min of injection. Acute NaB produced significant enhancement of the behavioural effects of muscimol in male and female mice, but was shorter acting in the females. GHBA-induced behaviour was significantly decreased after acute NaB administration to male, though not female, mice. Pentobarbital produced a dose-dependent enhancement of [^{3}H]-GABA binding in membrane fractions from naive and dependent animals with no significant difference between the two groups.

The results of the behavioural tests support electrophysiological and biochemical data showing augmentation of GABA inhibition by barbiturates. The enhancement of muscimol sedation by an acute sub-anaesthetic dose of NaB suggests that the same effect in dependent mice is due to the presence of the barbiturate itself. The results of the binding study are consistent with this hypothesis, though it does not rule out changes in number or sensitivity of GABA receptors after chronic barbiturate treatment.

P.L.G. is an M.R.C. scholar.

Asano, T. & Ogasawara, N. (1982) Eur. J. Pharmacol. 77, 355
Greenlee, D.V. et al (1978) Life Sci. 22, 1653
Olsen, R.W. et al (1981) Fed. Proc. 40, 309
Sivam S.P. et al (1982) J. Neurosci. Res. 7, 37
Willow, M. & Johnston, G.A.R. (1980) Neurosci. Lett. 18, 323

INSULIN ADMINISTRATION AND ETHANOL PREFERENCE IN LACG AND DIABETOGENIC C57BL STRAINS OF MICE

D.M. Connelly & P.V. Taberner, Department of Pharmacology, University of Bristol, Bristol BS8 1TD.

Diabetogenic C57BL mice, which prefer ethanol solution to water, respond to compulsory ethanol consumption by a fall in blood glucose level (BGL), which then corresponds to the BGL observed in ethanol avoiding LACG mice (Connelly et al, 1983). The oral antidiabetic drugs phenformin and glibenclimide also lower the BGL of C57 mice which then show aversion to ethanol (Connelly et al, 1983). Whilst a form of insulin resistance in C57 mice has been suggested to account for their mild diabetes (Goas et al, 1979) this has not been established or quantified. This investigation has compared the effects of acute insulin administration on behaviour, and chronic insulin administration on ethanol preference and plasma glucose levels (PGL) in C57 and LACG mice. PGL's were also monitored during withdrawal since we have previously shown that C57 mice lose their preference for ethanol following chronic ethanol drinking (Taberner & Unwin, 1981).

Adult LACG and C57 mice, of either sex, were rendered ethanol tolerant following the chronic drinking schedule previously described (Unwin & Taberner, 1980). PGL's were monitored by tail bleeding and assaying extracted plasma using a Beckman Glucose Analyser 2. Insulin zinc protamine (Wellcome) was administered acutely by i.p. injection. The depressant effects of insulin on behaviour were assessed by scoring in arbitrary units. Chronic insulin was administered by s.c. injection, (0.066 IU/g to 0.033 IU/g) at 24 hour intervals, during which preference for ethanol was monitored.

Acute low doses of insulin (0.17 IU/g, 0.10 IU/g) produced more sedation in ♀C57 than in LACG mice, the onset of sedation being quicker, more severe, and of longer duration. Chronic low doses of insulin (0.066 IU/g) reduced PGL of ♂C57 (10.70 ± 0.41 mM to 5.60 ± 0.26 mM, N=6, t-test $p < 0.001$) and ♀C57 mice (10.60 ± 0.38 to 4.63 ± 0.69, N=6, t-test $p < 0.001$) which then showed aversion to ethanol. Withdrawal of mice from chronic ethanol treatment caused acute hyperglycaemia in C57 and milder hyperglycaemia in LACG (see Table 1). PGL's of ♂C57, ♀C57 and ♂LACG were still significantly higher than before withdrawal, on day 22 ($p < 0.001$).

Table 1. Plasma Glucose levels (mM) Mean values ± SEM n ≥ 10

Days after withdrawal		0	2	7
C57	♂	8.58 ± 0.46	11.93 ± 0.51***	12.05 ± 0.71***
	♀	8.82 ± 0.35	12.19 ± 0.88***	10.75 ± 0.43**
LACG	♂	8.47 ± 0.27	10.69 ± 0.57**	10.03 ± 0.41**
	♀	8.50 ± 0.31	9.28 ± 0.25*	9.40 ± 0.32

Day 2 > Day 0 : * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$
Day 7 > Day 0 : ** $p < 0.01$ *** $p < 0.01$, correlated 2 tailed t-test

The results indicate **no** form of insulin resistance in C57 mice to account for their mild diabetes. Whether or not this diabetes stimulates ethanol consumption is uncertain, since insulin removes this preference, as does withdrawal from chronic ethanol treatment, which raises their PGL.

D.M.C. is an M.R.C. scholar

Connelly, D.M. et al (1983) Biochem. Pharmac. 32, 221
Goas, J.A. et al (1979) Pharmac. Biochem. & Behav. 10, 557
Taberner, P.V. & Unwin, J.W. (1981) Br. J. Pharmac. 74, 959P
Unwin, J.W. & Taberner, P.V. (1980) Neuropharmacology, 19, 1257

THE EFFECTS OF 17 β OESTRADIOL UPON (^{3}H)-MEPYRAMINE BINDING IN THE RAT BRAIN

J. Anuka & P.J. Thomas, Department of Pharmacology, Chelsea College, Manresa Road, London SW3 6LX.

Sex steroids have both long and short term effects upon the brain, modifying, for example, its ultra structure, the turnover rates of some neurotransmitters and the concentrations of high affinity binding sites for some monoamines (see Ani & Thomas, 1982) and for muscarinic ligands (Al-Dahan & Thomas, 1982). Little is known, however, about their effects on histamine sensitive pathways.

We describe here the binding of [^{3}H]-mepyramine (24.1 Ci/mmol) to membrane fragments prepared from the cerebral cortices, hypothalami and amygdalae of pro-oestrous, metoestrous and ovariectomised females as well as those of intact males and of ovariectomised females treated with oestradiol benzoate from operation to slaughter (125 µg/250g/day). Assay methods were essentially those of Hill & Young (1980) with minor modifications. Membrane fragments were prepared by centrifugation and were incubated with [^{3}H]-mepyramine in the presence and in the absence of an excess (5×10^{-6}M) unlabelled mepyramine as a control. Krebs-Henseleit buffer (pH 7.3) was used throughout. Bound and free were separated by centrifugation. After preliminary experiments to determine the time course of incubation, the IC50 value of unlabelled mepyramine, the effects of protein concentration and the effects of histamine, promethazine and triplrolidine on the reaction. Scatchard plots were obtained to estimate numbers and affinities of [^{3}H]-mepyramine binding sites in the different areas of the brain. In all cases, equilibrium dissociation constants of reaction (Kd) were in the order of 4nM. Numbers of sites, however, differed between brain regions and with hormonal status (Table 1).

Table 1.

(^{3}H mepyramine bound (pmols/g protein)

	Hypothalamus	Amygdala	Cerebral cortex
Male	106 ± 4	129 ± 16	66 ± 6
Female (M)	104 ± 10	95 ± 11	67 ± 8
Female (P)	57 ± 8	48 ± 5	37 ± 4
Female (ovx)	97 ± 10	93 ± 2	76 ± 3
Female (ovx + E_2)	65 ± 10	50 ± 10	48 ± 2

M = metoestrous, P = pro-oestrous
ovx = ovariectomised, ovx + E_2, = ovariectomised treated with oestradiol
n = 4 in each case

When oestrogen levels were low (male, metoestrous and ovariectomised females), binding was high. When oestrogen levels were high (pro-oestrous and oestrogen treated females), mepyramine binding was low. The fact that the cerebral cortex, as well as the classical target areas for oestrogens (hypothalamus and amygdala) was affected suggests that a direct membrane effect of the steroid may be involved in addition to its action via intracellular receptor systems.

Al-Dahan, M. & Thomas, P.J. (1981). Brit.J.Pharmacol. 74, 866-867P.

Hill, S.J. & Young, J.M. (1980). Brit.J.Pharmacol. 68, 687-696.

Thomas, P.J. & Ani, M. Molecular Aspects of Medicine 5, 1-61.

INHIBITION OF FOOD AND WATER INTAKE BY SOME ANTIDEPRESSANTS

D.K. Luscombe & S. McLennan, Division of Applied Pharmacology, Welsh School of Pharmacy, UWIST, Cathays Park, Cardiff CF1 3NU

Recent studies have shown that the acute administration of the tricyclic antidepressant agents imipramine (IMI) and desmethylimipramine (DMI) to deprived rats results in reduced intake of food (Blavet & De Feudis, 1982) and water (Zabik et al. 1977; O'Donnell et al. 1982). We now report on the influence of clomipramine (CLO) and (±)oxaprotiline (OXA), together with its (+) and (-) enantiomers, on food and water intake in satiated rats. For comparative purposes, the effects of IMI and DMI have also been studied.

Groups of 5 male Wistar rats (250-280g body weight) were housed individually and allowed food (rat breeding diet, Grain Harvesters) and water ad libitum, the animals being maintained on a 12 hour light/dark cycle. Drugs were injected intraperitoneally (2.5-20mg/kg) at the onset of the dark period (20.00h) and total food and water intake determined at 1,2,3,4 and 12h post drug administration. IMI, DMI, CLO and (±)OXA were all found to produce a dose related reduction in food and water intake which was detectable within the first hour of dosing and which persisted for more than 12 hours. The maximum inhibitory effect on food and water intake was attained 3h after dosing and the dose of each drug required to produce 50% inhibition (ID_{50}) of each parameter at this time, was calculated. Regarding food consumption the ID_{50} values for DMI, (±)OXA, IMI and CLO were found to be 4.6mg/kg, 5.6mg/kg, 8.3mg/kg and 16.0mg/kg respectively. A similar rank order of potency was observed for water intake, ID_{50} values being 3.0mg/kg, 5.4mg/kg, 7.0 mg/kg and 13.4mg/kg respectively. Interestingly, the (+) stereoisomer of OXA produced a greater diminution of food and water intake than the corresponding dose of (±)OXA, whereas an equivalent dose of (-)OXA had no effect on either feeding or drinking behaviour. Chronic studies involving the administration of DMI, IMI, CLO and (±)OXA (10mg/kg i.p.) twice daily for 14 days have shown a similar trend.

In common with DMI, (±)OXA has strong noradrenaline (NA) uptake inhibitor properties (Waldmeier et al. 1977), this action being stereospecific to the (+) enantiomer, the (-) isomer being relatively inactive (Waldmeier et al. 1982). In contrast, IMI is less potent than DMI in inhibiting neuronal NA uptake while CLO is known to be a selective inhibitor of 5-HT uptake possessing only weak NA uptake inhibitor activity. Thus, the rank order of potency of the drugs in feeding and drinking behaviour appears to reflect their activity as NA uptake inhibitors. Whilst DMI, (±)OXA, CLO and IMI all display some degree of H_1 receptor antagonism and possess mild sedative activity, these properties are also common to both the (+) and (-) enantiomers of OXA (Delini-Stula et al. 1982) whereas inhibition of feeding and water intake is clearly not. It is therefore unlikely that these properties contribute to the influence of antidepressants on food and water intake. Likewise a cholinergic component is unlikely since (±)OXA has no anticholinergic activity (Delini-Stula et al. 1982). Our findings therefore appear to indicate the involvement of noradrenergic systems in the action of antidepressants in reducing food and water intake in rats following acute and chronic administration.

We thank CIBA-GEIGY for the kind gift of (±)oxaprotiline and its enantiomers.

Blavet, N. & De Feudis, F.V. (1982) Pharmac. Res. Comm. 14, 663
Delini-Stula, A. et al (1982) in "Typical & Atypical Antidepressants: Molecular Mechanisms" Eds. E. Costa & G. Racagni. Raven Press. p265
O'Donnell, J.M. et al (1982) Psychopharmac. 77, 129
Waldmeier, P.C. et al (1977) Eur. J. Pharmac. 46, 387
Waldmeier, P.C. et al (1982) Biochem. Pharmac. 31, 2169
Zabik, J.E. et al (1977) Neuropharmac. 16, 267

CHARACTERISATION OF INCREASED NEUROTRANSMITTER RELEASE FROM BRAIN PREPARATIONS OBTAINED FROM ETHANOL-TOLERANT RATS

J.M. Littleton & M.A. Lynch, Department of Pharmacology, King's College, Strand, London WC2R 2LS.

As compared to brain slices from control animals, brain preparations obtained from animals which have received ethanol chronically release a greater fraction of stored neurotransmitter when stimulated electrically (Clark et al, 1977) or by K^+ depolarisation (Lynch et al, 1983). Since a similar result is obtained when release is stimulated by the Ca^{2+}-ionophore A23187 (Lynch & Littleton, 1983) we have suggested that increased Ca^{2+}-sensitivity may be the cause. An alternative explanation is that the resting intraneuronal Ca^{2+} concentration is higher in these ethanol-treated tissues and consequently is closer to the threshold at which neurotransmitter release can be initiated.

