THE INTERACTION OF SOME N-SUBSTITUTED HOMOLOGUES OF NORMETAZOCINE WITH THE μ-, δ- AND κ-BINDING SITES

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Recently, it has been shown that metazocine (5,9-dimethyl-2'-hydroxy-2-methyl-6,7-benzomorphan is a fairly selective ligand for the μ-binding site. However, replacement of the N-methyl group by N-tetrahydrofurfuryl gives Mr 2034 which is more active at the κ-binding site than the μ-binding site (Magnan et al, 1982). It was of interest to study the interaction at the μ-, δ- and κ-binding sites of homologues of normetazocine with different N-substituents.

The potency of the compounds to inhibit the binding of selective tritiated ligands was measured in homogenates of guinea-pig brain at 25°C. [^{3}H]-[D-Ala^2, MePhe^4,Gly-ol^5]enkephalin (1 nM) was used as μ-ligand, [^{3}H]-[D-Ala^2,D-Leu^5] enkephalin (1 nM) as δ-ligand and [^{3}H]-(±)-ethylketazocine (0.65 nM) as κ-ligand after suppression of binding at the μ- and δ-sites by the addition of 100 nM each of unlabelled [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin and [D-Ala^2,D-Leu^5]enkephalin (Magnan et al, 1982). The relative affinity of the compounds at the μ-, δ- and κ-sites was determined as K_I^{-1} at $x/(K_I^{-1}$ at μ + K_I^{-1} at δ + K_I^{-1} at κ).

When the N-substituent was 2-ethoxyethyl (Mr 2237), 2-methoxyethyl (Mr 2261) or 2-methoxypropyl (Mr 2549), the compounds were more active at the μ-site than the κ-site. Mr 2237 had the highest relative selectivity for the μ-site, having relative affinities at the μ-, δ- and κ-binding sites of 0.80, 0.02 and 0.18, respectively. In contrast, if the N-substituent was either a furfuryl- or furyl-methyl- ring, the compounds were more active at the κ-site than the μ-site. The compound with the highest relative selectivity for the κ-site was the N-furfuryl-homologue (Mr 1029); it had relative affinities at the μ-, δ- and κ-binding sites of 0.25, 0.03 and 0.72, respectively.

Thus, the presence of a ring structure as the N-substituent in the 5,9-dimethyl-2'-hydroxy-6,7-benzomorphan series results in relatively κ-selective compounds whereas a straight chain N-substituent yields relatively μ-selective compounds. The relative affinity for the δ-binding site is not greatly affected by the N-substituent.

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Magnan, J. et al (1982) Naunyn-Schmiedeberg's Arch.Pharmac. 319, 197-205

A HIGHLY SELECTIVE LIGAND FOR THE κ-BINDING SITE (U-50,488H)

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Evidence for the subdivision of the opioid binding sites into μ-, δ- and κ-subtypes has been obtained from the comparison of the binding of tritiated ligands and their differential inhibition by unlabelled compounds. However, characterization of the κ-binding site has been difficult due to the high degree of cross-reactivity of the ketazocine-like compounds with the μ- and to a lesser extent with the δ-binding sites (Kosterlitz et al, 1981; Magnan et al, 1982).

Recently it has been reported that U-50,488H (trans-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)cyclohexyl)-benzeneacetamine; The Upjohn Company) may be a selective ligand for the κ-binding site (Piercey et al, 1982). This compound has now been tested in both binding and pharmacological assays.

The potency of U-50,488H to displace the binding of selective tritiated ligands was measured in homogenates of guinea-pig brain at 25°C. Binding at the μ-site was determined with [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (1 nM), at the δ-site with [^{3}H]-[D-Ala^2,D-Leu^5]enkephalin (1 nM) and at the κ-site with [^{3}H]-(-)-bremazocine (0.15 nM) after suppression of binding at the μ- and δ-sites with 100 nM each of unlabelled [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin and [D-Ala^2,D-Leu^5] enkephalin (Magnan et al, 1982).

The compound was tested for agonist and antagonist activity in the vasa deferentia of the rat and rabbit. The rat vas deferens has a very low sensitivity to κ-ligands whereas in the rabbit vas deferens only κ-ligands but not μ- or δ-ligands are inhibitory (Corbett et al, 1982).

