REGIONAL HETEROGENEITY OF BENZODIAZEPINE BINDING SITES IN RAT BRAIN

R.W. Horton, S.A. Prestwich and S.R. Stapleton, Department of Pharmacology, St. George's Hospital Medical School, London SW17 ORE.

Benzodiazepines (BZs) are thought to produce their pharmacological effects by interacting with specific binding sites in the CNS. Recent evidence suggests the existence of two distinct sub-classes of BZ binding sites which differ in their pharmacological specificity and in their distribution within the brain. (Squires et al 1979; Klepner et al 1979; Sieghart and Karobath, 1980). BZs have equal affinities for these sub-classes of binding sites, whereas several groups of drugs, structurally unrelated to benzodiazepines including esters of β-carboline-3-carboxylic acid and a series of triazolopyridazines, such as CL 218,872 have different affinities for the sub-classes (Klepner et al 1979; Nielsen and Braestrup, 1980). Regional studies indicate the presence of mainly one class of BZ sites in cerebellum (BZ1) and two classes in the hippocampus (BZ1 and BZ2). (Braestrup and Nielsen, 1981).

We have compared the ability of diazepam, ethyl β-carboline-3-carboxylate (βCCE) and CL 218,872 to displace (^{3}H) flunitrazepam ((^{3}H) FNM), which labels both BZ1 and BZ2 sites and (^{3}H) propyl β-carboline-3-carboxylate ((^{3}H) PrCC), a selective label for the BZ1 sub class at low concentrations, from rat hippocampal and cerebellar membranes. The K_i values (mean of 3 experiments) are shown in Table 1.

Table 1. K_i values (nM) for displacement of (^{3}H) FNM and (^{3}H) PrCC.

	(^{3}H) FNM	(^{3}H) PrCC	$\frac{K_i\ (^3H)\ FNM}{K_i\ (^3H)\ PrCC}$
Hippocampus			
Diazepam	11.5	8.6	1.34
βCCE	4.4	1.0	4.4
CL 218,872	1660	236	7.0
Cerebellum			
Diazepam	10.4	11.8	0.9
βCCE	0.8	0.6	1.35
CL 218,872	336	204	1.65

Diazepam displaced both ligands with almost equal potency in both regions. βCCE and CL 218,872 were more potent at displacing (^{3}H) PrCC than (^{3}H) FNM in both brain regions. βCCE and CL 218,872 were 5-6 times more potent at displacing (^{3}H) FNM in the cerebellum than the hippocampus whereas there was much less regional difference in the displacement of (^{3}H) PrCC. The slope coefficients of βCCE and CL 218,872 displacement of (^{3}H) PrCC were not significantly lower than 1 in either brain area. The slope coefficients for the displacement of (^{3}H) FNM by βCCE and CL 218,872 were significantly less than 1 in the hippocampus but not in the cerebellum. Thus βCCE and CL 218,872 displace from the BZ1 sub class with high affinity and the BZ2 with lower affinity. The effect of GABA (100μM) and NaCl (200mM) clearly distinguishes βCCE and CL 218,872. The potency of CL 218,872 to displace both ligands in both brain areas was increased whereas the potency of βCCE was unchanged. The present results further emphasise regional and pharmacological heterogeneity of BZ binding sites in rat brain.

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DEVELOPMENT OF $GABA_B$ BINDING SITES IN THE CEREBRAL CORTEX AND CEREBELLUM OF THE RAT

R.W. Horton and C.C.Sykes, Department of Pharmacology, St. George's Hospital Medical School, London SW17 ORE.

The pharmacology of (^{3}H) GABA or (^{3}H) muscimol binding sites in crude cerebral membranes (in the absence of Na^+ ions) is consistent with the labelling of the classical GABA receptor. The potency of GABA agonists to displace such binding correlates with electrophysiological and behavioural tests of GABA agonist activity and the binding is bicuculline sensitive. In contrast a novel GABA receptor (termed the $GABA_B$ site), which is relatively insensitive to GABA agonists and antagonists has been described (Hill & Bowery, 1981). Baclofen, p-chlorophenyl GABA, is stereospecifically active at the $GABA_B$ site, whereas it is devoid of activity at the classical $GABA_A$ site. We report the development of $GABA_B$ binding sites in the rat cerebral cortex and cerebellum, and for comparison $GABA_A$ binding sites in the cerebral cortex.

Crude membranes were prepared from Porton strain rats (Costa et al, 1978) at various ages between 4 and 56 days of age. $GABA_B$ binding sites were determined by (^{3}H) GABA binding to well-washed membranes in 50mM Tris-HCl buffer pH 7.4, containing 2.5mM $CaCl_2$ and 40µM isoguvacine at room temperature (Hill & Bowery, 1981). Specific binding was defined as radioactivity displaceable by 10^{-4}M (±) baclofen. $GABA_A$ sites were determined by (^{3}H) GABA binding to Triton treated well-washed membranes in 50mM Tris-citrate pH 7.1 at 0°C. Specific binding was defined as radioactivity displaceable by 10^{-5}M muscimol. Scatchard analysis of $GABA_B$ binding gave linear plots at all ages studied whereas curved Scatchard plots were apparent for $GABA_A$ site binding at all ages. K_D (nM) and R_T (p moles/mg protein) for $GABA_B$ sites (means ± s.e.m. of experiments repeated on at least 3 occasions) are shown in Table 1.

Table 1. Development of $GABA_B$ binding sites in rat cortex and cerebellum.

	Cortex		Cerebellum	
Age (days)	K_D	R_T	K_D	R_T
4–5	75 ± 16	0.53 ± 0.07		
9–10	64 ± 8	0.62 ± 0.08	104 ± 14	0.30 ± 0.03
13–14	68 ± 4	0.93 ± 0.02	88 ± 22	0.67 ± 0.12
18–19	85 ± 8	1.07 ± 0.05	151 ± 38	1.35 ± 0.22
23–24	118 ± 18	1.09 ± 0.08	137 ± 32	1.20 ± 0.18
35–36	190 ± 37	1.40 ± 0.18	150 ± 16	1.04 ± 0.08
56	225 ± 25	2.05 ± 0.16	155 ± 40	1.30 ± 0.26

The number of $GABA_A$ sites in the cortex (not shown) and $GABA_B$ sites in the cerebellum showed a similar development. At 9–10 days of age they were present at 30–45% of the adult level and increased rapidly such that the adult level (56 day) was attained at 18–19 days of age, corresponding to the period of rapid synaptogenesis in the rat brain. K_D values showed no particular trend over this period. The development of $GABA_B$ sites in the cortex was slower. They were present at 30% of the adult level at 9–10 days of age, 52% at 18–19 days of age and showed a marked increase between 35–36 and 56 days of age. The K_D values of $GABA_B$ binding in the cortex increased during development particularly between 18–19 days (85 ± 8 nM) and 56 days (224 ± 25 nM).

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FURTHER IN VIVO EVALUATION OF A STABLE TRH ANALOGUE, RX77368

J.D. Kemp, T. Priestley and M.J. Turnbull, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG

There have been several reports in the literature suggesting that thyrotropin releasing hormone (TRH) may be of value in the treatment of depression (Prange et al., 1972; Kastin et al., 1972). The availability of stable TRH analogues has further stimulated interest in the "extra-endocrine" pharmacology of these tripeptides. We have studied one such analogue, RX77368 (L-pyroglutamyl-L-histidyl-L-3,3 dimethylprolineamide), which has been reported to share similar properties with the tricyclic antidepressants in standard antidepressant tests but to lack the inhibitory effect on amine uptake and MAO activity typically associated with "classical antidepressants" (Metcalf 1980, unpublished).

We were particularly interested in evaluating the potential antidepressant activity of RX77368 on step-down avoidance behaviour in the olfactory bulbectomised rat, since antidepressants have been reported to reduce the number of avoidance responses in these animals (Cairncross et al., 1978). In addition we have assessed the potential stimulant properties of RX77368 by examining its ability to produce stimulus generalisation to amphetamine in rats trained to discriminate amphetamine from saline. The ability of TRH analogues to release dopamine in the CNS (Heal & Green, 1981; Sharp et al., 1981) suggests that these compounds may possess unwanted stimulant properties.

The methodology for the step-down avoidance procedure was essentially that described by Cairncross et al. (1978). RX77368 significantly corrected the bulbectomy-induced learning deficit when administered at a dose of 0.5mg/kg i.p. or p.o. twice daily for 7 days. Surprisingly, a similar, but less pronounced effect was seen when RX 77368 (0.5mg/kg i.p.) or amitriptyline (5.0mg/kg i.p.) were administered acutely, 1 hour prior to testing.

A separate group of rats were trained in a standard, 2-lever, operant procedure to discriminate between injections of amphetamine sulphate (1mg/kg i.p., 30 mins pretreatment) or saline (1ml/kg i.p.). When animals reached criterion performance (10 consecutive daily sessions in which the correct lever was selected within the first 12 responses) the training drug was randomly substituted (30 mins prior to testing) for either RX77368 (1 & 2mg/kg i.p.), nomifensine (0.1, 0.5 & 1.0mg/kg i.p.), desmethylimipramine (25mg/kg i.p.) or fenfluramine (3 & 5 mg/kg i.p.). Nomifensine produced a dose related generalisation to the amphetamine stimulus, whereas RX77368, desmethylimipramine and fenfluramine induced saline-appropriate responding.

The results show that RX77368 was active in the olfactory bulbectomy model after oral or parenteral administration, suggestive of antidepressant activity. Since RX77368 generalised to saline at the doses used, it appears unlikely that the compound possesses amphetamine-like stimulant properties.

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EFFECTS OF NEUROTENSIN AND N-TERMINAL FRAGMENTS ON TRH-INDUCED WET-DOG SHAKING

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

Some of the behavioural actions of the neuropeptides thyrotrophin-releasing hormone (TRH, a tripeptide) and neurotensin (NT, a tridecapeptide) in the CNS are mutually antagonistic (Griffiths et al, 1981). We have investigated this antagonism in relation to the structure of NT by examining the ability of NT and 3 of its amino-terminal fragments to reduce TRH-induced wet-dog shaking (WDS).

A guide cannula for intracerebral microinjection was implanted above the periaqueductal grey region (PAG) in 30 anaesthetized male Sprague Dawley rats (180-200 g). After a 6 day recovery period, peptides were injected through a cannula into the PAG (A 0.6, H +0.5, L 0.45; König & Klippel, 1963). Peptides were dissolved in 0.9% saline and the volume injected was 1 µl. WDS were counted for 30 min. The injection site was verified histologically in every animal.

TRH (0.1 - 10 µg) in PAG produced dose-related WDS scores. TRH-NT interactions were studied using a standard dose (1 µg) of TRH. NT (1,5 µg) injected in PAG together with TRH reduced WDS (Figure 1).

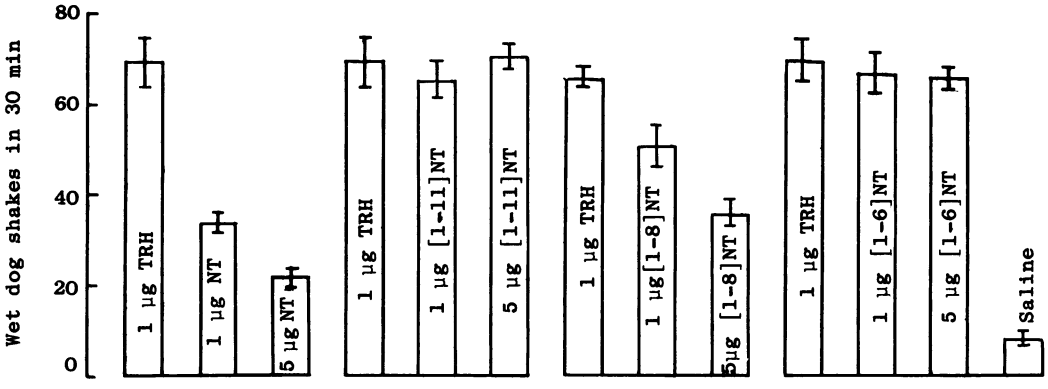

Figure 1. Effects of NT and NT fragments on wet-dog shaking induced by TRH in rat brain periaqueductal grey area.

Of the NT fragments investigated, neither [1-11]NT nor [1-6]NT had any effects on TRH-induced WDS. By contrast, [1-8]NT (1,5 µg) antagonised WDS. NT and NT fragments alone in PAG produced no significant WDS when compared with saline. Thus appreciable NT-like, anti-TRH activity resides in the [1-8]NT fragment which is also the main stable metabolite of NT in the CNS (McDermott et al., 1981). Biotransformation of NT to [1-8]NT may contribute to the central actions of the tridecapeptide.

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NEUROTOXICITY OF KAINATE AND L-GLUTAMATE IN THE STRIATUM

Gethin J. McBean and Peter J. Roberts, Department of Physiology and Pharmacology, University of Southampton, Southampton SO9 3TU.

Striatal kainate (KA) neurotoxicity is dependent on an intact cortico-striatal (glutamatergic) pathway (McGeer et al., 1978; Biziere & Coyle, 1978, 1979) and, following decortication, toxicity to KA may be restored by co-injection of L-glutamate. Although toxicity of KA may occur through a mechanism involving glu, direct injections of this latter substance result in only minimal neuronal loss (Olney and de Gubareff, 1978). Since in the hippocampus, removal of glutamatergic afferent terminals confers vulnerability of hippocampal neurones to glu injections (Köhler & Schwarcz, 1981), it is possible that rapid removal of glu from the site of administration by high- and/or low-affinity uptake sites may account for the weak toxicity that is normally observed.

In this study, we have investigated the effects of modifying uptake, either by decortication or by direct injection of a potent glu uptake inhibitor, DL-threo-3-hydroxyaspartate, on glu toxicity in the striatum. Stereotaxic injections (coordinates, AP + 7.5, ML + 2.2 and DV - 5.3) of KA (10 nmol) and glu (1 μmol) in 1 μl 0.1 M PBS (pH 7.4) were made 2 weeks after fronto-parietal decortication (Roberts et al., 1982), and animals allowed to recover for up to 1 week. For DL-threo-3-hydroxyaspartate (170 nmol) all assays were performed two weeks after injection. Striata were assessed neurochemically by assay of choline acetyltransferase (CAT) activity, and sodium-dependent ^{3}H-GABA uptake into homogenates. For histology, brains were perfusion-fixed, embedded in wax and 6 μm coronal sections stained with thionin.

Following decortication, KA failed to elicit any signs of neurotoxicity. One week after co-administration of KA and glu however, CAT activity and ^{3}H-GABA uptake were reduced by 30 and 35% respectively. Injection of glu alone, 2 weeks after decortication, resulted in similar decreases. Histological examination revealed an area of pronounced neuronal degeneration surrounding the needle tract, with intense glial proliferation. Two weeks following striatal injections of threo-3-hydroxyaspartate, neurotoxicity was again apparent, with reduction in CAT (30%) and ^{3}H-GABA uptake (40%) compared with controls. Histological examination confirmed the presence of neuronal degeneration, which was confined to the area around the injection site.