To investigate these possibilities we have made experiments on rat synaptosomes in which we have attempted to modify intracellular Ca^{2+} concentrations pharmacologically. In one series we superfused rat cortical synaptosomes with ouabain (10^{-4} M) which by inhibiting the Na^+ pump should promote Na^+ and Ca^{2+} exchange and thus increase intrasynaptosomal Ca^{2+} (Baker et al, 1969). In the other series we attempted to lower intrasynaptosomal Ca^{2+} by superfusing with EGTA (1 mM) and A23187 (30 μM). After these manoevres the releasing stimulus (30 μM A23187 in the presence of 2 mM Ca^{2+} or Krebs solution containing 2 mM Ca^{2+}) was presented to the synaptosomal preparation in the superfusing fluid and the fraction of previously uptaken [^{3}H]-noradrenaline released was measured. Synaptosomal preparations from control rats were compared with those from animals which received ethanol chronically by inhalation for 6 days. The activities of Na^+ K^+ dependent and Ca^{2+} Mg^{2+} dependent ATPase activities were also measured. If the increased neurotransmitter release is a consequence of increased intraneuronal free Ca^{2+} then it should (a) be mimicked by procedures (e.g. ouabain superfusion) which also increase intracellular Ca^{2+} (b) be abolished by procedures which reduce intracellular free Ca^{2+} (e.g. superfusion with EGTA and A23187).

The synaptosomal uptake of [^{3}H]-noradrenaline was similar in control and ethanol-tolerant preparations. The release of ^{3}H by A23187 was entirely dependent on external Ca^{2+} suggesting that it is indeed acting as an ionophore in this situation (Akerman & Nicholls, 1981). Superfusion of synaptosome with ouabain produced a small but significant increase in the fraction of ^{3}H released by A23187. The fraction of ^{3}H released was however very much greater from synaptosomes of ethanol-treated rats than controls. This argues that the marked alteration in release characteristics produced by ethanol in vivo is not mimicked by procedures which increase intracellular Ca^{2+}. Similarly, in the second series of experiments a much greater fraction of ^{3}H was released by Ca^{2+} in the synaptosomes from ethanol-treated rats and this was not altered by prior superfusion with EGTA and A23187. Thus this procedure which should reduce intracellular free Ca^{2+} to the same level in control and ethanol-treated synaptosomes, did not prevent the increased neurotransmitter release associated with ethanol tolerance. These findings all suggest that increased intracellular Ca^{2+} is not likely to be the only cause of increased transmitter release In addition the activity of Na^+ K^+ ATPase was found to be within normal limits whereas Ca^{2+} Mg^{2+} ATPase activity was significantly increased in synaptosomes from ethanol-treated animals. This change would be expected to reduce rather than increase cytosolic Ca^{2+}.

These experiments were supported by the Medical Council on Alcoholism.

Akerman, K.E.O. & Nicholls, D.J. (1981) Eur. J. Biochem. 115,67-73. Baker, P.F. et al (1969) J. Physiol. 200, 431-458. Clarke, J.W. et al (1977) Can. J. Physiol. 55, 758-768. Lynch, M. et al (1983) Brain Research (in press). Lynch, M. & Littleton, J.M. (1983) Nature, 303, 175-176.

DIFFERENTIATION BETWEEN PLATELET AND CORTICAL (^{3}H)-IMIPRAMINE BINDING SITES

A. Davis[1], A. Dumbrille-Ross and S.W. Tang. Psychopharmacology section, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario, M5T 1R8, CANADA.

Previous studies have shown [^{3}H] imipramine to exhibit maximal binding at 4°C. This is true in rat cortical and hippocampal homogenates and in fresh, outdated and solubilized human platelet membranes. The loss of binding observed at higher temperatures is completely reversible (Dumbrille-Ross et al., 1983; Davis et al., 1983a,b). In platelets, it is characterized by a loss in affinity; in the cortex, there is an additional loss in the number of sites. [^{3}H] Ro 11-2465 labels all imipramine binding sites in platelets (Dumbrille-Ross & Tang, 1983) but only a subpopulation in the cortex. Unlike [^{3}H] imipramine, this ligand shows only the affinity change in both tissues upon raising the temperature.

To further study these phenomena, the effects of N-ethylmaleimide (NEM) were studied. In platelets, [^{3}H] imipramine binding is decreased by NEM with a greater sensitivity at 23°C ($t_{\frac{1}{2}}$ at 4°C of 2h, 0.25h at 23°C). At 4°C the decrease was pseudo-first order, whereas at 23°C the logarithmic plot was curvilinear (Davis, 1983). In the cortex, however, the findings were different. At 4°C binding was relatively insensitive to NEM whereas at 23°C it was decreased with a $t_{\frac{1}{2}}$ of 2h.

To investigate possible different synaptic localizations, the [^{3}H] imipramine binding in subcellular fractions of rat cortical homogenates was studied. Temperature-sensitive binding was observed in both synaptosomal and microsomal fractions. No saturable binding was observed in the nuclear fraction.

A tentative model is proposed to explain these results (see Table): One binding (sub) site contains sulphydryl bonds and, with increased temperatures, loses affinity for both [^{3}H] Ro 11-2465 and [^{3}H] imipramine. In the cortex there is an additional (sub) site, not labelled by [^{3}H] Ro 11-2465, that does not contain sulphydryl bonds and exhibits a decrease in B_{max} at higher temperatures. Both sites appear to have identical pharmacological specificity.

Table

	IMI_A	IMI_B
Labelled by:	[^{3}H] Imipramine [^{3}H] Ro 11-2465	[^{3}H] Imipramine
NEM sensitivity	High	Low
Regional localization	Platelets & cortex	Cortex
Subcellular location	Synaptosomal and Microsomal	
Temperature sensitivity	Affinity loss	B_{max} loss

Dumbrille-Ross, Morris, Davis and Tang et al. (1983). Eur. J. Pharmac., in press.
Davis, Morris and Tang (1983a). Eur. J. Pharmac. 86, 353-359.
Davis, Morris and Tang (1983b). Eur. J. Pharmac., in press.
Davis (1983). J. Neurochem **S109, in press.**
Dumbrille-Ross and Tang (1983). Molec. Pharm. 23 607-613.
Supported by the Connaught Foundation, the C.K. Clarke Psychiatric Foundation and the Hospital for Sick children.

[1]Present address Boehringer Ingelheim, 977 Century Drive, Burlington, Ontario, L7L 5J8, CANADA.

THE TIME COURSE OF RAT CEREBROCORTICAL β-ADRENOCEPTOR DOWN-REGULATION FOLLOWING RS-21361 AND DESMETHYLIMIPRAMINE TREATMENT

A. Dye, C.M. Page and R.L. Whiting, Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

RS-21361 is a selective α_2-adrenoceptor antagonist as determined in both the pithed rat (Michel et al, 1981) and the isolated rat vas deferens (Michel & Whiting, 1981) preparations.

Chronic (1-3 weeks) administration of α_2-adrenoceptor antagonists and a number of antidepressants including tricyclics and monoamine oxidase inhibitors (Sellinger-Barnett et al, 1980) as well as electroconvulsive therapy (Bergstrom & Keller, 1979) are known to cause down regulation of cerebrocortical β-adrenoceptors. This prolonged treatment mimics the slow onset of therapeutic antidepressant effect in patients and suggests that the beneficial action of these drugs is partly due to a progressive desensitisation of β-adrenergic receptors (Sulser, 1979).

The present study investigates the effects of chronic RS-21361 or desmethyl-imipramine (DMI) treatment over a time course of 1-10 days on β-adrenoceptor binding sites in the rat cerebral cortex.

Male Sprague Dawley rats were orally dosed three times daily with RS-21361 (100 $mg.kg^{-1}$), DMI (20 $mg.kg^{-1}$) or distilled water (10 $ml.kg^{-1}$) for 1, 3, 5, 7 or 10 days. The animals were killed 8 h after the last dose and the cerebral cortex removed. The densities of β-adrenergic receptor sites were determined by a modification of the ligand binding assay described by Greenberg et al (1976). The prepared membranes were incubated with 7 concentrations (0.1-4 nM) of [^{3}H]-dihydroalprenolol (DHA) for 30 min at 25^{0}C. Total binding was determined in triplicate and non-specific binding in duplicate in the presence of 5×10^{-4} $mol.litre^{-1}$ isoprenaline. The results were analysed by the method of Scatchard (1949) using computer modelling techniques to calculate the binding parameters B_{max} (maximum number of receptor sites) and Kd (the affinity of the ligand).

Table 1

		B_{max} (fmol.mg protein^{-1}) ± SE of mean		
Day	n	Control	RS-21361	DMI
1	4	133.17 ± 8.13	135.33 ± 13.94	140.00 ± 28.35
3	4	132.36 ± 16.85	149.50 ± 14.18	143.46 ± 20.63
5	4	138.02 ± 20.84	106.64 ± 10.56	141.66 ± 8.22
7	6	135.45 ± 8.45	*83.96 ± 6.55	108.27 ± 7.29
10	8	126.41 ± 9.57	*66.41 ± 4.59	*75.41 ± 6.51

*$p < 0.001$

Statistical analysis shows that RS-21361 produces a more rapid onset of β-adrenoceptor desensitisation. No significant difference was seen in the affinity value of [^{3}H]-DHA between the 3 groups on any day of measurement.

RS-21361, a selective α_2-adrenoceptor antagonist, is therefore potentially useful as an antidepressant with the possibility of a more rapid onset of activity than standard tricyclic agents.

Bergstrom, D.S. & Keller, M.J. (1979) Nature 278, 464.
Greenberg, D.A. et al (1976) Life Sci. 19, 68-76.
Michel, A.D. & Whiting, R.L. (1981) Br.J.Pharmac. 74, 255P.
Michel, A.D. et al (1981) Br.J.Pharmac. 74, 845P.
Sellinger-Barnett, M.D. et al (1980) Neuropharmac. 19, 447.
Sulser, F. (1979) T.I.P.S. 1, 92-94.

BEHAVIOURAL EFFECTS OF UNILATERAL AND BILATERAL INTRAPALLIDAL INJECTIONS OF OPIATE RECEPTOR AGONISTS

D.Dewar, P.Jenner & C.D.Marsden, University Department of Neurology, Institute of Psychiatry & The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5 8AF, U.K.

The function of opiate receptors in globus pallidus is unknown but bilateral intra-pallidal injection of D-Ala^2, D-Leu^5-enkephalin (DADLE) increases locomotor activity (Joyce et al, 1981). We now report the behavioural effects of unilateral and bilateral intrapallidal injections of DADLE, FK 33-824 (Tyr-D-Ala-Gly-NCH_3-Phe-Met-(O)-ol) and ethylketocyclazocine (EKC) which differentially affect the opiate receptor subtypes δ, u and k respectively.

Drugs were administered in 1 ul 0.9% saline from a 10 ul Hamilton syringe via guide cannulae positioned directly above the globus pallidus (A6.5, L2.5, Konig & Klippel, 1967). Saline injections into the same area served as controls. All injection sites were confirmed histologically.

Unilateral intrapallidal injection of DADLE (0.5-5 ug) or FK 33-824 (0.001-0.01 ug) did not produce postural asymmetry or circling behaviour. Unilateral intrapallidal injection of EKC (5-40 ug) resulted in dose-dependent ipsiversive rotation lasting 35-75 min (Table 1). The circling response induced by unilateral intrapallidal injection of EKC (20 ug) was abolished by prior treatment with naloxone (1 mg/kg s.c.; 10 min previously).

Bilateral intrapallidal injections of DADLE (0.5-5 ug) caused a dose-dependent increase in locomotor activity (Table 1). Bilateral intrapallidal administration of FK 33-824 (0.001-0.01 ug) also increased locomotor activity. EKC (5-40 ug) had no apparent effect on locomotor activity. Increased locomotor activity caused by bilateral injection of DADLE (1 ug) was abolished by prior administration of naloxone (1 mg/kg s.c.; 5 min previously). DADLE alone: mean activity counts, 851 $\pm$ 77.1; DADLE with naloxone, 219.9 $\pm$ 42.0; $p < 0.05$.

Table 1 Circling and locomotor activity responses to unilateral and bilateral injections respectively

Drug	Dose (ug)	n	Max.rate of ipsiversive rotation $\pm$ S.E.M.	% increase in locomotor activity
DADLE	0.5	14	0	520*
	5	11	0	733*
FK 33-824	0.001	16	0	115
	0.01	10	0	380*
EKC	5	11	4.0 $\pm$ 0.7	183
	40	13	7.2 $\pm$ 0.7	154

* $p < 0.05$ comparing drug with control injections using paired Student's t test

In summary, the putative δ- and u-opiate receptor agonists DADLE and FK 33-824 respectively had no effect on postural mechanisms but did affect locomotor activity whereas the opposite was true for the putative k-opiate receptor agonist, EKC. Different opiate receptor subtypes in globus pallidus may modulate basal ganglia outflow controlling locomotion and posture.

Joyce et al (1981) Brain Res. 221, 359-370.
Konig, J.F.R. & Klippel, R.A. (1963) The Rat Brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams & Wilkins, Baltimore.

INTERACTION OF 5-HT AGONISTS WITH GUINEA-PIG BRAIN STEM 5-HT RECEPTORS AND THE INDUCTION OF MYOCLONUS

P.Jenner, G.Luscombe & C.D.Marsden, University Department of Neurology, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill,London SE5 8AF,U.K.