U-50,488H was a highly selective ligand for the κ-binding site with a K_I of 7.5 ± 0.65 nM (n = 5) against the suppressed binding of [^{3}H]-(-)-bremazocine. The corresponding values at the μ- and δ-sites were 890 ± 105 nM (n = 4) and 9803 ± 1066 nM (n = 5), respectively.

In the bioassays, U-50,488H had no agonist or antagonist activity in the rat vas deferens. However, it had a potent agonist activity in the rabbit vas deferens (IC_{50} = 35.4 ± 10, n = 4), which was more readily antagonised by Mr 2266 than by naloxone.

Thus, in both binding and pharmacological assays U-50,488H is a highly selective ligand for the κ-binding sites.

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BUPRENORPHINE: STIMULUS GENERALISATION TO MORPHINE - EVIDENCE FOR AN ACTION AT THE OPIATE μ-RECEPTOR

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Buprenorphine is a novel oripavine derivative with potent long-lasting analgesic effects. In marked contrast to other opiates however, it does not appear to produce the characteristic abstinence syndrome when chronic treatment is abruptly discontinued (Cowan et al., 1977). The nature of the receptor mediating the analgesic action of buprenorphine has been the subject of some debate in the literature. Thus buprenorphine has been profiled as a prototypic mu (μ) receptor agonist (Cowan et al., 1977) or, in contrast, as a kappa (K) receptor opiate analgesic of the cyclazocine type (Byrant & Tyers, 1979; Tyers, 1980).

In an attempt to resolve this question we have examined the stimulus properties of buprenorphine using the technique of drug discrimination. Buprenorphine and the K-agonist ethylketocyclazocine (EKC) have been compared in their ability to mimic the discriminative stimulus complex or "cue" induced by morphine, a phenomenon previously shown to be highly specific (Colpaert et al., 1975).

Six male Alderley Park rats were trained in standard 2-lever, operant chambers to respond on one lever following morphine (10mg/kg i.p., 45 minutes pretreatment) and on the opposite lever following saline (1ml/kg i.p.). Correct responses were reinforced (on a fixed ratio(FR)10 schedule) with sweetened condensed milk. Incorrect responses were of no consequence to the animal. The criterion for reliable discrimination was the completion of 10 consecutive sessions in which the correct lever was selected within the first 12 responses (i.e. pre FR < 12). Generalisation to morphine was said to have occurred when a novel drug-induced responding on the lever associated with morphine during training.

Morphine-cue recognition was shown to be dose-related (ED_{50}: 4.2mg/kg; 95% confidence limits 2.8-6.5) and to be significantly reversed by the opiate antagonist naloxone (1mg/kg i.p.). Buprenorphine stimulus generalisation to morphine was also shown to be dose-related (ED_{50}: 0.07mg/kg; 95% conf. limits 0.02-0.20). However, naloxone (1mg/kg i.p.) failed to reverse the opiate cue induced by 1mg/kg buprenorphine. A larger dose (3mg/kg i.p.) of the antagonist resulted in a partial reversal which failed to reach statistical significance.

EKC (0.2mg/kg i.p.) induced saline appropriate responding in all animals tested. This dose has previously been shown to be above that required for significant analgesia (Skingle & Tyers, 1980). The failure of EKC to generalise to the morphine stimulus confirms previous findings (Teal & Holtzman, 1980) and demonstrates the specificity of this phenomenon.

Stimulus generalisation to morphine is consistent with an action at the opiate u-receptor and buprenorphine is clearly capable of producing such an effect. Whilst this does not rule out a possible role of the K-receptor in the analgesic actions of buprenorphine, such a dissociation between analgesic and stimulus properties would be unique in view of the excellent correlation previously shown to exist between these two pharmacological effects (Colpaert et al., 1976)

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COMPARISON OF THE CELLULAR LOCATIONS OF $GABA_A$ AND $GABA_B$ RECEPTORS IN THE CEREBELLUM USING NEUROLOGICALLY MUTANT MICE

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The density of classical bicuculline-sensitive GABA receptors ($GABA_A$ sites) within the mammalian cerebellum appears to be higher in the granule cell layer than the molecular layer (Olsen & Mikoshiba, 1978; Palacios et al. 1980; Wilkin et al. 1981). A distribution ratio of approximately 5:1 has been reported. By comparison, bicuculline-insensitive $GABA_B$ receptors have only been detected in the molecular layer (Wilkin et al. 1981). However the densities of $GABA_A$ and $GABA_B$ sites in the molecular layer are virtually the same (Wilkin et al. 1981). Previous studies have demonstrated that the granule cell perikaryon is the location of the major portion of bicuculline-sensitive GABA sites (Olsen and Mikoshiba, 1978; Palacios et al. 1980) but their location in the molecular layer in unclear.