Our data confirm the previous reports on the influence of cortico-striatal afferents on KA toxicity in the striatum. However, since we have also shown that glu alone may be neurotoxic following decortication, it is not at present clear whether co-administration of glu does in fact restore KA toxicity. The ability of DL-threo-3-hydroxyaspartate to induce cytotoxicity is also indicative of a glu-mediated mechanism of striatal degeneration.

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BINDING OF L-ASPARTATE TO RAT SPINAL CORD SYNAPTIC MEMBRANES

S.P. Butcher and P.J. Roberts, Department of Physiology and Pharmacology, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU.

Binding studies with excitatory amino acids have been utilized as a means of labelling their physiological receptors. While glutamate has been widely studied, (Roberts, 1981), the sites labelled by L-aspartate have received little attention (Sharif and Roberts, 1981; Foster et al., 1981; Di Lauro et al., 1982). Electrophysiological studies, performed largely in the spinal cord (Watkins et al., 1981) have suggested the presence of at least 3 populations of excitatory amino acid receptors. Of these, one is activated preferentially by N-methyl-D-aspartate (NMDA) , and this may be the L-aspartate receptor. However, previous binding studies in brain have failed to find any major interaction of NMDA with the asp binding site. In this study therefore, we have examined the binding of L-2,3-^{3}H-aspartate to synaptic membranes prepared from spinal cord.

Wistar albino rats (200-250g) of either sex were killed by decapitation and the spinal cords removed rapidly and homogenised in 20 vol 0.32M sucrose in 5mM HEPES buffer (pH 7.4). A P_2 pellet was obtained by differential centrifugation, which was then resuspended in sucrose and layered onto a discontinuous sucrose gradient. The synaptosomal fraction was lysed in hypotonic medium, extensively washed and finally resuspended in Na^{+}-free 50 mM HEPES buffer (pH 7.4).

Binding assays were usually performed using 60nM L-^{3}H-asp and 1mM L-asp to define specific binding. After a 10 min incubation at 37^{o}C, free and bound ligand were separated by rapid centrifugation and careful washing of the pellet, which was then dissolved and the radioactivity determined.

Binding was linear with membrane concentration (50-400 ug protein/assay) and was optimal under physiological conditions of temperature and pH. The binding was slowly saturable, reaching equilibrium over 30 min, and was totally reversible. Kinetic analysis indicated that in contrast to the single binding site detected in cerebellum (Sharif and Roberts, 1981), there were three apparent sites on spinal cord membranes with K_D's of 10 and 800 nM and 40 uM respectively. Because of the complex nature of this binding, it has not so far proved possible to investigate comprehensively, the ligand's structural requirements for each site. However, asp and glu invariably act as potent inhibitors of binding. NMDA however, while active, showed little concentration-dependence; this finding does not support the proposal that NMDA interacts with L-asp binding sites.

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TOXICITY OF EXCITANT AMINO ACIDS IN THE COCKROACH (PERIPLANATA AMERICANUS)

F. Castle & R.H. Evans, Department of Pharmacology, Medical School, University Walk, Bristol, BS8 1TD.

Kainate, quisqualate and N-methyl-D-aspartate are selective agonists for different excitant amino acid receptor types in the vertebrate central nervous system (Watkins & Evans, 1981). The present study concerns a comparison between the toxicity of these three amino acids and L-glutamate in the insect where receptors activated by excitant amino acids have a peripheral neuromuscular function (Usherwood & Machili 1968, Clements & May 1974).

ED_{50} levels (Litchfield & Wilcoxon 1949) for paralysis of cockroaches were measured as determined by failure of the animals to attain normal posture within 60sec of being placed upside down on a horizontal surface. Amino acids were dissolved in saline (27.5g NaCl, 0.375g KCl per litre) prior to intra-abdominal injection between the 5th and 6th abdominal sternites. Body weight of animals was in the range 0.5 - 1.5g. A constant injection volume of 50 µl was employed throughout and the quantities of amino acids in this volume were adjusted with respect to the body weight. The tests were carried out at an ambient temperature of 25°C. Each compound was tested at 4 dose levels distributed, except in the case of N-methyl-D-aspartate, about the ED_{50}. Seven animals were treated at each level.

ED_{50} values (µmole per kg body weight), 95% confidence limits in parenthesis, were as follows: quisqualate 14.4 (6.6-31.8), kainate 42.8 (33.3-54.5), L-glutamate 1412 (1112-1793) and N-methyl-D-aspartate > 3500. Treatment of animals with either 50 µl saline or 1M NaCl had no effect on righting behaviour.

The respective excitatory potencies reported for these compounds at arthropod skeletal muscle fibres are 260 (Shinozaki & Shibuya 1974) : 0.0008 (Clements & May, 1974) : 1 : 0 (Usherwood & Machili 1968). The toxic activity of kainate, observed in the present tests, would not have been predicted from these values. It is unlikely therefore that the toxic effect of this amino acid is mediated at skeletal muscle fibres in the cockroach.

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EFFECTS OF PHOSPHONIC ANALOGUES OF EXCITANT AMINO ACIDS ON BEHAVIOUR IN THE IMMATURE RAT

M.L. Cutts & R.H. Evans, Department of Pharmacology, Medical School, University Walk, Bristol, BS8 1TD.

Apart from motor effects of (±)2-amino-5-phosphonovalerate (APV) which have been observed following mesencephalic application (Dawbarn & Pycock, 1981) there is little information on behavioural effects of phosphonic analogues of excitant amino acids. These compounds are very polar and thus unlikely to penetrate the blood brain barrier to any significant extent. In the present study this problem has been circumvented to some extent by the use of 3-8 day old rats in which the blood brain barrier is immature.

The actions of APV and (±)2-amino-4-phosphonobutyrate (APB) were compared with urethane which has been shown to have amino acid antagonist properties (Evans & Smith 1982). ED_{50} levels were measured for effects on righting behaviour or motor responses to squeezing or immersion of the tail in water at 49°C following intraperitoneal injection of neutral solutions of the test compounds. Each compound was tested on 4 groups of 27 animals at doses distributed around the ED_{50}. Effects were observed over 2 to 4 fold dose ranges distributed about the ED_{50}. Animals were tested from 1 to 5 hours following separation of litters from the mother and the ambient temperature was 20 ± 2°C. The results are summarized in Table 1 and compared with levels reported to depress dorsal root evoked ventral root potentials (DR-VRP) by 50% in immature rat or frog isolated spinal cord preparations (Evans et al, 1981a). Polysynaptic components of the DR-VRP are mediated at N-methyl aspartate (NMA) receptors whereas the monosynaptic component is mediated at kainate or quisqualate receptors (Evans et al, 1981b). Tests were made from 10 min after drug application and failure to respond within 30 sec of stimulation was recorded as no response. Peak effects usually occurred within 20 min of drug application.

Table 1

	ED_{50} in behavioural test in mmole/Kg			50% depression of DR-VRP mmole/l	
	Righting	tail pressure	tail heat	monosynaptic	polysynaptic
APV	0.57	>1.0	>1.0	>1.0	0.02
APB	0.12	0.07	>0.1	0.1	1.0
Urethane	13.6	15.1	>20.0	60	60

The weak effects of APV, relative to the polysynaptic blocking activity of this compound, suggest that NMA receptors do not predominate in pathways which mediate responses to vestibular or noxious stimuli (cf. Salt & Hill 1981 and Anis et al, 1982). However, the actions of APB and urethane suggest that kainate or quisqualate receptors are involved in behavioural responses to vestibular and mechanical, but not thermal, stimuli (cf. Salt & Hill 1981).

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TRANSMITTER-LIKE ACTIONS OF L-PROLINE AND GLYCINE IN RAT STRIATUM

R. Mitchell (Introduced by G.W. Arbuthnott) MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ.

A great variety of striatal transmitters have now been shown to exert potential presynaptic control of DA release from nigro-striatal terminals. These include GABA (whose action is probably indirect (Giorguieff et al., 1978)) and more recently glycine (Martin & Mitchell, 1979; Kerwin & Pycock, 1979). The potencies of various inhibitory amino acids in this action have rarely been rigorously compared.

The release of recently accumulated [^{3}H]DA from small (0.1mm) prisms of rat striatum was examined using a continuous superfusion system described in detail previously (Mitchell & Martin, 1978). Effects of amino acids and antagonists were studied on both basal and stimulus-evoked (15mM K^+) release of [^{3}H], which during stimulation represented some 84% authentic DA without any Monoamine Oxidase Inhibitor.

A number of putative transmitter amino acids elicited a facilitation of K^+-evoked, but not basal DA release, but concentration-response data revealed marked differences between them. GABA and taurine produced small increases only in excess of 300μM whilst β-alanine was slightly more potent causing 30% facilitation at 370μM. Equivalent responses however were produced by glycine at 105μM and L-proline at only 20μM. There was no significant effect of D-proline even at 900μM, demonstrating receptor-like stereospecificity. The L-proline and glycine responses were reproduced using synaptosomal preparations, indicating a direct presynaptic site of action. Experiments on the antagonism of these responses revealed a similar profile with L-proline (100μM) and glycine (150μM) being antagonised by strychnine, 50μM (75.6%* and 80.6%* respectively) and weakly by picrotoxinin, 50μM (40.4%* and 56.3%*), but not by bicuculline methiodide, 50μM. (*$p < 0.05$). (The bicuculline-sensitive glycine responses reported previously, (Martin & Mitchell, 1979) were apparent only at higher agonist concentrations, in excess of 300μM). Their similar profile of antagonism suggested the possibility that both L-proline and glycine responses may be mediated via the same site, presumably related to a transmitter function for L-proline on the basis of its 5 fold greater potency. However, experiments with [^{3}H] L-proline and [^{3}H] glycine revealed separate high affinity uptake systems in striatum for both (K_m's 6μM and 25μM respectively) and with the characteristic substrate specificities described for each in other regions. Furthermore, these recently accumulated [^{3}H] transmitters could both be released by depolarising stimuli (50μM K^+) in a largely Ca^{2+}-dependent fashion (proline, 99% and glycine 44% increase over basal efflux).

These experiments therefore provide biochemical evidence for individual transmitter roles for L-proline and glycine in striatum and demonstrate that especially L-proline, but probably both, may have an important regulatory role over terminals of the nigro-striatal DA system.

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MODIFIED PHARMACOLOGICAL RESPONSES TO MORPHINE IN THE RAT FOLLOWING ADMINISTRATION OF WY 26002 OR ZIMELIDINE

Linda Bowditch and Allan Fletcher (Introduced by R J Stephens) Department of Pharmacology, Wyeth Laboratories, Huntercombe Lane South, Taplow, Maidenhead,Berks

Central 5-hydroxytryptamine (5-HT) systems are thought to play an important role in the pharmacological actions of morphine (eg. see Messing and Lytle, 1977). Although drugs previously used to manipulate 5-HT metabolism have suffered from lack of specificity, selective inhibitors of 5-HT re-uptake are now available - Wy 26002 (Moser et al 1981) and zimelidine (Ross and Renyi 1977). We have therefore examined the effects of these drugs on some of the behavioural responses to morphine in rodents.

Antinociception in rats (male, Sprague-Dawley, 100-120g) was assessed in the hot-plate (55°C) procedure, and in mice (female, Tuck, 18-22g) using the hot-plate (55°C) and hot water tail-flick (51°C) procedures. Spontaneous motor activity (open field) and catalepsy (scored 0-4 for each animal) were also examined in rats. All behavioural assessments were performed blind. Drug doses are in $mg.kg^{-1}$ of base and were administered orally either 60 min. (Wy 26002), 90 min. (zimelidine) or 30 min. (morphine sulphate, subcutaneously) before testing.

Both Wy 26002 and zimelidine were devoid of antinociceptive activity in any of the methods employed. In the mouse, neither drug (at doses known to cause significant inhibition of in vivo 5-HT re-uptake) significantly modified the antinociceptive response to morphine (2 or 4 $mg.kg^{-1}$). Both uptake inhibitors potentiated the antinociceptive response to medium/high doses (3-6 $mg.kg^{-1}$) of morphine in the rat { mean reaction times in s ± S.E.M, n=10 : experiment 1 :- controls 3.9 ± 0.3 ; morphine (3$mg.kg^{-1}$) 7.1 ± 1.9 ; zimelidine (40 $mg.kg^{-1}$) + morphine 23.7 ± 6.3 ($p < 0.05$ cf. morphine alone). Experiment 2 :- controls 4.1 ± 0.4 ; morphine (6 $mg.kg^{-1}$) 14.5 ± 3.5 ; Wy 26002 (20 $mg.kg^{-1}$) + morphine 34.3 ± 5.7 ($p < 0.01$ cf. morphine alone) }. In open-field experiments, both Wy 26002 and zimelidine were found to have a synergistic effect with morphine in reducing spontaneous motor activity { mean number of lines crossed/2min. ± S.E.M, n=10 :- controls 71 ± 6 ; morphine (3$mg.kg^{-1}$) 68 ± 5 ; Wy 26002 (20$mg.kg^{-1}$) 66 ± 5 ; zimelidine (40 $mg.kg^{-1}$) 61 ± 7 ; Wy 26002 + morphine 37 ± 7 ($p < 0.01$) ; zimelidine + morphine 17 ± 6 ($p < 0.001$ cf. morphine alone) }. The degree of catalepsy induced by morphine was also increased by 5-HT uptake inhibitors { catalepsy scores, expressed as group (n=10) totals : experiment 1 :- controls (morphine 5 $mg.kg^{-1}$) 8 ; Wy 26002 (5 $mg.kg^{-1}$) + morphine, 20 ($p < 0.05$). Experiment 2:- controls (morphine 5 $mg.kg^{-1}$) 5; zimelidine (40 $mg.kg^{-1}$) + morphine, 21 ($p=0.002$) }.

These findings, in addition to the observation that neither uptake inhibitor potentiated the antinociceptive action of morphine in the mouse, a species in which morphine (in the dose range used in our experiments) does not induce catalepsy or pronounced sedation, lead us to conclude that the apparent potentiation of morphine-induced antinociception in the rat by 5-HT uptake inhibitors may be a secondary effect of an enhancement of opiate-mediated motor depression and catalepsy.

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MEPTAZINOL BINDS TO OPIATE AND NON-OPIATE SITES

Pamela A. Blurton, J. Fox, M.D. Wood & M.G. Wyllie, Biochemical Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire, S16 OPH.

The existence of multiple opiate receptors has been invoked to explain the difference in pharmacological, therapeutic and adverse reaction profiles of opiate and opiate-like drugs. Whereas the action of currently available centrally -acting analgesics can be explained by an interaction with such receptors, the effects of the new hexahydroazepine derivative, meptazinol, are not easily explained by assuming an interaction solely at opiate receptors. Meptazinol is thought to be a centrally acting analgesic (Stephens et al, 1978;) with opiate antagonist properties which displays only a low affinity interaction at opiate receptors (Bill et al, 1981; Blurton et al, 1982). We describe here the binding of {^{3}H}-meptazinol (22Ci/mmol, Amersham) to sites in rodent CNS.

Meptazinol binding in vitro was measured in Tris-HCl (50mM, pH 7.4). Microsomal or other material was incubated in the presence of {^{3}H} meptazinol for 15 min at 25°C and 8-20h at 0°C. The reaction was terminated by centrifugation (250,000g for 30 min). The resultant pellets were washed and solubilised prior to counting.