5-Hydroxytryptamine (5HT) precursors and indole-containing 5HT agonists induce dose-related myoclonus in guinea pigs which originates from brain stem (Chadwick et al,1978; Luscombe et al,1982). In contrast, 5HT agonists containing a piperazine moiety do not induce myoclonus in pharmacologically effective doses (Luscombe et al,1982). We now report the relationship between the ability of a range of 5HT agonists to induce myoclonus and their ability to displace ^{3}H-5HT and ^{3}H-spiperone from 5HT-1 and 5HT-2 receptors in guinea-pig brain stem preparations.

Brainstem (minus cerebellum) preparations from female guinea pigs (250-500 g) were used for ligand binding assays. ^{3}H-5HT (16.6 Ci/mmole; 0.5-64 nM) or ^{3}H-spiperone (21 Ci/mmole; 0.25-32 nM) were incorporated into 10 tissue incubates (1.0 ml; 10mg tissue) and specific binding defined by the incorporation of 5HT (10^{-5} M or d-lysergic acid diethylamide (d-LSD) (10^{-6} M). Saturation analysis of ^{3}H-5HT binding revealed two sites: a high affinity site (Bmax 1.97 $\pm$ 0.01 pmoles/g wet weight of tissue; K_D 3.7 $\pm$ 1.0 nM) and a low affinity site (Bmax 9.2 $\pm$ 2.2 pmoles/g wet weight of tissue; K_D 29.1 $\pm$ 4.0 nM). Specific binding of ^{3}H-spiperone to guinea pig brain stem preparation was very low and could not be reliably measured. This data would suggest that 5HT-1 receptors (Peroutka & Snyder, 1979) predominate in this region of guinea-pig brain.

The specific binding of ^{3}H-5HT (4 nM) to guinea pig brain stem preparations was potently displaced by indole-containing 5HT agonists (Table 1; range IC_{50}: 3.4-519 nM) but only weakly displaced by piperazine-containing 5HT agonists (Table 1; range IC_{50}; 408-42,551 nM).

Table 1 Displacement of specific ^{3}H-5HT binding by indole-containing and piperazine-containing 5HT agonists

Indole compounds	IC_{50} (nM)	Piperazine compounds	IC_{50} (nM)
d-LSD	3.4 $\pm$ 1.7	1-(m-trifluoromethyl-phenyl)piperazine	408 $\pm$ 124
Bufotenine	23 $\pm$ 12	m-Chlorophenylpiperazine	3261 $\pm$ 2738
RU 24969	30 $\pm$ 6	Quipazine	7241 $\pm$ 2714
5-Methoxy-N,N-dimethyltryptamine	158 $\pm$ 24	Phenylpiperazine	7476 $\pm$ 2725
Psilocin	241 $\pm$ 81	p-Chlorophenylpiperazine	8729 $\pm$ 4303
N,N-Dimethyltryptamine	280 $\pm$ 105	6-Chloro-2-(1-piperazinyl) pyrazine (MK 212)	17260 $\pm$ 9714
Psilocybin	519 $\pm$ 82	2-(1'-piperazinyl) quinoxaline	42551 $\pm$ 21,742

The ability of 5HT agonists to induce myoclonus in guinea pigs is related to the capacity to displace the specific binding of ^{3}H-5HT to 5HT-1 receptors in guinea pig brain stem.

Chadwick,D. et al (1978) J.Neurol.Sci.32,157.
Luscombe,G.et al (1982) Life Sci.30,1487.
Peroutka,S.J. & Snyder,S.H. (1979) Molec.Pharmacol.16,687.

CHRONIC IMIPRAMINE TREATMENT ANTAGONIZES THE INHIBITORY EFFECT OF 5-HYDROXYTRYPTAMINE IN THE RAT HIPPOCAMPAL SLICE

R. Anwyl & M.J. Rowan, Departments of Physiology and Pharmacology, Trinity College, University of Dublin, Ireland.

5-Hydroxytryptamine (5-HT) is believed to be one of the main neurotransmitters involved in depression and its treatment (Charney et al, 1981). Depending on the CNS region being studied, the long-term administration of antidepressants to animals has been shown biochemically to produce a down-regulation of 5-HT receptors or have no effect. In contrast electrophysiological research where spontaneous unit activity was monitored in vivo has shown an enhanced post-synaptic response to iontophorically applied 5-HT receptor agonists. The present study was designed to examine electrophysiologically the effect of subchronic imipramine treatment on 5-HT induced responses in vitro. In these experiments the effects of known concentrations of 5-HT were assessed on synaptically evoked neuronal firing in the rat isolated hippocampal slice.

Male rats received a single daily injection of 10 mg/kg imipramine HCl or saline for a period of 4 weeks. Animals weighing about 200-300g were used 24 hours after their last injection. Similar untreated rats were also studied. Transverse hippocampal slices of about 350 μm thickness were submerged in artificial cerebrospinal fluid maintained at about 34°C. Extracellular electrodes were placed in the stratum radiatum in order to stimulate afferent nerve fibres and in the pyramidal cell body layer of the CA1 region for recording. Input-output curves for the synaptically evoked population spike were obtained and a response which was approximately 50% of the maximum was chosen to study the effects of 5-HT. 5-HT was applied at various concentrations via the perfusion medium.

The effect of 5-HT on the synaptically evoked population spike was found to be mixed in nature. In general, concentrations of between 10^{-6}M and 10^{-5}M produced an initial increase followed by a decrease of the population spike amplitude in a dose dependent manner. On occasions a pure decrease or a pure increase in amplitude was observed. In these cases a value of zero was assigned to the effect when measuring the degree of enhancement or inhibition respectively. Under these measurement conditions there did not appear to be any difference between the experimental groups for the excitatory effect of 10^{-5}M 5-HT. In the case of the inhibitory effect of 10^{-5} 5-HT, there was no obvious difference between saline treated and untreated rats. These results were therefore pooled. Chronic treatment with imipramine was, however, found to greatly reduce the inhibitory effect of 5-HT (% inhibition ± S.E.M.: (a) for control rats 54 ± 9%, n = 13; (b) for chronic imipramine rats: 24 ± 8%, n = 7; $p < 0.05$, Student's t-test).

The reduced inhibitory response to 5-HT following long-term treatment by imipramine may be due to a decrease in the responsiveness of the pyramidal cells to 5-HT. Alternatively the effect may be at least partly explained by an adaptive increase in the uptake of 5-HT (Barbaccia et al, 1983). The apparent disagreement between our results and those of previous reports on the electrophysiological effects of chronic imipramine treatment in the hippocampus (e.g. De Montigny and Aghajanian, 1978) is probably due to differences in techniques.

We thank the St. Patrick's Hospital-T.C.D. fund for financial support.

Barbaccia, M.L., Brunello, N., Chuang, D.M. & Costa, E. (1983) Neuropharmacol. 22, 373-383.

Charney, D.S., Menkes, D.B. & Heninger, G.R. (1981) Arch. Gen. Psychiat. 38, 1160-1180.

De Montigny, C. & Aghajanian, G.K. (1978) Science 202, 1303-1306.

TYR-D-PEN-GLY-PHE-L-PEN AND TYR-D-PEN-GLY-PHE-D-PEN ARE SELECTIVE LIGANDS FOR THE δ-BINDING SITE

A.D. Corbett, M.G.C. Gillan, H.W. Kosterlitz, A.T. McKnight & S.J. Paterson, Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen AB9 1AS

Opioid binding sites have been subdivided into μ-, δ- and κ-subtypes on the basis of the binding of tritiated ligands and their differential displacement by unlabelled compounds. Characterization of the three binding sites has been difficult due to the high degree of cross-reactivity of the available ligands. At present, there are highly selective ligands for the μ-binding site, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (Kosterlitz & Paterson, 1981), and for the κ-binding site, dynorphin A (1-9) (Corbett et al, 1982) and U 50,488H (VonVoigtlander et al, 1983). However, the available δ-ligands display a significant affinity for μ-binding sites.

It has been reported that [D-Ser^2,L-Leu^5]enkephalyl-Thr (Fournié-Zaluski et al, 1981) and [D-Thr^2,L-Leu^5]enkephalyl-Thr (Zajac et al, 1983) are more selective than [D-Ala^2,D-Leu^5]enkephalin for the δ-binding site. We have compared the binding and pharmacological profiles of these enkephalin analogues with [D-Pen^2,L-Pen^5]enkephalin and [D-Pen^2,D-Pen^5]enkephalin (Dr. H. Mosberg, University of Arizona) where Pen = β,β dimethyl cysteine.

The potency of the peptides to displace the binding of tritiated ligands was measured in homogenates of guinea-pig brain at 25°C. [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (1 nM) was used as μ-ligand, [^{3}H]-[D-Ala^2,D-Leu^5]enkephalin (0.7-1 nM) as δ-ligand after suppression of μ-binding by the addition of 30 nM unlabelled [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (Gillan & Kosterlitz, 1982), and [^{3}H]-(-)-bremazocine (0.1-0.15 nM) as κ-ligand after suppression of μ- and δ-binding by the addition of 100 nM unlabelled μ- and δ-ligand.

The compounds were also tested for activity in the guinea-pig ileum myenteric plexus and the vasa deferentia of the mouse, rabbit and rat (McKnight et al, 1983).

In binding assays, [D-Pen^2,L-Pen^5]enkephalin and [D-Pen^2,D-Pen^5]enkephalin were highly selective compounds for the δ-binding site with a ratio of (K_I for μ)/(K_I for δ) of 235 and 262, respectively. The corresponding ratios for [D-Ala^2,D-Leu^5]enkephalin, [D-Thr^2,L-Leu^5]enkephalyl-Thr and [D-Ser^2,L-Leu^5]enkephalyl-Thr were 6.7, 12.8 and 21.9

[D-Pen^2,L-Pen^5]enkephalin and [D-Pen^2,D-Pen^5]enkephalin were potent agonists in the mouse vas deferens and weak agonists in the guinea-pig ileum. The ratios of IC_{50} in the guinea-pig ileum/IC_{50} in the mouse vas deferens were 12 for [D-Ala^2,D-Leu^5]enkephalin, 190 for [D-Ser^2,L-Leu^5]enkephalyl-Thr, 570 for [D-Pen^2,L-Pen^5]enkephalin and 835 for [D-Pen^2,D-Pen^5]enkephalin. [D-Pen^2,L-Pen^5]enkephalin and [D-Pen^2,D-Pen^5]enkephalin were both inactive in the rat vas deferens and in the rabbit vas deferens.

Thus, on the basis of both binding and pharmacological assays, [D-Pen^2,L-Pen^5]enkephalin and [D-Pen^2,D-Pen^5]enkephalin were highly selective ligands for the δ-binding site.

Supported by grants from the MRC and U.S. NIDA.

Corbett, A.D. et al (1982) Nature 299, 79-81
Fournié-Zaluski, M.C. et al (1981) Mol.Pharmac. 20, 484-491
Gillan, M.G.C. & Kosterlitz, H.W. (1982) Br.J.Pharmac. 77, 461-469
McKnight, A.T. et al (1983) Eur.J.Pharmac. 86, 393-402
Kosterlitz, H.W. & Paterson, S.J. (1981) Br.J.Pharmac. 73, 299-300
VonVoigtlander, P.F. et al (1983) J.Pharmac.exp.Ther. 224, 7-12
Zajac, J.M. et al (1983) Biochem.Biophys.Res.Commun. 111, 390-397

THE EFFECT OF SALBUTAMOL ON THE ACTIVITY OF THE OLFACTORY BULBECTOMIZED RAT IN THE 'OPEN FIELD' APPARATUS

Bernadette Earley & B.E. Leonard, Pharmacology Department, University College, Galway, Republic of Ireland.

Bilateral ablation of the olfactory bulbs of the rat causes an increase in locomotor activity when the animals are placed in the stressful environment of the 'open field' (Earley & Leonard, 1983). In order to investigate the effects of changes in adrenoceptor activity on the stress induced behaviour of the bulbectomized rat, we have studied the effects of the specific β_2-adrenoceptor agonist salbutamol; this drug has been shown to be a clinically effective antidepressant (Lecrubier et al., 1980).

Male-Sprague-Dawley rats (250-280g; N = 8 per group) were bilaterally bulbectomized as described elsewhere (Jancsár and Leonard, 1981). The rats were allowed to recover from surgery for 14 days before the commencement of drug treatment. The following drugs were administered for 14 days: salbutamol (5 mg/kg i.p. twice daily), DL-propranolol (10 mg/kg i.p.), salbutamol + DL-propranolol and saline (controls). The atypical antidepressant drug mianserin (5 mg/kg i.p.) was also administered so that the effect of salbutamol could be compared to a standard drug. The behaviour of the rats in the 'open field' apparatus (Gray & Laljee, 1971) was observed for 3 min. following 11 days of drug administration. All rats were decapitated after 14 days of drug treatment and the concentrations of MHPG, noradrenaline, dopamine and 5-hydroxytryptamine determined fluorimetrically (Earley & Leonard, 1978) in acid extracts prepared from the amygdaloid cortex and mid-brain.

The effects of the various drug treatments on the locomotor activity of the sham operated and bulbectomized rats is shown in Table 1. It can be seen that both mianserin and salbutamol attenuate the hyperactivity of the bulbectomized rats; the effect of salbutamol was antagonized by the concurrent administration of Dl-propranolol. The ambulation scores of sham operated rats were unaffected by drug treatment. The noradrenaline concentration was reduced following bulbectomy and normalized by mianserin and salbutamol treatment. Salbutamol and propranolol when administered separately, increased the levels of 5-hydroxytryptamine in the amygdaloid cortex and mid-brain. The combination of salbutamol and propranolol, reversed this effect. It is concluded that salbutamol is active in this animal model of depression and that its activity may be related to its agonistic effect on β_2 adrenoceptors.

