SELECTIVE INTERACTIONS OF BUSPIRONE, WB 4101 AND PRAZOSIN WITH 8-OH-DPAT AND RU 24969 IN THE RAT

Ruth Clague & Michael Spedding, Syntex Research Centre, Riccarton, Edinburgh, EH14 4AS, Scotland.

Buspirone and 8-OH-DPAT [8-hydroxy-2-[di-n-propylamino]tetralin] interact selectively with 5-HT1A receptors; affinity for this site may be related to anxiolytic effects, but it is unclear whether buspirone acts as a full antagonist. RU24969 is an agonist at 5-HT1 sites with some selectivity for the 5-HT1B site [Tricklebank et al, 1986] so challenge with 8-OH-DPAT or RU24969 might be used to distinguish selectivity at 5-HT1 subtypes. Male Sprague-Dawley rats [150-220g] were placed in plastic cages on activity meters for 18min after challenge with 8-OH-DPAT or RU24969 [s.c.] and scored for the "5-HT behavioural syndrome" of fore-paw treading, head-weaving and flat body posture [Tricklebank et al, 1985]. Locomotion was monitored on the activity meters between 3 and 18 min.

8-OH-DPAT [0.1-3mg/kg] induced the 5-HT behavioural syndrome [Tricklebank et al, 1985] and the effects of 8-OH-DPAT [0.3mg/kg] were antagonized by buspirone [1-5mg/kg, s.c. 30min previously] and WB4101 [0.5-5mg/kg, s.c.]. Prazosin [0.5mg/kg, s.c.] did not affect flat body posture but abolished head-weaving, fore-paw treading and ambulation, consistent with the adrenergic component of some of these behaviours [Tricklebank et al, 1985]

In contrast, RU24969 [3mg/kg] caused only slight fore-paw treading and flat body posture, but markedly increased head-weaving and locomotion. The slight degree of fore-paw treading was dose-dependently inhibited by buspirone [1-5mg/kg] and WB4101 [0.5-5mg/kg], indicating a 5-HT1A component. Furthermore, a subthreshold dose of 8-OH-DPAT [0.05mg/kg] selectively increased forepaw treading in response to RU24969. Low doses of buspirone [1mg/kg] or WB4101 [0.5mg/kg] markedly increased flat body posture, which is consequent to 5-HT1A receptor activation [Tricklebank et al, 1986]; the marked increase in flat body posture following buspirone and WB4101 may therefore be due to partial agonist effects at 5-HT1A receptors. The drugs caused slight dose-dependent inhibition of head-weaving and locomotion. Prazosin [0.5mg/kg] caused slight inhibition of all parameters except flat-body posture which was slightly increased.

None of the effects of 8-OH-DPAT or RU24969 were sensitive to alpha2 adrenoceptor antagonists [yohimbine, idazoxan, SKF86466; 0.5mg/kg, i.p.]. Thus although the selectivity of RU24969 for the 5HT1B site is low, comparison of the effects of RU24969 with those of 8-OH-DPAT in the presence of drugs with selectivity for 5-HT1 sites may yield an index of selectivity for the receptor subtypes and indicate the possibility of "partial agonist" effects.

Tricklebank M.D., Forler C. & Fozard J.R. [1985]. Eur. J. Pharmacol., 106,271-282. Tricklebank M.D., Middlemiss D.N. & Neill J. [1986]. Neuropharmacol., in press.

THE PHARMACOLOGICAL CHARACTERIZATION OF 5-HT MEDIATED PHOSPHOINOSITIDE TURNOVER IN MOUSE CORTEX

P.P. Godfrey, D.J.Heal[1], S.J. McClue and M.M. Young, MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE and [1]The Research Department, The Boots Company PLC, Nottingham NG2 3AA.

5-Hydroxytryptamine (5-HT) has recently been shown to increase phosphatidylinositol (PI) metabolism in rat cortical slices and this effect is probably mediated via 5-HT_2 receptors (Conn and Sanders-Bush, 1985; Kendall and Nahorski, 1985). In mice, 5-HT_2 receptors are also widely distributed in the brain; high concentrations have been found in cortex (Heal et al, 1985) and also in hindbrain and spinal cord where the head-twitch behaviour is almost certainly initiated (see Green and Heal,1985). In view of this, the effect of 5-HT on PI turnover has been studied in mouse cortical slices to determine whether this response is mediated via 5-HT_2 receptors.

Adult male C56/B1/601a mice (Olac, Bicester) weighing 25-30g were used throughout. Cortical slices from 4 mice were pooled and PI turnover was determined by the method of Brown et al (1984). The slices were incubated with ^{3}H-inositol (0.5µCi/ml) for 30min, and for a further 45min in the presence of agonist and 10mM LiCl. Antagonists were added 10min prior to agonist. The reactions were stopped with chloroform/ methanol (1:2) or 10% perchloric acid and ^{3}H-inositol phosphates (IP) were separated by anion exchange chromatography.

The addition of 5-HT ($3x10^{-8}$M-10^{-5}M) produced a dose-dependent increase in IP accumulation (ED_{50} 800nM). Analysis showed that 100µM 5-HT produced large increases in IP_1 (140%) and IP_2 (95%) with lesser effects on IP_3 (51%) and IP_4 (46%). 5-HT (100µM) induced IP accumulation was dose-dependently inhibited by the non-selective 5-HT antagonists methysergide, metergoline and spiperone (IC_{50} 260, 890, 600 nm respectively). The selective 5-HT_2 antagonist ketanserin also inhibited the response (IC_{50} 220nM) and additions of ketanserin (3, 10, 30 nm) caused progressive rightward shifts of the 5-HT dose-response curve. The beta-adrenoceptor antagonists propranolol and pindolol, which have been shown to have reasonable affinity for the 5-HT_1 receptor subtype (Nahorski and Willcocks, 1983), had no effect on 5-HT induced IP accumulation at concentrations up to 10uM. Additionally atropine, prazosin and mepyramine (1µM) were without effect.

In conclusion PI turnover was dose-dependently increased in mouse cortical slices by 5-HT and this probably results from stimulation of 5-HT_2 receptors. These data are supported by the earlier report of a high density of 5-HT_2 receptors in the cortex of C57/B1/601a mice and are also in good agreement with earlier findings obtained in rat brain (Conn and Sanders-Bush, 1985; Kendall and Nahorski, 1985).

Brown, E. et al (1984) J. Neurochem. 42, 1379
Conn, P.J. & Sanders-Bush E. (1985) J. Pharm. exp. Ther. 234, 195
Green, A.R. & Heal, D.J. (1985) In: Neuropharmacology of Serotonin. (Green, A.R. ed) p.329, Oxford University Press, Oxford
Heal, D.J. et al (1985) Neuropharmacology 24, 1201
Kendall, D.A. & Nahorski, S.R. (1985) J. Pharm. exp. Ther. 233, 473
Nahorski, S.R. & Willcocks, A.L. (1983) Br. J. Pharmac. 78, 107P

TOPOGRAPHICAL DOPAMINE AND DOPAC DISTRIBUTION AND DOPAMINE TURNOVER IN CONTROL AND MPTP-TREATED COMMOM MARMOSETS

Barnes, N.J.G., Barnes, N.M., Costall, B. and Naylor, R.J., Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces a severe parkinsonian syndrome in humans and non-human primates. The neurotoxin has been shown to selectively destroy cells in the substantia nigra leading to marked reductions in striatal dopamine (DA) content (Jacobowitz et al, 1984). In the present study we examine the regional distribution of DA and its metabolite dihydroxyphenylacetic acid (DOPAC) in the marmoset caudate nucleus (CN) and putamen (PUT.) in control and MPTP-treated marmosets.

Common marmosets (Callithrix jacchus, male, 350-400g) were given (i.p.) MPTP, 4 mg/kg on day 1; 2 mg/kg on days 2, 3 and 4, or vehicle (n=3). 6 months post-MPTP treatment marmosets were anaesthetised (pentobarbitone 15 mg/kg i.p.) and then killed by decapitation. The brains were removed and rapidly frozen on dry ice and sliced coronally into 2 mm segments. Slices were then mounted on a freezing microtome stage and discs of tissue (0.75 mm diameter) were removed using a 1 gauge serum needle with an internal wire plunger for removal of the tissue sample. Punches were taken at 3-4 rostro-caudal levels. The tissue samples were sonicated in perchloric acid and stored in liquid nitrogen until assayed. DA and DOPAC were measured by high pressure liquid chromotography (HPLC) with electrochemical detection (ECD)

Table 1. Topographical analysis of the modification by MPTP of dopamine/metabolite levels in the caudate nucleus and putamen.

Brain region/ Coordinate*	DA (ng/mg tissue) Control	DA (ng/mg tissue) MPTP	DOPAC (ng/mg tissue) Control	DOPAC (ng/mg tissue) MPTP	DA:DOPAC Control	DA:DOPAC MPTP
CN						
A 13.0-12.0	25.2±1.1	0.31±0.19	2.45±0.18	0.07±0.03	10.2	4.4
A 11.5-9.5	22.5±1.4	0.51±0.08	1.57±0.32	0.18±0.11	14.7	2.8
A 9.5-7.0	23.3±3.2	0.15±0.01	1.07±0.26	0.03±0.01	22.3	5.0
A 7.0-4.0	12.4±1.2	0.05±0.01	0.58±0.35	0.01±0.01	21.3	5.0
PUT						
A 11.5-9.5	24.9±2.7	0.82±0.27	2.13±0.39	0.15±0.09	11.6	5.4
A 9.5-7.0	18.9±1.3	0.15±0.04	0.99±0.55	0.04±0.01	19.0	3.7
A 7.0-4.0	8.0±1.2	0.15±0.08	0.21±0.02	0.01±0.01	38.1	15

All reduction in DA and DOPAC caused by MPTP are significant to $P<0.001$. (Student's t-test.) *Atlas of Stephan et al (1980).

The data shows a topography in levels of DA and in the DA:DOPAC ratio in both the CN and PUT of normal marmosets with the former decreasing and the latter increasing in the rostro-caudal direction, with 2-3 fold differences being recorded. The MPTP treatment reduced levels of DA by 98-99% and the DA:DOPAC ratio by 54-81% to mask the normal topography (Table 1), and to indicate a greater turnover of DA in the remaining neurones.

This work was supported by the Parkinson's Disease Society and by Glaxo Group Research.

Jacobowitz, D.M. et al (1984) Psychopharmac. Bull. 20, 416-422
Stephan, M. et al (1980) The Brain of the Common Marmoset (Callitrix jacchus) A stereotaxic Atlas, New York: Springer Verlag

CIRCLING BEHAVIOUR FOLLOWING LESION OF THE MRN IS MODIFIED BY DRUG ACTION ON 5-HT SYSTEMS

Brenda Costall, R.J. Naylor & E.S. Onaivi Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP

Contralateral circling and body asymmetry follow asymmetric lesion of the medial raphe nucleus (MRN) in the rodent (Pycock, 1978). The circling/asymmetry are considered to reflect a disruption to the ascending 5-hydroxytryptamine (5-HT) systems, although the precise forebrain structures mediating the response have not been established. In the present study we investigate drug action in the nucleus accumbens (ACB) to modify the circling/asymmetry following MRN lesions.

Male albino mice (BKW) weighing 30-40g were subject to standard stereotaxic surgery to lesion asymmetrically the MRN. Animals showing a marked contralateral circling (11-17revs/min) and asymmetry (score 3, on a 0-4 scale) were selected and implanted with chronically indwelling guide cannulae to allow subsequent delivery of drug or vehicle into the centre of the nucleus accumbens. n = 5-10 in all experiments.

The contralateral circling/asymmetry caused by the MRN lesions persisted for at least 20 weeks. The unilateral injection of 0.1µg fluphenazine into the ACB of the lesioned side reduced the circling/asymmetry by 35 and 51% respectively ($P<0.001$). 1.0µg fluphenazine reversed the contralateral responding to a modest ipsilateral circling (2-3revs/min) and asymmetry (score 1). The unilateral injection of fluphenazine (0.1 and 1.0µg) into the ACB of the non-lesioned side failed to cause any reduction in asymmetry but reduced circling to 64 and 12% of control values ($P<0.001$), with no reversal of response.

The unilateral injection of ICS 205-930 (0.1µg) into the ACB of the lesioned side enhanced contralateral circling behaviour to an intense response of 22-24revs/min ($P<0.001$); contralateral asymmetry was also increased to 120-130% of control values ($P<0.05$). A higher dose of 1.0µg ICS 205-930 reduced both circling (to 28%) and asymmetry (to 40%). The injection of ICS 205-930 (0.1 and 1.0µg) into the other ACB failed to consistently modify asymmetry, but circling was reduced by 53% at the higher dose ($P<0.001$).

The unilateral injection of 2-methyl-5-HT (0.1 and 1.0µg) into the ACB of the side of the MRN lesion abolished the contralateral circling/asymmetry and caused a brief reversal of both circling/asymmetry to an ipsilateral response. The injection of 2-methyl-5-HT (0.1 and 1.0µg) into the other ACB failed to consistently modify the contralateral asymmetry and reduced circling by 31 and 57% ($P<0.01$-$P<0.001$) at 0.1 and 1.0µg respectively.

It is concluded that the ACB may be an important structure to contribute to the development of circling/asymmetry following MRN lesions. The contralateral circling/asymmetry was most effectively antagonised by drug injection into the ACB of the lesioned side where the antagonism by fluphenazine indicates the importance of dopamine to mediate the circling/asymmetry. The potent antagonism exerted by the selective 5-HT M receptor agonist 2-methyl-5-HT and the enhancement of contralateral circling by the 5-HT M receptor antagonist ICS 205-930 indicates the importance of a 5-HT involvement. The specificity of drug action on the 5-HT receptors is being assessed to determine the value of the circling model to characterise the specificity of 5-HT agonist/antagonist activity.

Pycock, C.J. (1978) Neuroscience 5, 461-514.

IN VIVO PHARMACOLOGY OF ICI 169,369- A NEW 5-HT_2 ANTAGONIST

T.P. Blackburn, B. Cox, R.J. Pearce and C. W. Thornber. ICI Pharmaceuticals Division, Research Department 2, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG.

The in vivo pharmacology of ICI 169,369 (2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride) was investigated in a variety of species in order to determine its selectivity for the 5-HT receptor. In two central tests indicative of activity at 5-HT_2-receptors, 5-HTP-induced head twitches in the mouse and fenfluramine-induced hyperthermia in the rat (Blackburn et al., 1983), ICI 169,369 produced a dose related inhibition with ID_{50} (inhibitory dose 50%) values of 1.6 (1.0-2.5)mg/kg i.p. and 1.2 (0.3-4.3)mg/kg p.o. respectively (numbers in parenthesis indicate 95% confidence limits). ID_{50} values for a number of other classical 5-HT antagonists were also determined in these models (eg ketanserin 0.5(0.02-0.9)mg/kg i.p. and 0.1(0.02-0.3)mg/kg p.o. respectively, ritanserin 0.08(0.03-0.18)mg/kg i.p. and 0.3(0.02-5.8)mg/kg p.o. respectively). ICI 169,369 was also active against 5-HT-induced presssor responses in the pithed rat (ID_{50} 0.1mg/kg i.v.) and was inactive against noradrenaline-induced pressor response (ID_{50} >1.0 mg/kg i.v.) in this preparation. Tests of activity at other receptor sites were; histamine-induced bronchospasm in the guinea-pig Konzett-Rossler test (H1-receptor), oxotremorine-induced tremor and salivation in the mouse (muscarinic M_1 receptor) amphetamine-induced stereotypy in the rat (dopamine D_2 receptor), noradrenaline and electrically stimulated cat nictitating membrane preparation (α_1 & α_2 receptors) and isoprenaline-induced tachycardia and vasodilation in the dog (β_1 & β_2 receptors). ICI 169,369 was found to be inactive in all of these tests at 10-100 times the ID_{50} value for 5-HT antagonism. Thus results from the in-vivo studies with ICI 169,369 show that the compound has a marked selectivity for 5-HT receptors in vivo, with no apparent affinity for other classical neurotransmitter receptors.