The highest level of specific binding of meptazinol was located in cerebral cortex and spinal cord. Relatively little binding was found in midbrain which is considered to be rich in opiate receptors. In contrast to naloxone and buprenorphine binding which was associated with the synaptosomal fraction, specific meptazinol binding was mainly associated with microsomal material. In addition, substantial non-specific binding occurred in myelin fragments.

Scatchard analysis revealed the existence of two microsomal binding sites for meptazinol (Bill et al, 1981). Neither naloxone, buprenorphine nor morphine (10μM) caused a significant displacement of meptazinol from either of these sites. In contrast, meptazinol (10μM) displaced bound naloxone.

The administration of 32 mg.kg^{-1} meptazinol s.c. (16 mg.kg^{-1} = dose to double tail flick latency) to mice 30 min prior to the preparation of brain fractions, induced a 50% displacement of ^{3}H-meptazinol (1μCi) in vivo binding to cortical and spinal sites. Naloxone (0.2, 1.0 & 5.0 mg.kg^{-1} s.c.) pretreatment did not reduce ^{3}H-meptazinol binding in any brain region. The highest dose of antagonist used, however, was sufficient to cause an 80% reduction in the antinociceptive response to meptazinol and to substantially reduce the in vivo binding of ^{3}H-naloxone (1μCi).

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INTERACTION OF MEPTAZINOL WITH MULTIPLE OPIATE RECEPTORS

Pamela A. Blurton, J. Fox, M.D. Wood & M.G. Wyllie, Biochemical Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead.

Following the isolation of the naturally occurring opiod peptides, evidence has accumulated in favour of the existence of at least four opiate receptors (inter alia Gilbert & Martin , 1976; Gillan et al. 1980; Zhang & Pasternak, 1981). We have studied the binding of the opiod ligands ^{3}H-naloxone, ^{3}H- ethylketocyclazocine (EKZ) and ^{3}H-D-ala^{2}-D-leu^{5}-enkephalin (DADL) to multiple sites in rat CNS, and also characterised the interaction of the analgesic agent, meptazinol, with these sites.

Binding of tritiated ligands was carried out according to the method of Snyder (Childers et al, 1979). Membranes were incubated in Tris-HCl (pH7.7, 50mM) for 30 min in the presence of radioligand and displacing agent. The reaction was terminated by filtration through glass-fibre filters (GF/B).

Naloxone bound, with differing affinity, to three distinct sites as revealed by Scatchard analysis. The computed affinity constants (KD's) were 0.64nM, 2.5nM and 12.0nM with Bmax's of 15.5, 130 and 314 fmol/mg original wet weight of tissue, respectively. Drug displacement studies revealed that morphine and naloxone were more potent against the super-high affinity site (KD < 1nM), whereas nalorphine and buprenorphine were more potent against the high affinity site (KD approx. 2.5nM). These findings are consistent with the postulated sub-division of the morphine (μ) binding site into μ_i (super-high) and μ_2 (high affinity) sites (Zhang & Pasternak, 1981). The low affinity site (KD approx. 12 nM) has been suggested to represent binding to the enkephalin (δ) receptor ; the high binding capacity of this low affinity naloxone component militates against this.

Scatchard analysis revealed that EKZ binding consisted of two components. The high affinity component (KD 0.1nM Bmax 10fmol/18mg tissue was sensitive to displacement by bremazocine but not morphine, and may therefore represent a κ site. The low affinity component (KD 3nM, Bmax 250 fmol/18mg tissue) may represent binding to a μ site.

Meptazinol displayed only a low-affinity interaction with these sites, being inactive against the κ & δ sites, and having KI's of 1.8 $\pm$0.2 x 10^{-6}M (5) against μ_2 binding and 1.9$\pm$0.4 x 10^{-7}M (5) against μ_1 binding. Even the interaction with the μ_1 sites was of low affinity compared to other opiate analgesics. However, antinociceptive doses of meptazinol result in brain concentrations of 10-20μM which could result in substantial interactions with both μ sites.

Using the "sodium ratio" as an index of efficacy, a ratio of 1.4 for meptazinol suggests a predominantly antagonist action at the μ receptor. This is substantiated by studies in morphine - sensitive isolated tissues (Bill et al, 1981).

These studies provide further evidence for the multiplicity of opiate receptors/ binding sites in rat CNS, and demonstrate that meptazinol displays only a low affinity interaction with these sites.

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EFFECT OF ACUTE AND CHRONIC BARBITONE TREATMENT AND WITHDRAWAL ON MOUSE BRAIN MONOAMINE METABOLISM

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

Acute barbiturate administration has been reported to decrease catecholamine turnover in whole rat brain (Corrodi et al, 1966), but more recent studies of catecholamine turnover in discrete brain areas following chronic barbiturate treatment and withdrawal have given rise to some discrepancies (Morgan et al, 1978; Nabeshima et al, 1981): although both groups reported increased noradrenaline turnover in some brain areas during barbiturate withdrawal, a rise in dopamine turnover in withdrawal remains controversial, as does a decrease in dopamine turnover after chronic barbiturate treatment. Chronic barbiturate administration has also been reported to decrease 5-HT synthesis in tolerant mice (Nabeshima & Ho, 1981). The present study was designed to examine possible concurrent changes in dopamine and 5-HT turnover in whole mouse brain following acute and chronic barbiturate treatment and withdrawal using the steady state levels of metabolite versus neurotransmitter to determine relative turnover.

Male LACG mice were treated acutely with barbital (250mg/kg i.p.) 90 minutes prior to sacrifice, or chronically by administration of barbital in powdered food in a schedule of increasing dosage known to induce tolerance and physical dependence, for a total of 28 days. One group of ten mice was given barbital-containing food until the time of death; another group was withdrawn for 48 hours prior to sacrifice. Naive mice were either given acute saline or received no treatment. Mice were killed by cervical fracture and whole brains (excluding brain stem and olfactory lobes) were used for assay of levels of catecholamines, 5-HT and metabolites. Analysis was carried out by HPLC with electrochemical detection according to the method of Mefford (1980) with minor modifications.

Noradrenaline was significantly raised during barbital feeding and withdrawal, and dopamine was significantly decreased in withdrawal. There was no significant difference in 5-HT or dihydroxyphenylacetic acid (DOPAC) after barbital treatment. 5-Hydroxyindoleacetic acid (5-HIAA) was significantly raised after acute barbital and lowered in withdrawal. Homovanillic acid (HVA) was significantly raised by acute barbital and acute saline administration. 5-HT turnover was decreased in barbiturate withdrawal ($p < 0.005$, Student's t-test), whereas dopamine turnover was increased in withdrawal when calculated as HVA/Dopamine or DOPAC/Dopamine ($p < 0.05$). Turnover rates were unchanged during barbital feeding, but HVA/Dopamine ratio was increased after acute barbital ($p < 0.01$).

Since neurotransmitter turnover is thought to provide an index of neuronal activity, the present results suggest decreased serotonergic function in barbital withdrawal and concomitant increase in dopaminergic function. Further studies are in progress to investigate these perturbations in barbiturate withdrawal.

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ACTION OF DOPAMINE AND PROSTAGLANDIN E_1 ON STRIATAL CYCLIC AMP LEVELS IN MORPHINE INJECTED ANIMALS

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

Previous experiments on the action of opiate agonists on intracellular adenosine cyclic 3',5' monophosphate (cyclic AMP) levels have yielded contradictory results. Whereas Puri et al (1975) reported a rise in striatal cyclic AMP in the presence of opiates, Collier and Roy (1974) reported a fall and Bonnet (1975) found no change. The concept that opiates may modulate dopamine and prostaglandin E_1 stimulated adenylate cyclase has also been investigated, with similar conflicting results. In the present communication we describe the action of morphine, dopamine and prostaglandin E_1 on cyclic AMP levels in striatal slices prepared from adult male rat brain; furthermore, we have examined the effect of these substances on striatal slices prepared from rats injected with morphine (acute, chronic, naloxone withdrawn after chronic treatment) to determine whether such treatment modifies their action.

The treated animals were injected with morphine, acutely (20 mg morphine hydrochloride/kg, thirty minutes before cervical dislocation) and chronically (according to the method of Collier et al (1972)). Dependence was tested independently using behavioural changes (10 mg naloxone/kg injected i.p.) and tolerance (10 mg/kg morphine hydrochloride, i.p.) was tested by the tail flick method for analgesic effect. In the chronically treated animals withdrawal was initiated with naloxone (10 mg/ml/kg) twenty minutes before cervical dislocation. Striatal slices were prepared by the modified method of Quik et al (1979) and striatal homogenates according to the method of Kerwin et al (1980). Activation of adenylate cyclase and measurement of cyclic AMP levels were determined by the method of Brown et al (1971).

We observed no significant difference in both the basal activation of adenylate cyclase in striatal membranes and basal levels of cyclic AMP in striatal slices between normal and morphine injected rats (acute, chronic, and naloxone withdrawn groups). Morphine (10^{-4} to 10^{-6}M) when applied to rat striatal slices, produced no significant change in basal cyclic AMP. As anticipated, dopamine (10^{-3}M to 10^{-6}M) produced a significant increase in cyclic AMP levels in striatal slices of untreated animals. However, in the morphine injected rats (acute, chronic and naloxone withdrawn chronic groups), dopamine (10^{-4} to 10^{-5}M) appeared to be less effective in raising cyclic AMP. In contrast, prostaglandin E_1 (10^{-5} - 10^{-6}M) caused a significant increase in cyclic AMP levels in striatal slices prepared from normal and morphine injected animals (acute, chronic and naloxone withdrawn chronic groups). The effect of prostaglandin E_1 on striatal cyclic AMP was not modified in experiments in which the striatal slices were preincubated with morphine (10^{-4} to 10^{-6}M) for thirty minutes.

Morphine did not activate striatal adenylate cyclase or alter cyclic AMP levels. In morphine treated animals, the ability of dopamine to increase striatal cyclic AMP seemed to be reduced whereas the significant stimulation produced by prostaglandin E_1 was unaffected.

Sarah Pay is an SERC student.

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MODIFICATION OF 24 HOUR VARIATIONS IN BRAIN TRYPTOPHAN AND 5HT CONCENTRATIONS BY IMIPRAMINE

K.F. Martin & P.H. Redfern; Pharmacology Group, School of Pharmacy & Pharmacology, University of Bath, Bath, Avon, BA2 7AY.

In several studies antidepressant drugs have been shown to modify circadian rhythms in rodents (e.g. Wirz-Justice & Wehr, 1979) and in man (Wehr & Goodwin, 1979). We have previously reported the effects of clomipramine and zimelidine on 24 hour variations of tryptophan (TRY) and 5-hydroxytryptamine (5HT) concentrations in rat brain; chronopharmacological effects of Imipramine; a tricyclic antidepressant with a less selective effect on the 5HT uptake mechanism, are now reported.

Imipramine HCl., 50,100 or 200 mg/l was administered to male Wistar rats (University of Bath strain) for 2 or 14 days via the drinking water. Experimental design and assay methods were as previously described (Martin & Redfern 1982a).

The effect of imipramine on the 24 hour variation in TRY and 5HT concentration is shown in Table I. In brief, it can be seen that apart from short exposure to the lowest dose, which significantly raised 5HT concentrations, the overall effect was to lower TRY and 5HT levels, particularly after 14 days. Consistent with the results previously obtained with clomipramine and zimelidine (Martin & Redfern, 1982 a & b), TRY concentrations were affected much more than 5HT levels, and the influence of imipramine on TRY and 5HT levels was significantly greater in the middle of the light period and beginning of the dark period when the normal rhythm of 5HT is at its peak.

Thus, the responses to all three antidepressants, irrespective of differences in specificity or mode of action, showed a marked tendency towards attenuation of diurnal rhythms, especially in TRY, observed in untreated animals. These results provide further evidence for an effect of antidepressants on the central circadian generator as reported by Lighton et al (1982), although, at this stage, we cannot rule out the possibility that the apparent attenuation in amplitude of the rhythms is in fact caused by a shift in acrophase, as suggested by the work of Wirz-Justice et al (1980).

TABLE 1

TIME	0100		0700		1300		1900	
TREATMENT	TRY	5HT	TRY	5HT	TRY	5HT	TRY	5HT
CONTROL	2.01±0.19	0.50±0.06	2.25±0.12	0.74±0.08	2.39±0.09	0.74±0.06	3.12±0.31	0.61±0.05
IMIPRAMINE HCl 50 mg/L 2/70	0.99±0.11	1.1 ±0.13	1.46±0.24	1.19±0.08	1.33±0.13	0.88±0.07	1.32±0.08	1.06±0.11
- " - 50 mg/L 14/7	1.34±0.09	0.36±0.03	1.33±0.19	0.48±0.09	1.46±0.12	0.55±0.06	1.27±0.07	0.59±0.04
- " - 100 mg/L 2/7	1.50±0.15	0.55±0.06	1.61±0.10	0.73±0.02	1.20±0.09	0.53±0.05	1.18±0.10	0.45±0.03
- " - 100 mg/L 14/7	1.56±0.22	0.31±0.03	1.78±0.08	0.49±0.02	1.72±0.10	0.48±0.02	1.44±0.18	0.45±0.03
- " - 200 mg/L 2/7	1.62±0.17	0.34±0.02	1.52±0.11	0.48±0.06	1.29±0.10	0.45±0.02	1.81±0.23	0.53±0.02
- " - 200 mg/L 14/7	1.90±0.35	0.43±0.03	1.62±0.13	0.69±0.06	1.22±0.07	0.54±0.03	1.32±0.07	0.56±0.02

Concentrations are expressed(means±s.e.m.)as µg/g wet wt. (n=4 5 or 6)

K.F.M. is a S.E.R.C. scholar.

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BRAIN CATECHOLAMINE RECEPTOR BINDING AND CHOLINE TRANSPORT IN SHORT TERM OXYPERTINE TREATMENTS IN RATS

T. Fakouhi, A. Morgan‡ A.B. Norman & A.K. Prince, Department of Pharmacology, King's College London, Strand, London WC2R 2LS and Sterling Winthrop Research and Development‡ St. Mark's Hill, Surbiton, Surrey, KT6 4PH.

The amine-depleting drug oxypertine has been reported useful in the treatment of the negative symptoms of schizophrenia (Van Praag & Korf, 1975) and the tardive dyskinesias caused by other neuroleptics (Freeman et al, 1980). Single doses (5-35 mg/kg s.c.) comparable with those used clinically caused greater depletions of noradrenaline than of dopamine (DA) in rat whole brain, effects of 4 to 16 h duration (Hassler et al, 1970). Oxypertine was also a more potent antagonist of binding to α-receptors than to DA-receptors in rat brain (Nakahara et al, 1980). We investigated the effects of 3-4 week treatments of rats with oxypertine.