TABLE 1. Effect of salbutamol and mianserin on the locomotor activity of rats in the 'open field' apparatus.

	GROUP			
Drug Treatment	Sham + Saline	SHAM + Drug	OB + Saline	OB + Drug
Mianserin	92 ± 6	75 ± 9	131 ± 10*	106 ± 9•
Salbutamol	86 ± 8	96 ± 4	141 ± 4 *	112 ± 6•
Salbutamol+Propranolol	103 ± 8	89 ± 4	139 ± 10*	176 ± 11•
Propranolol	108 ± 9	104 ± 6	130 ± 7 *	120 ± 8

* $P<0.05$ versus Sham + Saline • $P<0.05$ versus OB + Saline. Results expressed as number of squares crossed per 3 min. period of observation.

Acknowledgement. The authors thank Allen & Hambury's Ltd. (UK) and Organon International B.V. (Holland) for the gifts of salbutamol and mianserin respectively used in these studies.

Earley, B & Leonard, B.E. (1983). Ir. J. Med. Sci. (In press).
Earley, C. & Leonard, B.E. (1978). J. Pharmacol. Meth. 1, 67 - 69.
Gray, J.A. et al. (1974). Anim. Behav., 22, 856 - 861.
Jancsár, S.M. & Leonard, B.E. 1981). Neuropharmac., 20, 1341 - 1347.
Lecruber, Y. et al. (1980). Br. J. Psychiatry. 136, 354 - 358.

AMINE OXIDASE ACTIVITIES IN ISOLATED BROWN FAT CELLS AND IN FAT CELL MEMBRANE PREPARATIONS

Margery A. Barrand, B.A. Callingham and Sheila A. Fox. Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD.

Two separate amine oxidase activities, a flavin-dependent monoamine oxidase (MAO) and an enzyme resistant to clorgyline but sensitive to carbonyl reagents (CRAO), have been identified in homogenates of brown adipose tissue (BAT) (Barrand & Callingham, 1982). The physiological function of CRAO is unknown but clues to its action may be derived from greater knowledge of its cellular location. Histochemical methods to visualise these enzymes in BAT at the ultrastructural level have indicated that CRAO may be present along the outer membranes of the fat cells but not in the endothelial cells of associated blood vessels (Barrand et al, 1983). Further studies have now been undertaken to extend these observations.

Brown fat cells isolated from other cell types have been prepared and investigated for their content of amine oxidase activity. The method of isolation was taken from that of Bukowiecki et al, 1980. Fresh, finely chopped BAT was incubated with collagenase, filtered and the isolated floating fat cells were separated from other cell types by repeated washing and centrifugation. The purity of the isolated fat cell preparations was monitored by light microscopy and samples were also examined by electron microscopy. Enzyme activity in these cells after osmotic shock was assayed and compared with the activity found in homogenates of whole tissue. ^{3}H-5-hydroxytryptamine (5-HT, 500µM) and ^{14}C-benzylamine (BZ, 50µM) were used as substrates for radiochemical assay of MAO-A and CRAO activities respectively. Specific deaminating activities measured as nmoles of product.h^{-1}mg protein^{-1} for the two enzymes were found to be:

Substrate	Whole tissue	Isolated fat cell preparation
BZ (n = 9)	41.68 ± 5.01	47.34 ± 11.03
5-HT (n = 6)	22.90 ± 4.20	30.84 ± 5.26

Outer cell membranes have also been prepared from isolated fat cells by borate extraction (Warley & Cook, 1973) and centrifugation through 35% sucrose. Preparations were examined by electron microscopy and monitored for enrichment of plasma membranes by assay of various enzyme markers. A 5 to 6-fold increase in activity of CRAO and of the plasma membrane enzyme, phosphodiesterase I, was detected in these preparations.

A second method was attempted in which isolated cells were bound to polycation coated beads (Jacobson, 1980), disrupted to release intracellular contents and the plasma membrane fragments remaining on the beads assayed for enzyme activity. Enrichment of activity, expressed as the ratio of specific activity in isolated membranes to specific activity in cell suspensions, was found in 5 separate preparations to be: phosphodiesterase I, 5.58 ± 1.11; CRAO, 1.88 ± 0.25; MAO-A, 1.86 ± 0.19. Evidence from these two methods that CRAO is located on the outer cell membrane is thus conflicting. Reasons for this have yet to be found but may be associated with the "sidedness" of the membrane.

This work was supported by the British Heart Foundation.

Barrand, M.A. & Callingham, B.A. (1982) Biochem.Pharmac. 31, 2177-2184
Barrand, M.A. et al (1983) Br.J.Pharmac. in press
Bukowiecki, L. et al (1980) Am.J.Physiol. 238, E552-E563
Jacobson, B.S. (1980) Biochim.Biophys.Acta 600, 769-780
Warley, A. & Cook, G.M.W. (1973) Biochim.Biophys.Acta 323, 55-68

CHANGES IN BRAIN α-ADRENOCEPTORS WITH AGE

C.A. Hamilton, C.A. Howie*, J.L. Reid, D.J. Sumner, Departments of Materia Medica and Statistics, University of Glasgow, Scotland.

Many biochemical and morphological changes occur as the brain ages. Decreases in the binding of both beta (De Blasi et al 1982) and dopaminergic (Misra et al 1980) ligands have been reported with age. We have examined specific binding of the α_1 adrenoceptor ligand ^{3}H prazosin and the α_2 ligand ^{3}H clonidine in brain membranes from rabbits aged 1-36 months and related changes in maximum number of binding sites (Bmax) and dissociation constant (K_D) with changes in adrenoceptor function in vivo. Binding studies were carried out as previously described (Berthelot et al 1982). Bmax ± S.D. and K_D ± S.D. were estimated for forebrain and hindbrain from each rabbit using a rearrangement of the Scatchard equation - Free/Bound = K_D/Bmax + Free/Bmax. Free concentration can be regarded as constant but the bound fraction varies between animals and is subject to experimental error. The plot Free/Bound v Free was preferred to the more usual Bound/Free v Bound as the variable Bound only appears on one axis. As the estimates of Bmax and K_D were of varying degrees of precision a model which included terms for group mean, inter-rabbit variability and the precision of the estimate was used for comparison of age groups. The equality of the means was tested using the Generalised Likelihood Ratio test and Bonferroni confidence intervals constructed to investigate where the differences lay. Both prazosin and clonidine binding in fore and hindbrain decreased with age, but no age related changes in K_D were observed.

Table 1 Changes in the maximum number of binding sites with age
fmoles/mg protein (n = 6)

Tissue	Ligand	Age (Months) 1-1.5	2-3	6-8	24-36
Forebrain	[^{3}H] Prazosin	118±34	112±30	63± 6^{x+}	42±10^{x+}
Hindbrain	[^{3}H] Prazosin	172±58	124±58	72±27^{x}	43± 7^{x+}
Forebrain	[^{3}H] Clonidine	137±19	144±19	76±20^{x+}	56± 8^{x+}
Hindbrain	[^{3}H] Clonidine	160±59	151±31	156±17	49±17$^{x+\Delta}$

Significantly different from ^{x}1-1.5 $^{+}$2-3 $^{\Delta}$6-8 month group at 95% level

The depressor response to intracisternal clonidine 0.1 μg/kg is mediated by α_2 adrenoceptors in the brain stem. The maximum fall in mean arterial pressure after clonidine was similar in rabbits aged 2-3 and 24-36 months (27 ± 5 and 30 ± 3 mmHg) but occurred significantly earlier in the younger group, 5-15 mins after injection compared to 25-40 mins after injection in the older group. The changes in prazosin binding may be part of a generalised aging phenomenon as similar changes have also been observed in the spleen and heart (Dalrymple et al 1982). The clonidine binding data suggest that α_2 adrenoceptors may 'age' at different rates. The decrease in clonidine binding could not be related to changes in responses to intracisternal clonidine. Study of more localised brain regions will be required to confirm that α_2 adrenoceptor number is altered in the brain stem regions involved in circulatory control.

Berthelot A, Hamilton CA, Petty MA, Reid JL. J. Cardiovasc. Pharmacol. 4, 567-574, 1982.
Dalrymple HW, Hamilton CA, Reid JL. Br. J. Pharmac. 77, 322p, 1982.
De Blasi A, Cotecchia S, Mennini T. Life Sci 31, 335-340 1982.
Misra CH, Shelat HS, Smith RC. Life Sci 27, 521-526, 1980.

IS 5-HT UPTAKE REGULATED BY THE 5-HT AUTORECEPTOR?

M. Briley and Chantal Moret, Biochemical Pharmacology Department, Centre de Recherches Pierre Fabre, 81106 Castres Cédex, France.

The depolarisation-induced release of ^{3}H-5HT in the rat brain is reduced by presynaptic 5HT autoreceptor agonists and increased by the 5HT autoreceptor antagonist, methiothepin (Göthert, 1982). The inhibition by LSD of the electrically evoked release of ^{3}H-5HT from rat hypothalamic slices is prevented by the presence of the 5HT uptake inhibitor, citalopram (Langer and Moret, 1982). Thus it was suggested that the 5HT uptake site might modulate the 5HT autoreceptor. The aim of the present study was to determine whether the inverse interaction also exists, namely whether 5HT uptake is modulated by the 5HT autoreceptor.

5HT uptake was measured in 0.4 mm slices of the rat hypothalamus, the same tissue as for the 5HT release, by a modification of the method of Langer & al. (1980). The tissue sample (one slice, 3.58 ± 0.24 mg wet weight, n = 12) was incubated in oxygenated Krebs solution at 37°C with various concentrations of the test drugs. After this preincubation period, ^{3}H-5HT was added at a final concentration of 50 nM. Following 5 minutes incubation, the uptake was terminated by filtration through glassfibre filters and washing twice with 2 ml of cold Krebs solution. A parallel experiment was carried out with 100 µM citalopram as a control for passive diffusion. The radioactivity in the tissue was determined by liquid scintillation spectrometry and the inhibition of uptake was calculated.

LSD, at 1 µM, a concentration which strongly stimulates presynaptic 5HT autoreceptors, did not modify 5HT uptake. Methiothepin, in excess of 0.1 µM, a concentration which blocks the 5HT autoreceptor, inhibited 5HT uptake in a concentration-dependent manner (IC_{50} = 1.5 ± 0.2 µM, n = 4). The potency of methiothepin on ^{3}H-5HT uptake was however not significantly altered by the addition of LSD at 1 µM (IC_{50} = 2.3 ± 0.3 µM, n = 4).

In addition, LSD, at 1 µM, did not modify the inhibition of 5HT uptake by citalopram (IC_{50} = 18 ± 2 nM, n = 4, for citalopram ; IC_{50} = 25 ± 4 nM, n = 4, for citalopram in the presence of LSD 1 µM). Similarly, the inhibition of 5HT uptake by citalopram was not modified by the addition of methiothepin at 1 µM (IC_{50} = 20 ± 3 nM, n = 3, for citalopram ; IC_{50} = 22 ± 7 nM, n = 3, for citalopram in the presence of methiothepin 1 µM).

Thus the 5HT autoreceptor agonist, LSD, modifies neither 5HT uptake nor the potency of the 5HT uptake inhibitor, citalopram. Although the 5HT autoreceptor antagonist, methiothepin, inhibits the 5HT transport process, LSD does not modify this inhibitory effect suggesting that 5HT autoreceptor blockade is not involved. Furthermore, methiothepin does not modify the potency of citalopram on 5HT uptake.

Although previous results suggested that the 5HT uptake site modulates the 5HT autoreceptor (Langer and Moret, 1982), here it is clear that stimulation or blockade of the 5HT autoreceptor does not modulate the 5HT uptake process. Thus if any interaction exists between the 5HT autoreceptor and the 5HT uptake site, the functional control is unidirectional.

Göthert, M. (1982) TIPS, 437-440.
Langer, S.Z., Moret, C., Raisman, R., Dubocovich, M.L. and Briley,M. (1980) Science, 210, 1133-1135.
Langer, S.Z. and Moret, C. (1982) J. Pharmacol. Exp. Ther., 222, 220-226.

ON THE 5-HYDROXYTRYPTAMINE CONTENT OF RAT SENSORY GANGLIA

C.J. Bojkowski, M.A. Kai-Kai, P. Keen & I.C. Kilpatrick[1], Departments of Pharmacology and Anatomy[1], University of Bristol, Bristol BS8 1TD.

The occurrence of peptides in sensory ganglia is well-documented but little is known about the distribution of monoamines in these tissues (Owman & Santini, 1966; Price & Mudge, 1983). The present study examines the origin and distribution of 5-HT in dorsal root ganglia (DRG) and trigeminal ganglion (TG) of the rat.

Female Wistar rats (250-300g) were anaesthetised with chloral hydrate (375 $mg.kg^{-1}$ i.p.), perfused through the ascending aorta with ice-cold Krebs solution and kept on ice while TG and DRG L4-6 were removed under a dissecting microscope. Tissues were homogenised (0.4M $HClO_4$, 130μM EDTA), centrifuged (25,000g; 10 min; 4°C) and the supernatant applied to an Ultrasphere-ODS HPLC column, (mobile phase: 0.1M sodium acetate/0.02M citric acid (pH 4.1), 130μM EDTA, 230μM sodium octyl sulphate, 5-13% methanol; $1ml.min^{-1}$). The eluate was passed through a BAS LC-17 electrochemical detector (carbon-paste electrode; +650 mV).