Blackburn,T.P., Cox,B., Heywood,B. and Kemp,J.D. (1983) Br. J. Pharmac. 79,223P.

THE ANXIOLYTIC ACTIVITY OF GR38032F IN THE MOUSE AND MARMOSET

Brenda Costall, Annette M. Domeney, C.A. Hendrie, M.Elizabeth Kelly, R.J. Naylor & M.B. Tyers[1], Postgraduate Schools of Studies in Pharmacology and Psychology, University of Bradford, Bradford, BD7 1DP, and [1]Neuropharmacology Department, Glaxo Group Research Ltd., Ware, SG12 0DJ.

A simple test has been described for detecting anxiolytic action in the mouse (Costall et al, 1986). Briefly, the apparatus consists of an open-topped box one third painted black and illuminated with dim red light and partitioned, with an access opening, from the remainder of the box painted white and brightly illuminated. The floor is lined into 9cm sqaures. Naive mice (male, BKW) from a dark environment are placed in the centre of the white area and subsequent rearings and line crossings in the white and black sections measured (n=5-10).

Diazepam (0.125-5mg/kg i.p., 45 min) significantly increased rearing in the white section (42.6±4.2/5 min, compared with 12.7±1.2/5 min for control animals) with corresponding decreases in the black (12.6±1.4/5 min; control values 32.1±4.1/5 min, $p<0.001$). Similarly, line crossings were elevated in the white section (82.7±8.6/5 min, control values 49.7±5.0/5 min, $p<0.001$) and decreased in the black section (26.7±2.9/5 min, control values 61.1±6.3/5 min, $p<0.001$). At a higher dose of diazepam (10mg/kg) marked sedation lead to depression of rearing and line crossings in both the black and white sections. The $5HT_3$ receptor antagonist GR38032F (Brittain et al, this meeting) altered mouse behaviour in a manner similar to diazepam, but without sedation. Thus GR38032F (0.00005-0.01mg/kg i.p., 45 min) elevated rearing behaviour in the white section (33.2±3.1/5 min, control values 16.7±1.5/5 min, $p<0.001$) and decreased this behaviour in the black (21.1±2.3/5 min to 14.4±1.6/5 min, control values 32.4±3.4/5 min, $p<0.01$-$p<0.001$), and caused increased line crossings in the white section (84.7±8.6/5 min to 88.4±8.9/5 min, control values 41.2±43/5 min, $p<0.001$), and decreased in the black (37.6±3.7/5 min, control values 59.7±6.1/5 min, $p<0.01$-$p<0.001$).

Common marmosets (Callithrax jacchus) exhibit characteristic behaviours of apprehension and anxiety when an experimenter presents a 'threat' by standing in front of the housing cage. The most characteristic changes were in the amount of time the marmosets spent on the front of the cage observing and posturing at the experimenter, the number of aggressive postures made and the time spent vocalising (associated with alarm or threat). Marmosets were observed for 10 min when the number of jumps to and from the cage front ranged from 0-4 (mean 2.9±0.6/2 min, n=7). Diazepam (0.025mg/kg b.d.) elevated these values to a mean of 7.6±0.8/2 min, (n=4): a similar change (mean 8.0±0.8/2 min, n=4) was seen after treatment with GR38032F (0.001mg/kg b.d.). In diazepam-treated animals the number of aggressive postures reduced from 10.4±1.1/2 min to 2.3±0.2/2 min, and for GR38032F these were reduced to 6.1±0.5/2 min (but with a reduction to 2.1±0.7 on the 12th day of treatment). Both diazepam and GR38032F reduced the amount of time spent vocalising by at least 25%.

Thus, in both the mouse and marmoset (as in the rat and monkey; Jones et al, this meeting) the selective $5HT_3$ receptor antagonist GR38032F causes similar effects to diazepam in situations of aversion, anxiety or apprehension, and it is suggested that this agent may represent the first of a new class of non-sedative anxiolytic agents.

Costall et al (1986) Neuropharmacology, in press

ALCOHOL TREATMENT AND WITHDRAWAL IN A MOUSE MODEL OF ANXIETY

Brenda Costall, M. Elizabeth Kelly and R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP.

The ability of alcohol to modify locomotor activity is well established (Waller et al, 1986) and the present study investigates (a) whether changes in exploratory activity might reflect an anxiolytic profile during alcohol intake using a simple model of anxiety in the mouse and (b) the effects of diazepam and tiapride on the anxiogenic consequence of alcohol withdrawal.

Naive male albino BKW mice were allowed free access to drinking water containing 8%v/v alcohol. After 7 days treatment, and 2 days after withdrawal of a 14 day treatment, mice were placed in a testing box, 1/3 painted black and illuminated with red light and partitioned (with an access opening) from a brightly illuminated white area. The floor area was lined into squares. Mice taken from dark lighting conditions are averse to the white area and a reduction in anxiety is reflected as increased line crossings and rearings in the white section with corresponding decreases in the black, the converse being true for an anxiogenic response (Costall et al, 1986). These two parameters were observed by remote video recordings in the 5 min period following placement of mice individually in the white area.

Table 1. Modification of rearing behaviour and line crossings in the white and black areas during (D) and after withdrawal (W/D) of alcohol.

Drug treatment	White section		Black section	
	Rearings	Line crossings	Rearings	Line crossings
Control	23.0±2.4	32.4±3.0	39.8±4.1	52.1±4.3
Alcohol (D)	57.5±5.8*	70.7±6.7*	17.6±2.1+	18.5±2.0+
Alcohol (W/D)	7.6±0.2+	11.5±1.1+	72.8±8.9*	76.9±7.1*
Diazepam	42.3±3.9*	64.6±7.1*	14.2±1.4+	30.0±2.8+
Diaz. + alcohol	47.5±4.0°	72.0±7.3°	12.8±1.7°	20.5±2.2°
Tiapride	40.8±3.9*	74.5±7.4*	16.0±1.1+	19.1±2.3+
Tiap. + alcohol	44.4±4.2°	50.8±5.6°	14.7±1.8°	18.9±1.7°

n = 5 for drug treatments, n = 40 for control values. Significant increase or decrease relative to control values shown as *P<0.001 and +P<0.001 respectively; antagonism of effects of alcohol (W/D) significant to °P<0.001 (Dunnett's t test). Diazepam (10 mg/kg i.p.) and tiapride (40 mg/kg i.p.) were administered twice daily during withdrawal.

During alcohol intake mice showed markedly reduced aversion to the brightly-lit compartment of the test system in which exploratory behaviour was increased. The intensity of this anxiolytic action was at least as great as that previously recorded for established anxiolytic agents such as diazepam (Costall et al, 1986). Within 48h of withdrawing alcohol mice showed an intense anxiogenic responding with markedly elevated activity in the black section of the test box. This anxiogenic responding equated to that recorded following abrupt withdrawal of long-term treatment with low or high dose diazepam (Costall et al, unpublished) and required high dosage treatment with diazepam or tiapride for prevention. The data indicates a role for the non-benzodiazepine anxiolytic agent, tiapride, in the treatment of the anxiogenesis associated with alcohol withdrawal.

Costall et al (1986) Neuropharmacology, in press
Waller et al (1986) Pharmac.Biochem.Behav. 24, 617-623

THE AUTORADIOGRAPHIC LOCALISATION OF NEUROKININ A BINDING SITES IN PRIMATE BRAIN

A.R. Crossman, S.M. Farmery, A.M. Stuart, Experimental Neurology Group, Department of Cell and Structural Biology, University of Manchester Medical School, Oxford Road, Manchester M13 9PT.

The neuropeptide neurokinin A (substance K) belongs to a group of mammalian tachykinins which includes substance P. Neurokinin A (NKA) is widely distributed throughout the central and peripheral nervous systems and high affinity binding sites for radiolabelled NKA have been described in several species. In the present study the regional distribution of NKA binding sites in the primate brain has been determined by in vitro autoradiography.

Frozen brain tissue from three macaques killed by barbiturate overdose was blocked stereotactically. Sections (20um) were cut on a cryostat and thaw-mounted onto gelatin-coated glass slides. The sections were pre-incubated for 15 min at 20°C in 50mM Tris HCl, pH 7.4, containing 0.2mg/ml bovine serum albumin. They were incubated for 30 min at 20°C in the same medium to which had been added 3mM MnCl2, 2ug/ml chymostatin, 4ug/ml leupeptin, 40ug/ml bacitracin and 70pM (^{125}I)-NKA. The specific binding was defined as that displaced by 1uM NKA. The sections were then washed briefly in ice-cold buffer, air dried and apposed to tritium-sensitive film for 20 days.

The distribution of NKA binding to primate brain showed many regional variations. The table below indicates the optical density values corresponding to several brain regions as determined using a microcomputer-based image analysis system.

Brain Region	O.D.	Brain Region	O.D.
Amygdala	+	Hippocampus	o
Caudate nucleus	++	Dentate gyrus	++
Putamen	++	Interpeduncular nucleus	+++
Globus pallidus	o	Habenula	+++
Cerebral cortex	+	Locus coeruleus	+++
Cerebellum	o	Substantia nigra	o

O.D. : Optical Density

The distribution of NKA binding sites observed in the present study is very similar to the distribution of Substance P binding sites previously reported (Crossman et al, 1985). The low binding values in the substantia nigra contrast with the findings of Quirion and Dam (1985) who reported high densities of NKA binding sites in the guinea-pig substantia nigra. Further experiments are being undertaken to investigate tachykinin pathways in the primate brain.

Crossman, A.R., Griffiths, E.C., Mitchell, I.J., Slater, P., Stuart, A.M. (1985) Neurosci. Lett. Suppl. 22, S181.
Quirion, R., Dam, T-V. (1985) Neuropeptides 6, 191.

GABA RECEPTORS ON AN IDENTIFIED INSECT MOTORNEURONE

J.A.David, R.D.Pinnock, D.B.Sattelle & K.A.Wafford., A.F.R.C. Unit of Insect Neurophysiology & Pharmacology, Dept of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ.

In the CNS of vertebrates there is good evidence for the existence of two GABA receptors. Much less is known of the properties and pharmacology of the invertebrate GABA receptors. Recent ligand binding studies show that the CNS GABA receptors of the cockroach Periplaneta americana are not readily classified as either the $GABA_A$ or the $GABA_B$ subtypes found in vertebrate CNS (Lummis & Sattelle 1986). We have therefore examined the actions of GABA and a range of pharmacological agents on an identified motorneurone (D_f) in the CNS of the cockroach Periplaneta americana using electrophysiological techniques. Motorneurone D_f was impaled with two recording electrodes. Drugs were applied by ionophoresis or bath application in the perfused saline (David & Sattelle 1984).

The response to GABA was a hyperpolarization with an increase in conductance. Under voltage-clamp conditions the GABA induced current reversed direction at -80.0±3.2mV (mean ± s.e.m. n=6), close to the chloride equilibrium potential. A non-linear current-voltage relationship at hyperpolarized potentials was evident for the GABA-induced response. The current amplitude in response to increasing ionophoretic doses of GABA increased to a maximum of about 30nA. Hill plots yielded slopes of 2.3±0.1 (mean ± s.e.m. n=6), suggesting that binding of more than one GABA molecule to the receptor channel complex is required for channel opening. GABA agonists were bath-applied to the preperation. Agonist dose response curves gave the following EC_{50} values: isoguvacine; 1.5×10^{-5}M, GABA; 7.0×10^{-5}M, muscimol; 7.0x10-5M and 3-APS 1.0x10-4M. Baclofen was inactive at 1.0×10^{-4}M (4 neurones). Picrotoxin was an effective noncompetitive voltage-independent antagonist of the GABA responses at doses higher than 1.0×10^{-7}M. At 1.0×10^{-8}M picrotoxin block was voltage-dependent. Bicuculline (1.0×10^{-4}M) was without effect on the GABA responses of 4 neurones recorded at resting potential. Pitrazepin produced a weak (50%) block of the GABA response at 1.0×10^{-4}M. This antagonism was not competitive. 1.0×10^{-4}M RU5135 caused a 20% reduction in the response to a low dose of GABA, however, higher submaximal responses to GABA were not affected at this concentration.

The pharmacological profile of the GABA receptor on motorneurone D_f is distinct from that of the vertebrate $GABA_A$ and $GABA_B$ receptor. For example the potent competitive antagonists of the $GABA_A$ receptor, bicuculline, pitrazepin and RU5135 (Simmonds and Turner 1985; Kemp et al. 1986) are not antagonists at the insect GABA recognition site. Furthermore, the agonist potency series for the $GABA_A$ site (Kemp et al. 1986) is different to that reported here and the $GABA_B$ receptor agonist baclofen is not active on the insect motorneurone.

David, J.A. and Sattelle, D.B. (1984) J.Exp. Biol. 108, 119-136.
Kemp,J.A.,Marshall,G.R. & Woodruff,G.N. (1986) Br.J.Pharmac.87,677-684.
Lummis,S.C.R. & Sattelle,D.B. (1986) Neurochem. Int. 9, 287-293.
Simmonds,M.A. & Turner,J.P. (1985) Br.J.Pharmac. 84, 631-635

BTS 54 524 , A MONOAMINE UPTAKE INHIBITOR EXHIBITING POTENT ACTIONS IN MODELS PREDICTIVE OF POTENTIAL ANTIDEPRESSANT ACTIVITY

W.R. Buckett, R.H. Hopcroft, G.P. Luscombe and P.C. Thomas, Research Department, The Boots Company PLC., Nottingham. NG2 3AA

BTS 54 524 (N-1-(1-(4-chlorophenyl)cyclobutyl)-3-methylbutyl-N,N-dimethylamine hydrochloride monohydrate) is a putative antidepressant which rapidly and potently induces cortical β-adrenoceptor subsensitivity in the rat (Buckett et al, this meeting). We now report the activity of BTS 54 524 in a number of acute pharmacological tests designed to delineate its potential antidepressant activity and indicate its mode of action.

Male CD1 mice (18-30g) or male Sprague-Dawley rats (150-300g)(both Charles River) were used as appropriate. BTS 54 524, standard antidepressants (see Table 1) or vehicle were examined for their ability to reverse reserpine-induced hypothermia in mice, to prevent reserpine-induced ptosis in rats and to increase the mobility of mice in the Porsolt test, a putative model of depression (Porsolt, 1981). In addition, compounds were examined for their ability to block 14C-monoamine uptake into either rat cortical slices (noradrenaline (NA) and 5-hydroxytryptamine (5-HT)) or rat striatal synaptosomes (dopamine (DA)) in vitro.

The results show BTS 54 524 in general to be more potent than standard antidepressants in each of the reserpine models as well as in the Porsolt test (Table 1). Each of these three tests is more capable of detecting activity in compounds which preferentially inhibit catecholamine uptake rather than those having selectivity for 5-HT uptake (for example see Porsolt, 1981). This is consistent with the in vitro monoamine uptake data obtained for BTS 54 524 which show preferential inhibition of catecholamine uptake (Table 1).