Male Wistar rats (groups of 5 or 6, 100-150 g) were given oxypertine citrate in drinking water (ad. lib.) or by daily s.c. injection. Brain tissues were homogenised (5000 r.p.m., 5% w/v, 0°C), washed (3 x 30,000 g x 10 min), incubated (equivalent 10 mg original tissue, 2.5 ml, 37°, 10 min) with ^{3}H-spiroperidol (4 nM) or ^{3}H-WB-4101 (2 nM) in Tris-Krebs (Burgess et al, 1978) containing 0.1% w/v ascorbate and 10 μM-pargyline, then filtered (GF/C discs). Specific and non-specific binding were defined using (+)butaclamol (2 μM) or phentolamine (10 μM). Na-dependent transport of ^{3}H-choline was measured in fine slices of corpus striatum (Burgess et al, 1978) 1 and 3 days, binding 1 day, after ending treatment.

Specific bindings in the striatum and hippocampus were increased comparably (35 & 33%, $p < 0.002$) by s.c. oxypertine (35 mg/kg, Table 1). Via drinking water, 35 & 70 mg/kg also increased striatal binding (24 & 28%, $p < 0.05$). However increases in striatal choline transport (4-24%) after chronic oxypertine treatments (35 mg/kg, s.c.) and 1-21 h after a single dose (35 or 70 mg/kg) were not statistically significant ($p > 0.05$).

Table 1

Oxypertine-treatment daily:	Binding† (pmol/g tissue) of: 3H-spiroperidol in corpus striatum specific	non-specific‡	3H-WB4101 in hippocampus specific	non-specific‡
35 mg/kg s.c. injection:	22.0, 0.66	14.9, 0.89	6.24, 0.26	4.86, 0.32
	29.8, 0.45	14.8, 1.15	8.26, 0.40	5.20, 0.53
70 mg/kg intake via drinking water:	21.6, 0.82	12.4, 1.79		
	27.5, 1.98	11.0, 2.05		

† Mean values and standard errors presented for typical chronic treatments. Upper figures: values for vehicle-treated controls, lower figures: values for oxypertine treated, significance levels quoted in text; ‡ includes binding to filters which represented approx. 20% "non-specific" binding quoted.

It seems unlikely therefore that the selectivity of oxypertine is sufficient, at least at the larger clinical doses, to avoid increased striatal DA-receptor binding capacity in chronic treatments. However movement disorders may not result where DA is depleted. Thus striatal choline transport was unaffected after chronic treatments with oxypertine. However similar results in acute treatments were compatible with only small increases (28%) in striatal transport in acute reserpine-treatments (Burgess et al, 1978) and with transport regulation different from that in other areas of brain (Sherman et al, 1978).

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EVIDENCE FOR THE PRESENCE OF BOTH α_1 AND α_2-ADRENOCEPTORS IN RAT CORTICAL SLICES

Christine Ennis, Biochemical Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berks.

The existence of α_2 - adrenoceptors on noradrenergic nerve terminals in rat cortex has previously been reported (Taube, Starke and Borowski, 1977), Gothert, Huth and Schlicker (1981) have recently suggested that the α-adrenoceptors located on serotonin nerve terminals in the rat occipital cortex appear to resemble α_2-adrenoceptors. In the present study the α-adrenoceptors located on serotonergic nerve terminals in the frontal cortex of the rat have been compared with the α_2-adrenoceptors on noradrenergic nerve terminals.

The release of tritium from slices of rat frontal cortex preloaded with {^{3}H}-serotonin (5-HT) or from cortical slices preloaded with {^{3}H}-noradrenaline (NA) was measured using a superfusion system (Ennis and Cox, 1982).

Clonidine produced a dose-related inhibition of K^+-evoked tritium release from cortical slices preloaded with {^{3}H}-NA with a pD_2 value of 7.12 ± 0.05. Similarly BHT933 (azepexole) also inhibited K^+-evoked release with a pD_2 value of 6.28 ± 0.08. In contrast, phenylephrine and methoxamine had no effect in concentrations up to 10^{-5}M. Both methoxamine and phenylephrine however were potent inhibitors of K^+-evoked tritium release from slices of frontal cortex preloaded with {^{3}H} -5HT. The pD_2 values were 8.37 ± 0.07 and 8.12 ± 0.20 respectively. Clonidine and BHT933 only inhibited 5-HT release at very high concentrations (pD_2=5.22±0.40 and 4.21±0.30). Thus the rank order of potency for these agonists as inhibitors of K^+-evoked NA release was clonidine > BHT933 >> phenylephrine = methoxamine and that for the inhibition of K^+-evoked 5-HT release was methoxamine > phenylephrine >> clonidine > BHT933.

The potency of some α-adrenoceptor antagonists to reverse the inhibition of K^+-evoked 5HT release produced by methoxamine and the inhibition of K^+-evoked NA release produced by clonidine was compared using an Arunlakshana-Schild analysis. The pA_2 values are shown in Table 1. Thus the relative order of potency of the antagonists against clonidine was phentolamine > rauwolscine = yohimbine > WB4101 whilst against methoxamine the order was WB4101 > phentolamine > yohimbine > rauwolscine. These results suggest that, in contrast to the findings of Gothert et al (1981), the α-adrenoceptor modulating the release of 5-HT in the frontal cortex appears to be an α_1-adrenoceptor.

Table 1 pA_2 values for the antagonism of clonidine - inhibition of NA release. and methoxamine inhibition of 5-HT release.

	pA_2 ± S.E.M.	
	Clonidine	Methoxamine
WB4101	< 7.0	8.29 ± 0.09
Phentolamine	8.50 ± 0.60	7.96 ± 0.16
Yohimbine	7.70 ± 0.02	< 7.0
Rauwolscine	7.61 ± 0.30	6.07 ± 0.56

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SUBSENSITIVITY OF THE NORADRENALINE RECEPTOR-COUPLED ADENYLATE CYCLASE SYSTEM IN RAT LIMBIC FOREBRAIN INDUCED BY DOTHIEPIN

W.R. Buckett & G.L. Diggory, Research Department, The Boots Company PLC., Nottingham, NG2 3AA

Recent studies have demonstrated a decrease in the number of cortical β-adrenoceptor recognition sites (B_{max}) without alteration in receptor affinity (K_D) following chronic treatment of rats with some antidepressant drugs (Banerjee et al, 1977). However all known clinically effective drugs and ECT, administered over a clinically relevant time period, decrease the sensitivity of the noradrenaline (NA)-receptor coupled adenylate cyclase system in rat brain (Sulser, 1978). The present work was undertaken to determine whether dothiepin (Prothiaden, Dosulepin) also caused subsensitivity of the system as this antidepressant has previously been shown to reduce cortical β-adrenoceptor binding in rats after subchronic oral administration (Buckett & Thomas, 1982).

Male Sprague-Dawley (Charles River) rats (180 to 300 g; groups of 8) were treated with dothiepin HCl (100 mg/kg orally) once daily for 1, 3, 7 or 14 days and once or twice daily for 21 days. Desipramine (20 mg/kg orally) was given once daily for 21 days and control animals received vehicle alone. 24 h after the last dose, limbic forebrains were rapidly dissected out on ice and cyclic-AMP assayed by the method of Blumberg et al (1976) with modifications. Slices (0.3 mm^3) were incubated in buffer (37°C; 95% O_2: 5% CO_2) with or without 100 μM NA. Decanted slices were then immersed in liquid nitrogen, homogenised in 0.3 N perchloric acid, centrifuged (3000 g: 30 min; 0°C) and supernatants applied to H^+ Dowex columns (10 x 0.8 cm) to isolate c-AMP, which was then determined by a protein binding method.

Treatment with Dothiepin (100 mg/kg/day) for 14 days led to an attenuated increase of 8.7 ± 2.2 (s.e.m.) pmoles c-AMP/mg protein in comparison with the control increase of 14.2 ± 3.9 pmoles/mg. After treatment with dothiepin once daily for 21 days the increase was 9.7 ± 2.5 pmoles/mg whereas the control group value was 15.1 ± 3.0 pmoles/mg. A reduction of greater significance was found after dothiepin had been given twice daily at 100 mg/kg orally for 21 days, when the change in c-AMP production was only 4.8 ± 3.8 pmoles/mg compared with control increase of 14.1 ± 4.4 pmoles/mg. In this experiment desipramine (20 mg/kg/day orally) for 21 days led to an attenuated increase of 6.7 ± 3.0 pmoles c-AMP/mg protein. In contrast, dothiepin treatments of 1, 3 or 7 days duration were insufficient to effect a reduced increase in c-AMP in response to NA. The results indicate that subsensitivity of the NA receptor-coupled adenylate cyclase system has occurred after subchronic oral dothiepin or desipramine treatment for at least 14 days and support the hypothesis that this is a common property of clinically useful antidepressant agents. On the other hand, more rapid adaptive changes have been observed with β-adrenoceptor binding using ^{3}H-dihydroalprenolol, when significant subsensitivity was found after 3, 7 and 14 days of dothiepin treatment (Buckett & Thomas, 1982). This may be a general phenomenon of recognition site adaptation taking place more rapidly than adaptive changes in functional responses or it may be related to the different brain regions used in each study.

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REVERSAL OF RESERPINE-INDUCED AKINESIA IN MICE BY PRODRUGS OF L-DOPA

D.R.Cooper, P.Jenner, C.Marrel[1], C.D.Marsden & B.Testa[1], University Department of Neurology, Institute of Psychiatry and King's College Hospital Medical School, London,SE5,UK and [1] School of Pharmacy, University of Lausanne, CH-1005 Lausanne, Switzerland.

Chronic treatment of Parkinson's disease with L-DOPA results in a shortening of duration of drug action so as to produce "end of dose deterioration" (Marsden 1980). The change in response to L-DOPA therapy may reflect altered pharmacokinetic handling. Prodrug esters of L-DOPA may increase the bioavailability of L-DOPA or prolong its duration of action. We have studied the ability of L-DOPA and of a series of more lipophilic ester prodrugs to reverse akinesia induced by reserpine in mice.

Male Tuck No.1 mice (18-23 g) were reserpinised (5 mg/kg ip 18 h previously) and pretreated with carbidopa (α-methyldopahydrazine; 25 mg/kg ip 1 h previously). Animals then received either L-DOPA or an ester prodrug of L-DOPA (in doses equivalent to 200 mg/kg ip L-DOPA on an equimolar basis). Locomotor activity of batches of 3 mice was monitored over the following 4 h period using automated activity meters.

L-DOPA (200 mg/kg ip) caused a marked reversal of reserpine akinesia (activity counts: reserpine treated control animals 424±157; L-DOPA treated animals 8382± 2197; $p < 0.05$). Administration of the methyl, ethyl and tetrahydropyran-2-methyl esters reversed reserpine akinesia in an idential manner to L-DOPA (Table 1). In each case total locomotor activity, peak activity and duration of action were the same. Total locomotor activity produced by 2-methoxyisopropyl ester was not different over a 4 h period compared to that produced by L-DOPA but peak activity was only 76% of that of L-DOPA. Analysis of variance showed the time profile of activity of the 2-methoxyisopropyl ester to be prolonged compared to L-DOPA. All other prodrugs examined were less active in reversing reserpine-induced akinesia than L-DOPA (Table 1). Relative activity compared to L-DOPA ranged from 23-79% of that found for L-DOPA.

Table 1 Reversal of reserpine akinesia in mice by prodrug esters of L-DOPA

Ester	% Relative total activity of ester (L-DOPA = 100%)	% Relative peak activity of ester (L-DOPA = 100%)	Duration (h) (L-DOPA 3.5-4 h)
Tetrahydropyran-2-methyl	109	99	4
2-Methoxyisopropyl	106	76	> 4+
Ethyl	106	97	4
Methyl	95	98	4
n-Propyl	79	101	4
p-Methoxyphenylethyl	79	97	3.5-4
Phenylethyl	78	70	4
Trifluoromethylbenzyl	48*	61	3
Cyclohexyl	41*	49	3
p-Chlorophenylethyl	30*	58	2-2.5
Benzyl	23*	40	3.5-4

* $p < 0.05$ compared to L-DOPA Student's t test
\+ $p < 0.05$ compared to L-DOPA Analysis of variance

With the possible exception of the 2-methoxyisopropyl derivative,none of the esters examined provided a means of increasing the duration of action of L-DOPA.

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A COMPARISON OF DA-DEPENDENT DYSKINESIAS CAUSED IN THE GUINEA-PIG BY 14 DAYS TREATMENT WITH HALOPERIDOL OR L-DOPA PLUS BENSERAZIDE

Brenda Costall, R.J. Naylor & V. Nohria, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP.

Long-term treatment with either L-dopa or neuroleptic agents can cause dyskinesias in the clinic (Marsden et al, 1975). The development of dyskinesias to both dopamine (DA) agonist and DA- antagonist therapy are generally, somewhat paradoxically, associated with development of supersensitivity of cerebral dopaminergic mechanisms (Klawans & Margolin, 1975; Clow et al, 1980). We have previously demonstrated that a 14-day treatment with L-dopa plus benserazide (LD+B) causes dyskinesias in the guinea-pig (Costall et al, 1981). In the present study, we report the behavioural consequences of a 14-day treatment with haloperidol (H) in guinea-pigs and make comparisons with behavioural effects of a 14-day treatment with LD+B.

Female Dunkin-Hartley guinea-pigs (500-550 g) were stereotoxically implanted with guide cannulae to allow for drug injections into a striatal area sensitive to dyskinesia induction (Ant.8.0, Lat.± 2.5, Vert.8.2 mm below the skull surface; see Costall et al, 1980). Animals were divided into three groups which received vehicle, H(2.0 mg/kg i.p.) or LD(100 mg/kg i.p.) plus B(50 mg/kg i.p.) daily for 14 days. The behaviour of guinea-pigs was monitored throughout treatment and for a further 16 days after its withdrawal. Both treatment with H and LD+B caused the development of dyskinesias which were characterised by intense grimacing, munching and licking and tongue protusion with some guinea-pigs also exhibiting dyskinesias of the head and limbs. Dyskinesias in the LD+B group were most marked during the drug treatment whilst those of the H group were more apparent after drug withdrawal. Both the intra-striatal administration of DA (100 µg) and the peripheral administration of 2- (N,N-dipropyl) amino-5,6-dihydroxy-tetralin (0.00625 mg/kg s.c.) exacerbated the dyskinesias during and after both treatment regimes (these doses of DA-agonists caused only a mild behavioural response in vehicle treated animals). In contrast, the ability of 2- (N,N-dipropyl) amino - 5,6 - dihydroxytetralin (0.00625 mg/kg s.c.) to cause sedation in guinea-pigs (a possible presynaptic effect?) decreased during LD+B treatment whilst its ability to cause locomotor hyperactivity was exaggerated following H treatment. Furthermore, tiapride was 2-4 times more effective to reduce dyskinesias in the H group than in the LD+B group.

The present studies suggest that although the behavioural consequences of long-term treatment with both a DA-antagonist (haloperidol) or DA-agonist (L-dopa + benserazide) are similar, the underlying pathophysiological mechanisms may be different.

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EFFECT OF AGING ON CHOLINERGIC RECEPTOR SUBTYPE IN RAT BRAIN

R.S. Briggs, P. Cook[1] & M. Petersen (introduced by G.N. Woodruff), Department of Geriatric Medicine, University of Southampton, and [1] Department of Medicine, Royal Free Hospital, London.