Extracts of both DRG and TG yielded a peak ($k' = 9-15$) which co-chromatographed with authentic 5-HT. DRG contained 3.12 ± 0.72 ng 5-HT per ganglia triplet and individual TG 6.22 ± 0.78 ng 5-HT. 48 hrs after a dose of the tryptophan hydroxylase inhibitor p-chlorophenylalanine (p-CPA, 300 $mg.kg^{-1}$, i.p.), which depleted lumbar spinal cord 5-HT by 97%, DRG 5-HT levels were unchanged and those of TG reduced by only 20% ($p > 0.4$). Regional analysis revealed that the peripheral halves of both DRG and TG contained more 5-HT (472 ± 34 and 995 ± 61 $ng.g^{-1}$) than the central halves (200 ± 44 and 610 ± 143 $ng.g^{-1}$ respectively). Whereas the 5-HT content of the mixed nerve immediately peripheral to the DRG (398 ± 49 $ng.g^{-1}$) was similar to that of the peripheral part of DRG, the dorsal and ventral spinal nerve roots contained only 5% of this amount (19 ± 2 and 23 ± 3 $ng.g^{-1}$ respectively). The lack of effect of p-CPA together with the preferential location of 5-HT in peripheral sectors suggested that 5-HT was both turning over slowly and possibly of mast cell origin. Therefore the mast cell depletor, 48/80, was administered over 3 days to a cumulative dose of 17 $mg.kg^{-1}$ i.p. and this reduced the 5-HT content of DRG and TG by 53% and 34% respectively ($p < 0.05$), confirming that at least part of the ganglionic 5-HT was contained in mast cells. The possibility that some of the indole was also contained in neurones was investigated with p-chloroamphetamine (80 $mg.kg^{-1}$, i.p., a 5-HT terminal depletor) following chlorpromazine pretreatment (10 $mg.kg^{-1}$, i.p. 1h; Meek, 1978). One day later, cerebral cortex 5-HT was reduced by 96% whilst DRG and TG were depleted by 63% and 52%, respectively ($p < 0.05$).

Complementing these neurochemical data, which suggested that 5-HT was contained in both neuronal and mast cell compartments, immunohistochemistry using a 5-HT antiserum (Immunocorp), localised 5-HT to mast cells and to certain neuronal perikarya in both L5 DRG and TG. When ganglia were incubated with [^{3}H]-5-HT (1μM in the presence of 10μM (-)noradrenaline) autoradiography showed an accumulation of radioactivity in the cell bodies of a discrete population of small neurones.

Thus rat sensory ganglia contain 5-HT, a proportion of which is localised in neuronal cell bodies. The amount of the amine contained in mast cells, however, precludes examination of the neuronal 5-HT compartment by conventional neurochemical techniques.

M.A.K-K. is supported by the Medical Research Council.

Meek, J.L. (1978) Ann. N.Y. Acad. Sci. 305, 190-197
Owman, C. & Santini, M. (1966) Acta physiol. Scand. 68, 127-128
Price, J. & Mudge, A.W. (1983) Nature (Lond.) 301, 241-243

EFFECTS OF A POTENT 5-HT AGONIST, RU 24969, ON THE MESOCORTICO-LIMBIC AND NIGROSTRIATAL DOPAMINE SYSTEMS

C. Oberlander (introduced by M. Worcel). Centre de Recherches Roussel-Uclaf, BP.9, 93230 Romainville, France.

RU 24969 (5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H indole, succinate) is a potent 5-hydroxytryptamine (5-HT) receptor agonist (Hunt and Oberlander, 1981). In rats it induces hyperlocomotion by itself like the 5HT-precursors tryptophan and 5-hydroxytryptophan or the 5HT agonist 5-methoxytryptamine but in the presence of a monoamine oxidase inhibitor. Unlike these compounds however it causes a dopamine (DA) independent contralateral circling behaviour in rats unilaterally lesioned in the striatum with kainic acid (Oberlander and Boissier, 1981).

In the present work the sites of interaction of RU 24969 with central DA systems have been studied by the use of local intracerebral injection techniques.

On systemic injection, at a threshold dose (2mg/kg i.p.) RU 24969 strongly potentiated the hyperlocomotion induced by d-amphetamine or ergometrine injected bilaterally into the nucleus accumbens (10 and 5µg respectively). RU 24969 by itself had no effect when injected into the nucleus accumbens (10µg bilaterally). After injection into the ventral tegmental area, RU 24969 (5ug bilaterally induced a long-lasting hyperlocomotion and markedly increased the hyperlocomotor activity induced by d-amphetamine (1.5mg/kg i.p.) or led to a potent and long-lasting state of catalepsy in rats treated with a threshold dose of haloperidol (0.5-0.75mg/kg i.p., horizontal bar test). RU 24969 (0.5-10mg/kg i.p.) neither antagonized nor potentiated the cataleptigenic action of haloperidol.

On systemic injection RU 24969 (1-2mg/kg i.p.) was able to increase the contralateral circling behaviour induced by unilateral intranigral injection of the GABA agonist muscimol (10ng) in intact rats or the ipsi and contralateral rotations respectively induced by d-amphetamine (2mg/kg i.p.) apomorphine (0.025mg/kg s.c.) or pergolide (0.05mg/kg i.p.) in rats with unilateral 6-OHDA lesion. In lesioned rats at higher doses (10mg/kg i.p.), RU 24969 induced ipsilateral rotations easily blocked by haloperidol (0.5mg/kg i.p.). Bilateral intrastriatal injections of RU 24969 (20µg) were behaviourally ineffective. In the substantia nigra RU 24969 (5µg bilaterally) induced a relatively short-lasting phase of hyperactivity and reversed the haloperidol (5mg/kg i.p.) induced catalepsy.

It is concluded that systemic injection of RU 24969 functionally activates the mesocorticolimbic DA system while injection into the ventral tegmental area, leads to an activation at the mesocorticolimbic DA system and an inhibition of striatal DA-ergic functions. On systemic or intranigral injection, RU 24969 activates the nigrostriatal DA neurons and their nigral output pathway.

Hunt, P. & Oberlander, C. (1981) Serotonin, In: Adv. in exp. med. and biol., 133, 547-561, Plenum, New York
Oberlander, C. & Boissier, J.R. (1981) Proc. 8th Int. Congr. Pharmacol., Tokyo.

DIFFERENT PRE- AND POST-SYNAPTIC DOPAMINERGIC EFFECTS OF 3PPP ENANTIOMERS

C. Bardelay, D. Cousty and P. Hunt (introduced by M. Worcel).
Centre de Recherches Roussel-Uclaf, BP.9, 93230 Romainville, France

The dopamine (DA) analogue, 3-(3-hydroxyphenyl) N-n-propyl piperidine (3-PPP) has been reported as a central DA agonist with selectivity for autoreceptors (Hjorth et al., 1981). The present communication concerns the activity of the two separate enantiomers of 3-PPP on both pre- and post-synaptic DA receptors in rat corpus striatum.

Two criteria were used for an action on pre-synaptic DA receptors : "in vivo",the inhibition of DOPA accumulation after γ-butyrolactone (GBL) blockade of DA impulse flow and "in vitro", the effect on DA release from field-stimulated striatal slices. Post-synaptic DA effects were investigated "in vivo" by measuring striatal acetylcholine (ACh) levels.

Both enantiomers inhibited DOPA accumulation after GBL treatment thus confirming pre-synaptic DA agonist activity. (+)3PPP, however, was about 7 times more active than (-)3PPP, the respective doses (s.c.) causing 50% inhibition being 1.9 $\pm$ 0.3 and 15.0 $\pm$ 3.7mg/kg (n=7).

The (+)-enantiomer also showed pre-synaptic DA agonist activity "in vitro", inhibiting stimulation evoked release of radioactivity from superfused striatal slices preloaded with ^{3}H-DA. Maximal inhibition (71%) was observed at a concentration of 10^{-6}M. (-)3PPP,on the other hand, had no effect on DA release at concentrations up to 10^{-6}M.

With respect to striatal ACh, (+)3PPP caused significant ($p < 0.01$) increases in levels from a dose (s.c.) of 10mg/kg (control value : 47.1$\pm$2.8 ; treated, 10mg/kg : 60.8$\pm$2.8, 50mg/kg : 76.8$\pm$3.8nmol/g tissue, n=7) indicating post-synaptic DA agonist activity. At the same doses (-)3PPP behaved as a DA blocker, significantly ($p < 0.05$ and < 0.01 respectively) decreasing ACh levels (10mg/kg : 37.7$\pm$2.4, 50mg/kg : 32.0$\pm$2.4nmol/g tissue, n=7).

These results show that both 3PPP enantiomers behave as DA agonists at presynaptic receptors, but (+)3PPP is clearly more active than (-)3PPP. Their action however is not selectively pre-synaptic. (+) 3PPP shows post-synaptic agonist properties with respect to striatal ACh while (-)3PPP is an antagonist.

One possible conclusion is that pre- and post-synaptic DA receptors have different specificities.

Hjorth, S. et al. (1981) Life Sci. 28, 1225.

THE EFFECTS OF OESTROGEN ON THE FATTY ACID CONTENT OF THE BRAIN

P.J. Thomas & Patricia Tuohy, Department of Pharmacology, Chelsea College, Manresa Road, London, SW3 6LX.

The mechanisms by which prostaglandins, neurotransmitters and ovarian steroids interact to affect the hypothalamic-amygdaloid control of ovulation are complex and ill understood, as are the mechanisms by which ovarian steroids affect prostaglandin metabolism. Since 17β oestradiol reduces adenylate cyclase activity in the brain (Ani et al 1980) and since 3'5' c AMP depresses Δ5 and Δ6 desaturase activity in the periphery (see Brenner, 1981) we postulated that an early step in the action of the hormone might be to modify the relative abundance of prostaglandin precursors in the brain.

We report preliminary results on the total fatty acid composition of the hypothalamic-amygdaloid region of ovariectomised rats compared with oestrogen treated controls. Adult rats were ovariectomised under ether 10 days before sacrifice: the experimental group was treated with oestradiol benzoate (20 µg/250g daily from ovariectomy to slaughter. Rats were beheaded and their hypothalamic-amygdaloid regions (with the optic chiasma removed) rapidly dissected into ice cold chloroform:methanol (2:1, v:v) under nitrogen. They were homogenised (Teflon-glass) and stored for two hours. After vigorous shaking with salt reagent, phosolipids were absorbed onto a silicic acid slurry and eluted with methanol. After alkaline hydrolysis, fatty acids were methylated by boiling (2 mins) with boron trifluoride reagent (BDH), extracted with chloroform and washed with water. Methyl esters were dried under nitrogen and stored in dark (4°) before estimation by gas chromatography, using a 10% polyethylene glycol adipate column (180°, isothermal), with a flame ionisation detector at 250°. Peaks were integrated using an Apple II computor and identified by comparison of retention times with those of standard methyl esters of fatty acids. Fatty acid compositions, expressed as percentages of total, are shown below (n=5 in both cases).

Table 1	+ Oestradiol	- Oestradiol	U
16:0	21.1 ± 3.9	24.7 ± 5.5	7
18:0	24.3 ± 1.4	24.4 ± 1.9	9
18:1	27.5 ± 2.0	27.6 ± 2.2	12
18:2	1.9 ± 2.2	1.4 ± 0.7	10
18:3	0.03 ± 0.06	1.3 ± 0.8	1*
20:4	9.2 ± 0.8	10.3 ± 7.2	12
22:4	6.2 ± 6.5	1.4 ± 0.5	4
22:6	9.6 ± 2.2	8.3 ± 2.8	8

U = U value obtained from Mann Whitney U-test
* significant difference (<0.01)

Linolenic acid (18:3) which is not normally present in detectable amounts (Sprecher, 1981) made up 1.3% of the total in oestrogen free animals but less than 0.05% in treated animals. Furthermore, our results suggest that oestradiol causes an increase in docasatetraenonic acid (22:4). We are unable to say from our results whether the linolenic acid detected in oestrogen free animals is of the ω3 or ω6 series but as 22:4 is only produced from ω6 fatty acids we feel that our results are consistent with an oestrogen induced increase in desaturase activity in the brain.

We thank Dr.J.Littleton for helpful advice.

Ani, M., Butterworth, P.J. & Thomas, P.J. (1980). Brain Research 183, 341-355.
Brenner, R.F.(1981). Prog.Lipid Res. 20, 41-47.
Sprecher (1981). Prog.Lipid Res.20, 13-22.

INTERACTION OF MEPTAZINOL WITH MUSCARINIC BINDING SITES

E. Brennan and R.S. Briggs, Department of Geriatric Medicine, University of Southampton

Meptazinol is a centrally acting analgesic with some characteristics of an opiate antagonist (Stephens et al 1978). Despite analgesic properties which are reversible by naloxone, meptazinol does not appear to cause dependence nor peripheral opiate effects such as respiratory depression and constipation. Ligand binding studies with meptazinol have shown a relatively low affinity for opiate sites (Blurton et al 1982). These anomalies suggest that meptazinol may also act at non-opiate sites, and central cholinergic synapses have been postulated as a result of experiments in isolated tissues and in conscious animals. This report describes the interaction between meptazinol and muscarinic ligand binding in rat brain.