Table 1 Activity of BTS 54 524 and standard antidepressants in behavioural models indicative of antidepressant efficacy and against in vitro 14C-monoamine uptake

Compound	Reserpine reversal (ED50)	Reserpine prevention (ED50)	Porsolt test (LED)	In vitro uptake inhibition NA (IC50)	DA (IC50)	5HT (IC50)
BTS 54 524	1.8	0.6	10	2.2	11	477
Amitriptyline	5.8	70	10	0.9	8.7	2.1
Desipramine	6	1.8	30	1.0	6.3	19.7
Dothiepin	67	159	100	1.5	8.1	4.6
Imipramine	17	10	30	0.7	18.0	2.1
Nomifensine	2.2	1.1	10	0.18	0.24	>20

Mouse reserpine reversal and rat reserpine prevention values are the oral ED50 doses (mg/kg) required to inhibit the effect of reserpine by 50% (n=8-10 animals/dose).
Porsolt test value is the lowest effective oral dose (mg/kg) to increase mobility by 50% compared to concurrent control mice (n=10-15 mice/dose).
NA, DA and 5HT (in vitro uptake) values are the IC50 concentrations (μM) required to inhibit 14C-monoamine uptake by 50% (n=3-4 experiments).

The potent activity of BTS 54 524 in acute behavioural models predictive of potential antidepressant activity appears to be largely a consequence of catecholamine uptake inhibition, and is probably responsible for its potent and rapid down-regulation of rat cortical β-adrenoceptors (Buckett et al, this meeting).

Buckett,W.R. et al (1987) This meeting
Porsolt, R.D. (1981) in "Antidepressants. Neurochemical, Behavioural and Clinical Perspectives". Ed. S.J. Enna et al. Raven Press p.121

EFFECTS OF CHRONIC DIAZEPAM AND TETRAZEPAM ON TOLERANCE AND DEPENDENCE IN MICE

A. Bachy, K. Bizière*, P. Keane, M. Morre and J. Simiand, Sanofi Recherche, 195 route d'Espagne, Toulouse, France, and * Sanofi Recherche, rue du Pr Joseph Blayac, Montpellier, France.

Tolerance and physical dependence have been described after chronic administration of benzodiazepines (BZDs) to animals or man. We have investigated the relationships between these two phenomena in mice. Tetrazepam (70 mg/kg twice daily) and diazepam (10 mg/kg twice daily) were administered orally for 17 days to groups of 12 mice. Drug treatment schedules assured a 24-hour coverage of drug binding to brain BZD receptors. A control group received the vehicle (1 % carboxymethyl cellulose). Muscle relaxant activity in the horizontal wire test was assessed 30 minutes after each dose. On the 18th day, the BZD receptor antagonist Ro 15-1788 (10 mg/kg i.v.) was administered to half the mice in each group, and withdrawal symptoms were noted over the next 90 minutes (Cumin et al, 1982). The other 6 mice in each group were used to evaluate the effects of these chronic treatments on brain BZD receptors. The brains were removed and homogenised; the membranes were extensively washed to remove residual drug retained in the tissue, and BZD receptor binding assayed (Rosenberg and Chiu, 1981).

Tolerance to the muscle relaxant effects of tetrazepam and diazepam was significant ($P<0.05$) after a single dose and muscle relaxant activity disappeared completely by Day 5. Mice treated with diazepam exhibited a marked withdrawal syndrome after injection of Ro 15-1788 : Straub tail in 5 mice ($p<0.05$), jumping in 3 ($p<0.05$), clonic seizures in 3 ($p<0.05$) and stretching in 3 ($p<$. In contrast, only one clonic seizure was seen in tetrazepam treated mice. Brain BZD receptor binding was reduced in both diazepam (-17 % ; $p<0.05$) and tetrazepam (-16 % ; $p<0.05$) treated mice, suggesting that this change may be related to tolerance rather than to dependence.

These results suggest that it is possible to dissociate the phenomena of tolerance and dependence after BZD treatment and that dependence producing potential does not appear to be equal for all BZDs.

The authors wish to thank Dr C. Servens, Institut du Pin, Bordeaux, France, for the synthesis of Ro 15-1788.

R. Cumin et al, Experientia 38 : 833-834 (1982).
H.C. Rosenberg and T.H. Chiu, Eur. J; Pharmacol. 70 : 453-460 (1982).

AN ANALYSIS OF SEX DIFFERENCES IN THE OPEN FIELD AND TESTS OF EXPLORATION AND ANXIETY

F. Farabollini, S.E. File[1], A.L. Johnston[1] & C.A. Wilson[2], Department of Human Physiology, University of Siena, Italy, [1] MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX and [2] Department of Obstetrics and Gynaecology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE.

There is evidence that in the open field, adult male rats show less ambulatory and rearing activity and defaecate more than adult female rats; this difference is thought to occur because of the organisational effect that testosterone exerts on the neonatal male brain (Blizard & Denef, 1973).

Wistar rats were tested as adults in the open field, the holeboard (which provides a measure of exploration; File & Wardill, 1973) and the elevated plus-maze (a test of anxiety; Pellow et al, 1985). The following groups were investigated: intact females, females ovariectomised as adults, neonatally androgenised females, intact males, males castrated as adults and neonatally castrated males. Testing took place during the dark period of the animals' light cycle.

In the open field, rats with female brains (i.e. intact females, ovariectomised females and neonatally castrated males) were more active (they crossed more squares and reared more) than rats with male brains (i.e. intact males, males castrated as adults and androgenised females). Analysis of the ratio of inner to outer segments crossed suggests that rats show a high ratio if they have a female brain and oestrogen present at the time of testing (e.g. intact females), but show a lower ratio in the absence of either of these factors and in the presence of androgen (e.g. intact males). In the holeboard, rats with female brains were more active (crossed more squares and reared more) than rats with male brains. Exploration (head-dipping) was high when a rat had a female brain and oestrogen present during the test and was reduced by the absence of these factors and by the presence of androgen. In the elevated plus-maze rats with female brains were less anxious than rats with male brains.

We conclude, that intact females are less anxious, more active and explore more than intact males. Our results also suggest that anxiety and general activity are determined mainly by the brain sex of the rat but that exploration is determined by both the brain sex and the hormones present at the time of testing. In the open field, the ratio of inner to outer segments crossed was also dependent on both the brain sex and the hormonal make-up of the rat; we therefore suggest that this measure is more likely to reflect exploratory activity rather than emotionality or anxiety.

S.E.F. is a Wellcome Trust Senior Lecturer. A.L.J. is supported by a post-graduate studentship from the Dept. of Education N. Ireland.

Blizard, D. & Denef, C. (1973) Physiol. Behav. 11, 65-69.
File, S.E. & Wardill, A.G. (1975) Psychopharmacologia 44, 53-59.
Pellow, S., Chopin, P., File, S.E & Briley, M. (1985) J Neurosci Methods 14, 149-167.

LOW DOSES OF BENZODIAZEPINE RECEPTOR INVERSE AGONISTS ENHANCE, AND HIGH DOSES IMPAIR, PASSIVE AVOIDANCE LEARNING IN RATS

Sandra E. File and Sharon Pellow. MRC Neuropharmacology Research Group, The School of Pharmacy, 29-39 Brunswick Square London WC1N 1AX.

Benzodiazepine (BDZ) receptor inverse agonists have behavioural effects generally in the opposite direction to those of BDZs i.e. they are anxiogenic, and either convulsant or proconvulsant. It is also possible that inverse agonists may have opposite effects to BDZs on learning and memory. Studies in man (Lister 1985) and animals (Thiebot 1985) have shown that BDZs impair learning of a wide range of tasks. The effect is not state-dependent, and is seen in long-term memory tasks.

We investigated the effects of the proconvulsant partial inverse agonist FG 7142 and the convulsant inverse agonist DMCM on passive avoidance learning in the rat. The selected doses ranged from anxiogenic doses to proconvulsant doses (FG 7142) or subconvulsant doses (DMCM). Male hooded Lister rats were injected with each drug according to the following schedule: Gp.I (acquisition): injected 20 min before training session; Gp.II (consolidation): injected immediately after training; Gp.III (retrieval): injected 20 min before test session; Gp.IV (state-dependency): injected 20 min before both training and testing. All rats received vehicle (i.p.) injections at the times when they did not receive drug. Training was as follows: each rat was placed in the light compartment of a 2-compartment box and allowed to explore freely for 2 min. The latency to enter the dark compartment and the time spent there were recorded. The rat was then removed, and immediately trapped in the black compartment where he was given a 0.4 mA, 0.5-sec shock to the feet. 24h later animals were tested by placing them in the light compartment where they were again allowed to explore freely for 2 min, the same measures being taken.

No significant drug effects were obtained on consolidation or retrieval (Gps. II and III). Effects on acquisition (Gp.I) were dose-related. At low doses FG 7142 (5 mg/kg) and DMCM (0.1mg/kg) facilitated acquisition i.e. on testing rats had a longer latency to enter the black and spent less time there than controls. At high doses FG 7142 (40 mg/kg) and DMCM (0.5 mg/kg) impaired acquisition. In all cases the effects obtained were also observed when rats also received drug on the test day (Gp.IV).

Our results with low doses of DMCM are in accord with those of Venault et al (1986) who found that the inverse agonist B-CCM facilitated acquisition of passive avoidance learning. We have extended this finding to non-convulsant, partial inverse agonists such as FG 7142. The amnesic effect of high doses of inverse agonists has not previously been reported.

Lister, R. (1985). Neurosci. Biobehav. Rev. 9, 87-94.
Thiebot, M.-H. (1985). Neurosci. Biobehav. Rev. 9, 95-100.
Venault, P., Chapouthier, G., Prado de Carvalho, L., Simiand, J., Morre, M., Dodd, R.H. and Rossier, J. (1986). Nature 321, 864-866.

EFFECTS OF COMPOUNDS ACTING AT CNS 5-HYDROXYTRYPTAMINE SYSTEMS ON ANXIETY IN THE RAT.

S.E. File, A.L. Johnston & S. Pellow, MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX.

Brain 5-hydroxytryptamine (5-HT) pathways have long been implicated in the control of anxiety, although studies to date have provided no conclusive evidence that these systems are critical. However, the recent development of more specific 5-HT receptor ligands has led to a resurgence of interest in the link between 5-HT and anxiety. The effects of a number of compounds that affect 5-HT neurotransmission have been investigated in a test of anxiety in the rat. They were: 8-OH DPAT, TVX Q 7821 and buspirone (ligands for the 5-HT_{1A} receptor); RU 24969 (agonist at the 5-HT_1 receptor); quipazine (non-selective 5-HT receptor agonist); ritanserin (5-HT_2 receptor antagonist); (-)-propranolol (5-HT_1 receptor antagonist); and metergoline (non-selective 5-HT receptor antagonist); and BTG 1501 (reported to reduce tryptophan hydroxylase activity in the brain; Boadle-Biber & Phan, 1986).

The test of anxiety was the elevated plus-maze (Pellow et al., 1985): two open and two enclosed arms of a plus-maze were elevated to a height of 50 cm. The percentage of entries onto and of time spent on the open arms is a measure of anxiety; being elevated by anxiolytic drugs and reduced by anxiogenic drugs. The total number of arm entries provides a measure of overall activity.

Buspirone (0.5-20 mg/kg), 8-OH DPAT (0.0625-0.25 mg/kg) and TVX Q 7821 (2.5-10 mg/kg) had no specific effects on anxiety, although buspirone (20 mg/kg), 8-OH DPAT (0.25 mg/kg) and TVX Q 7821 (all doses) caused a general depression in activity. RU 24969 (0.1875-1.5 mg/kg) significantly reduced the percentage of time spent on the open arms without affecting the percentage of entries made onto the open arms; (-)-propranolol (5 & 10 mg/kg) significantly reduced only the percentage of entries made onto the open arms. Ritanserin (0.25-10 mg/kg) displayed an anxiogenic profile by reducing both measures of anxiety. Quipazine (2 & 4 mg/kg) showed signs of anxiogenic activity, while metergoline (4mg/kg) showed signs of anxiolytic activity; however, neither of these effects was consistently replicable.

BTG 1501 (10-80 mg/kg) was without effect, but 100 mg/kg produced a partial anxiolytic profile by elevating the percentage of entries made onto the open arms. This effect was observed after both acute and chronic (daily injection for 7 days) treatment; however, at this dose BTG 1501 also reduced the total number of arm entries.

We are grateful to the British Technology Group for financial support. S.E.F. is a Wellcome Trust Senior Lecturer. A.L.J. is supported by a post-graduate studentship from the Dept. of Education N. Ireland.

Pellow, S., Chopin, P., File, S.E. & Briley, M. (1985). J. Neurosci. Methods 14, 149-167.
Boadle-Biber, M.C. & Phan, T.H. (1986). Biochem. Pharmacol. 35, 1521-1526.

THE USE OF [Glp^6, L-PRO^9]SP-(6-11) AND [Glp^6 , D-PRO^9] SP-(6-11) TO DISTINGUISH RECEPTORS FOR TACHYKININS

A.E. Fletcher, A.T. McKnight and J.J. Maguire, Merck Sharp and Dohme Research Laboratories, Harlow, England.

The existence of three types of receptor for tachykinins (SP-P or NK1, SP-E or NK2, SP-N or NK3) has been proposed on the basis of experiments in smooth muscle preparations, but with the lack of potent and selective antagonists, the only means of distinguishing receptor types is to compare rank orders of potencies of agonists. Recent results from our laboratory suggest that [Glp^6, L-Pro^9] SP-(6-11) and its D-Pro^9 homologue (Piercey et al., 1985) may be useful tools in this regard (Lee et al., 1986), and we have compared the potencies of these, and other agonists in pharmacological assays for the three receptor types.

Table 1 Relative potencies of tachykinins in pharmacological assays

	guinea-pig ileum	rat portal vein	rat vas deferens	rat ileum
Substance P	100	100	100	100
Eledoisin	57	2400	2730	4300
Neurokinin A	9	340	9090	23000
Neurokinin B	60	24000	2200	7300
[Glp^6, D-Pro^9]SP-(6-11)	7	50	1970	120
[Glp^6, L-Pro^9]SP-(6-11)	35	2	<10	<0.1
Substance P-methyl ester	65	<0.01	<10	<0.1

The selectivity of substance P-methyl ester for NK1 receptors was confirmed; this peptide was a potent agonist in the guinea-pig ileum, but was essentially inactive in the other tissues. [Glp^6, L-Pro^9]SP-(6-11) also exhibited a degree of selectivity for the NK1 receptor, and was 5 times more potent than the D-Pro^9 analogue. In the other tissues the pattern was reversed, with the L-Pro^9 analogue being virtually inactive. From the results in the vas deferens [Glp^6, D-Pro^9]SP-(6-11) is a potent and selective agonist at the NK2 receptor in this tissue, but this result is not borne out by the observed low potency in the rat ileum although the order of potencies of the natural tachykinins in this tissue is that for the NK2 receptor (neurokinin A > neurokinin B >> substance P). At the putative NK3 receptor in the portal vein the D-Pro^9 analogue is the more potent, but is itself only one five-hundredth the potency of neurokinin B (table 1).

We conclude that a comparison of the relative potencies of [Glp^6,L-Pro^9] SP-(6-11) and its D-Pro^9 homologue may be useful in characterising tachykinin receptors, particulaly in the case of the NK2 receptor where the L-Pro^9 analogue is inactive, but the D-Pro^9 analogue may be a potent agonist.

Lee, C.-M., Campbell, N.J., Williams, B.J. & Iversen, L.L. (1986) Eur. J. Pharmac. in press.
Piercey, M.F., Dobry-Schreur, P.J.K., Masiques, N. & Schroeder, L.A. (1985) Life Sci. 36, 777-780

DSP-4 ALTERS THE EFFECT OF d-AMPHETAMINE ON OPERANT BEHAVIOUR

C.M. Bradshaw, M.J. Morley and E. Szabadi, Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT.