Post-mortem neurochemical studies in man have demonstrated a loss of choline acetyltransferase (ChAT) activity with "normal" aging of the cerebral cortex, accentuated in senile dementia of Alzheimer type (SDAT). The reduction in this presynaptic cholinergic marker correlates with the degree of morphological and intellectual change. Although cortical muscarinic receptors decline in number with age in man, there is no additional loss in SDAT (Perry et al, 1978), so that the use of cholinergic agonist drugs or anticholinesterases has been suggested as a potential therapy for senile dementia. Muscarinic receptors are heterogeneous: high- and low-affinity agonist binding sites can be differentiated with varying proportions being found in different brain regions and, in the rat, developing at different rates in ontogeny. The present study, which confirms the decline in presynaptic (ChAT activity) and postsynaptic (muscarinic binding) cholinergic markers with age in rat brain, was undertaken to define the effects of such age-related cholinergic deficit on muscarinic receptor subtype.

Male Wistar Porton rats, reared under identical conditions, were killed at 6 months (young) or 24 months (old). ChAT activity was determined in whole brain homogenates by the method of Fonnum (1975). Receptor binding assays were performed with the muscarinic ligand [^{3}H]L-(-)-quinuclidinyl benzilate, specific binding being that displaced by 1 μM atropine and representing > 90% of total binding. Total receptor number (Bmax) and affinity (K_D) were determined by Scatchard analysis of saturation data. High and low affinity binding sites were differentiated by agonist displacement using carbachol and an iterative curve-fitting procedure (Briggs et al, 1981). There was a decline in mean (± SEM) Bmax from 949 ± 31 to 800 ± 25 fmol.mg protein^{-1} ($p < 0.002$) and also in ChAT activity from 7.0 ± 0.24 to 6.2 ± 0.17 nmol.mg protein^{-1} hour^{-1} ($p < 0.02$) between young and old animals. However, there was no change in either Ki or proportion of high and low affinity sub-types.

These findings suggest quantitative loss of cerebral cholinergic synapses with age, but are consistent with the hypothesis that remaining synapses are qualitatively unchanged.

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SALMON CALCITONIN AND CENTRAL ACETYLCHOLINESTERASE ACTIVITY

R.F.L. Bates, G.A. Buckley, R.M. Eglen, C.A. McArdle and R.J. Strettle*, Department of Life Sciences, Trent Polytechnic, Nottingham and *Division of Biology, Preston Polytechnic, Lancashire.

Nakhla and Majumdar (1978) reported that intramuscular administration of porcine calcitonin decreases plasma tryptophan and increases central 5-hydroxytryptamine (5-HT) in rats; this rise is thought to mediate an increase in central acetylcholinesterase (AchE) activity. Either of these changes might be associated with the anti-nociceptive action of calcitonin (Pecile et al, 1975; Bates et al, 1981).

To test this hypothesis, groups of 10 CFLP mice (♂&♀,30g) or Wistar rats (♂&♀, 250-300g) were given drugs or appropriate vehicle. One hour later (15 min in the case of eserine) the brains were removed. Cortex, cerebellum and the remaining tissue were homogenised separately in 10ml, 0.1M phosphate buffer at the appropriate pH. The protein concentration of the homogenates was determined according to the method of Lowry et al (1951) and AchE activity was determined according to Ellman et al (1961) at pH 7.4°C and 21°C for mouse brain or pH 8 and 37°C for rat brain. The results are shown in Table 1.

Table 1 AchE activity of brain homogenates

Treatment	Cerebellum	Cortex	Remainder
Tryptophan 300mg.kg^{-1} s.c.	1.32 ± 0.05	6.50 ± 0.50	5.18 ± 0.46
(Control Mice)	1.36 ± 0.23	6.07 ± 0.22	5.84 ± 0.29
5-HT 0.67mg.kg^{-1} i.c.v.	1.57 ± 0.09	6.11 ± 0.23	4.91 ± 0.19
(Control Mice)	1.75 ± 0.09	6.54 ± 0.21	5.37 ± 0.19
p-chlorophenylalanine 300mg.kg^{-1} p.o.	1.24 ± 0.07	5.17 ± 0.17	4.53 ± 0.14
(Control Mice)	1.39 ± 0.10	5.20 ± 0.29	4.45 ± 0.27
Salmon calcitonin 20 IU.kg^{-1} s.c.	1.02 ± 0.12	4.15 ± 0.21	3.90 ± 0.05
(Control Mice)	1.05 ± 0.08	4.37 ± 0.27	3.91 ± 0.18
Salmon calcitonin 50 IU.kg^{-1} i.c.v.	1.45 ± 0.14	6.44 ± 0.30	5.48 ± 0.18
(Control Mice)	1.76 ± 0.19	6.45 ± 0.32	5.18 ± 0.20
Salmon calcitonin 20 IU.kg^{-1} s.c.	4.41 ± 0.21	7.53 ± 0.34	7.07 ± 0.32
(Control Rats)	4.33 ± 0.27	7.18 ± 0.37	7.23 ± 0.43
Eserine 2mg.kg^{-1} s.c.	1.77 ± 0.20	3.30 ± 0.25**	3.16 ± 0.24*
(Control Mice)	2.06 ± 0.24	5.65 ± 0.25	4.48 ± 0.41

Results expressed in μ.mol.hr^{-1} (mg protein)$^{-1}$, $\bar{x}$ ± s.e., n = 7-10, *P<0.02, **P<0.001.

No significant change in AchE activity was observed after any of these treatments except for eserine, which was used as a control for the assay system.

In conclusion, we are unable to provide evidence either that agents which modify 5-HT metabolism influence AchE activity in the brain or that the central anti-nociceptive action of salmon calcitonin is likely to be associated with changes in AchE activity.

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A POSSIBLE CAUSE FOR DIFFERENCES IN HEART RATE EFFECTS OF HYDRALAZINE AND SODIUM NITROPRUSSIDE IN DOGS

D. B. Yates, Research Department, The Boots Company PLC, Nottingham, NG2 3AA

Although hydralazine-induced tachycardia has long been considered to be due to reflexes induced by hypotension, more recent evidence suggests that the tachycardia bears little relation to the hypotension. Spokas and Wang (1980) have speculated on the involvement of the Bainbridge reflex and aspects of this have been investigated.

Intra-venous administration of hydralazine to pentobarbitone (30 mg kg^{-1} i.v.) anaesthetised dogs produced a hypotension (-3.5% ± 1.1 in mean blood pressure 1 min after end of infusion; -3.4% ± 1.4 5 min after; -9.6% ± 2.9 15 min after.) and an increased cardiac output (+21.5% ± 6.9 at 1 min; +51.7% ± 7.5 5 min after; 57.8% ± 13.2 15 min after) and heart rate (+6.5% ± 4.6 at 1 min; 24.7% ± 5.3 at 5 min; 42.1% ± 6.5 at 15 min.) Hind limb volume, making due allowance for changes in arterial flow, decreased by 31% ± 10.8 1 min after and 17.2% ± 13.6 5 min after. These data contrast with sodium nitroprusside 4.0 μg kg^{-1} min^{-1} infusions where there was a much smaller increase in heart rate (16.4% ± 5.1) for a greater fall in mean pressure (-14.0% ± 1.9); limb volume increased (+61.5% ± 11.5).

Intra-arterial infusion of hydralazine to the dog hind-limb also results in a decrease in limb volume. Sodium nitroprusside in the same preparation gave dose-related increases in limb volume ranging from 60% at 1.0 μg kg^{-1} to 145% at 8.0 μg kg^{-1}.

In conscious, renal hypertensive dogs (n = 5), hydralazine 0.3 mg kg^{-1} i.v. produced a mean increase in heart rate of 73 beats min^{-1} for decreases in systolic and diastolic pressure of 28.5 and 15.5 mm Hg. Sodium nitroprusside 10.0 μg kg^{-1} min^{-1} i.v. gave a 44 beats min^{-1} rise in heart rate for a systolic decrease of 39.1 mm Hg and a diastolic decrease of 17.3 mm Hg. Thus in conscious dogs there was a tendency for nitroprusside to produce less tachycardia for a given fall in pressure than hydralazine. The data suggests that hydralazine may cause a venoconstriction in the anaesthetised dog hind-limb; if this is generalised much of the tachycardia due to hydralazine may be caused by the Bainbridge reflex. Dilatation of peripheral veins by sodium nitroprusside would not trigger this reflex since venous return would tend to reduce. The use of a mixed arterio-venous vasodilator might prove more acceptable than purely arterial dilators in terms of heart rate effects.

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SELECTIVE INHIBITION OF THE BEZOLD-JARISCH EFFECT OF 5-HT IN THE RAT BY ANTAGONISTS AT NEURONAL 5-HT RECEPTORS

J.R. Fozard & Martine Host, Centre de Recherche Merrell International, 16, rue d'Ankara 67084 Strasbourg-Cedex, France.

Although numerous in vitro studies have shown 5-hydroxytryptamine (5-HT) to excite a variety of neurones in the mammalian peripheral nervous system through activation of neuronal 5-HT receptors (Wallis, 1981), few interactions of this type have been carried out in vivo. We considered that a potentially useful response in this respect might be the Bezold-Jarisch effect (BJE) which is the reflex cardiac slowing seen in a number of species following activation by 5-HT of sensory neurones in the region of the heart and lungs (Paintal, 1973). In this report we describe the effects of several compounds with selective blocking activity at the 5-HT receptors present on the terminal sympathetic fibres of the rabbit heart (Fozard et al. 1979) on the BJE induced by 5-HT in the rat.

Male Sprague-Dawley rats (Charles River, France) weighing 260-340 g were anaesthetized with urethane, 1.25 g/kg i.p. Blood pressure and heart rate were recorded by standard techniques and injections were made into a jugular vein.

Rapid, bolus injections of 5-HT (0.25-8 μg/kg) elicited abrupt, dose-related falls in cardiac rate of short duration (< 10s). Blood pressure initially fell, associated with the fall in cardiac rate, then rose transiently before showing a relatively sustained (2-5 min) fall with no associated change in heart rate. The rapid cardiac slowing was abolished by atropine, 0.1 mg/kg, i.v., or by bilateral section of the vago-sympathetic trunk. The compounds to be tested were injected i.v. 5 min before challenge with 5-HT and the dose was derived which reduced a submaximal response to 5-HT (usually 2 μg/kg) by 50%.

Table 1. Inhibition of the BJE o 5-HT by neuronal 5-HT receptor antagonists.

Compound	ED50 (μmol/kg)	n	Rabbit heart* pA_2 vs 5-HT	n
metoclopramide methiodide	0.57 ± 0.08	4	7.38 ± 0.04	3
metoclopramide	1.12 ± 0.08	8	7.28 ± 0.09	3
Benzoylpseudotropine	0.76 ± 0.18	4	6.96 ± 0.14	4
(+)-cocaine	1.04 ± 0.12	3	6.90 ± 0.07	3
(-)-cocaine	3.26 ± 0.56	7	6.24 ± 0.08	4
procaine	6.41 ± 1.36	4	5.58 ± 0.07	3

Mean values with standard errors are presented. * Taken in part from Fozard & Mobarok Ali (1978) and Fozard et al (1979).

At the doses specified, none of the compounds reduced the response to submaximal electrical stimulation of the vagus nerves or inhibited the direct effects of 5-HT on blood pressure.

The results are consistent with selective blockade by these agents of the receptors for 5-HT present on the sensory neurones subserving the afferent limb of the BJE. Moreover, the generally good agreement between the potencies of these compounds as antagonists of 5-HT in the rabbit heart and the BJE in vivo in the rat encourages speculation that the receptors mediating responses at the two sites are similar.

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TRYPTAMINE-INDUCED VASOPRESSOR RESPONSES IN PITHED RATS ARE NOT PREDOMINANTLY MEDIATED VIA 5-HYDROXYTRYPTAMINE RECEPTORS

W. Feniuk, P.P.A. Humphrey & M.J. Perren, Department of Pharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 ODJ.

Previous studies have suggested that central 5-hydroxytryptamine (5HT) and tryptamine (TRY) receptors are different (Cox et al., 1981; Jones, 1982). The present study was undertaken to determine whether 5HT and TRY-induced vasopressor responses in pithed rats are mediated via common or different receptor types.

Femoral arterial blood pressure was recorded from artificially respired, pithed male Wistar rats (weighing 200-450g) which had been pretreated with atropine (0.5 mg/kg i.p.). Those rats subsequently receiving TRY were additionally treated with ciorgyline (0.1 mg/kg i.v.) 15 min beforehand. All drugs were administered into a femoral vein. Dose-response curves for increases in diastolic blood pressure to 5HT, TRY or phenylephrine (PE) were obtained before and 15 min after cumulative doses of antagonist (methysergide, cyproheptadine and phentolamine). The antagonistic potencies of these drugs were determined by calculating the DR_{10} value according to the method of Daly et al., 1975 and the results are summarised in Table 1.

Table 1 : Antagonistic potency of methysergide, cyproheptadine and phentolamine against 5HT, TRY and PE-induced increases in diastolic blood pressure in pithed rats.

	Methysergide		Cyproheptadine		Phentolamine	
	*DR_{10} (mg/kg)	+Slope	DR_{10} (mg/kg)	Slope	DR_{10} (mg/kg)	Slope
5HT	0.007 (0.005-0.010)	1.24 (0.80-1.93)	0.027 (0.016-0.44)	1.36 (1.01-1.84)	> 3.0	-
TRY	> 0.10	-	> 0.10	-	> 3.0	-
PE	> 0.10	-	> 0.10	-	0.9 (0.5-1.8)	1.1 (0.93-1.31)

All values are geometric mean (95% confidence limits) from at least 4 determinations. *DR_{10} - dose of antagonist required to cause a 10 fold rightward shift of the agonist dose-effect curve. +Slope - of regression obtained from plot of log (agonist dose ratio-1) against log (antagonist dose).

Both methysergide and cyproheptadine were potent selective and competitive antagonists of 5HT induced pressor responses. In contrast phentolamine selectively antagonised PE vasopressor responses. These results demonstrate that 5HT and PE mediate their vasopressor effects by stimulation of 5HT D-receptors and α-adrenoceptors respectively. In contrast high doses of methysergide (0.1 mg/kg) cyproheptadine 0.1 mg/kg) and phentolamine (3 mg/kg) caused only an approximate threefold rightward displacement of the TRY-vasopressor dose-effect curve. Our findings therefore indicate that TRY-induced vasopressor responses in the pithed rat involve a different mechanism and this is under further investigation.

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EFFECTS OF β-ADRENOCEPTOR ANTAGONISTS ON CENTRAL TURNOVER OF 5-HYDROXYTRYPTAMINE IN SPONTANEOUSLY HYPERTENSIVE RATS

K.G. Charlton & I.W. Rodger[1], Department of Pharmacology, Sunderland Polytechnic, Sunderland SR1 3SD and [1]Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

5-Hydroxytryptamine (5-HT) is known to play a role in the control of blood pressure (Jarrott et al, 1975). In receptor binding studies Middlemiss et al (1977) have demonstrated an interaction between β-adrenoceptor antagonists and 5-HT receptors. We were interested, therefore, to examine and compare the effects of the β-adrenoceptor antagonist Ro-03-9807 [4-〈3-(tertbutyl-amino)-2-hydroxypropoxy〉-7-hydroxy-2-benzimidazolinone] and (±) propranolol on the steady state levels and turnover of brain 5-HT in spontaneously hypertensive (SH) Okamoto rats.

Concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in whole brain from female SH rats were assayed spectrophotofluorometrically. Turnover of 5-HT was assessed by two non-isotopic methods. In the first, levels of 5-HIAA were measured 90 min after 200 $mgkg^{-1}$ probenecid i.p. (Neff et al, 1967). In the second, 5-HT levels were determined 60 min after 75 $mgkg^{-1}$ pargyline i.p. (Tozer et al, 1966). Propranolol (5 $mgkg^{-1}$ i.p.) or Ro-03-9807 (10 μgkg^{-1} i.p.) in a dose that significantly lowered diastolic blood pressure for up to 3 h was administered 15 min prior to pargyline or probenecid treatment. The results are summarised in Table 1.

Table 1 Effects of (±) propranolol and Ro-03-9807 on turnover of 5-HT. Values are the mean ± s.d. mean (n = 3-9 determinations).

Treatment	Brain 5-HIAA levels (μgg^{-1}, wet weight)	Brain 5-HT levels (μgg^{-1}, wet weight)
Saline control	0.30 ± 0.03	0.54 ± 0.07
(±) Propranolol control	0.31 ± 0.05	0.55 ± 0.04
Ro-03-9807 control	0.28 ± 0.03	0.51 ± 0.04
Saline + Probenecid	0.54 ± 0.08*	
(±) Propranolol + Probenecid	0.55 ± 0.06*	
Ro-03-9807 + Probenecid	0.57 ± 0.09*	
Saline + Pargyline		1.01 ± 0.17*
(±) Propranolol + Pargyline		1.00 ± 0.12*
Ro-03-9807 + Pargyline		1.18 ± 0.15*

* Significantly different from appropriate control treatment ($P < 0.01$)

Treatment with probenecid and pargyline significantly elevated levels of both 5-HIAA and 5-HT in all groups compared with saline alone. However neither (±) propranolol nor Ro-03-9807 significantly altered either the steady state levels of 5-HIAA or 5-HT or the increased levels occurring after treatment with pargyline or probenecid.

These data demonstrate that the hypotensive effects of propranolol and Ro-03-9807 are not accompanied by an alteration in 5-HT turnover. These results, however, do not exclude the possibility that changes in 5-HT turnover, secondary to the hypotensive effect, may occur outwith the measurement period used in these experiments.

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α-TOCOPHEROL PROTECTS AGAINST THE EFFECTS OF HYPOXIA IN GUINEA-PIG PORTAL VEIN, EVEN IN THE ABSENCE OF AN ENERGY SOURCE

M.J. Kelly, G.S. Lovett, W. Richardson and A.J. Sweetman, School of Health and Applied Sciences, Leeds Polytechnic, Leeds, LS1 3HE.

α-Tocopherol (vitamin E) has been shown to alleviate the deleterious effects of hypoxia in rabbit heart (Guarnieri et al, 1978) and in guinea-pig atria and portal vein (Kelly and Richardson, 1981). The nature of the protective action is unknown: one possibility is that α-tocopherol activates an anaerobic, energy-producing metabolic pathway. To test this hypothesis, we have studied the effect of α-tocopherol on noradrenaline-induced contractions in guinea-pig portal vein preparations, using modified Krebs-Henseleit solutions (KHS).

Spirally cut guinea-pig portal veins were suspended vertically under 1-2 g tension in tissue baths containing KHS, bubbled with 5% CO_2 in N_2, at 37°C. Tension was induced by the cummulative addition of noradrenaline^{-2}(10 nM - 160 μM), and the resulting tension changes were measured using a force displacement transducer coupled to an oscillograph. In some experiments, the KHS was modified by replacing the glucose (11.4 mM) with either sucrose or sodium succinate, at the same concentration.

Responses to noradrenaline in control experiments, performed with glucose in the KHS, gave a maximum developed tension of 789 ± 15 mg (n = 50). Preincubation with α-tocopherol (167 μM) for 30 min, followed by the cummulative addition of noradrenaline, increased the maximum tension to 1081 ± 148 mg (n = 17). The mechanical responses in the presence of α-tocopherol were significantly greater ($P < 0.005$) than the control responses. Treatment with α-tocopherol (167 μM) had no effect on the resting tension of the tissue. The glycolysis inhibitor, iodoacetic acid (100 μM) completely abolished responses to noradrenaline in both the control experiments, and when α-tocopherol was included in the KHS (n = 5, in both cases).

Replacement of the glucose in the KHS with sucrose or sodium succinate, compounds which were unlikely to generate energy-rich molecules in the absence of oxygen, markedly reduced the responsiveness of the tissue to noradrenaline. Maximum responses in the presence of sucrose were 41 ± 17 mg (n = 20), and with succinate 64 ± 23 mg (n = 20). These responses were increased in the presence of α-tocopherol (167 μM) to 172 ± 30 mg (n = 25) in the sucrose-KHS, and 180 ± 24 mg (n = 25) with succinate-KHS. Both these results were significantly greater ($P < 0.001$) than the responses obtained in the absence of α-tocopherol. The enhanced responses to noradrenaline in both sucrose-KHS and succinate-KHS, in the presence of α-tocopherol (167 μM), were completely abolished by the inclusion of iodoacetic acid (100 μM) in the KHS (n = 5, in both cases).

The findings are consistent with the hypothesis that the protective effect of α-tocopherol against hypoxia in the guinea-pig portal vein is mediated through activation of an iodoacetate-sensitive pathway.

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LANTHANUM AND VERAPAMIL: NEGATIVE INOTROPIC EFFECTS ON OUABAIN- AND ISOPRENALINE-TREATED ATRIAL PREPARATIONS

J. Halliday & J.S. Williams, Department of Pharmacology, King's College, Strand, London WC2R 2LS.

Calcium influx via voltage-dependent calcium channels, which occurs with each action potential, is recognised as an important determinant of the level of contractile force in mammalian myocardial tissues, and it seems likely that a significant part of the positive inotropic action of β-agonists can be attributed to a cAMP-mediated increase in this calcium influx (Reuter & Scholz, 1977). There is, however, still debate as to whether cardiac glycosides also act to a significant extent by increasing this voltage-dependent calcium entry. The present study, amplifying, and to an extent modifying, earlier findings by Halliday and Harding (1979), examines this question, indirectly, by utilizing two agents known to depress voltage-dependent calcium entry in cardiac muscle, namely, the trivalent cation lanthanum (Sanborn & Langer, 1970) and the organic calcium "channel blocker" verapamil (Fleckenstein et al, 1969), to inhibit contractile force in control, ouabain-, and isoprenaline-treated tissues. A difference in the extent to which the two positive inotropic effects (produced by the cardiac glycoside and β-agonist, respectively) were antagonized by the negative inotropic agents could point to different final mechanisms of producing a positive inotropic effect.

Bisected left atria from male guinea-pigs (weight range 300-600 g) were mounted in an organ bath on platinum electrodes (2 g resting tension) and electrically stimulated (500 μs, 3Hz, supramaximal voltage). Bathing solutions were Krebs (Krebs & Henseleit, 1932) at 30°C or, in the lanthanum experiments, HEPES (Mayer et al, 1972) at 37°C. Preparations were allowed to stabilize for 2h then ouabain (0.25 μM) or isoprenaline (0.01 μM) were added to the appropriate baths. When the maximum positive inotropic effect had been achieved, successive and cumulative doses of either lanthanum (5-300 μM) or verapamil (0.1-0.6 μM) were added to treated and control tissues, each dose being allowed to produce a stable effect before the next was added. These concentration ranges of lanthanum and verapamil produced 5-70% decreases in force of contraction in the tissues.

Covariance analysis of the percentage inhibition of contractile force produced by verapamil in control, ouabain-, and isoprenaline-treated tissues showed that this agent decreased contractile force to a similar degree in control and isoprenaline-treated tissues, consistent with the hypothesis that in both of these cases, calcium influx is by means of a calcium channel sensitive to "blockade" by verapamil. In ouabain-treated tissues however, contractile force was less effectively reduced by verapamil compared with controls ($p < 0.05$). This could suggest that the glycoside achieves its inotropism by some mechanism relatively insensitive to verapamil's action.

In contrast, ouabain-stimulated tissues were inhibited by lanthanum to a degree not significantly different from controls whereas isoprenaline-stimulated tissues were more sensitive to the negative inotropic action of this agent compared with controls ($p < 0.05$). These findings indicate that lanthanum antagonises β-mediated inotropism by mechanisms additional to calcium channel "blockade".

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INACTIVATION OF BARIUM INDUCED AUTOMATICITY BY MALEATE IONS

L. Patmore (introduced by R.L. Whiting), Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

Automaticity can be induced in normally quiescent ventricular tissue under certain conditions, including electrical depolarisation, lowering extracellular potassium or calcium ion concentrations and the application of barium ions (Antoni, 1975). The latter method was used in this study.

Continuously perfused guinea pig papillary muscle preparations were used as described previously (Patmore & Whiting, 1982). In the presence of 4mmol.litre^{-1} barium chloride, single pulse electrical stimulation induces automatic activity which can be sustained at a constant rate (mean = 0.7 Hz) at 30^{0}C for several hours.

Barium depolarised the membrane potential to around -60mV which is attributed to a decrease in potassium conductance. At this potential an outward K^+ current becomes apparent which resembles the I_{X1} current responsible for pacemaker activity at depolarised potentials in Purkinje fibres (Katzung & Morgenstern, 1976). The rate of activity was dependent on the extracellular barium ion concentration ($(Ba^{2+})_o$). Threshold and maximal $(Ba^{2+})_o$ were 0.5 and 10mmol. litre^{-1} respectively.

The role of Na^+ and Ca^{2+} in the action of barium is not entirely clear. Increasing extracellular Ca^{2+} ($(Ca^{2+})_o$) has been reported to suppress automaticity (Toda, 1970) which was confirmed in these experiments. Hiraoka et al (1980) found that automaticity could also be induced in fibres treated with 1.5 x 10^{-8}mol.litre^{-1} tetrodotoxin (TTX) but was abolished in Na free solutions.

In the present experiments 3 x 10^{-6}mol.litre^{-1} TTX partially inhibited automaticity in a fashion where activity was inhibited for several seconds then re-activated at a slightly lower frequency for several seconds followed by similar periods of inactivation and re-activation. TTX at 1 x 10^{-5}mol.litre^{-1} abolished automaticity. Automaticity induced by barium in this preparation was dependent on sodium channel transport but inversely dependent on $(Ca^{2+})_o$.

Maleate ions (as sodium maleate) at 10^{-6} to 10^{-4}mol.litre^{-1} caused a concentration dependent decrease in the rate of automaticity. At higher concentrations, 1 x 10^{-3}mol.litre^{-1} maleate had a similar effect to TTX and caused transient inactivation. Maleate at 5 x 10^{-3}mol.litre^{-1} abolished automaticity.

In fibres depolarised by 20mmol.litre^{-1} potassium, slow action potentials can be induced by electrical stimulation which are dependent on $(Ca^{2+})_o$ and are a measure of the slow inward current (Patmore, 1982). Maleate ions at concentrations up to 5 x 10^{-3}mol.litre^{-1} had no effect on these responses.

The inhibitory effects of maleate ions may be attributed to a blockade of sodium channels or alternatively a direct interaction with Ba^{2+} affecting the suppression of potassium conductance.

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EFFECTS OF MEPTAZINOL AND NALOXONE UPON REGIONAL BLOOD FLOW IN RATS SUBJECTED TO HAEMORRHAGIC HYPOTENSION

E. Chance and J.F. Waterfall. Department of Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire

The analgesic agent meptazinol, m-(3-ethyl-1-methyl-hexahydro-1H-azepin-3yl) phenol hydrochloride, and the opiate antagonist naloxone reverse the hypotension associated with haemorrhagic shock in rats (Chance et al, 1981). The effects of meptazinol and naloxone on the haemodynamics and regional blood flows of rats subjected to haemorrhagic hypotension have been compared using radiolabelled microspheres.

Four groups of 5 female Sprague Dawley rats (222-274g) were anaesthetised with halothane and cannulae implanted in the left femoral vein for drug administration and in the left femoral artery for the withdrawal of reference blood samples. A cannula was implanted in the aortic arch for administration of the microspheres and for monitoring of mean arterial pressure (MAP) and heart rate (HR). On completion of the operative procedures halothane was discontinued and anaesthesia maintained with urethane and chloralose. Rats were stabilised for 30 min before regional blood flow measurements were made.

In one group of rats ^{57}Co labelled microspheres were injected via the carotid cannula to determine cardiac output and regional blood flows prior to haemorrhage. Ten minutes later 20% of the blood volume was removed as previously described (Chance et al, 1981) and MAP and HR monitored for a further 20 min. Regional flows were then reassessed using Sn labelled microspheres.

In the other groups the Co labelled microspheres were injected 20 min after haemorrhage and 10 min later saline vehicle (1ml/kg i.v.) meptazinol (17.4 mg/kg im) or naloxone (10mg/kg iv) were administered. Regional blood flows were reassessed with Sn microspheres at 5 min after drug administration.

The rats were killed and the organ samples removed for weighing and counting in a gamma counter (Packard). Organ blood flows and cardiac output (CO) were calculated using the formula of Saini and Somani (1979). Results were analysed using paired t tests.

Haemorrhage evoked significant reductions in MAP, HR and CO but there was no significant change in total peripheral resistance (TPR). Blood flows to the heart, skin, skeletal muscle, kidneys,spleen and liver were significantly decreased but perfusion of the brain and small intestine was not significantly changed.

Following haemorrhage, meptazinol and naloxone increased MAP and TPR but did not alter HR or CO. Further decreases in liver flows occurred in the drug and vehicle treated groups, Meptazinol also reduced skeletal muscle flow.

These results confirm that naloxone and meptazinol reverse the hypotension associated with haemorrhagic shock. Both drugs showed similar profiles of activity with respect to haemodynamic and regional blood flow changes and neither drug increased blood pressure at the expense of reduced cerebral,cardiac or renal blood flow. Naloxone has been used clinically in the treatment of shock (Peters et al, 1981) and the present study suggests that meptazinol would also be effective whilst providing the added benefit of analgesia.

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INVESTIGATION INTO THE CARDIOREGULATORY PROPERTIES OF INDORAMIN, AN α_1-ADRENOCEPTOR ANTAGONIST

Vanessa Pierce and J.F. Waterfall, Department of Pharmacology, Wyeth Laboratories, Huntercombe Lane South, Taplow, Maidenhead, Berks.

The antihypertensive activity of indoramin, an alpha$_1$-adrenoceptor antagonist, is not accompanied by reflex tachycardia in either man or animals (Carballo et al, 1974; Baum et al, 1973). The currently accepted view is that indoramin has a direct action on cardiac muscle and that the absence of tachycardia is the result of this membrane stabilizing action. (Algate et al, 1981). Other factors such as central and baroreceptor effects cannot be discounted however, and the cardioregulatory actions of indoramin have therefore been further investigated.