Whereas classical muscarinic antagonists bind to an apparently homogeneous population of receptor sites, agonist displacement of labelled antagonist binding deviates from mass action kinetics (Birdsall et al 1978, Hulme et al 1978). Meptazinol was compared with the classical muscarinic antagonist atropine and the agonists oxotremorine and carbachol as inhibitors of [^{3}H] L (-)quinuclidinyl benzilate (L-QNB) binding to rat brain. Homogenates were prepared in 50mM Tris-HCl buffer (pH 7.8) from cortex, striatum and cerebellum. Data from displacement curves were fitted using a computer-assisted iterative procedure, arriving at the best-fit parameters by minimization of the sum of squares (Briggs et al 1982).

Displacement of L-QNB binding by oxotremorine and carbachol was typical of that caused by agonists: the competition curves fitted an interaction with two affinity states of the muscarinic receptor, the proportion of high- and low-affinity sites varying with brain region. The proportion of high-affinity sites in cortex striatum and cerebellum respectively was 12%, 13% and 61% for oxotremorine and 29%, 40% and 62% for carbachol. In contrast, competitive inhibition by atropine and meptazinol was consistent with the law of mass action for a single class of receptor. The mean ($\pm$ SEM) inhibition constant of atropine was 1.48 ($\pm$ 0.40) x 10^{-10}M, whilst that of meptazinol was 1.56 ($\pm$ 0.57) x 10^{-5}M.

Thus meptazinol showed the same qualitative interaction with the L-QNB binding site as the classical muscarinic antagonist atropine, though with an affinity five orders of magnitude lower. In contrast, experiments in whole animals (Bill et al 1983) or isolated tissues (Stephens et al 1978) have been interpreted as suggesting that meptazinol has cholinergic agonist properties, but are complicated by concomitant effects at opiate receptors. A more direct insight into the functional significance of the muscarinic binding properties of meptazinol would be achieved by studying a biochemical response coupled to the central muscarinic receptor.

Bill, D. et al (1983) Br. J. Pharmac (in press)
Birdsall, N.J.M. et al (1978) Molec. Pharmac. 14, 723-736
Blurton, P.A. et al (1982) Br. J. Pharmac. 77, 508P
Briggs, R.S. et al (1982) Neurobiol. Aging 3, 259-261
Hulme, E.C. et al (1978) Molec. Pharmac. 14, 737-750
Stephens, R.J. et al (1978) Gen. Pharmac. 9, 73-78

BINDING OF (^{3}H)-TIOTIDINE IN MOUSE VAS DEFERENS

J.A. Calvete, R.A.H. Harvey, T.P. Sloan, Department of Pharmacology, St Mary's Hospital Medical School, London W2 1PG.

It has previously been reported that strain differences exist in histamine H2-receptor characteristics in the mouse vas deferens (MVD), (Lush et al., 1982). To further characterise these postulated receptor differences we have evaluated the binding properties of [^{3}H]-tiotidine ([^{3}H]-T) with respect to both particulate and solubilised protein preparations obtained from MVD. Attempts to study [^{3}H]-T binding to particulate protein from MVD using conventional filtration assays were unsuccessful, owing to high levels of ligand binding to the filters used, (25% of ligand in incubation mixture). This effect was observed with celotate, fluoropore and glass fibre filter types. In addition, binding to filters could be displaced by the untritiated histamine H2-ligands ranitidine (R), cimetidine, dimaprit (D) and histamine (H) when present in concentrations > 10uM.

Binding of [^{3}H]-T dependent on the presence of protein could, however be demonstrated in a solubilised protein preparation from MVD. Vasa from TO strain mice (20-25g) were homogenised in ice-cold 10mM phosphate buffer (pH 7.4), and the homogenate centrifuged at 1500g for 10 min. The resulting supernatant was centrifuged again at 30000g for 60 min. The pellet was solubilised in 1% Triton X-100 in 10mM phosphate buffer (pH 7.4). Following centrifugation at 75000g for 20 minutes aliquots of supernatant were assayed for [^{3}H]-T binding capacity by incubation for 45 min at 37^{o}C with 50nM ligand. The incubation was terminated by addition of 10% activated charcoal and 4% BSA, followed by centrifugation at 5000g for 20 min. Bound ligand in the supernatant was determined by liquid scintillation spectrometry. The effects of untritiated histamine H2-specific competing ligands on [^{3}H]-T binding are summarised in Figure 1. Such competitors elicited a 2-5 fold

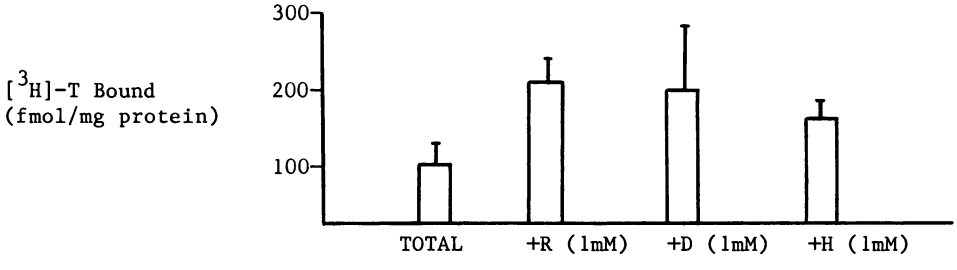

Figure 1: Effects of untritiated H2-receptor ligands on the binding of [^{3}H]-T to solubilized protein preparations from MVD. (Mean ± S.D., n=9).

increase in radioligand binding, indicating the presence of at least one other tiotidine binding component in addition to the histamine H2-receptor in this preparation. Resolution of the tiotidine binding components has been attempted using Sepharose 6B gel filtration chromatography. Preliminary studies have identified a tiotidine binding protein of molecular weight approximately 75000 It is apparent from these studies that partial purification of the histamine H2-receptor in MVD will be necessary for radioligand binding studies.

This work was supported by the Medical Research Council.

Lush, I.E., Sloan, T.P, Smith, R.L. (1982) Brit. J. Pharmacol., 76, 196P.

THE EFFECT OF α- AND β-ADRENOCEPTOR ANTAGONISTS ON RAT HEPATIC MITOCHONDRIAL CALCIUM UPTAKE

I. Cameron, I. Ebenezer, P. McDougall and A. Markham. Department of Pharmacology, Sunderland Polytechnic, Sunderland, SR1 3SD, U.K.

In recent years model systems using mitochondria isolated from heart muscle (Dhalla and Lee, 1977) and sacroplasmic reticulum isolated from either heart tissue (Scales and McIntosh, 1968) or white muscle (Noack et al, 1978) have been used to study the effect of β-adrenoceptor antagonists with respect to their ability to alter calcium ion transport into these organelles.

In this study both α and β-adrenoceptor antagonists were found to inhibit calcium ion uptake into tightly coupled rat hepatic mitochondria in a concentration dependent manner. Calcium ion uptake was studied indirectly by measuring proton ejection from mitochondria following the addition of 100-500 nmol $CaCl_2$ to the reaction medium containing either succinate (5mM), or glutamate (5mM) plus malate (5mM), according to the method of Carafoli and Azzi (1972).

IC_{50} values (concentrations required to reduce uptake by 50%) give a rank order of potency for the antagonists of dihydroergotamine (0.08±0.02mM), sotalol (0.10± 0.01mM) L-propranolol (0.30±0.04mM) and practolol (0.40±0.05mM), all n=5. The β-adrenoceptor antagonist oxprenolol (1mM) and the α-adrenoceptor antagonist phentolamine (0.5mM) were found to cause only 12% and 25% inhibition respectively, while the β-adrenoceptor agonist isoprenaline (0.01 to 2mM) had no significant effect (P>0.05) on either calcium uptake, or the level of uptake inhibition caused by practolol (0.4mM).

Concentrations of dihydroergotamine, isoprenaline, oxprenolol, practolol, L-propranolol and sotalol between 0.01 to 1mM were found to have no significant effect (P>0.05) on either mitochondrial state 3 (ADP present, with substrate and oxygen in excess) or State 4 (ADP absent, but substrate and oxygen in excess) respiratory rates. Difference spectra measurements confirmed that L-propranolol (0.5mM) prevented the reoxidation of the respiratory chain cytochromes a and a_3, with total inhibition occurring after 10 min. These findings suggest that α- and β-adrenoceptor antagonists do not cause inhibition of calcium uptake by preventing the synthesis of ATP, and must, therefore, act via a specific interaction with the mitochondrial membrane carrier system responsible for calcium ion transport.

The unphysiological concentrations of antagonists required to inhibit calcium uptake and the potencies obtained, indicate that the pharmacological properties of the antagonists are not totally related to their ability to prevent mitochondrial calcium uptake.

Carafoli, E. and Azzi, A. (1972) Experienta, 27, 906.
Dhalla, N. and Lee, S.L. (1977) Br. J. Pharmac., 57, 215
Noack, E. et al (1978) J. Pharmac. Exp. Ther. 206, 281.
Scales, B. and McIntosh, D.A.D. (1968) J. Pharmac. Exp. Ther. 160, 261.

ASSESSMENT OF AGONIST ACTIVITY OF CERTAIN DRUGS AT THE POST-JUNCTIONAL α-ADRENOCEPTORS OF THE RAT ISOLATED SEMINAL VESICLE

S. D. Gokhale & S. I. Sharif (introduced by J. Crossland).
Department of Pharmacology, Garyounis Medical School, Benghazi, Libya.

Parameters of agonist activity provide reliable data for classification of receptors. Of these, agonist potency ratios and the maximum response (Emax) are most commonly used. Based on determination of these parameters, it was concluded that the postjunctional α-adrenoceptors of the rat seminal vesicle are of the "classical or α_1-type (our unpublished observations). However, the EC_{50} value of an agonist may not represent a reliable estimate of the true affinity of that agonist, especially if a significant receptor reserve exists (Furchgott, 1972). A more reliable method of characterising receptors would then be a comparison of the equilibrium dissociation constants of agonists. Another important parameter of agonist activity is the efficacy (Stephenson, 1956) which represents the ability of the agonist to activate the receptors. While a determination of absolute efficacies is not possible, it is possible to determine the efficacy of one agonist relative to another standard agonist usually noradrenaline.

In the present investigation we have determined pD_2 and $-\log K_A$ values as estimates of affinity and Emax (maximum response relative to noradrenaline) and er (relative efficacy) values as estimates of efficacy for three α-adrenoceptor agonists, adrenaline, noradrenaline and phenylephrine, in an attempt to further characterize the postjunctional α-adrenoceptors of the rat isolated seminal vesicle and to see how these parameters of agonist activity correlate in this tissue. Seminal vesicles removed from young Sprague Dawley rats were employed as described previously (Gokhale & Sharif, 1982).

Dissociation constant (K_A) of agonists were determined by the technique of Furchgott & Burztyn (1967) using phenoxybenzamine (5nM for 60 min) to produce partial irreversible receptor inactivation. Efficacies of agonists relative to noradrenaline were determined from the respective K_A values by the procedures described by Furchgott & Burztyn (1967) and Basse & Furchgott (1976).

Table 1. Parameters of α-adrenoceptor agonist activity in the rat isolated Seminal Vesicle.

Agonist	n	pD_2	Relative Potency	$-\log K_A$	Relative Affinity	Emax	er
Adrenaline	7	7.09 ± 0.22	5.5	5.87 ± 0.28	1.57	1.15	4.24
Noradrenaline	7	6.19 ± 0.14	1	5.38 ± 0.13	1.0	1.0	1.0
Phenylephrine	8	5.76 ± 0.01	0.21	6.30 ± 0.09	9.45	1.13	0.15

n = Number of observations. pD_2 and $-\log K_A$ values are expressed as mean ± s.e. mean. The results indicate that for all the agonists tested, relative potency correlates well with relative efficacy ($r = 0.99$, $P = 0.001$) but has no correlation with relative affinity. In general the findings support the α_1- subclassification of the receptors involved.

Basse, J. C. & Furchgott, R. F. (1976). J. Pharmac. Exp. Ther. 197, 66-78.
Furchgott, R. F. (1972). Handbk. Exptl. Pharmac. 33, 258-289.
Furchgott, R. F. & Burztyn, P. (1967). Ann. N. Y. Acad. Sc. 144, 882-898.
Gokhale, S. D. & Sharif, S. I. (1982) Br. J. Pharmac. 77, 415 P.
Stephenson, R. P. (1956). Br. J. Pharmac. 11, 379-393.

EFFECT OF CHEMICAL SYMPATHECTOMY ON THE RESPONSE OF THE HEPATO-SPLANCHNIC BED TO PHENOBARBITONE IN THE RAT

C.R. Hiley & A.C. Wilson, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD.

Phenobarbitone (PB) increases liver blood flow in a dose-dependent manner by increasing the fraction of the cardiac output (CO) going to the organs draining into the portal vein (Yates et al. 1978). Both the hepatic and the splanchnic beds are innervated by sympathetic nerves which appear to control blood flow through these vascular beds. Since PB has ganglion blocking activity (Exley, 1954) it is possible that part of its effect on liver blood flow is mediated by changes in sympathetic activity. Accordingly we have investigated the effect of chemical sympathectomy on the distribution of CO and the response to PB in the rat.