The effect of d-amphetamine on operant behaviour maintained by variable-interval schedules of positive reinforcement can be described as a reduction in the values of two constants, R_{max} and K_H, of the hyperbolic equation relating response rate, R, to reinforcement frequency, r:

$$R = R_{max} .r/(K_H + r)$$

(Morley et al., 1985). In the present experiment we have used the neurotoxin DSP4, which selectively destroys the dorsal noradrenergic pathway (Jonsson et al., 1981), in order to investigate the possible role of this pathway in mediating the effect of d-amphetamine on variable-interval performance.

Twenty-two female Wistar rats were randomly assigned to two groups and treated with either DSP4 (two injections of 50 mg/kg separated by 7 days) (n=10) or vehicle (n=12). They were maintained at 80% of their free-feeding body weights and trained to press a lever for sucrose reinforcement (0.6M, 0.05 ml). In Phase I half the rats from each group were trained under variable-interval 1-min and the other half under variable-interval 12-min; in Phase II the schedule conditions were reversed. In each phase, following 30 preliminary daily training sessions, the rats received intraperitoneal injections of d-amphetamine sulphate (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 mg/kg) or vehicle (distilled water). Each dose was administered twice to each rat, the order of presentation of the doses being balanced across rats. The effect of d-amphetamine was expressed as proportional change in response rate, compared to vehicle control, and the data were analysed by a three-factor analysis of variance (treatment with DSP4/vehicle, schedule of reinforcement, dose of d-amphetamine), with repeated measures on the second and third factors. At the end of the experiment the rats were sacrificed and the concentrations of noradrenaline and dopamine in the frontal and parietal cortices, hippocampus, corpus striatum, nucleus accumbens, septum, hypothalamus and cerebellum were determined by high performance liquid chromatography with electrochemical detection.

d-Amphetamine had a dose-related suppressant effect on response rate ($F(5,100)= 35.8$, $P<0.01$), the degree of suppression being greater in the case of variable-interval 12-min ($F(1,20)= 9.9$, $P<0.01$). Treatment with DSP4 enhanced the suppression of response rates by lower doses (0.2, 0.4 mg/kg) and attenuated the effect of higher doses (0.8-3.2 mg/kg) ($F(5,100)=3.0$, $P<0.05$). This effect of DSP4 did not differ between the two schedules ($F<1$). The concentration of noradrenaline in the parietal cortex, hippocampus and cerebellum was reduced to approximately 15% of control values in the DSP4-pretreated rats (t-test, $P<0.01$ in each case); the levels of noradrenaline in the other regions and the levels of dopamine in all regions tested were not significantly altered.

The greater suppressant effect of d-amphetamine on performance maintained by the higher reinforcement frequency is in agreement with previous findings and indicates an effect of the drug on both R_{max} and K_H (Morley et al., 1985). The pattern of effect of DSP4 on the dose-response curves for d-amphetamine is consistent with the effect of the drug on both constants being attenuated following treatment with the neurotoxin. Thus the results suggest that the dorsal noradrenergic pathway may be involved in mediating the effect of d-amphetamine on operant behaviour.

This work was supported by the Sir Jules Thorn Charitable Trust

Jonsson, G. et al., (1981) Eur. J. Pharmac., 72, 173-188.
Morley, M.J. et al., (1985) Psychopharmacology, 87, 207-211.

IN VIVO MONITORING OF EXTRACELLULAR 5-HT IN RAT FRONTAL CORTEX USING MICRODIALYSIS COMBINED WITH SMALL-BORE HPLC-EC

A.Carlsson[1], H.G.Series, T.Sharp and U.Ungerstedt, Department of Pharmacology, Karolinska Institute, Box 60 400, S-104 01 Stockholm and [1]Department of Analytical Chemistry, University of Uppsala, Uppsala, Sweden.

Intracerebral microdialysis is a brain perfusion method which, in combination with HPLC and electrochemical detection (EC) assays, enables constant monitoring of extracellular monoamine neurotransmitters in rat brain in vivo. A recent advance in the dialysis methodology has been the application of a highly sensitive, miniaturized HPLC-EC system based on small-bore HPLC columns for the analysis of dopamine and its metabolites (Carlsson et al., 1986). Here we report the development of a small-bore HPLC-EC assay for the measurement of 5-hydroxytryptamine (5-HT) in rat brain dialysates and illustrate its use to study drug-induced changes in cortical 5-HT release.

The HPLC-EC system comprised an LKB 2150 HPLC pump, a Rheodyne 7143 injector (5 µl internal loop) and a BAS LC-4B electrochemical detector. 5-HT and its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) were separated on a 250 x 1 mm HPLC column packed with Spherisorb ODS-1 5 µm particles (Scantec, Partille, Sweden), using 0.1M acetate-citrate buffer, pH 4.0, containing 0.1 mM EDTA and 10% (v/v) methanol, at a flow-rate of 60 µl/min. The indoleamines were detected with a conventional glassy carbon working electrode (BAS TL-5A) held at +0.75V. The dead volume between the column and detector surface was minimised using a fused silica capillary connector and a 23 µm electrode spacer.

With a thorough injection port and syringe washing procedure, and a 10 µl load volume, the HPLC assay showed a high degree of reproducibilty (0.037 relative SD). The limit of detection for 5-HT was about 4 fmol/5 µl sample (0.8nM). Dialysates were collected every 10 min from the frontal cortex of the halothane-anaesthetized rat using a stereotaxically implanted microdialysis probe (2 x 0.4 mm, Carnegie Medicin, Solna, Sweden) perfused at 1 µl/min with Ringer's solution. After a 1 h control perfusion period, levels of 5-HT and 5-HIAA were stable over 3 h. Basal amounts of 5-HT and 5-HIAA in the cortical perfusates were 12.75±3.25 fmol/5 µl (mean±SEM, n=9) and 2.05±0.18 pmol/5 µl, respectively (uncorrected for recovery). The 5-HT uptake blocker, citalopram (0.5-5 mg/kg s.c.), produced a dose-related increase of 5-HT in the cortical perfusates (maximal response, +172% 40 min after the highest dose, n=5). Also, the 5-HT releasing agent, p-chloroamphetamine (2 mg/kg s.c.), induced a marked rise of 5-HT in the perfusates (+2119% 40 min post-drug ,n=3). Two animals pretreated with citalopram did not show a 5-HT response to p-chloroamphetamine, which is further evidence that the releasing agent must enter the 5-HT neuron via the 5-HT uptake site to elicit its action.

In conclusion, the combination of intracerebral microdialysis with a newly developed small-bore HPLC-EC assay provides a sensitive method for studying drug action on brain 5-HT release in vivo.

We thank the SERC, Karolinska Institute and Carnegie Medicin for financial support and Lundbeck, Copenhagen for their generous supply of citalopram.

Carlsson, A., Sharp, T., Zetterström, T. and Ungerstedt, U., (1986) J.Chromatography, in press.

THE DOPAMINE D-$_2$ RECEPTOR AGONIST, LY 171555, CAUSES A TRANSIENT INCREASE OF DOPAMINE RELEASE IN RAT STRIATUM *IN VIVO*

T.Sharp, U.Ungerstedt and T.Zetterström, Department of Pharmacology, Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden.

There is considerable evidence from biochemical studies in the rat that the activity of nigro-striatal dopamine (DA) neurons is autoregulated by inhibitory DA receptors. It is established that the release of DA evoked from preparations of rat striatum *in vitro* is reduced by the presence in the incubation medium of DA agonists acting directly on the DA D-2 receptor (eg. Lehman et al., 1983). Also, we have recently shown using intracerebral dialysis that systemic administration of D-1 and D-2 receptor agonists decreases the release of DA in rat striatum *in vivo* (Zetterström et al., 1986). Here we present follow-up experiments to the latter study and note that the D-2 agonist LY 171555 causes an unexpected increase in release of DA in striatum *in vivo*.

The *in vivo* brain perfusion method, intracerebral dialysis, is described in detail elsewhere (see Zetterström et al., 1986). Briefly, rats were anaesthetized with halothane and a dialysis probe (short loop type) was implanted unilaterally into striatum. The probe was continuously perfused (2 µl/min) with Ringer's solution and perfusates were collected every 20 min and analysed for DA and its main metabolite dihydroxyphenylacetic acid (DOPAC) using HPLC with electrochemical detection. Once stable baseline measurements were established (100-120 min), drugs were administered subcutaneously and perfusates were collected for a further 2 h.

At low doses (0.005, 0.01 and 0.05 mg/kg), LY 171555 produced a dose-dependent decrease of DA in striatal perfusates over the 2 h post-drug period. The DA response to 0.01 mg/kg LY 171555 was blocked by 20 min pretreatment with the D-2 antagonist sulpiride (10 mg/kg). Higher doses of LY 171555, 0.5 and 5 mg/kg, caused a dose-related increase of striatal DA (+110 %, n=5 and +355 %, n=6, respectively) in the first 20 min perfusate post-drug, which was followed by a marked fall of DA below preinjection values. Both the increase and decrease of DA induced by 0.5 mg/kg LY 171555 were inhibited by sulpiride (10-100 mg/kg). In comparison, perfusate levels of DOPAC declined in a dose-dependent and sulpiride-sensitive manner after LY 171555 (0.005-5 mg/kg).

The results indicate that over a certain dose range the DA agonist LY 171555 releases DA in rat striatum *in vivo*. This unexpected finding is supported by a recent study showing that LY 171555-induced behavioural excitation in rats is prevented by brain DA depletion (Barone et al., 1986). Furthermore, our data suggests that the mechanism underlying the central DA releasing action of LY 171555 involves D-2 DA receptor activation.

We wish to thank Anna-Karin Collin for her expert technical assistance and Elli Lilly & Co. and Delagrange for their generous gift of drugs. This work was supported by grants from the SERC, Karolinska Institute and Swedish MRC.

Barone, P., Davis, T.A., Braun, A.R. and Chase, T.N. (1986) Eur.J.Pharmacol. 123, 109-114.
Lehman, J., Briley, M. and Langer, S.Z. (1983) Eur.J.Pharmacol. 88, 11-26.
Zetterström, T., Sharp,T. and Ungerstedt,U. (1986) N.S.Arch.Pharmacol. in press.

THE EFFECTS OF CHRONIC BENZODIAZEPINE TREATMENT ON THE HYPOTHERMIC ACTIONS OF BENZODIAZEPINE INVERSE AGONISTS

R. Gale[1], H. J. Little[2], D. J. Nutt[3], B. Standing & S. C. Taylor[1], [1]University Department of Pharmacology, Oxford, [2]Department of Pharmacology, University Walk, Bristol, [3]NIAAA, 9000, Rockville Pike, Bethesda, MD 20205, U.S.A.

Benzodiazepine inverse agonists have effects in most tests which are opposite to those of the benzodiazepines; they are anxiogenic and convulsant or proconvulsant. However both groups of compounds decrease body temperature in rodents (Taylor et al, 1985). We reported previously that chronic treatment with benzodiazepine agonists increased the convulsive effects of inverse agonists, such as CGS 8216, FG7142 and β-CCM (Little et al, 1986a). In order to examine further whether the increases in inverse agonist action are due to precipitated withdrawal or to changes in the intrinsic effects of the inverse agonists, we have now studied the effects of such treatment on the hypothermic actions of inverse agonists.

Male CD1 mice (Charles Rivers), 30 - 35g were given flurazepam (FZ), 40 mg kg^{-1}, i.p., once daily for seven days; controls received saline injections (S). 24h or 48h later the hypothermic effects of inverse agonists, at i.p. doses in the middle of the effective ranges, were measured using a rectal probe. The effects of CGS 8216 and FG7142 were significantly increased 24h and 48h after the benzodiazepine treatment, *P<0.05, **P<0.01 (Mann-Whitney 'U' test). No change was seen after the antagonist Ro 15-1788, or in the hypothermic effects of β-CCM or DMCM, inverse agonists with high efficacy. Tolerance occurred to the hypothermic action of flurazepam. Saline injections raised temperatures in flurazepam treated mice.

Acute inj. (mg kg^{-1}) Temps °C: 15 and 30 min after injection, mean ± s.e.m.,(n=8-10)
FG7142(40), 48h 15min: S 36.0±0.35 FZ 35.1±0.2*, 30min: S 36.1±0.35 FZ 35.1±0.2*
CGS8216(10),24h 15min: S 36.8±0.13 FZ 35.4±0.25**, 30min: S 37.2±0.11 FZ 35.0±0.31**
Ro15-1788(5) 24h 15min: S 37.7±0.13 FZ 37.6±0.16, 30min: S 37.5±0.11 FZ 37.5±0.17
Saline, 48h 15min: S 38.1±0.12 FZ 38.5±0.1, 30min: S 38.1±0.05 FZ 38.5±0.1**

The occasional seen convulsions after CGS8216 or FG7142 did not appear to alter the body temperatures. DMCM and β-CCM, however, caused convulsions in almost all animals and this may have affected the results. The hypothermic actions of the partial inverse agonists were consistently increased. For several reasons, this was unlikely to be due to precipitation of withdrawal. The latter could only occur if there was residual benzodiazepine in the CNS when the inverse agonists were given. We found no measurable flurazepam or metabolites 24h or 48h after the last dose of flurazepam (Little et al, 1986b). As a small amount of compound may have remained bound to the receptor sites, we measured *in vivo* and *in vitro* [^{3}-H]-flunitrazepam binding after the flurazepam treatment (Nicholass et al, in preparation). No significant differences were found in either case at the 48h interval, although there was a significant decease *in vivo* at 24h. Taken together these results indicate that residual benzodiazepine was present at 24h but not 48h. In addition, residual benzodiazepine would have decreased the effects of inverse agonists on body temperature as the hypothermic effects of these two groups of compounds are mutually antagonistic (Little, 1986). The intrinsic effects of partial inverse agonists may therefore be increased during benzodiazepine withdrawal, a change not due to precipitation of withdrawal. The lack of change after Ro15-1788 and the rise in temperature when saline was given during the withdrawal support this hypothesis.

Little, H. J., Nutt, D. J. & Taylor, S. C. (1986a) B.P.S. Hatfield Polytechnic.
Little, H. J., Nutt, D. J. & Taylor, S. C.(1986b) Pharmacol. Biochem. Behav. In Press
Little, H. J. (1986) Br. J. Pharmacol., In Press
Taylor, S. C., Nutt, D. J., Sellars, N. & Little, H. J., (1985) Neuropharm., 24, 69 -73

ON THE MECHANISM OF HISTAMINE POTENTIATION OF ADENOSINE-STIMULATED ADENYLATE CYCLASE IN GUINEA-PIG CEREBRAL CORTEX

Sonye K. Danoff & J.M. Young, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

Histamine H_1-agonists potentiate adenosine-stimulated accumulation of cyclic AMP in guinea-pig cerebral cortex (references in Daum et al., 1982). The nature of the interaction has become of particular interest following the demonstration that histidine decarboxylase and adenosine deaminase may be present in the same nerve fibres in brain (Senba et al., 1985). The mechanism of the potentiating effects of H_1-agonists has been generally assumed to involve a rise in free intracellular Ca^{2+} (Schwabe et al., 1978), but more recently evidence has been presented that in some systems cyclic AMP accumulation can be potentiated by phorbol esters. In particular, Hollingsworth et al. (1985) have reported that PMA (phorbol 12-myristate-13-acetate) enhances 2-chloroadenosine-elicited cyclic AMP accumulation in guinea-pig cerebral cortex. However, very much higher concentrations of PMA were necessary ($\geq$ 2μM) than those normally required for protein kinase C activation (1-10nM) (Nishizuka, 1986). We have examined the effect of PMA on the response to adenosine and investigated whether stimulation of cyclic AMP accumulation can be obtained at lower concentrations of PMA if activation of the other arm of the inositol phospholipid pathway, Ca^{2+} release induced by IP_3, is mimicked by the presence of the calcium ionophore A23187.