Groups of 4-6 female Sprague Dawley rats (250-300g) were anaesthetised with α-chloralose (80mg.kg^{-1} i.p.) and pentobarbitone sodium (6mg.kg^{-1} i.p.) Blood pressure was recorded from the left femoral artery and heart rate derived from the pulse pressure signal using a tachograph. Drugs were administered via a cannula into the left femoral vein. Pithed rats were artificially respired (60 strokes. min^{-1}, 1 ml.100g^{-1}).

The effect of indoramin (0.8-25.6 mg.kg^{-1} i.v.) or vehicle on the heart rate of anaesthetised rats was examined after the following treatments:- anaesthetic alone, pithing, atropine (1mg.kg^{-1} i.v.) vagotomy,guanethidine (1 and 5 mg.kg^{-1} i.v.) and reserpine (5mg.kg^{-1} s.c., 24 hours before experimentation).

Data were statistically analysed using a 2-way analysis of variance from which t-ratios were derived. All comparisons were made within groups before and after treatments.

In pithed rats indoramin (0.8-6.4 mg.kg^{-1}i.v.)did not significantly decrease heart rate. In contrast in anaesthetised animals a significant sustained decrease of 70 beats.min.$^{-1}$ was noted over the same dose range. Higher doses (12.8 and 25.6 mg.kg^{-1} i.v.) evoked significant decreases in both preparations. Catecholamine depletion by reserpine or guanethidine did not affect the bradycardia evoked by indoramin in anaesthetised animals whereas vagotomised animals and those pretreated with atropine (1 mg.kg^{-1}i.v.) did not exhibit significant bradycardia until a cumulative dose of 6.4 mg.kg^{-1} indoramin was reached.

The current experiments suggest that indoramin has at least one other property, in addition to its direct action, that could be important in the control of heart rate. This action appeared to involve cholinergic pathways since it was sensitive to atropine and was modified by vagotomy but not by catecholamine depletion (reserpine and guanethidine pretreatment).

Experiments performed on anaesthetised cats have shown that indoramin (1-3mg.kg^{-1} i.v.) reduces blood pressure, heart rate and preganglionic sympathetic nerve activity (Ramage, 1982). These experiments also suggest that indoramin can act to lower heart rate by a mechanism involving the central nervous system.

The relative importance of the neurally mediated effects of indoramin compared with its direct cardiac action has not yet been established.

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EVIDENCE THAT METOCLOPRAMIDE BLOCKS α_2 ADRENOCEPTORS ON HUMAN PLATELETS IN VITRO

A.F. Esmail, Beecham Pharmaceuticals, Research Division, Biosciences Research Centre, Epsom, Surrey, KT18 5XQ.

Metoclopramide acts as a dopamine receptor antagonist in the brain (Peringer et al, 1976) and in peripheral vasculature (Kohli et al, 1978). Recently, Spedding (1980) has shown that metoclopramide at concentrations in the range 28-280 μM, blocks α_2 adrenoceptors in the rat isolated vas deferens - a preparation used extensively to characterize α adrenoceptor agonists and antagonists. However, metoclopramide does not inhibit α_2 receptor mediated responses in the pithed rat in vivo (Clapham and Hamilton, 1980). Although human platelets possess both α_1 and α_2 adrenoceptors, adrenaline induced aggregation is mediated via the α_2 receptor subtype and platelet α_1 adrenoceptors have no significant role in the response to adrenaline (Grant and Scrutton, 1980). Inhibition of adrenaline induced aggregation of human platelets can therefore be used to study actions of compounds as α_2 antagonists.

Platelet aggregation in human whole blood was studied as described elsewhere (Nunn, 1981). Briefly, 0.5 ml aliquots of citrated whole blood were incubated at 37°C for 3 min in the presence of the antagnoists or saline. Each sample was stirred with adrenaline (1 min) or ADP (30 s). Platelet aggregates were fixed with 25 μl phosphate-buffered glutaraldehyde. Fall in single platelet count was determined using an Ultra-Flo 100 whole blood platelet counter. Dose ratios were constructed from the EC50 values for adrenaline and ADP in the presence and absence of the antagonists. Metoclopramide (30-300 μM) inhibited adrenaline induced aggregation in a concentration dependent manner without affecting the response to ADP. Yohimbine, an α_2 antagonist, also selectively inhibited the response to adrenaline (Table 1). These results suggest that metoclopramide blocks α_2 adrenoceptors on human platelets. However, the plasma concentrations of metoclopramide in man after a single dose of 10-20 mg are about 1000 fold lower than those used in present experiments (Bateman et al, 1980).

Table 1 Effects of metoclopramide and yohimbine on platelet aggregation in human whole blood in vitro

Antagonist	Dose ratio for	
	Adrenaline	ADP
Metoclopramide		
30 μM	1.9 ± 0.7 (3)	
100 μM	5.6 ± 0.6 (5)	1.0 ± 0.2 (3)
300 μM	18.0 ± 6.5 (5)	1.1 ± 0.1 (3)
Yohimbine		
0.5 μM	4.8 ± 0.1 (3)	
10.0 μM		1.1 ± 0.02 (3)

Values are mean ± s.e. mean (n)

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THE β-ADRENOCEPTOR PRESENT ON HUMAN AND RAT PLATELETS IS A β_2-ADRENOCEPTOR

Roger Kerry and Michael C. Scrutton, Department of Biochemistry, King's College, London.

Previous studies have shown that isoprenaline can inhibit the response of human platelets to ADP and collagen and causes an increase in the level of platelet cyclic-3',5'-AMP. These effects are prevented by propranolol thus suggesting the presence of β-adrenoceptors (Mills & Smith, 1971; Mills & Smith, 1972). We have confirmed that isoprenaline inhibits the aggregatory response to a wide range of excitatory agonists (ADP, thrombin, U-46619, 5HT, vasopressin) and that this inhibition is blocked in the presence of 2',5'-dideoxyadenosine, an inhibitor of adenylate cyclase.

The inhibitory effect of isoprenaline on the response of human platelets to excitatory agonists is also blocked by selective β_2-adrenoceptor antagonists such as ICI-118,551 (IC_{50} = 35 ± 18 nM (3)) and butoxamine (IC_{50} = 80 ± 10 nM (3)) over a concentration range similar to that observed for (-)propranolol (IC_{50} = 23 ± 9 nM (3)). In contrast selective β_1-adrenoceptor antagonists such as atenolol, practolol and metoprolol are ineffective at concentrations as high as 100 μM. In accord with these observations the aggregatory response of human platelets to vasopressin can be inhibited by selective β_2-adrenoceptor agonists such as salbutamol (EC_{50} = 65 ± 17 nM (3)) and terbutaline (EC_{50} = 83 ± 12 nM (3)) over a concentration range similar to that over which inhibition by (-)isoprenaline is observed (EC_{50} = 70 ± 7 nM (7)), whereas selective β_1-adrenoceptor agonists such as prenalterol and tazolol are ineffective at concentrations up to 100 μM. The efficacy of the selective β_2-adrenoceptor agonists in inhibiting the aggregatory response to vasopressin is however 35-50% of that observed for isoprenaline.

Very similar results have been obtained in studies of this type performed on rat platelets except that in this species no inhibitory response can be observed on addition of the selective β_2-adrenoceptor agonists.

We conclude therefore that the platelet β-adrenoceptor is of the β_2-subtype.

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FURTHER EVIDENCE THAT RENIN RELEASE FROM RABBIT KIDNEY SLICES IS MEDIATED BY A β_1 ADRENOCEPTOR

Derkx, F.H.M.* McDonald, R. and Reid, J.L., Department of Materia Medica, University of Glasgow, Stobhill General Hospital, Glasgow, G21 3UW.

A major role for the beta adrenoceptor mechanisms in the control of renin release from the juxtaglomerular cell has been established. Although the beta receptor appears to be of the beta$_1$ sub-type (Johns, 1981), this has not been established conclusively (Keeton, 1980). Studies have often used non-selective agonists (isoprenaline) and antagonists (propranolol). Recently two new drugs have become available to study β-receptor mediated release processes. Prenalterol is a selective β_1 adrenoceptor agonist (Carlsson, 1977) while ICI 118,551 is a relatively selective β_2-adrenoceptor antagonist (Bilski, 1981). We have studied renin release in-vitro using the rabbit cortical slice preparation (Weinberger, 1975). The following drugs were used; β-adrenoceptor agonists, (isoprenaline (β_1+β_2), prenalterol (β_1), salbutamol (β_2) and antagonists (propranolol (β_1+β_2), atenolol (β_1), ICI 118,551 (β_2), Ouabain and dibutyrul-cyclic AMP were also examined. Three of four rabbit kidney slices (10-20 mg) dry weight 0.5-1.0mm thick were prepared from the outer cortex of each kidney from male New-Zealand white rabbits. The slices were incubated in a shaking water bath at 37°C in 5 ml of a medium containing NaCl 119 mmol/L, $NaHCO_3$ 25 mmol/L KCl 4.7 mmol/L, Na_2HPO_4 1.2 mmol/L, $MgCl_2$ 1.2 mmol/L, $CaCl_2$ 2.75 mmol/L, glucose 5.65 mmol/L and ascorbic acid 6 mmol/L. The pH of the medium was adjusted to 7.4 and incubated for four successive 20-minutes periods. The study drug was added during the third period. Renin release did not change during period - 1 to 4 in untreated slices.

Dibutyrylcyclic-AMP 10^{-4} mol/L gave a 109 ± 12 per cent (mean ± S.D.) increase in renin release ($p < 0.001$). This effect was not blocked by propranolol 6×10^{-5} mol/L. Isoprenaline and prenalterol gave a dose dependent increase in renin release (ED_{50} 3×10^{-7} and 2×10^{-6} mol/L respectively). Salbutamol had no significant effects on renin release. Propranolol, atenolol and ICI 118,551 in a concentration of 6×10^{-5} mol/L had no significant effect on basal renin release. Propranolol and atenolol were about 200 times more active in blocking the isoprenaline-renin release than ICI 118,551. Ouabain 10^{-5} mol/L blocked basal renin release by 66 ± 6 per cent (mean ± S.D. $p < 0.01$) and isoprenaline response (10^{-6} mol/L) during ouabain was reduced 61 ± 6 per cent (mean ± S.D. $p < 0.01$).

In the rabbit kidney slice preparation beta adrenoceptor mediated renin release results from activation of a beta$_1$-type of adrenoceptor. Inhibition of the Na-k-ATP-ase by ouabain interferes not only with basal renin release but also with the responses to isoprenaline.

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COMPARATIVE EFFECTS OF α_2-ADRENOCEPTOR ANTAGONISTS AT PERIPHERAL AND CENTRAL α_2-ADRENOCEPTORS IN THE RAT

J. C. Doxey, B. Gadie, A. C. Lane and I. F. Tulloch. Department of Pharmacology, Reckitt and Colman, Dansom Lane, Hull, HU8 7DS.

Substantial evidence now exists showing the presence of α_2-adrenoceptors in a wide variety of tissues (Starke, 1981). The question arises therefore of whether these receptors are pharmacologically identical in different tissues. This study attempts to address this question by comparing the relative potencies of α_2-adrenoceptor antagonists at identified peripheral and central α_2-adrenoceptors in the rat.

In the periphery, α_2-adrenoceptor antagonist potency was assessed *in vitro* against clonidine-induced inhibition of the electrically-stimulated vas deferens (Doxey et al., 1977). Antagonist concentrations (Ke) required to produce a clonidine dose-ratio shift of 2 were determined. In the CNS, antagonists were tested for their ability to inhibit saturable binding of the selective α_2-adrenoceptor antagonist ^{3}H-RX 781094 to cortical membranes (Howlett et al., 1982). Competition binding assays were performed in a physiological salt solution (Starke and Montel, 1973; excluding ascorbic acid and dextrose) at 25^{0}C. Data were analysed using a Hill plot and inhibitor constants (Ki) were derived from IC_{50} values. Central antagonist potency was also assessed *in vivo* against α_2-adrenoceptor agonist-induced mydriasis in pentobarbitone-anaesthetised rats (Berridge et al., 1982). Maximal pupil dilation was induced by guanoxabenz (0.3 mg/kg, i.v.) and antagonist potency is expressed as the cumulative i.v. dose (uM/kg) causing a 50% reversal of the mydriasis (AD_{50}).

Regression analysis of the data (Table 1) revealed significant positive correlations between antagonist potencies in the three tests : A vs B ($r=0.98$; $P<0.001$); A vs C ($r=0.99$; $P<0.001$); B vs C ($r=0.97$; $P<0.01$).

Table 1 Antagonist potencies at α_2-adrenoceptors

Antagonist	Vas deferens Ke(nM)	Cortex Ki(nM)	Mydriasis AD_{50}(uM/kg)
RX 781094	3.0 ± 0.3	3.1 ± 0.4	0.2 ± 0.05
Yohimbine	8.0 ± 0.7	40.0 ± 9.5	2.2 ± 0.04
Rauwolscine	16.0 ± 3.0	43.0 ± 8.2	3.2 ± 0.2
Piperoxan	20.0 ± 1.6	34.0 ± 6.5	3.1 ± 0.2
Mianserin	53.0 ± 12.0	82.0 ± 2.4	10.0 ± 0.9
RS 21361*	107.0 ± 5.0	193.0	16.2 ± 2.5

* Michel and Whiting, 1981 Values are means ± s.e.m.

These results indicate that these previously characterised α_2-adrenoceptors in the vas deferens and CNS are pharmacologically similar.

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EFFECTS OF RX 781094 AND YOHIMBINE ON THE RESPONSES TO UK 14304 AT VARIOUS α_2-ADRENOCEPTORS IN RATS

J.C.Doxey, A.G.Roach, Diane A. Strachan and Narinder Virdee, Pharmacology Department, Reckitt and Colman, Pharmaceutical Division, Hull, HU8 7DS

Recent evidence suggests that α_2-adrenoceptors are not confined to prejunctional sites but are also present postjunctionally on vascular smooth muscle. Stimulation of postjunctional receptors by selective $\alpha_2$adrenoceptor agonists increases blood pressure in pithed rats; these responses are antagonised by selective antagonists such as RX 781094 and yohimbine but resistant to prazosin (Berridge et al., 1982; Timmermans et al., 1980). The present study compares the antagonist effects of RX 781094, a new potent, selective α_2-adrenoceptor antagonist (Chapleo et al., 1981) and yohimbine on the responses to UK14304, a selective α_2-adrenoceptor agonist (Cambridge, 1981) at prejunctional α_2-adrenoceptors in the vas deferens and heart and postjunctional α_2-adrenoceptors mediating vasoconstriction in pithed rats.

Male Sprague-Dawley normotensive rats (300-350g) were pithed, vagotomised and blood pressure measured from a carotid artery. Heart rate (HR) was also monitored. Drugs were injected into a femoral vein. HR was elevated by 90-110 beats/min by continuously stimulating (0.1-0.3 Hz, 0.5 ms, 60V) the thoracic spinal cord whereas contractions of the vas deferens were elicited by stimulating (6Hz, 50us, 40V for 2s every 30s) the lumbar spinal cord. The cumulative i.v. doses of UK14304 needed to reduce the sympathetic tachycardia or contractions of the vas deferens by 50% or increase diastolic blood pressure (DBP) by 50 mmHg (ED_{50} values) were obtained in separate groups of rats (n=5-8/group). All rats were pretreated with atropine (1.0 mg/kg) and stimulated rats additionally received d-tubocurarine (1.0 mg/kg). UK14304 dose-response curves were constructed 5 min after giving the antagonists.