Male Wistar rats (250-300g) were anaesthetized with ether and sympathectomised (sympex) by injection of 6-hydroxydopamine (50 mg/kg; Sigma) into a tail vein. This was repeated 16 and 24h later and the animals were maintained by the addition of 5% (w/v) glucose to the drinking water. Sham-treated animals received injections of vehicle (0.9% NaCl with 1 mg/ml ascorbic acid) at the same time. 16h after the last injection, treatment with PB (BDH; 40 mg/kg i.p. twice daily for 5 days) was started and CO distribution was determined 16-24h after the last dose using 15 μm diameter microspheres (3M Co.) as described previously (Yates et al.1978). Control sham-sympex or sympex rats received i.p. saline (2 ml/kg) twice daily for 5 days.

Five days after chemical sympathectomy there were no changes in CO (27.1±2.2 & 28.0 ±2.8 ml/min/100g body wt), hepatosplanchnic flow (8.00±0.45 & 8.35±0.74 ml/min/100g body wt) or total peripheral resistance (1.81±0.17 & 1.90±0.27 mm Hg.min/ml). Certain organ blood flows did change; there were increases in flow in the hepatic artery (0.13±0.03 & 0.30±0.08 ml/min/g liver; $P<0.05$) and the epididymides (0.18±0.04 & 0.45±0.05 ml/min/g tissue; $P<0.05$) and there were decreases in the percentage of CO reaching the testes (2.3±0.1% & 1.6±0.3%; $P<0.05$) and small intestine (6.7±0.5% & 5.0±0.5%; $P<0.05$). All values are mean ± s.e.m. with that for the sham sympex group given first; n=4 for the sympex group and P values were assessed by Student's t-test.

The amount of CO reaching the liver increased from 21.0±1.2% in saline-pretreated sympex rats to 27.3±1.3% ($P<0.01$) and 29.1±1.2% ($P<0.001$) in PB-treated sympex and sham-sympex rats respectively (P from saline-treated rats by analysis of variance; n=7 for all groups). Hence the increase in hepatosplanchnic blood flow was similar for both PB treated groups, from 4.68±0.21 ml/min/100g body wt (control) to 6.38± 0.65 and 6.35±0.61 ml/min/100g body wt. in the PB-treated sympex and PB-treated sham-sympex groups respectively ($P<0.05$ relative to control for both PB groups). Liver weight increased from 3.32±0.06 g/100g body wt. (saline sympex) to 4.15±0.10 g/100g body wt. (PB treated sympex; $P<0.001$) and 4.59±0.12 g/100g body wt. (PB treated sham-sympex; $P<0.001$).

The lack of effect of 6-hydroxydopamine pretreatment on the liver blood flow response to PB suggests that this response is not due to the known interactions of PB with the sympathetic nervous system. However it is possible that during PB treatment sufficient recovery of nerve activity occurred to restore normal function (Finch et al. 1973).

ACW was an MRC scholar. This work was supported by the British Heart Foundation.

Exley, K.A. (1954) Br.J.Pharmac. 9, 170-181
Finch, L. et al (1973) Br.J.Pharmac. 48, 59-72
Yates,M.S. et al (1978) Biochem.Pharmac. 27, 2617-2621

THE RESPONSE OF HUMAN VAS DEFERENS TO DRUGS MODIFYING NORADRENERGIC NEUROTRANSMISSION

R.J. Hayes & T.P. Sloan, Department of Pharmacology, St Mary's Hospital Medical School, London W2 1PG.

Noradrenaline released from sympathetic nerve endings is believed to activate presynaptic α-adrenoreceptors mediating feedback inhibition of further transmitter release (Westfall, 1977). A large proportion of the evidence for this concept is based on studies of vasa deferentia from mouse,rat and guinea pig (Farnebo & Malmfors, 1971; Stjarne, 1973). Drugs acting as agonists on presynaptic α-adrenoreceptors, such as clonidine reduce [^{3}H]-noradrenaline efflux and inhibit contractile responses to neuronal stimulation in these tissues, whilst presynaptic αadrenoreceptor antagonists such as yohimbine and phentolamine produce the reverse effects. Despite evidence that neurotransmission in human vas deferens is noradrenergic (Anton & McGrath, 1977), there is little information as to whether or not significant autoregulation of the process occurs in man.

Accordingly, we have studied the effects on human vas deferens of agents known to modify noradrenergic autoregulation. Human vasa (3-4 cm portions surplus to histological requirement, taken from the epididymal region) were obtained from 5 patients (25-49y) undergoing elective vasectomy. Tissues were mounted under 1g resting tension in 2ml organ baths in Krebs solution preheated to 37°C and gassed with 95% O_2/5% CO_2. Drugs were administered directly into the lumen of the tissue via 0.2mm i.d. a Portex cannula and removed by continuous perfusion with Krebs solution using a Gilson Minipuls 2 peristaltic pump, (perfusion rate 0.5ml/min). Field stimulation was applied as trains of 2 pulses (2 ms, 20Hz, 64V) at a train rate of 0.05Hz. These stimulation parameters resulted in a contractile response of consistent amplitude, stimulation rates in excess of 20Hz resulted in a single large contraction followed by contractions reduced in amplitude by 80-90%. The responses of human vas to the drugs studied are summarised in Table 1. The effects

TABLE 1: Effects of α-adrenoreceptor agonists on the response of human and murine vas deferens to electrical field stimulation.

DRUG	EFFECT ON HUMAN VAS	EFFECT ON MOUSE VAS
Clonidine (50-200ng)	70-500% potentiation	90-95% inhibition
Tyramine (50ng)	150% potentiation	50% inhibition
Phenylephrine (100ng)	300% potentiation and baseline contraction	as human

of clonidine on human tissue were abolished by both guanethidine (10ug) and prazosin (0.5ng). The effects of tyramine were abolished by guanethidine (10ug) and partially antagonised but not completely abolished by prazosin (0.5ng). The effects of phenylephrine on the response to field stimulation were abolished by both guanethidine and prazosin, but the drugs ability to elicit a baseline contraction was unaffected by either compound. As a preliminary explanation of these findings, we propose that the actions of clonidine on noradrenergic neurotransmission in human vas deferens are facilitatory rather than inhibitory, in contrast to the actions clonidine exerts in vasa deferentia from other species.

We are grateful to Mr. C. Kennedy (Dept. of Urology) for obtaining human vasa.

Anton, P.G. & McGrath, J.C. (1977), J. Physiol. 273, 45.
Farnebo, L.O. & Malmfors, T. (1971), Acta Physiol. Scand. Suppl. 371.
Stjarne, L. (1973), Nature 241, 190.
Westfall, T.C. (1977), Physiol. Rev. 57, 569.

A COMPARATIVE STUDY OF THE ACTIONS OF α-TOCOPHEROL AND VERAPAMIL ON THE GUINEA-PIG PORTAL VEIN

Wendy Richardson & A. J. Sweetman, School of Health and Applied Sciences, Leeds Polytechnic, Leeds LS1 3HE.

Both verapamil (Nayler et al, 1976) and α-tocopherol (Kelly and Richardson, 1981) have been shown to protect cardiac and vascular tissue against the deleterious effects of oxygen deprivation. In the following experiments we have examined whether verapamil and α-tocopherol have a common mode of action. The approach we used was to study the effect of both agents on spontaneous activity and calcium-induced tension changes in guinea-pig portal vein.

Spirally cut portal veins were suspended vertically in tissue baths containing Krebs-Henseleit solution bubbled with 5% CO_2 in O_2 at 37°C with a resting tension of 1-2g. Tension changes were measured isometrically with a force displacement transducer and recorded on an oscillograph. Mean tension changes were determined over 5 min periods for each set of conditions. Hypoxia was produced by changing to a gas mixture containing 5% CO_2 in N_2. Responses to calcium were obtained with a cumulative dosing schedule in calcium-free Krebs-Henseleit solution containing an increased level of KCl (50 mM), to depolarise the tissue. All values are the mean ± s.e.mean of from 3-5 determinations using different preparations.

Spontaneous activity. In hypoxic conditions spontaneous activity was approximately half that observed in the presence of oxygen. α-Tocopherol (41.8 μM) restored this activity to the levels observed in normoxic controls. In contrast, verapamil (0.1 μM) further reduced the level of activity (see Table 1). The protective action of α-tocopherol was antagonised by verapamil.

Table 1. Effect of α-tocopherol and verapamil on spontaneous activity

	mean tension (mg ± s.e.mean)		
	Normoxic control	Hypoxic control	Hypoxia + drug
α-Tocopherol (41.8 μM)	253.2 ± 40.5	140.8 ± 22.5	240.2 ± 34.8
Verapamil (0.1 μM)	231.2 ± 14.2	96.9 ± 21.7	33.4 ± 9.1
α-Tocopherol plus verapamil	203.6 ± 29	105.7 ± 16.6	37.8 ± 12.7

Each hypoxic control was significantly different from its normoxic control ($P<0.05$), and each drug treatment was significantly different from its hypoxic control ($P<0.05$).

Calcium-induced tension changes. Cumulative additions of calcium (1-6 mM) produced concentration-dependent tension changes which were unaffected by the inclusion of α-tocopherol (2.6-41.8 μM) in the bathing solution. Verapamil (0.1 uM) completely abolished responses to calcium, except at the highest dose of calcium (6 mM), when a 76% reduction in the response was observed. A similar pattern of activities was found in hypoxic conditions. It is concluded that the protective action of α-tocopherol is not due to a verapamil-like action.

Kelly, M.J. & Richardson, W. (1981) Br. J. Pharmac., 74, 941P.
Nayler, W.G. et al. (1976) Cardiovasc. Res., 10, 650-662.

THE CALCIUM DEPENDENCE OF EVOKED NORADRENALINE RELEASE FROM SYMPATHETIC NERVES: EVALUATION USING D600, NIFEDIPINE, DANTROLENE

K. Callanan & A.K. Keenan, Department of Pharmacology, University College Dublin.

The secretion of noradrenaline (NA) from central and peripheral noradrenergic neurones requires extracellular calcium (Trifaro, 1977). However it is not clear to what extent calcium released from intracellular stores also contributes to evoked NA release. In this study, comparison was made between the effects of dantrolene and the slow channel blockers D600 and nifedipine on evoked ^{3}H-NA release from isolated rat atria. Dantrolene is thought to inhibit calcium release from intracellular binding sites (Desmedt & Hainaut, 1979). The direct effect of these drugs on neuronal calcium uptake was assessed using rat brain synaptosomes.

Isolated rat atria were loaded with ^{3}H-NA, suspended between silver electrodes in an organ bath containing gassed Locke's solution and subjected to three periods of electrical stimulation (S_1-S_3) as described previously (Callanan & Keenan, 1980). Drug-induced changes in evoked release were measured as relative fractional release (Δt) between S_3 and S_1.

Rat brain synaptosomes, prepared by the method of Gray & Whittaker (1962), were preincubated for 20 min. with or without drug. Stimulated ^{45}Ca uptake was measured by difference, following 1 min. incubations in 5 mM or 45 mM K solution.

The effects of D600, nifedipine and dantrolene on evoked ^{3}H-NA release and K-stimulated ^{45}Ca uptake are summarised in Table 1.

Table 1. Inhibitory effects of D600, nifedipine and dantrolene.

		Evoked ^{3}H-NA release S_3/S_1, % of control^{+}	K-stimulated ^{45}Ca uptake % of control^{+}
D600 (μM)	20	53 ± 6***(8)	62.5 ± 6.3**(3)
	50	ND	39.1 ± 9.5**(3)
	100	6 ± 6***(4)	26.5 ±10.4**(3)
Nifedipine (μM)	20	77 ± 4***(5)	73.7 ±12.3* (3)
	50	ND	39.8 ± 6.8**(3)
	100	67 ± 6** (6)	47.2 ± 6.1**(3)
Dantrolene (μM)	20	70 ± 9** (4)	103.3 ±11.0 (5)
	50	ND	95.1 ± 8.9 (5)
	100	75 ±10** (5)	63.8 ±13.1* (5)

$^{+}$in the absence of drug; *$p<0.05$; **$p<0.01$; ***$p<0.001$; ND not determined.

D600 and nifedipine (20-100 μM) inhibited evoked release and similarly inhibited K-stimulated ^{45}Ca uptake. These findings are consistent with the reported slow channel blockade observed with these agents in smooth and cardiac muscle. (Triggle & Swamy, 1980). Dantrolene (20-100 μM) inhibited ^{3}H-NA release, the effect being maximal at 20 μM. In contrast, 20-50 μM dantrolene did not inhibit K-stimulated ^{45}Ca uptake, while 100 μM dantrolene reduced uptake to 63.8% of control.

Since dantrolene inhibited evoked release at a concentration not associated with inhibition of ^{45}Ca uptake, it can be inferred that calcium released from intracellular binding sites contributes to the evoked release of ^{3}H-NA.

This work was supported by the Medical Research Council of Ireland.

Callanan, K. & Keenan, A.K. (1980) Br. J. Pharmac. 72, 191-192P.
Desmedt, J.E. & Hainaut, K. (1979) Biochem. Pharmac. 28, 957-964.
Gray, E.G. & Whittaker, V.P. (1962) J. Anat. 96, 79-87.
Trifaro, J.M. (1977) Ann. Rev. Pharmac. Tox. 17, 27-47.
Triggle, D.J. & Swamy, V.C. (1980) Chest, suppl. 78, 174-179.