Cyclic AMP accumulation in cross-chopped slices (300 x 300μM) of guinea-pig cerebral cortex was measured as described previously (Daum et al., 1982). PMA was dissolved in dimethylsulphoxide and A23187 in ethanol. Vehicle alone had no significant effect on cyclic AMP accumulation.

Histamine (100μM) routinely produced a 2-3 fold stimulation of cyclic AMP accumulation induced by a 10 min incubation with 100μM adenosine. The EC_{50} for histamine was 8.4 ± 1.9μM and the effect was practically abolished by 1μM mepyramine. PMA (1-100nM) had no stimulatory effect on the response to 100μM adenosine. On the contrary there was a progressive inhibition which was statistically significant at 100nM PMA. This effect did not appear to be on cyclic AMP breakdown, since PMA (10nM - 1μM) did not reduce levels of cyclic AMP elevated by forskolin. Preincubation of slices with PMA (1-100nM) for 20 min before addition of adenosine also failed to produce a stimulation. A23187 (0.1 - 10μM) similarly produced no stimulation of the adenosine response, again only an inhibitory action was observed. PMA (10nM) and A23187 (0.1μM) in combination failed to cause stimulation.

There is no indication in these results that protein kinase C activation has an important role in the mechanism of the potentiation of adenosine-stimulated accumulation of cyclic AMP by histamine.

This work was supported by the M.R.C. S.K.D. is a Winston Churchill Foundation Scholar.

Daum, P.R. et al (1982) Br. J. Pharmac. 77, 347
Hollingsworth, E.B. et al (1985) FEBS Lett. 184, 339
Nishizuka, Y. (1986) J. Natl. Cancer Inst. 76, 363
Schwabe, U. et al (1978) Naunyn Schmiedeberg's Arch. Pharmac. 302, 141
Senba, E. et al (1985) J. Neurosci. 5, 3393

THE EFFECT OF NICOTINE ON CORTICAL EVENT-RELATED SLOW POTENTIALS IN THE CONSCIOUS RAT

I.S. Ebenezer (Introduced by J.W. Thompson), Department of Pharmacological Sciences, Medical School, University of Newcastle upon Tyne, NE2 4HH.*

Event-related slow potentials (SPs), similar to the human contingent negative variation (CNV), have been described in the rat (see Ebenezer, 1986a). An interesting characteristic of these slow potentials is their sensitivity to centrally active drugs (Ashton et al., 1980; Ebenezer, 1986b). One drug that has been extensively studied in this respect is nicotine. Ashton et al. (1980) have shown that nicotine produces dose-related biphasic changes in the magnitude of the human CNV. The present study was undertaken to see if nicotine caused similar changes in the SP responses in the rat.

Male Wistar rats were chronically implanted with electrodes, as described previously (Ebenezer, 1986c). The rats were subsequently trained in a paradigm in which a 2ms click (S_1) was followed 2s later by the appearance of a retractable lever (S_2) in the conditioning chamber which the rat had to press for a food reward. EEGs were recorded by means of high gain a.c. amplifiers (time constant 15s). A negative SP shift developed during the S_1 - S_2 interval and persisted with overtraining. Forty trials were presented to a rat in a single session, and only one session was conducted per day. Artefact free trials were averaged off-line on a PDP8 computer.

Nicotine (0.05, 0.1, 0.2, 0.4, and 0.8 mg/kg) was administered by s.c. injection to each rat (n=9) that had developed steady SP responses. Two saline control sessions were conducted on the 2 days prior to drug administration. Only 1 dose of nicotine was given to a rat in any one week. The area of the SPs measured 25 - 55 min after nicotine was compared with the area of the control SP recorded on the day preceding a drug session.

Nicotine produced complex biphasic alterations in the magnitude of the SPs. In some rats (n=5) nicotine caused maximal reductions in SP magnitude at the lower doses and maximal increases at the higher doses, while in other rats (n=3) the drug had the opposite effect. Nicotine caused reductions in SP magnitude at all doses in 1 rat. The individual differences in the response of the rats to nicotine made it impossible to construct a standard dose response curve (see Ashton et al., 1980 for discussion). Instead, 2 points were chosen to demonstrate the biphasic effects statistically: the <u>maximum</u> and the <u>minimum</u> responses. The <u>maximum response</u> occurred at a mean dose ± s.e.m. of 0.37 ± 0.12 mg/kg and the maximum increase was 26.6% ($P<0.01$; paired t-test). The <u>minimum response</u> occurred at a mean dose ± s.e.m. of 0.16 ± 0.04 mg/kg and the maximum reduction was 40.5% ($P<0.01$).

The results of this study broadly agree with those of Ashton et al. (1980) and show that nicotine causes dose-related biphasic changes in the magnitude of rat SPs. These changes may simply reflect the stimulant and depressant properties of nicotine (Ashton et al., 1980). However, recent evidence suggests that the effects of the drug on SPs may be secondary to the release of other central neurotransmitters (e.g. ACh: muscarinic action). These possibilities are discussed more fully elsewhere (Ebenezer, 1986a).

I wish to thank Professor J.W. Thompson for his help during this study. *<u>Present address</u>: AFRC Institute of Animal Physiology & Genetics Research, Babraham, Cambridge.

Ashton, C.H. et al. (1980) Br. J. clin. Pharmac. 10, 579-589
Ebenezer, I.S. (1986a) IRCS Med. Sci. 14, 509-514
Ebenezer, I.S. (1986b) Eur. J. Pharmac. 120, 371-374
Ebenezer, I.S. (1986c) Neuropharmacology, 25, 639-643

MDL 72832: A POTENT, SELECTIVE AND STEREOSPECIFIC LIGAND FOR 5-HT_{1A} RECEPTORS

J.R. Fozard, M. Hibert, E.J. Kidd, D.N. Middlemiss, A.K. Mir and M.D. Tricklebank, Merrell Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg Cedex, France.

8-[4-(1,4-Benzodioxan-2-ylmethylamino)butyl]-8-azaspiro[4,5]decane-7,9-dione, (±)MDL 72832, and its R(+) and S(-) enantiomers were synthesized as part of a programme aimed at the design of molecules with high affinity and selectivity for central 5-HT_{1A} receptors. We here describe the affinities of these molecules for a number of brain neurotransmitter recognition sites and their mixed agonist and antagonist properties at both central and peripheral 5-HT_{1A} receptors.

Radioligand receptor binding studies were carried out using rat brain membranes as previously described (Fozard et al., 1986). The transmurally stimulated guinea-pig ileum was used to quantify activity at the 5-HT_{1A} receptors on the enteric cholinergic neurones (Fozard and Kilbinger, 1985). Cardiovascular effects were evaluated in normotensive pentobarbitone-anaesthetized rats (Fozard et al., 1986). The effects of the compounds were also investigated in animals trained to differentiate 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) from saline in a drug discrimination paradigm (Tricklebank et al., 1986).

(±)MDL 72832 showed high affinity for the 5-HT_{1A} recognition sites and was 20-fold and some 1000-fold more active at this site than at the α_1-adrenoceptor or the other 5-HT receptor subtypes, respectively. The interaction with the 5-HT_{1A} site was stereospecific in that (-)MDL 72832 was 32 times more active than (+)MDL 72832.

Table 1. Affinity of MDL 72832 and its enantiomers for central neurotransmitter recognition sites. (pIC_{50} ± SEM ; n=3-6)

	5-HT_{1A}	5-HT_{1B}	5-HT_2	α_1	α_2	D_2
(±) MDL 72832	9.1±0.1	6.2±0.1	6.2±0.1	7.8±0.1	6.4±0.1	6.8±0.1
(-) MDL 72832	9.2±0.1	6.1±0.4	6.7±0.1	8.0±0.1	6.3±0.1	7.1±0.3
(+) MDL 72832	7.7±0.2	5.3±0.1	6.1±0.1	7.2±0.1	6.2±0.1	5.6±0.1

(±)MDL 72832, (-)MDL 72832 and (+)MDL 72832 blocked inhibition of the enteric cholinergic neurones evoked by 8-OH-DPAT with -log K_B values of 9.5, 9.9 and 8.4, respectively. At higher concentrations the compounds elicited weak agonist activity in this preparation. (±)MDL 72832 (0.006-0.1 mg/kg s.c.) blocked dose-dependently the cardiovascular effects of 8-OH-DPAT (32 µg/kg i.v.) in anaesthetized rats without modifying an equieffective response to clonidine (2 µg/kg i.v.). (-)MDL 72832 was slightly more active than (±)MDL 72832 in this respect, whereas (+)MDL 72832 displayed threshold activity only at 1 mg/kg s.c. In rats, both MDL (±)72832 and (-)MDL 72832 generalized to the 8-OH-DPAT cue at doses from 0.01 to 0.1 mg/kg s.c. ; (+)MDL 72832 had no effects at doses up to 0.3 mg/kg s.c.

These results characterise MDL 72832 as a potent, selective and stereospecific ligand for 5-HT_{1A} recognition sites. In functional tests, the interactions with the putative 5-HT_{1A} receptor are manifested as antagonist and/or agonist activity. The similar stereospecific requirements evident from the binding assay and the functional tests provides strong evidence that the 5-HT_{1A} recognition site is a biologically important receptor.

Fozard, J.R. et al (1986) J.Cardiovasc.Pharmacol. in press.
Fozard, J.R. & Kilbinger, H. (1985) Brit.J.Pharmacol. 86, 601P.
Tricklebank, M.D. et al (1986) Eur.J.Pharmacol. in press.

IS THE 5-HT RECEPTOR WHICH MODULATES [^{3}H]-DOPAMINE RELEASE IN RAT STRIATUM A 5-HT$_{1A}$ RECEPTOR?

Christine Ennis, Wyeth Research (UK) Ltd, Huntercombe Lane South, Taplow, Berks.

It has previously been reported that 5-HT can inhibit the K^+or electrically-evoked release of |^{3}H|-dopamine (DA) from slices of rat striatum (Ennis et al, 1981, Westfall and Tittermary, 1982). The 5-HT receptor mediating this effect is not the same as the 5-HT autoreceptor (Ennis & Cox, 1982) in that the effect can be blocked by a number of 5-HT antagonists, in particular methysergide, which are devoid of activity at the autoreceptor. In view of the recent finding that 5HT$_1$ receptors are comprised of 3 subtypes, the present study was performed to investigate whether the 5-HT receptor on dopaminergic terminals in the rat striatum may be classified as 1A, 1B or 1C (Pazos et al, 1985).

Slices of rat striatum were preloaded with |^{3}H|-DA (specific activity 50 Ci mmol^{-1}, Amersham International) and superfused at a rate of 0.4 ml min^{-1} with oxygenated Krebs-Henseleit solution containing chlorimipramine (1.0 µM). Two 4 min pulses of Krebs containing 25mMK^+ were administered at 68 (S_1) and 92 (S_2) min after the start of the superfusion. Drugs were added to the superfusing medium immediately after S_1. The increase in radioactivity above basal levels during S_1 and S_2 was measured and the results were expressed as the ratio S_2/S_1.

The 5HT-1A selective agonists 8-hydroxy-2 (di-n-propylamino) tetralin (8OHDPAT), isapirone (TVX-Q7821) and carboxamidotryptamine (CT) all produced an inhibition of K^+-evoked |^{3}H|-DA release. It was not possible to obtain a pD_2 value for 8-OHDPAT since at concentrations above 0.03 µM it produced an increase in basal release which may be due to an interaction with dopamine receptors. The pD_2 values for isapirone and CT were 7.98 ± 0.14 and 7.98 ± 0.06 (n=6) respectively. The effects of both compounds were antagonised by methysergide with pA_2 values of 9.27 ± 0.09 and 8.91 ± 0.26 respectively. The slopes of the Arunlakshana-Schild plots were not significantly different from 1.0.

In contrast the 5HT-1B selective agents, RU 24929 (Goodwin & Green, 1985) and tri-fluoromethylphenylpiperazine (TFMPP) were found to have no significant effect on K^+-evoked |^{3}H|-DA release in concentrations up to 1.0 µM.

In conclusion, the results suggest that the 5HT receptor located on dopaminergic terminals in the rat striatum may be of the 5HT-1A subtype and that 5-HT modulation of |^{3}H|-DA release may provide a useful model with which to further the study of the 5HT-1A receptor.

Ennis, C & Cox, B. (1982) Neuropharmacology, 21, 41-44.
Ennis, C., Kemp, J.D. & Cox, B. (1981) J. Neurochem., 36, 1515-1520.
Goodwin, G. M. & Green, A.R. (1985) Br. J. Pharmac., 84, 743-753
Pazos, A et al (1985) Eur. J. Pharmac., 106, 539-546
Westfall, T.C. & Tittermary, V. (1982) Neuroscience Letts., 28, 205-209

NOMIFENSINE ABOLISHES THE ACTIONS OF DOPAMINE RECEPTOR ANTAGONISTS ON STIMULATED STRIATAL DOPAMINE RELEASE: IN VIVO VOLTAMMETRIC DATA

J.A. STAMFORD and R.M. WIGHTMAN*, (introduced by Z.L. KRUK), Department of Pharmacology, The London Hospital Medical College, Turner Street, London E1 2AD, and *Department of Chemistry, Indiana University, Bloomington, IN 47405, USA.

In vitro studies of stimulated dopamine (DA) release have been widely used to evaluate the actions of drugs at DA autoreceptors (eg Starke et al, 1978). Some have shown that DA uptake blockers (particularly nomifensine) can attenuate the autoreceptor response to DA agonists (Cubeddu et al, 1983). Although a functional link between DA uptake and autoreceptors may exist, there has been little investigation of interactions between DA uptake blockers and receptor antagonists. We therefore examined, in vivo, the effects of DA uptake blockers on the potentiation of electrically stimulated DA release by metoclopramide, chlorpromazine and haloperidol.

Striatal DA release was measured in anaesthetised rats using computerised high speed cyclic voltammetry (-0.4 to +0.8 V vs saturated calomel reference electrode, 300 V/s scan rate : Kuhr and Wightman, 1986) at carbon fibre microelectrodes (Armstrong James & Millar, 1979). Two stimulations of the median forebrain bundle (100 biphasic pulses, 360 μA, 2 ms duration, 60 Hz) were performed 20 minutes apart. Nomifensine (13 mg/kg i.p.), benztropine (20 mg/kg i.p.) or d-amphetamine (1.5 mg/kg i.p.) was given 20 minutes prior to the first stimulation (S1). Metoclopramide (10 mg/kg i.p.), chlorpromazine (5 mg/kg i.p.), haloperidol (1 mg/kg i.p.) or saline was given immediately after S1. Drug effects on DA release were expressed as the S2:S1 ratio. The results are shown in Table 1.

	Saline	Metoclopramide	Chlorpromazine	Haloperidol
Controls	1.09 ± 0.16	1.80 ± 0.10**	2.97 ± 0.57*	2.24 ± 0.68*
Nomifensine	0.92 ± 0.08	0.63 ± 0.10	0.82 ± 0.10	0.99 ± 0.16
Benztropine	0.89 ± 0.08	1.65 ± 0.11***	1.60 ± 0.16**	2.08 ± 0.29**
d-Amphetamine	0.86 ± 0.09	0.96 ± 0.13	1.93 ± 0.32*	1.98 ± 0.25**

S2:S1 ratios. Means ± s.e.m. (n = 4-7).
* P < 0.02, ** P < 0.01, *** P < 0.001 vs saline (Students t-test).