α_2-ADRENOCEPTOR SYSTEM	UK 14304 ED_{50} VALUES (µg/kg ; iv)			
	SALINE 1·0ml/kg	RX 781094 0·1 mg/kg	RX 781094 1·0 mg/kg	YOHIMBINE 1·0 mg/kg
DIASTOLIC BP (POST)	4·6 ± 0·9	10·9 ± 2·0 (2·4)	57·3 ± 10·7 (12·4)	49·7 ± 6·4 (10·8)
CARDIAC HR (PRE)	1·9 ± 0·4	12·9 ± 4·2 (6·9)	65·5 ± 13·3 (35·0)	29·3 ± 4·0 (15·7)
VAS DEFERENS (PRE)	2·5 ± 0·4	33·3 ± 4·3 (13·3)	234·8 ± 35·9 (93·9)	35·0 ± 9·1 (14·0)

TABLE 1. UK14304 ED_{50} values after various treatments in pithed rats. Values in parenthesis indicate agonist dose-ratio shifts.

The control ED_{50} values for UK14304 in the 2 prejunctional α_2-adrenoceptor systems were significantly different ($P<0.05$) from the DBP ED_{50} value (Table 1). The 3 α_2-adrenoceptor responses to UK14304 were similarly antagonised by yohimbine. RX 781094 and yohimbine were equipotent against the postjunctional (DBP) effects of UK14304. However, the UK14304 prejunctional responses in the rat heart and vas deferens were shifted 3 and 8 times more by RX 781094 than the DBP responses. The differential antagonism produced by RX 781094 of the three responses to UK14304 may indicate that the α_2-adrenoceptors in the rat vasculature and on sympathetic nerves innervating the heart and vas deferens are different. However, the results with RX 781094 may simply reflect differences in tissue distribution of the antagonist.

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IN VITRO AND IN VIVO α_2-ADRENOCEPTOR SELECTIVITY PROFILES OF YOHIMBINE, RAUWOLSCINE AND CORYNANTHINE

J.C.Doxey, A.G.Roach and Narinder Virdee, Pharmacology Department, Reckitt and Colman, Pharmaceutical Division, Hull, HU8 7DS.

In functional studies using the rabbit pulmonary artery and rat vas deferens both yohimbine and its diastereoisomer rauwolscine were found to be selective α_2-adrenoceptor antagonists, rauwolscine displaying the greater selectivity (Weitzell et al., 1979; Michel and Whiting, 1981). In contrast, corynanthine preferentially antagonized α_1-adrenoceptors.
The prejunctional α_2- and postjunctional α_1-adrenoceptor antagonist potencies and selectivities of these three antagonists were assessed *in vitro* by determining pA_2 values against clonidine and noradrenaline in the rat vas deferens and anococcygeus muscle, respectively (Doxey et al., 1977). Antagonism of the inhibitory effects of cocaine (90 uM) on the rat vas deferens was also used to assess prejunctional α_2-adrenoceptor antagonist potency (Doxey and Roach, 1981). *In vivo* prejunctional α_2-adrenoceptor antagonist potency was determined in the pithed rat. Stimulation-evoked contractions of the vas deferens and anococcygeus muscle were abolished with clonidine (100 µg/kg, i.v.) and guanabenz (30 µg/kg,i.v.), respectively. The cumulative i.v. doses of the antagonists causing 50% reversal (ED_{50},mg/kg) of these inhibitions were determined (Chapleo et al., 1981).

The *in vitro* profiles of the antagonists are shown in Table 1.
TABLE 1: *In vitro* profiles of the antagonists at α_2/α_1-adrenoceptors.

ANTAGONIST	α_2-ADRENOCEPTORS		α_1-ADRENOCEPTORS	α_2/α_1 RATIO
	RAT VAS DEFERENS	RAT VAS DEFERENS	RAT ANOCOCCYGEUS	
	ED_{50} vs COCAINE (nM)	pA_2 vs CLONIDINE	pA_2 vs NORADRENALINE	
YOHIMBINE	28 ± 8	8·14 ± 0·05	6·49 ± 0·06	44·7
RAUWOLSCINE	82 ± 24	7·86 ± 0·06	7·02 ± 0·11	6·9
CORYNANTHINE	1200 ± 200	5·48 ± 0·10	6·65 ± 0·08	0·07

In the pithed rat, yohimbine and rauwolscine fully reversed the effects of clonidine in the vas deferens; the respective ED_{50} values (n=5) being 0.69 ± 0.13 and 0.54 ± 0.10 mg/kg. Reversal of guanabenz on the anococcygeus muscle is a demanding test for α_2-adrenoceptor selectivity. Only yohimbine (ED_{50} = 0.11 ± 0.03 mg/kg, i.v.) fully antagonized guanabenz in this preparation. Rauwolscine produced a reversal which only exceeded 50% in 3 out of 5 rats. In these experiments full reversal was never attained since higher doses inhibited the stimulation responses. The ED_{50} for rauwolscine was 0.51±0.24 mg/kg (n=3). Corynanthine, in doses up to 4.4 mg/kg,i.v., failed to produce reversal in both test situations.
In conclusion, yohimbine and rauwolscine were selective α_2-adrenoceptor antagonists; rauwolscine appearing less selective under the present experimental conditions. The present results demonstrate that different selectivities of antagonists for α_2-adrenoceptors may occur in different tissues and experimental conditions. The selectivity of corynanthine for α_1-adrenoceptors was confirmed.

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PHARMACOLOGICAL EVIDENCE FOR HIGH AND LOW AFFINITY SITES ON PRE-JUNCTIONAL α_2-ADRENOCEPTORS

D.R. Mottram, School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF.

It has previously been reported (Mottram, 1982) that yohimbine exhibits differential antagonistic activity against clonidine and α-methyl noradrenaline induced inhibitions of field stimulated rat vas deferens, adding weight to the suggestion by Ruffolo et al (1977) that imidazolines and phenethylamines act through different sites on the α-adrenoceptor. From these results it was suggested that α-methyl noradrenaline may be acting through a low affinity binding site on the α_2-adrenoceptor. In the present study it was intended to establish whether these observations applied equally to other agonists of the imidazoline and phenethylamine types and whether these differential properties extend to α_2-adrenoceptors other than those found pre-junctionally on adrenergic terminals in the rat vas deferens.

Stripped vasa deferentia from male wistar rats were set up as previously described (Mottram, 1982). Field stimulated guinea pig ileum preparations were set up according to the method of Drew (1978). On the rat vas deferens increasing concentrations of yohimbine produced a competitive antagonism (pA_2 7.76±0.23) of the selective α_2-adrenoceptor agonist B HT920 (Van Meel et al 1981) a member of the imidazoline group of drugs, whilst yohimbine failed to competitively antagonise, the phenethylamine type of α_2-selective agonist, adrenaline (Wikberg, 1978). The α_1-selective antagonist WB 4101 (Kapur & Mottram, 1978) whilst competitively blocking (pA_2 6.44±0.14) B HT920-induced inhibition of the twitch response, in accord with previous results on the WB 4101 antagonism of clonidine on α_2-adrenoceptors in rat vas deferens (Kapur & Mottram, 1978), did not block α-methyl noradrenaline induced inhibition indicating that the phenethylamines do not exert their pre-junctional effects via α_1-adrenoceptors.

In guinea pig ileum similar results to those obtained in the rat vas deferens were recorded. Yohimbine competitively antagonised clonidine (pA_2 7.74±0.24) but, as in the rat vas deferens, concentrations in excess of 3 μM did not produce a further significant shift in the dose-response curve to α-methyl noradrenaline.

Results therefore confirm that imidazolines and phenethylamines exert their effects through different sites on the α_2-adrenoceptor and that these effects are not unique to the pre-junctional α_2-adrenoceptors of the rat vas deferens. The possibility that the two sites for interaction are discrete rather than overlapping is proposed in light of the results of a set of experiments in which the competitive antagonism of B HT920 by yohimbine (1 μM) was not affected by attempted reversal of the yohimbine blockade by the intermediate administration of a cumulative addition of α-methyl noradrenaline.

It is therefore concluded that the results of the present study provide pharmacological evidence in support of the ligand binding studies which led to the suggestion that there are high and low affinity binding sites associated with the α_2-adrenoceptor (Hoffman et al, 1980; Rouot et al, 1980; Jarrott et al, 1982).

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EFFECT OF DL-α-MONOFLUOROMETHYLDOPA ON THE KINETICS OF NORADRENALINE AND DOPAMINE β-HYDROXYLASE RELEASE FROM THE PERFUSED HEART

E.Muscholl & F.-J.Spira, Department of Pharmacology, University of Mainz, Obere Zahlbacher Strasse 67, D-6500 Mainz

Rabbit isolated hearts were prepared according to a modification of the Langendorff method described by De Deckere & Ten Hoor (1977). Tyrode's solution containing atropine 500 nM and tetraethylammonium 10 mM was perfused at 20 ml/min and 35° C. Noradrenaline (NA) was determined fluorimetrically in the venous effluents. The transmyocardial fluid dripping from the apex of the heart was collected for determination of dopamine β-hydroxylase (DBH) according to Molinoff et al (1971). As shown previously, peak overflows of NA and DBH after brief depolarizing stimuli coincided, whereas the washout of NA and DBH was complete in 6 and 25 min, respectively (Jilg & Muscholl, 1980). Electrical field stimulation with pulses of 10 Hz, 7 ms, 220 mA for 1 min was carried out 6 times at intervals of 30 min (S1 - S6).

The DBH overflow S 1 - S6 declined monoexponentially with a rate constant (α) per stimulation period of 0.53 ± 0.06 (mean ± s.e.mean, n = 10). However, the decline of NA overflow was biexponential (α = 1.36 ± 0.24; β = 0.15 ± 0.03; n = 10). This was confirmed by additional experiments with 10 stimulations (α = 1.18 ± 0.04; β = 0.17 ± 0.03; n = 4). The biphasical decline of NA from S1 - S6 was unaltered in the presence of the neuronal uptake inhibitor, cocaine 18 μM (α = 1.03 ± 0.14; β = 0.17 ± 0.02; n = 4). When the NA synthesis in the heart was blocked by pretreatment of the rabbits with the irreversible inhibitor of DOPA decarboxylase (Kollonitsch et al, 1978), DL-α-monofluoromethyldopa (MFMD) 100 mg/kg i.v. 10 min before death, both NA (α = 0.67 ± 0.03; n = 6) and DBH (α = 0.64 ± 0.03; n = 3) declined monoexponentially from S1 - S6. Hence, the biexponential decline of NA overflow is due to resynthesis of amine which increasingly compensates for the loss of previously stored NA, thus disturbing an analysis which is based on steady-state kinetics. On the other hand, the agreement of α for NA and DBH after inhibition of NA synthesis indicates a stoichiometric release of previously stored amine and soluble DBH.

In the absence of synthesis inhibition, the sum of NA overflows S1 - S6 plus the NA remaining in the heart after S6 (11.3 ± 0.9 μg, n = 10) corresponded perfectly with the sum of NA represented by the area under the curve (AUC) constructed from the overflow values (11.7 μg). This was true also for the cocaine experiments (10.9 ± 1.9 μg vs. AUC, 11.1 μg) or after MFMD (9.3 ± 1.2 μg vs. AUC, 9.2 μg). This procedure was adopted to calculate the pool size of soluble DBH in the heart and to compare it with the soluble enzyme determined from subcellular fractionation. The overflow of DBH S1 - S6 was 182 ± 20 units (n = 4) compared to the AUC, 263 units. The difference (81 units) supposed to represent unreleased soluble DBH agreed with the DBH found in the 100,000 g supernatant (60 min) of heart homogenates (78 ± 5 units, n = 4). Supernatants of unstimulated hearts contained 277 ± 20 units DBH (n = 7) which was 18 % of the total (bound plus soluble) activity of the homogenate.

Similar balance experiments carried out after MFMD treatment showed that the depletion of NA and soluble DBH caused by S1 - S6 was 70 % in either case. The present method permits to quantitatively study the dynamics of NA and DBH release from the whole heart.

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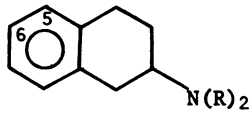
RELATIVE AGONIST POTENCIES OF A SERIES OF 2-AMINOTETRALIN DERIVATIVES AT PERIPHERAL PREJUNCTIONAL DOPAMINE RECEPTORS

R.A.Brown, Y.Crimp & S.E.O'Connor, Department of Pharmacology, Fisons plc, Pharmaceutical Division, Science & Technology Laboratories, Bakewell Road, Loughborough, Leicestershire, LE11 0QY.

Semi-rigid aminotetralin analogues of dopamine (DA) have been widely used to characterise DA receptors within the CNS, and recent reports have indicated that (contrary to earlier views) a catechol is not essential for agonist activity (Feenstra et al, 1980). We have examined a series of 5-OH, 6-OH and 5,6-diOH 2-aminotetralins at peripheral prejunctional DA receptors in the rabbit isolated ear artery preparation.

Rabbit ear arteries were set up as described previously (Brown & O'Connor, 1981). Prejunctional DA receptor agonist activity was defined as inhibition of stimulation-induced vasoconstriction sensitive to antagonism by metoclopramide (2.5×10^{-6}M), in the presence of cocaine (5×10^{-5}M) and propranolol (10^{-6}M). Agonists were administered cumulatively into the extraluminal bath fluid. Results are shown in Table I.

TABLE I



	5	6	R	Mean EC50 µM (n=4-6)	Relative equipotent molar conc. (DA=1)
I	OH	OH	n-C_3H_7	0.009	0.036
II	OH	-	n-C_3H_7	0.010	0.074
III	-	OH	n-C_3H_7	0.75	7.2
IV	OH	OH	H	0.29	1.6
V	OH	-	H	>10	>70
VI	-	OH	H	>10	>70

The exceptional potency of I was anticipated, but significantly the 5-OH analogue (II) was only marginally less potent than I and substantially more potent than DA, demonstrating that a catechol is not a prerequisite for activity at peripheral prejunctional DA receptors. The 6-OH analogue (III) was only weakly active indicating that the 5-OH (which corresponds to the meta position of DA) is the principal site of receptor interaction (as proposed by McDermed et al, 1979 for CNS DA receptors). The relative potencies of I, II and III at peripheral prejunctional DA receptors correspond closely to those described for DA receptor systems in the CNS, e.g.production of stereotyped behaviour (Feenstra et al, 1980), and this is a further illustration of the similarity between these models (Brown & O'Connor, 1981). The relatively weak activity of 5,6-ADTN (IV) and inactivity of V and VI, emphasises the significant enhancement of potency which results from N,N-di-n-propyl substitution and demonstrates that amongst primary amines a catechol moeity may still be necessary to achieve significant activity.

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