PROPERTIES OF THE CHICKEN ERYTHROCYTE CATECHOLAMINE-DEPENDENT ADENYLATE CYCLASE SYSTEM

A.K. Keenan & R. Murray, Department of Pharmacology, University College, Dublin.

In recent years a number of attempts have been made to reconstitute the catecholamine-sensitive adenylate cyclase system (Keenan et al, 1982) or its individual components (Citri & Schramm, 1980) solubilised from turkey erythrocyte membranes. In the present experiments adrenoceptor binding and adenylate cyclase activities were measured in the chicken erythrocyte with a view to its adoption as a model for reconstitution protocols. Parallel experiments were performed with turkey erythrocytes. All results quoted represent the mean± s.e. mean of three experiments.

Chicken erythrocyte membranes were prepared by the method used for turkey membranes (Steer & Levitzki, 1975), suspended in 50% sucrose; phosphate buffer to a protein concentration of 2.5 mg/ml and stored in liquid nitrogen until required. Binding of ^{125}I-cyanopindolol (^{125}I-CYP) was measured following dilution of membranes to 0.3 mg/ml in TME (50 mM Tris-HCl, 2 mM Mg, 1 mM EDTA, pH 7.4), incubation for 30 min. at 37°C and filtration through glass-fibre filters (GF/C). All data represent specific binding i.e. that binding displacable by 10 μM propranolol. Saturation curves were computer fitted using a nonlinear least squares curve fitting procedure (Metzler et al, 1974). Adenylate cyclase activity was measured by the method of Salomon et al (1974).

Chicken erythrocyte membranes bound ^{125}I-CYP with high affinity (K_D 22.2 ± 2.5 pM) this value compared favourably with the K_D of 70.6 ± 6.4 pM measured in turkey erythrocyte membranes under the same conditions. The maximal binding capacity of ^{125}I-CYP was 204.5 ± 6.7 f.mol/mg in chicken and 406 ± 16 f.mol/mg in turkey membranes which is less than previously published values for turkey (Keenan et al, 1982).

Adenylate cyclase activity was measured under different stimulating conditions and the results are summarised in Table 1. Both chicken and turkey cyclase were stimulated by the GTPanalogue, Gpp(NH)p. In both preparations fuller activiation was achieved with sodium fluoride. The extent of activation obtained with isoprenaline is a measure of receptor-cyclase coupling and was much less for chicken (3-fold increase over the basal) than for turkey (20-fold increase). In order to express the full activity of the system, membranes were "permanently activated" with Gpp(NH)p + isoprenaline.

In summary, we have shown that the catecholamine-dependent adenylate cyclase of chicken erythrocytes has properties in common with those of the turkey erythrocyte.

Table 1. Stimulation of adenylate cyclase activity (p.mol cAMP/min/mg protein).

Treatment	Chicken	Turkey
Basal	1.5 ± 1.03	2.7 ± 1.8
Gpp(NH)p 100 μM	22.3 ± 3.4	87.3 ± 5.0
Isoprenaline 10 μM	4.4 ± 2.3	54.5 ± 9.4
NaF 10 mM	38.9 ± 3.6	100.0 ± 1.4
Gpp(NH)p + isoprenaline	113.3 ± 3.0	288.7 ± 8.6

This work was supported by the Medical Research Council of Ireland.

Citri, Y. & Schramm, M. (1980) Nature, 287, 297-300.
Keenan et al (1982) Biochem. Biophys. Res. Commun., 105, 615-623.
Metzler, C.M. et al (1974) Biometrics, 30, 562.
Salomon, Y. et al (1974) Anal. Biochem., 58, 541-548.
Steer, M.L. & Levitzki, A. (1975) J. Biol. Chem., 250, 2080-2084.

DOMPERIDONE AND BASAL AND TRH-STIMULATED PROLACTIN SECRETION IN THE OVARIECTOMIZED RAT

P. Gilna and F. Martin, Department of Pharmacology, University College Dublin, Dublin 4, Ireland.

The effect of the dopaminergic antagonist domperidone on basal and TRH stimulated prolactin secretion in the oestrogen primed anaesthetized rat has been studied.

Groups of oestradiol-primed ovariectomized adult rats were anaesthetized with urethane (0.8 ml. 100 g^{-1}, 14% solution (Wt/V in saline)) and cannulae implanted in the jugular vein and carotoid artery. One hour later a basal blood sample (arterial (100 µl)) was taken and TRH (50ng in 200µl saline) administered intravenously. A further blood sample was taken 5 min post TRH administration. Domperidone at three doses was infused (rate 2.0 ml. h^{-1}) in three different experiments such that 50µg was delivered in 1h, 5.0µg in 30 min and 1.0µg in 30 min. Further basal and 5-min post TRH blood samples were taken (as above) at 30 min intervals both during and up to 2h after the infusion ended. Plasma was separated and prolactin levels determined by radioimmunoassay. Differences arising during and post-infusion were investigated using a two-way analysis of variance.

Domperidone (50µg, infused over 1h) markedly increased basal plasma prolactin levels (Control,12 ±1.6ng/ml,DOM,146.9±6.9ng/ml n=6, p 0.0005) and these levels were still significantly greater than control 2h post infusion. Plasma prolactin levels 5 min post TRH were also significantly increased over control (52.3±13.0ng/ml, n=6) both during (145.2±5.3ng/ml, n=6, $p < 0.005$) and up to 2h post infusion (128.88 ±4.6ng/ml, n=6, $p < 0.0005$ cf control). However, at the end of the infusion period and in 3 of the 4 post infusion TRH tests no net secretory response to TRH was seen. Over this period basal prolactin levels were ≃10-fold control.

Domperidone (5µg, infused over 30 min) similarly affected basal plasma prolactin levels and 5 min post TRH levels and the effects were maintained in the post infusion period. Also, the net secretory response to TRH at the end of this infusion period was significantly increased over control (control, 49.3±12.2 ng/ml n=7, DOM,182.4±15.8 ng/ml, n=7, $p < 0.0005$) but this effect was not maintained in the post infusion period. However, the secretory response to TRH expressed as a fraction of the basal prolactin level was less than control both during and post domperidone infusion in both these experiments. Domperidone (1µg, infused over 30 min) showed a similar trend to increase basal and post TRH plasma prolactin levels but only the post TRH change was significant. There was no change in the net secretory response to TRH.

Domperidone strikingly increased basal plasma prolactin when doses ≥ 5µg were infused and the effect was partially maintained in the post-infusion period. However, only at the end of the 5µg infusion was the net TRH secretory response significatnly increased over control and in no case was the TRH response, expressed as a fraction of basal levels, increased. We conclude, therefore, that reversal of the dopaminergic control of basal prolactin secretion does not universally potentiate responses to TRH and can impair them.

A SINGLE ADMINISTRATION OF TAMOXIFEN POTENTIATES OESTRADIOL-INDUCED PROLACTIN SECRETION IN THE MALE RAT.

P. Gilna and F. Martin, Department of Pharmacology, University College Dublin, Dublin 4, Ireland.

The interaction of tamoxifen (administered both acutely and continuously) with oestradiol has been studied in relation to its effects on basal plasma prolactin levels.

Male wistar rats 140 ± 10 g were used. In each experiment oestradiol or tamoxifen or mixtures thereof were administered in saline (containing 10% ethanol) as a single or single daily subcutaneous injections. The animals were given water and pelleted food ad libitum. Forty eight hours after the last injection the animals were anaesthetized with urethane (0.8 ml. 100 g^{-1}, 14% solution (wt/V in saline)) and the right jugular vein cannulated. One hour later a basal blood sample (100µl) was taken via the jugular cannula and TRH (50ng in 200µl saline) administered intravenously. A further blood sample was taken 5 min post TRH administration. The procedure was repeated after 25 min with the collection of another basal blood sample and a 5 min post-TRH sample. The animals were then killed, the anterior pituitary removed and homogenized in phosphosaline buffer, pH 7.4. Plasma was separated and all samples were assayed immediately for rat prolactin by radioimmunoassay. Differences between treatments were investigated using a one way analysis of variance.

Tamoxifen (60µg) administered simultaneously with oestradiol (10µg) potentiated the increases in basal plasma prolactin (OE_2, 1.8±0.1ng/ml, n=6, OE_2 + TAM; 4.4±0.7 n=5; $p < 0.0005$), TRH - stimulated prolactin secretion (OE_2, 3.0±0.6ng/ml, n=6, OE_2+TAM; 9.5 ± 1.9 ng/ml, n=5; $p < 0.0005$) and radioimmunoassayable pituitary prolactin (OE_2, 1.8 ± 0.1µg/mg, n =6, OE_2+TAM; 4.4±0.7 n=5; $p < 0.05$) seen in response to a single subcutaneous injection of oestradiol. Tamoxifen (60µg administered as a single injection 3 or 24h prior to oestradiol similarly potentiated the basal and TRH-stimulated plasma prolactin levels seen with oestradiol alone. However, if the tamoxifen was administered sequentially i.e. 48 and 24h before or 48 and 24h before and simultaneous with the oestradiol the potentiation was completely reversed. Single or multiple administrations of tamoxifen alone had no suppressive effects on plasma prolactin levels or pituitary prolactin.

Van Doorn, Poortman, Thijssen and Schwartz (1981) have shown that 5-androstene-3β, 17β-diol, an oestrogen of low potency, will potentiate oestradiol induced uterotrophic responses in the rat. This steriod elevates the level of oestradiol in the uterine cytosol and causes a second wave of oestrogen receptor complex translocation to the nucleus 6h after steriod administration. Acute tamoxifen may act in this system by a similar mechanism. In contrast, repeated administration of tamoxifen must reverse this potentiation by its classical antioestrogenic action.

Doorn, L.G. van, Poortman, J., Thijssen, J.H.H. and Schwartz, F. (1981) Endocrinol. 108, 1587-1593)

INHIBITORY ACTIONS OF SOMATOSTATIN ON INTRALUMINAL PRESSURE MEASUREMENTS IN THE GUINEA-PIG ISOLATED STOMACH

A.S. Thamia and D. Wood (introduced by J.M. Foy), Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

The tetradecapeptide somatostatin (SST) has been shown to inhibit gastric motility and tone in anaesthetised (Tansy et al, 1979) and conscious (Ormsbee et al, 1979; Bueno et al, 1982) animals of several species. This study investigates the mechanism of action of SST in an isolated preparation free from central influences.

Starved Duncan-Hartley derived guinea pigs (180-250 g) of either sex were killed. Stomachs were excised following ligation of the gastro-oesophageal junction and cannulation via the duodenum. After flushing the gastric lumen, the preparations were immersed in Krebs' solution at 37°C, gassed with 5% CO_2 in O_2. Each stomach was inflated (15-20 ml Krebs' solution) and intraluminal pressure recorded via the cannula and attached pressure transducer. Within 5 min of inflation the basal pressure fell to 4-8 mmHg, a level which could be maintained for several hours. The preparation was allowed to equilibrate for 30 min before drug addition to the bath. For all experiments, $n \geq 4$.

In these preparations, two patterns of basal motor activity occurred, either singly or in combination: a fast rhythm (2-4 cycles min^{-1} with an amplitude of 1-2 mmHg and a slower rhythm (0.5-1 cycles min^{-1}) with an amplitude of 2-4 mmHg. Tetrodotoxin (TTX, 10 nM) or atropine (30 nM) almost completely inhibited the normal contractile activity and both produced falls in basal pressure (2.0 ± 0.3 and 3.0 ± 0.5 mmHg respectively).

SST (0.6 - 60 nM) reduced the amplitude of both forms of contractile activity in a dose-dependent manner, the maximum reduction being 80-90%. Similar doses produced falls in basal pressure, with a maximum reduction of 0.9 ± 0.2 mmHg. Noradrenaline (3-300 nM) also reduced the amplitude of the contractile activity (maximum 90-100% reduction) and produced a fall in basal pressure (maximum fall 1.9 ± 0.2 mmHg). Neither agonist affected the frequency of the contractile activity. The fall in basal pressure produced by SST was abolished by either TTX or atropine, whereas the reduction in pressure produced by noradrenaline was not significantly affected by these agents ($p>0.05$ cf. control).

These results suggest that the intrinsic tone and activity of this preparation are largely under cholinergic control, confirming the findings of Paton & Vane (1963) and Beani et al, (1971). Secondly, whilst noradrenaline appears to produce a reduction in basal pressure through a direct action upon the smooth muscle, as reported by Bülbring & Gerson (1967), SST apparently decreased basal pressure through interference with cholinergic mechanisms.

A.S.T. is a S.E.R.C. CASE student with Ciba-Geigy Ltd.

Beani, L. et al (1971) J. Physiol. 217, 259-279
Bueno, L. et al (1982) Gastroenterol. 82, 1395-1405
Bülbring, E. & Gershon, M.D. (1967) J. Physiol. 192, 823-846
Ormsbee, H.S. et al (1978) Am. J. Dig. Dis. 23, 781-788
Paton, W.D.M. & Vane, J.R. (1963) J. Physiol. 165, 10-46
Tansy, M.F. et al (1979) J. Pharmaceut. Sci. 68, 1107-1113