Metoclopramide, chlorpromazine and haloperidol all elevated the S2:S1 ratio in controls. However, in animals pretreated with nomifensine the DA antagonists had no effect. Benztropine (20 mg/kg i.p.) did not prevent the actions of the DA antagonists. D-amphetamine (1.5 mg/kg) reduced only the effect of metoclopramide. This indicates that neuronal DA uptake blockade is unlikely to be the mechanism by which nomifensine exerts its effect.

Further studies are required to establish whether there is a functional linkage between DA uptake and autoreceptors. Nevertheless the results suggest that the use of nomifensine in studies of DA autoreceptors should be avoided.

This research was supported by grants to JAS from the Wellcome Trust and the Royal Society.

Armstrong James, M. & Millar, J. (1979). J. Neurosci. Meth. 1, p279-87.
Cubeddu, L.X. et al (1983). J. Pharmac. Exp. Ther. 226, 88-94.
Kuhr, W.G. & Wightman, R.M. (1986). Brain Res. (in press).
Starke, K. et al (1978). Naunyn Schmiedebergs. Arch. Pharmac. 305, 27-36.

THE IN VITRO PROFILE OF THE NOVEL 5-HT ANTAGONIST ICI 169,369 IN TISSUES CONTAINING DIFFERENT 5-HT RECEPTOR SUB-TYPES

T.P. Blackburn, B. Cox, J.W. Growcott* and R. Hatton, ICI Pharmaceuticals Division, Research Department 2, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Although sub-types of 5-HT receptors have been recognised for almost 30 years (Gaddum & Picarelli, 1957) and despite the recent introduction of a number of antagonists and agonists, claimed to be selective for various 5-HT receptor sub-types, there is still no universally accepted classification. Attempts have recently been made to form a consensus view of this complex area (Bradley et al. 1986). However, although this consensus does go some way in helping to clarify the situation it is largely based on data obtained using agonists and antagonists of dubious specificity.

The aim of this study was to examine the effects of a novel 5-HT antagonist - ICI 169,369 (2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride) on three tissues that have been claimed to contain different sub-types of 5-HT receptor. Ketanserin (5-HT_2 antagonist) and methysergide (mixed 5-HT_1 and 5-HT_2 antagonist) were used for comparative purposes.

Isotonic contractions of rat fundic strips and spirally cut strips of rat caudal artery (Alderley Park Wistar strain weighing 150-250g) and segments of guinea pig ileum (Dunkin Hartley strain weighing 300-500g) were recorded from preparations suspended in 5ml organ baths containing Krebs-Henseleit solution (1μM atropine was added to the guinea-pig ileum and rat fundic strip preparations) aerated with 95% O_2; 5% CO_2 at 37°C. Concentration-effect curves were constructed for 5-HT in the presence and absence of antagonists. Antagonist activity, where measurable, was expressed as an equipotent dose-ratio.

In the rat caudal artery, ICI 169,369 (10^{-7}M) and ketanserin (10^{-7}M) produced parallel rightward shifts in the 5-HT dose-response curve (dose ratios 16.9 ± 6.6 and 30.6 ± 5 respectively), whereas, methysergide behaved as a non-competitive antagonist. In the guinea-pig ileum, both ICI 169,369 and ketanserin were non-competitive antagonists over the concentration range of 10^{-7}M - 10^{-5}M, whereas methysergide was inactive (>10^{-6}M). In the rat fundic strip, ICI 169,369 and methysergide produced non-competitive antagonism (10^{-7}M to 10^{-5}M) of 5-HT, whereas ketanserin was only weakly active at concentrations greater than 10^{-6}M. Thus, ICI 169,369 behaved like ketanserin, a 5-HT_2 antagonist, in the rat caudal artery and guinea-pig ileum. In contrast, unlike ketanserin, ICI 169,369 appeared to have affinity for the 5-HT receptor in the rat fundic strip, a tissue recently classified as "5-HT_1-like" (Bradley et al. 1986).

We have previously shown that ICI 169,369 is selective for 5-HT receptors using in vitro and in vivo functional tests (Blackburn et al, 1986 a & b). The present studies demonstrate that ICI 169,369 has affinity not only for 5-HT_2 receptors on rat caudal artery and the smooth muscle for the guinea-pig ileum, but also for the as yet ill defined 'D' (5-HT_1-like?) receptor of the rat fundic strip. Thus ICI 169,369 may prove a useful agent in helping to further characterise the 5-HT receptors on smooth muscle.

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. and Saxena, P.R. (1986) Neuropharmacol. 25, 563-576.

Gaddum, J.H. and Picarelli, Z.P.(1957), Br. J. Pharmac. Chemother. 12: 323-328

Blackburn, T.P., Cox, B., Thornber, C.W. and Pearce, R.J. (1986 a & b) Abst. Soc. Neurosci. Washington.

IN VITRO PHARMACOLOGY OF ICI 169,369 A NEW 5-HT_2 ANTAGONIST

T.P. Blackburn, B Cox, R.J. Pearce and C. W. Thornber. ICI Pharmaceuticals Division, Research Dept. 2, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG

We have studied the effects of ICI 169,369 (2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride on a variety of isolated tissue preparations in order to determine its selectivity for 5-HT receptors and to assess its affinity for other classical receptor sites.

Isolated tissue preparations were set up for recording of isotonic tension in 5-ml organ baths containing Krebs-Henseleit solution at 37°C aerated with 95% O_2, 5% CO_2. In the case of the mouse vas deferens the tissue was stimulated electrically (duration-100m.sec, frequency-0.1/sec, pulse frequency-50Hz, supramaximal voltage 50-60V). Agonist concentration effect curves were constructed in the presence and absence of ICI 169,369 in these preparations. ICI 169,369 was a potent selective surmountable antagonist of 5-HT on a variety of vascular preparations (rat caudal artery, rabbit aorta and pig coronary artery) which are believed to contain the 5-HT_2 receptor-subtype. The threshold concentration for antagonism was approximately 10^{-8}M. In most of these tissues there was a parallel rightward shift of the dose response curve with no depression of the maximum response and the slope of Arunlakshana-Schild (1959) plot was not significantly different from 1. In addition the compound also antagonised 5-HT on the D-receptor sub-type on the smooth muscle of the rat fundic strip and guinea-pig ileum, with a similar potency to that shown at the 5-HT_2 receptor. The compound had no apparent affinity for the M-receptor in the latter preparation. ICI 169,369 showed neither agonist or antagonist activity on a variety of tissues used to measure affinity for the H_1(guinea-pig ileum), H_2 (guinea-pig right atrium), α_1 mouse vas deferens), α_2 (field stimulated mouse deferens), β_1 (guinea-pig right atrium), β_2 (guinea-pig tracheal chain), and muscarinic-receptor (guinea pig ileum). Non-selective inhibition was only observed at very high concentrations (>10^{-6}M) relative to its effects on the 5-HT receptor. Radioligand binding studies were also performed to measure the ability of ICI 169,369 to displace a variety of ligands from their specific binding sites in CNS tissues. Ki-values were calculated for ICI 169,369 against each receptor binding site i.e. 5-HT_1(1.58x 10^{-6}M), 5-HT_2(1.79x 10^{-8}M) α_1(5.6 x 10^{-7}M), α_2(1.37x 10^{-6}M), β_1(3.72x 10^{-6}M), β_2(2.8 x 10^{-6}M), D_2 (1.34x 10^{-7}M) and muscarinic-receptor (M1) (3.3 x 10^{-6}M). Thus, in conclusion, results from isolated tissue and ligand binding studies indicate that ICI 169,369 is a potent selective 5-HT antagonist devoid of significant effects at other neurotransmitter receptors.

Arunlakashana, O. and Schild, H.O. (1959) Br. J. Pharmac. 14, 48-58.

AZIRIDINIUM INHIBITION OF CHOLINE TRANSPORT IN RAT CORPUS STRIATUM, CEREBRAL CORTEX AND HIPPOCAMPUS AFTER DEPOLARISATION IN VITRO

G C Ormandy & A K Prince, Dept Pharmacology, King's College, Strand, London WC2R 2LS, UK

Irreversible inhibition of high affinity choline uptake by ethylcholine mustard aziridinium (ECMA) is dependent on the activity of the choline carriers. Uptake in fine slices of corpus striatum is 3-4 times that in cortex or hippocampus and more extensively inhibited by ECMA. However inhibition by phenoxybenzamine (PB) does not differ between the tissues (Ormandy & Prince 1986). It was concluded that inhibition by ECMA may include an action from within the nerve terminals after transport, although PB inhibits only from the external medium. Despite greater resting activity, choline uptake in striatum was less stimulated after tissue depolarisation than was uptake in cortex or hippocampus (Sherman et al 1978). We therefore examined the relation of carrier activity and sensivity to inhibition by ECMA or PB, after tissue depolarisation with K^+.

Fine slices (0.1 x 0.1 x approx 1 mm) were preincubated with KCl (5 or 60 mM, 5 min) washed twice by centrifugation, reacted with 3 µM ECMA or 100µM PB (2 min), washed twice , and assayed for choline uptake (^{3}H-choline 1µM, 1µCi, 4 min) using 1µM hemicholinium-3 to define high affinity uptake (reactions throughout were in Na-Tris Krebs' solution, 37°C).

In resting tissue inhibition of choline uptake by ECMA was greatest in striatum (Table, 5mM K^+).Uptake in striatum was less stimulated by prior depolarisation of the slices (Table, K^+- stimulation) and inhibition by ECMA was also less stimulated (Table, 60 mM K^+). Under these conditions inhibition was identical in the three tissues. Inhibition by PB did not differ between tissues and was unchanged by depolarisation.

% EFFECTS OF K^+ , ECMA & PB ON HIGH AFFINITY CHOLINE UPTAKE*

	PREINC K^+ (mM)	STRIATUM	HIPPOCAMPUS	CORTEX
K^+- stimulation:	60 vs 5	23.9,3.6,11	101.3,5.1,11	60.8,6.1,9
Inhibition by ECMA after:	5	64.3,4.2,6	49.1,4.0,6	39.8,4.2,6
	60	72.1,1.1,6	68.4,2.4,6	66.1,2.5,5
Inhibition by PB after:	5	37.9,3.2,5	34.3,4.4,5	29.5,9.8,3
	60	31.4,2.8,5	34.3,4.3,5	35.6,9.1,3

* mean % controls, SE mean % controls, number of independent estimates of means. Resting activities: striatum, hippocampus, cortex: 24690, 7860, 6870 cpm/10 mg tissue/4 min.

Since depolarisation stimulates inhibition by ECMA most markedly where choline uptake is strongly stimulated (cortex and hippocampus) the action depends on carrier activity. The lesser stimulation in striatum further reflects the insensitivity of striatal choline uptake to depolarising conditions. However after depolarisation, even though striatal uptake remains twice that in the other tissues, inhibition by ECMA is equalised. Thus transport may be less important to inhibition than orientation of the carriers to the external medium. If so, at maximum activity of the carriers (i.e. after depolarisation) sensitivities to ECMA are identical in the three tissues. At rest therefore, the greater inhibition in the striatum solely reflects greater individual carrier activities, although the larger maximum activities suggest there also exist greater concentrations of choline carriers and/or cholinergic nerve terminals in the striatum than in the other tissues. If ECMA and PB both only have external actions, inhibition independent of carrier activity suggests that PB interacts at a different site.

Ormandy & Prince (1986) Proc Br Pharmac Soc 2nd-4th July, P80
Sherman et al (1978) Life Sci 23, 1863-1870.

ACTIVATION OF QUISQUALATE RECEPTORS INCREASES CALCIUM LEVELS IN SYNAPTONEUROSOMES FROM IMMATURE RAT BRAIN

J. Benavidés and B. Scatton, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.) 31, Av. P.V. Couturier, 92220- Bagneux, France.

It has been reported that glutamate and other excitatory amino acids and analogs increase Ca^{++} uptake by brain tissue both in vivo and in vitro(Riveros and Orrego,1986). This effect seems to be mediated by the interaction of these compounds with specific receptor subtypes, which have been termed according to their pharmacological specificity as N-methyl-D-aspartate (NMDA), quisqualate and kainate receptors. It is unknown, however, whether the stimulation of Ca^{++} uptake is directly linked to the activation of these receptors or is the consequence of cell depolarisation or other biochemical alterations elicited by receptor activation. In an attempt to solve this question we have studied by a fluorimetric technique(Grynkiewicz et al,1985), the effect of excitatory aminoacids and analogs on Ca^{++} levels in synaptoneurosomes.

Synaptoneurosomes (a brain particulate fraction enriched in synaptosomes with attached postsynaptic membrane fragments which have resealed in a vesicular fashion) were prepared from the whole brain of 9 day old rats by the technique of Hollingsworth et al(1985).For loading with fluorescent dye, they were resuspended in Krebs-bicarbonate buffer (118mM NaCl, 4.7mM KCl, 1.18mM $MgSO_4$, 1.2mM $CaCl_2$, 24.9mM $NaHCO_3$, 1.2mM KH_2PO_4 and 10mM glucose pH 7.4) at a protein concentration of 3mg/ml and incubated at 37°C in the presence of 0.5µM FURA II AM. After 10 min, the suspension was diluted 3 times with the same buffer and kept at room temperature under 95% O_2/5% CO_2. A 1ml aliquot of this suspension was centrifuged for 10 sec in an Eppendorf desk centrifuge and the pellet gently resuspended in 3ml Krebs-HEPES buffer. After 10 min equilibration at 37°C, fluorescence was measured at this temperature in a AMINCO SPF-500 spectrofluorimeter. An excitation wavelength of 380nm, at which fluorescence was inversely related to $[Ca^{++}]$, was selected because of a better signal to noise ratio. Emission wavelength was 500nm.

Basal Ca^{++} levels of this preparation were 150-200nM and the depolarisation induced by the addition of 30mM KCl elicited an increase in $[Ca^{++}]$ of about 100nM. L-glutamate also elevated $[Ca^{++}]$ with a threshold response (10nM increase in $[Ca^{++}]$) at 10^{-7}M and a maximal effect of 50nM at 10^{-4}M (EC_{50} =10µM). This L-glutamate response was detectable for at least 3 hours after FURA II loading. Increases in Ca^{++} levels were also induced by D-glutamate and L-and D-aspartate but at higher concentrations and the maximal increase in $[Ca^{++}]$ was never higher than 25nM. Compounds specific for the excitatory aminoacid receptor subtypes also elevated intracellular $[Ca^{++}]$ with the following order of potency: quisqualate AMPA≃ibotenate NMDA≃kainate. The quisqualate antagonist γ-glutamyldiethyl ester blocked the increase in $[Ca^{++}]$ elicited by L-glutamate.

Taken together these results suggest that glutamate can increase intrasynaptoneurosomal Ca^{++} levels by interacting with a quisqualate preferring receptor. The present results also indicate that FURA II loaded synaptoneurosomes are a sensitive and convenient preparation for studying the effect of neurotransmitters and drugs on intracellular Ca^{++} homeostasis. This model will provide further understanding of the coupling mechanisms between receptor activation and alterations in Ca^{++} levels in neuronal tissue.

Grynkiewicz, G., Poenie, M., Tsien, R.Y.(1985) J. Biol. Chem. 260, 3440-3445.
Hollingsworth, E.B., Mc Neal, E.T., Burton, J.L., Williams, R.J., Daly, J.W. and Creveling, C.R. (1985) J; Neurosci. 5: 2240-2253.
Riveros, N., Orrego, F. (1986) Neuroscience 17, 541-546.

INCREASED PERIPHERAL BENZODIAZEPINE BINDING SITE DENSITIES IN BRAIN AS AN INDEX OF EXCITOTOXIN-INDUCED NEURONAL DAMAGE

J. Benavides, C. Carter, D. Fage and B. Scatton, Laboratoires d'Etudes et de Recherches Synthélabo, 31 ave. P.V. Couturier, 92220 Bagneux, France.

Neuronal damage in brain is accompanied by microglial proliferation. These cells possess peripheral type benzodiazepine binding sites (PTBBS) which can be labelled with Ro5-4864 (Schoemaker et al, 1982). An increase in PTBBS density can theoretically be used to quantify the extent of neuronal damage, and we have tested the validity of this approach by comparing the effects of various striatally injected neurotoxins on striatal PTBBS density and on the more conventional markers of neuronal damage, choline acetyltransferase (CAT) and glutamate decarboxylase (GAD) activity.

Male Sprague-Dawley rats (200g, Charles River France) received unilateral striatal injection of various doses of kainate, quisqualate or N-methyl-D-aspartate, under chloral hydrate anaesthesia (A:+8.2; V:0; L:±2.7; König and Klippel, 1963) 4µl NaOH buffered saline (pH 7.4); 1µl/min +5 min). CAT and GAD activity (Fonnum, 1969; Albers and Brady, 1959) and PTBBS density were measured in control and lesioned striatal homogenates, 1 week after surgery. PTBBS were assayed in striatal membrane preparations essentially using the filtration assay of Schoemaker et al (1982). Membranes were incubated with 1nM ^{3}H-Ro5 4864 (81.5 Ci/mMole for 2h at 4°C. Non specific binding was defined with 1µM PK11195.

	nmoles	CAT	GAD	PTBBS	Correlation coefficients	
		% reduction		% increase	CAT/PTBBS	GAD/PTBBS
Kainate	2	-3	0	140*		
N=6	4	-36*	-28.5*	310*	-0.87	-0.84
	8	-66*	-61.5*	375*		
	12	-86*	-82.5*	340*		
Quisqualate	100	-4	0	75*	-	-
N=6	250	14*	-18*	210*		
NMDA	62.5	-2	-7	60*		
N=6	125	-7	-16	110*	-0.975	-0.998
	250	-25*	-25*	160*		
	500	-49*	-48*	320*		

(* $p < 0.05$)

Each neurotoxin produced a dose related decrease in CAT and GAD activities accompanied by an increase in PTBBS binding. The increase in PTBBS density was a more sensitive index of neuronal damage as significant changes were observed with each excitotoxin at doses below those producing significant reductions in CAT and GAD activities. There was also an excellent significant correlation between the increase in PTBBS density and the reduction in CAT or GAD activities. Coinjection of 2-amino-5-phosphonovalerate (3-30 nmoles) protected against the neurotoxic effects of 250 nmoles NMDA, which was reflected by a concomitant reduction of the decrease in CAT or GAD activities and of the increase in PTBBS density.

These results demonstrate the validity, sensitivity and usefulness of PTBBS assay as a marker for neurotoxin induced neuronal damage, and as a tool to monitor and quantify the protective effects of excitatory amino acid antagonists. Autoradiographic measurement of PTBBS will improve the spatial resolution of this type of index.

Albers, R.W. and Brady R.O. (1959) J. Biol. Chem. 234 : 926-930.
Fonnum, F. (1969) Biochem. J. 115 : 465-472.
König, J.F.R. and Klippel, R.A. (1963) Krieger, New York.
Schoemaker, H., Morselli, M., Deshmuckh, P. and Yamamura, M.I. (1982) Brain Res. 248 : 396-401.

THE KAPPA AGONISTS U50488 AND U69593 PRODUCE ANTINOCICEPTION AFTER ICV AND PERIPHERAL BUT NOT INTRATHECAL ADMINISTRATION IN THE RAT

R.G.Hill, J.Hughes, G.E.Leighton and R.E.Rodriguez, Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge, CB2 2QB

There is much evidence for antinociception mediated via an action at the kappa opioid receptor although a certain amount of controversy surrounds the question of whether this effect results from an action at a spinal or a supraspinal site(Satoh et al 1983,Hayes et al 1983,Schmauss & Yaksh 1984).This report describes a comparative study of the antinociceptive effects of the selective kappa agonists U50488 and U69593 following administration intravenously, into the cerebral ventricles or into the subarachnoid space of the lumbar region of the spinal cord.

Male Wistar rats (280-300g) were implanted either with intrathecal cannulae according to the method first described by Yaksh and Rudy(1976) under 2,2,2 tribromoethanol anaesthesia or with a cannula into the third ventricle using chloral hydrate as the anaesthetic.All cannulated animals were allowed a recovery period of at least seven days prior to behavioural testing. For the intravenous study male Wistar rats (80-100g) were used and test compounds were administered via a tail vein.

Peripheral injection of U50488(dose to produce 50% maximum possible antinociceptive effect(MPE50)=1.95mg/kg) and U69593 (MPE50=1.21mg/kg)produced clear antinociception as measured using a paw pressure test.This effect was reversed by naloxone(1mg/kg s.c) confirming that it is mediated through an action at an opioid receptor.Intracerebral administration of U50488(MPE50=33ug/rat) and U69593 (MPE50<11ug/rat) also produced naloxone reversible antinociception in the paw pressure test.In contrast to this observation intrathecal administration of either kappa agonist,up to a maximum dose of 100ug/rat,did not produce any antinociception.Morphine(5ug/rat) injected via the intrathecal route always produced antinociception thus confirming that using our experimental protocol we could detect the effects of a known analgesic .

Our results show that, under our experimental conditions, selective kappa agonists do not produce antinociception when injected directly into the lumbar region of the spinal cord.On the other hand a clear antinociceptive effect is observed following administration by an intravenous route or injection directly into the third ventricle suggesting a supraspinal primary site of action for these compounds in the rat.

Satoh A et al (1983) Life Sci 33 sup 1 :689-692
Schmauss C & T.L.Yaksh (1984) J.Pharmacol Exp Ther 228:1-12
Hayes A.G,M.Skingle & M.B.Tyers (1983)Life Sci 33 sup 1 :657-660
Yaksh T.L & T.R.Rudy (1976) Physiol & Behav 17:1031-1036

ELECTROPHYSIOLOGICAL STUDIES ON THE INTERACTION OF SUBSTANCE P, BOMBESIN AND CHOLECYSTOKININ-8 ON THE RAT SPINAL CORD IN VITRO

A. Davies, R.G. Hill, K.G. Meecham and K.M Pittaway, Parke-Davis Research Unit, Addenbrookes Hospital Site, Cambridge CB2 2QB.

A number of peptides have been suggested to have a role in neurotransmission within the spinal cord (for review see Salt and Hill, 1983) and some (e.g. Substance P (SP), cholecystokinin (CCK) and bombesin (BN)) have pronounced behavioural actions when injected intrathecally (Jurna and Zetler, 1981; O'Donohue et al., 1984). The study of the action of these peptides is hampered by the lack of potent and selective antagonists, and we have accordingly investigated their interactions on motor and interneurones in isolated rat spinal cord preparations in order to ascertain whether there are separate or common sites of action for SP and for BN or CCK respectively.

In the isolated, transverse spinal cord slice preparation (Willets et al., 1985) conventional techniques were used to obtain intracellular records from dorsal horn interneurones using 3M KCl filled micropipettes (40-100 MΩ). Drugs were applied to the preparation by superfusion in the bathing solution. In 14 of 21 neurones SP (0.1-10 μM) applied for 1 minute induced a slow depolarisation which was often accompanied by an increase in synaptic activity. Similar results were obtained with CCK (16 of 23 neurones) and with BN (9 of 14 neurones) using a similar concentration range and application time. In 5 neurones where recording conditions were sufficiently stable to allow repeated application of all three peptides, 2 responded to all 3 peptides whereas the other 3 neurones each responded to only one of the peptides. This finding resembles the results of Dodd and Kelly (1981) on hippocampal neurones. Responses to SP, BN and CCK were preserved in concentrations of tetrodotoxin (1 μM) sufficient to block synaptic activity.

In a second series of experiments, spinal cords were isolated from 1 to 6 day old rats and hemisected (Evans and Watkins, 1978). Motoneurone depolarizations were recorded from L3 to L5 ventral roots using Ag/AgCl wick electrodes. All three peptides were found to depolarize ventral roots when applied in concentrations greater than 0.1 μM in the superfusing fluid. Desensitization to the effects of these peptides was seen when the dose interval was less than 20-30 minutes. In three preparations desensitized to BN, the SP response was unaffected in one and reduced by 11 and 33% in the other two. In two further preparations desensitized to CCK, the response to the CCK analogue caerulein was lost, but that to SP was unaffected.

This lack of cross desensitization coupled with the ability of some dorsal horn neurones to respond to only one of the peptides applied, suggests that SP, BN and CCK have independent sites of action in the rat spinal cord.

Evans, R.H. and Watkins, J.C. (1978) Eur.J.Pharmac., 50, 123-129.
Jurna, I. and Zetler, G. (1981) Eur.J.Pharmac., 73, 323-331.
O'Donohue, T.L. et al. (1984) J.Neurosci., 4, 2956-2962.
Salt, T.E. and Hill, R.G. (1983) Neuroscience 10, 1083-1103.
Willetts, J. et al. (1985) Ann.N.Y.Acad.Sci., 448, 385-402.

THE EFFECTS OF THE KAPPA RECEPTOR AGONIST U50488 ON THE CEREBRAL BLOOD FLOW OF THE URETHANE ANAESTHETISED RAT

R.G. Hill, J. Hughes, K.G. Meecham and J.M. Wide, Parke-Davis Research Unit, Addenbrookes Hospital Site, Cambridge CB2 2QB.

The involvement of endogenous opioids in the pathophysiology of circulatory crisis was indicated by the work of Holaday (1983), who demonstrated beneficial effects for the specific opiate antagonist naloxone in circulatory shock. In apparent contrast to these observations, the selective kappa opiate receptor agonist U50488 was shown by Tang (1985) to be protective against cerebral ischaemia induced by temporary bilateral carotid occlusion in both rats and gerbils, though the mechanisms involved are unclear.

In this study we investigated the effects of U50488 on the cerebral blood flow (CBF) of the anaesthetised rat using laser doppler flowmetry. This technique allows blood flow to be continuously monitored in the cerebral microvasculature using a non-invasive procedure (Öberg et al., 1983).

Male Wistar rats (300-450g) were anaesthetised with urethane (1.25 g/kg i.p.). Blood pressure was recorded from right carotid or femoral arteries and drugs were administered as i.v. bolus injections. Rats were mounted in a stereotaxic frame and artificially respired. CBF was monitored in the left parietal cortex, with the laser probe placed directly above the cortex through a 2-3 mm diameter craniotomy.

Cumulative doses of U50488 (0.1 to 14.4 mg/kg) were administered to rats with in tact carotid arteries and to rats with unilateral carotid occlusions. No significant changes in CBF were seen in either model at doses below 14.4 mg/kg. At this dose the CBF in the non-occluded group was significantly decreased but the heart rate was also reduced by 34%.

In a second series of experiments both carotid arteries were repeatedly occluded for 4 min periods, with 10-15 min inter-occlusion intervals. The effects of 2.0 mg/kg U50488 on the immediate fall in CBF seen on occlusion and on the reperfusion response were measured. No significant effects were seen on the initial fall in CBF, but significant increases in the reperfusion response were seen, as measured by integration. In each of two separate experiments repeated control responses were obtained, followed by repeated responses in the presence of U50488. Combining the data, the area under the control reperfusion response was 209 ± 31 mm^2 (n=7), and in the presence of U50488 the area significantly increased to 443 ± 27 mm^2 (n=4) ($p<0.01$). Preliminary results suggest that this action of U50488 can be reversed by subsequent administration of 2.0 mg/kg naloxone.

It is clear that U50488 has no pronounced effects on baseline CBF, but does improve the level of reperfusion following temporary bilateral carotid occlusion. Further work will be required to determine whether this effect is directly linked with the protective properties of the compound observed by Tang.

Holaday, J.W. (1983) Biochem. Pharmac., 32, 573-585.
Öberg, P.A. et al. (1983) Acta Med Scand, Suppl 687, 17-24.
Tang, A.H. (1985) Life Sci., 37, 1475-1482.

EFFECT OF DESENSITISATION OF THE P_{2x}-PURINOCEPTOR ON THE PRESSOR RESPONSE TO DMPP IN THE RAT

M.J. Brown, C.R. Hiley, D. Peard, G.R. Thomas, Clinical Pharmacology Unit and Department of Pharmacology, Cambridge University Clinical School, Addenbrooke's Hospital, F & G Block, Hills Road, Cambridge. CB2 2QQ

The release of adenosine triphosphate (ATP) from sympathetic nerve endings as a co-transmitter with noradrenaline may contribute to the haemodynamic responses to sympathetic nerve stimulation. Recently, 2 sub-types of the P2-purinoceptor have been defined on the basis of differing agonist potencies, with the P2x and P2y receptors respectively mediating constriction and dilatation (Kennedy & Burnstock, 1985). In vitro, desensitisation of the P2x receptor by the selective P2x agonist, alpha, beta-methyleneATP (meATP) has been used in the absence of an antagonist to test for the role of endogenous ATP in causing vasoconstriction. We have investigated whether desensitisation can be achieved reproducibly in vivo, and employed this to establish whether ATP release might contribute to the pressor action of sympathetic nerve stimulation. Experiments were performed using direct arterial pressure monitoring in rats anaesthetised with thiobutabarbitone.

The desensitisation schedule comprised increasing bolus i.v. doses of meATP from 0.001 to 0.1 mg, with the highest dose repeated on 7 occasions at 5 min intervals. The peak response to diastolic BP was 35 mmHg, falling to 0 mmHg after desensitisation. In vivo, the pressor response to meATP was bimodal with an immediate, transient rise and fall in BP during the first min after injection, followed by a more sustained peak during the second and third min. The time course of recovery from desensitisation was studied in 3 rats. The sustained pressor response was not observed up to 90 min after desensitisation, whereas the transient early increase recovered within 15 min.

In 10 rats, the effect of desensitisation was investigated on the pressor response to the nicotinic agonist, demethylphenylpiperazinium (DMPP). An initial dose-response curve to DMPP was constructed using i.v. bolus doses between 0.003 and 0.1 mg, following which rats received 7 sequential bolus doses either of meATP 0.1 mg or vehicle (n=5 for each) followed after 5 min by a repeat DMPP dose-response curve. In the meATP-desensitised rats, DMPP raised diastolic BP by 55±11 and 57±13 mmHg respectively before and after meATP. In the vehicle treated rats, DMPP raised diastolic BP by 55±10 and 55±9 mmHg respectively at the end of the 2 dose-response curves.

DMPP stimulates the postganglionic sympathetic neurons to release noradrenaline. The pressor response to stimulation of these neurons does not appear to be attenuated by desensitisation of the P2x-purinoceptor.

This work was supported by the M.R.C.

Kennedy, C. & Burnstock, G. (1985) Eur. J. Pharmacol. 111, 49

IN THE FROG HEART, [^{3}H]-DESIPRAMINE LABELS THE NEURONAL TRANSPORTER FOR ADRENALINE

S.Z. Langer, Carmen Pimoule* and H. Schoemaker, Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France

It is now well established that ^{3}H-desipramine binding is closely associated with the neuronal noradrenergic transporter in central and peripheral tissues of the rat (Raisman et al., 1982 ; Lee et al., 1982). In the frog heart, the level of adrenaline (A) is much higher than that of noradrenaline (NA), and A but not NA is released after sympathetic stimulation (Azuma et al., 1965). It is likely, therefore, that the frog heart exclusively contains the neuronal transporter for A. In our study, we carried out experiments to see if binding of ^{3}H-desipramine in the frog heart could be associated with the neuronal transporter for A.

Frogs were sacrificed, hearts removed and stored at -80°C. The levels of NA and A were determined by high performance liquid chromatography (HPLC). Frog heart membranes were prepared by homogenisation in ice-cold buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH 7.4) and centrifuged twice at 48,000 x g for 20 min. The final pellet was resuspended in 15 volumes of the same buffer. ^{3}H-Desipramine binding was determined by incubating aliquots of the membrane suspension with ^{3}H-desipramine (S.A. 77 Ci/mmole, New England Nuclear) from 0.1 to 10 nM in a final volume of 250 µl. After 1 h incubation at 25°C, membranes were collected by vacuum filtration over Whatman GF/F glass fiber filters. Non specific binding was defined as residual binding observed in the presence of 100 µM nisoxetine, and it represented 60 % of the total binding at 2 nM. Competition curves were carried out at 0.5 nM ^{3}H-desipramine.

In the frog heart, the levels of A and NA were : 2.10 ± 0.18 µg/g tissue (n = 8) and < 0.075 µg/g tissue (n = 8), respectively. The specific binding of ^{3}H-desipramine was saturable and Scatchard analysis gave straight lines, indicating a single population of binding sites (Kd = 1.94 ± 0.39 nM ; Bmax = 816 ± 66 fmol /mg prot., n = 3). As found in the rat heart, (-)-NA, (-)-A, DA and 5HT were inactive to inhibit ^{3}H-desipramine binding in the frog heart. Nisoxetine and (±)-oxaprotiline, both potent inhibitors of NA uptake, were potent to inhibit ^{3}H-desipramine binding. Desipramine showed the same potency as in rat heart for inhibition of ^{3}H-desipramine binding. Interestingly, the atypical antidepressants, iprindol and mianserin, and the tricyclic antidepressant, imipramine, were much more potent in frog heart than in rat heart.

Drug	^{3}H-Desipramine binding IC_{50} (µM) frog heart	IC_{50} (µM) rat heart*
Nisoxetine	0.013	0.007
(+)-Oxaprotiline	0.040	0.020
Desipramine	0.0025	0.006
Iprindol	0.25	10.0
Mianserin	0.050	0.95
Imipramine	0.004	0.220

*Results from Lee et al. (1982) or Raisman et al. (1982).

Although species differences cannot be excluded, our data indicate that the pharmacological profile of ^{3}H-desipramine binding to the neuronal transporter for A may be different from that of the noradrenergic transporter.

Azuma, T. et al. (1965) Am. J. Physiol. 209, 1287.
Lee, C.M. et al. (1982) J. Neurosci. 2, 1515.
Raisman, R. et al. (1982) Eur. J. Pharmacol. 78, 345.

MONOAMINE NEUROTRANSMITTERS AND THEIR METABOLITES IN DEPRESSED SUICIDE VICTIMS

S.C. Cheetham[1], M.R. Crompton[2], C. Czudek, R.W. Horton[1], C.L.E. Katona[3] and G.P. Reynolds. Department of Pathology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, [2]Department of Pharmacology and Clinical Pharmacology[1] and Forensic Medicine[2], St George's Hospital Medical School, London SW17 ORE and Department of Psychiatry[3], Middlesex Hospital Medical School, London W1N 8AA.

Altered monoaminergic function (particularly of 5-hydroxytryptamine, 5HT and noradrenaline, NA) has long been implicated in depression and suicide. Reduced CSF levels of monoamine metabolites have been reported in depression but not consistently replicated. A correlation between low CSF 5-hydroxyindole acetic acid (5HIAA) concentrations and violent suicide attempts (Asberg et al, 1976) provides the strongest support for reduced 5HT function in some depressed patients. Post-mortem brain studies do not conclusively support a relationship between monoamines, their metabolites and suicide and/or depression.

We have measured monoamine transmitters and their metabolites in post-mortem brain samples from suicide victims (7M 3F, mean age $\pm$ s.e.m., 46.3$\pm$2.9 years) and a control group (7M 3F, mean age $\pm$ s.e.m., 45.2$\pm$3.4 years) dying suddenly from non-psychiatric non-neurological causes. The suicide group included only subjects in which a firm diagnosis of depression could be made retrospectively. 5HT, 5HIAA, NA, dopamine (DA) and homovanillic acid (HVA) were measured by reverse phase HPLC with electrochemical detection in frontal and temporal cortices, hippocampus, putamen and caudate nucleus.

The results for 5HT and 5HIAA in 3 brain areas are shown in Table 1.

Table 1. 5HT and 5HIAA in brains of depressed suicides & controls

Brain Region		Caudate		Hippocampus		Frontal Cortex	
Hemisphere		L	R	L	R	L	R
5HT	controls	247(111)	261(69)	86.2(26.5)	79.8(26.9)	15.6(9.6)	15(7.7)
	suicides	237(47)	235(80)	95.5(29.8)	88.6(20.3)	14.8(4.9)	14.6(5.4)
5HIAA	controls	463(247)	477(177)	242(31)	235(34)	62.1(14.4)	64.2(13.6)
	suicides	440(135)	388(135)	251(45)	255(57)	56.8(13.1)	59.6(14.5)

Values are means ($\pm$ sd) in ng/g tissue (n=8 to 10)

There were no significant differences in 5HT, or 5HIAA, NA, DA or HVA between the depressed suicides and controls in these or the other brain areas studied. No signficant differences were apparent between left and right hemispheres in controls or depressed suicides. Thus no dysfunction of monoamine neurotransmitters was identified in this small group of depressed suicides. The results are largely in agreement with the study of Owen et al, 1986. They did report a modest increase in hippocampal 5HIAA in suicide victims. Although we did not replicate this finding we have reported a lower number of hippocampal $5HT_2$ binding sites in depressed suicides (Cheetham et al, 1986).

We thank the Wellcome Trust for financial support. SCC is an MRC scholar.

Asberg, M. et al (1976) Arch. Gen. Psychiat. 33, 1193
Cheetham, S.C. et al (1986) B.P.S. Hatfield Meeting
Owen, F.A. et al (1986) Brain Res. 362, 185

RESOLVING $GABA_A$/BENZODIAZEPINE RECEPTORS: CELLULAR AND SUBCELLULAR LOCALIZATION IN THE CNS WITH MONOCLONAL ANTIBODIES

H. Möhler, J.G. Richards* & P. Schoch, Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, Switzerland

The distribution and density of CNS binding sites for benzodiazepine minor tranquillizers can be demonstrated by radiolabelling techniques in vitro and in vivo (Richards & Möhler, 1984; Glinz et al., 1986). Although such methods are highly sensitive and quantitative, they have only a limited resolution due mainly to radiation scatter and possible diffusion of the bound radioligand (Richards et al., 1986).

Monoclonal antibodies, raised against a purified $GABA_A$/benzodiazepine receptor complex from bovine cerebral cortex, have been used to visualize the cellular and subcellular distribution of receptor-like immunoreactivity in the rat CNS, cat spinal cord, bovine and post-mortem human brain. Two different antibodies have been used for these studies: bd-17 recognizes the β-subunit (Mr 55kD) in all the species tested, whereas bd-24 recognizes the α-subunit (Mr 50 kD) of bovine, cat and human but not rat tissues.

The general distribution and density of receptor antigenic sites in the rat CNS, were very similar to those of benzodiazepine binding sites radiolabelled with ^{3}H-Ro 15-1788 and of glutamate decarboxylase (GAD)-stained nerve terminals. The results demonstrate a very high receptor density (around neuronal cell bodies and processes or less discretely distributed) in the olfactory bulbs, cerebral cortex, ventral pallidum, islands of Calleja, globus pallidus, hippocampus, dentate gyrus, substantia nigra, geniculate nuclei, inferior colliculi, cerebellum, reticular formation, spinal cord and retina. In contrast, no receptors could be detected in white matter, pineal, pituitary, adrenals and superior cervical ganglia. Only among cerebellar layers did we observe a conspicuous difference between the staining intensity and the radiolabelling. In bovine and post-mortem human brain, e.g. hippocampus, dentate gyrus, cerebral cortex and substantia nigra, both antibodies produced very similar staining patterns indicating a homogenous receptor composition, at least in the brain areas investigated. Again, the same close correlation between the immunohistochemical and radiohistochemical findings was observed.

At the electron microscopical level, the immune reaction product in the rat substantia nigra and globus pallidus, for example, was localized to pre- and postsynaptic membranes of axodendritic and axosomatic synapses. Whether the presynaptic staining represents GABA autoreceptors will be discussed.

In the near future, the monoclonal antibodies will be used in double labelling experiments with GAD, to identify those GABAergic projections in the CNS that are modulated by benzodiazepine minor tranquillizers. Furthermore, they could also be used, in studies of post-mortem human brain, to diagnose receptor dysfunction possibly associated with CNS disorders such as epilepsy.

Glinz, R. et al (1986) This meeting
Richards, J.G. & Möhler, H. (1984) Neuropharmacol. 23, 233-242
Richards, J.G. et al (1986) Experientia 42, 121-126

ELECTRONMICROSCOPE AUTORADIOGRAPHIC LOCALIZATION OF ADENOSINE RECEPTORS IN THE FROG SCIATIC NERVE

L.R. Mata, J.A. Ribeiro[1] & A.M. Sebastião[1], Laboratory of Cell Biology and [1]Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781 Oeiras, Portugal.

It has been shown (Ribeiro & Sebastião, 1984) that adenosine and its stable analogue, L-N^6-phenylisopropyladenosine (L-PIA) inhibit nerve conduction in the frog sciatic nerve. The present work was undertaken to investigate in which part of the frog sciatic nerve |^{3}H|-L-PIA would preferentially bind.

The tissue was incubated during 30 min at 22°C with Ringer solution containing adenosine deaminase (0.2 IU/ml) to remove endogenous adenosine. |^{3}H|-L-PIA (46 Ci/mmol) in a concentration of 1.5 nM was either used alone or in the presence of 10 uM of unlabelled L-PIA for 45 min at 22°C. The incubation was terminated by fixation with 50% Karnovsky's fixative in 0.1 M sodium cacodylate buffer for 2 h at 4°C. After washing the tissue, this has been post-fixed in 1% OsO_4 using the same buffer for 1 h at 4°C, dehydrated and embedded in epon. Thin sections cut at approximately 90 nm were collected on Formvar coated grids, double stained, carbon coated and covered with a monolayer of Ilford-L_4 emulsion. The grids were photographically processed after a 30-day exposure and studied in a JEOL 100CX electronmicroscope. The distribution of silver grains was studied by morphometric analysis at a final magnification of x12,000 in the following compartments: axon, axonal membrane (area included within 5 half-distances) and Schwann cell.

It was found that in the absence of cold L-PIA the autoradiographic silver grains were more frequent over or close to the axonal membrane of the frog sciatic nerve fibres. The relative grain density (% of silver grains: % of area) was greater than 1 over the axonal membrane and lower than 1 over the other compartments. In the control experiments, i.e. experiments in which excess of unlabelled L-PIA has been used, the relative grain density was lower than 1. The distribution of the grains in the absence of unlabelled L-PIA was significantly different from that observed in the control experiments (X^2 probability < 0.0005).

These results suggest that |^{3}H|-L-PIA binds specifically to the axonal membrane of the frog sciatic nerve, and are consistent with the presence of functional adenosine receptors in these membranes (cf. Ribeiro, 1986).

Ribeiro, J.A. (1986) Pflugers Archiv. 407, Suppl. 1, S24.
Ribeiro, J.A. & Sebastião, A.M. (1984) Br.J.Pharmac. 83, 485-492.

CARDIAC ADRENOCEPTOR RESPONSIVNESS IN HYPOTHYROID RATS

R. Chess-Williams and D.J.P. Critchley, Department of Pharmacology & Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

Hypothyroidism has been claimed by several investigators to produce a depression of cardiac β- and potentiation of cardiac α-adrenoceptor mediated responses (Kunos et al., 1977). However the influence of thyroid hormone on cardiac adrenoceptors has been questioned by some workers (Levey, 1971). The present study examines cardiac adrenergic responsiveness in the hypothyroid rat.

A hypothyroid state was induced in rats by pretreating with 6-propylthiouracil (PTU) (15 $mgKg^{-1}$ daily) administered via the drinking water for 8-10 weeks. Left atria and papillary muscles from PTU-treated and untreated rats were set up in oxygenated Krebs at 32°C. Tissues were paced at 1Hz and isometric developed tension recorded. Following 30 mins equilibration, cumulative concentration-response curves to isoprenaline, methoxamine, carbachol and calcium were constructed in the presence of metanephrine (10μM) and desipramine (1μM).

Table 1: EC_{50} values with 95% confidence limits

Tissue	Agonist	Untreated	PTU-pretreated	'P' value
Left	isoprenaline	1.0 (0.6-1.7)nM	179.9 (49.6-653.4)nM	<0.001
Atria	methoxamine	58.8 (23.5-147.2)μM	17.3 (7.2-42.1)μM	<0.05
	carbachol	186.7 (156.7-222.3)nM	211 (127.2-349.9)nM	N.D.
	calcium	0.99 (0.5-1.8)mM	1.0 (0.53-2.0)mM	N.D.
Papillary	isoprenaline	103.4 (57.0-187.1)nM	134.4 (37.0-488.4)nM	N.D.
Muscle	methoxamine	57.5 (28.7-115.3)μM	4.9 (2.4-9.9)μM	<0.001
	carbachol	97.5 (65.6-144.7)nM	112.7 (82.0-155.1)nM	N.D.
	calcium	1.3 (1.0-1.7)nM	1.2 (0.6-2.3)mM	N.D.

PTU-pretreatment resulted in a depression of left atrial sensitivity to isoprenaline (Table 1). The maximum developed tension to isoprenaline was unchanged (0.86 ± 0.19g (LA) and 0.72 ± 0.21g (PM) for controls; 0.81 ± 0.23g (LA) & 0.68 ± 0.17g (PM) for pretreated tissues). Responses to methoxamine however were potentiated by PTU pretreatment. EC_{50} values were reduced (Table 1) and the maximum methoxamine response relative to that for isoprenaline was increased from 69.5 ± 4.4% to 92.4 ± 5.7% for left atria ($P < 0.05$) and from 71.1 ± 3.0% to 97.0 ± 5.3% for papillary muscles ($P < 0.01$).

Carbachol responses were unaffected by treatment with similar EC_{50} values (Table 1) and similar maximum reductions in developed tension in control (68.9 ± 6.6%) (LA); 19.5 ± 3.1% (PM)) and treated tissues (64.6 ± 5.8% (LA); 16.8 ± 2.3% (PM)). Inotropic responses to calcium were also unaltered by PTU-pretreatment, with similar EC_{50} values (Table 1) in both groups and similar maximum responses to calcium (0.57 ± 0.05g (LA); 0.65 ± 0.05g (PM)) in controls and 0.58 ± 0.04g (LA); 0.60 ± 0.06g (PM) in PTU pre-treated animals).

These results confirm previous reports of a depression of β- and potentiation of α-adrenoceptor mediated responses in the hearts of hypothyroid animals.

This work is supported by the British Heart Foundation and Bristol-Myers.

Kunos, G. (1977) Br. J. Pharmac. 59: 177-189.
Levy, G.S. (1971) Am. J. Med. 50 (4): 413-420.