The neurologically mutant mouse with specific genetic lesions within the cerebellum provides a system of deletion for determining the cellular locations of these sites. We have therefore used four types of mutants for this study: Weaver (wv/wv 20-30 days old, n=5), Staggerer (sg/sg 18-20 days, n=4), Lurcher (Lc/+ 75-89 days, n=4) and Stumbler (stu/stu 20 days, n=7). The Weaver and Staggerer are agranular with a secondary reduction of Purkinje cell dendrites. The Lurcher is devoid of Purkinje cells and has about 20% of the normal number of granule cells. The Stumbler has Purkinje cells with stunted dendrites but with no changes in the other cell types.

$GABA_A$ and $GABA_B$ sites in cerebellum were labelled by incubation of 10 µm thick sections of the tissue in ^{3}H-GABA (50 nM) for 15 minutes at 20°C. The incubation conditions were altered to allow the separate detection of each receptor type as described by Wilkin et al. (1981). The radiolabelled sections were then subjected to a dry mounting autoradiographic technique (Wilkin et al. 1981) and comparisons between $GABA_A$ and $GABA_B$ locations made within the same animal as well as with normal littermates. Data obtained in Weaver and Staggerer mutants confirm the reduction of $GABA_A$ sites when granule cells are absent. However, some $GABA_A$ and $GABA_B$ sites remain in these mutants. By contrast, $GABA_B$ sites are 95% depleted in the molecular layer of the Lurcher mutant and $GABA_A$ sites are reduced in the granule cell layer concomitant with the reduction in granule cells. In the Stumbler mouse, $GABA_A$ sites are reduced by <5% in the granule cell layer whereas like $GABA_B$ sites they are decreased by >50% in the molecular layer.

We conclude that $GABA_A$ sites are on the granule cells and Purkinje cell dendrites whilst $GABA_B$ sites are also present on the Purkinje cell dendrites. Comparative autoradiographs will be shown to demonstrate these points.

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EFFECTS OF BACLOFEN, MUSCIMOL AND GHBA ON THE ANTICONVULSANT ACTION OF PHENOBARBITAL AND DIPHENYLHYDANTOIN

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There is evidence that derangements in GABA-ergic transmission may be associated with epilepsy and convulsive states. Consequently, drugs able to cause a reduction in GABA function are epileptogenic and agents either enhancing GABA-ergic transmission or potentiating GABA-mediated inhibition show anticonvulsant activity (Meldrum, 1979). It was therefore of interest to study the effect of baclofen ($GABA_B$ agonist), muscimol ($GABA_A$ agonist) and γ-hydroxybutyric acid (GHBA) on the protective action of diphenylhydantoin (DPH) and phenobarbital (PB) against maximal electroshock-induced seizures.

Male Albino Swiss mice weighing 18-22 g were used. Electroconvulsions were produced with the use of corneal electrodes and alternating current (50 mA; 50 Hz), the stimulus duration being 0.2 s. PB (Polfa) was dissolved in sterile saline and DPH (Polfa) was suspended in a 3% solution of Tween 81 - both anticonvulsants were injected i.p. 60 min before the test. Muscimol, baclofen (Polfa) and GHBA (Sigma) were brought into solution with sterile saline and injected i.p. - baclofen 60 min and the remaining drugs 30 min before electroconvulsions. Muscimol was also administered intracerebroventricularly (i.c.v.) according to Lipman and Spencer (1980) in a volume of 1 μl 5 min before the test. ED_{50} values (in mg kg^{-1}) were calculated for DPH and PB alone or combined with either muscimol, baclofen or GHBA.

The anticonvulsant action of PB was potentiated by both baclofen and GHBA but not by muscimol injected by two different routes. Specifically, baclofen in doses of 5, 10 and 15 mg kg^{-1} decreased ED_{50} value of PB from 32 mg kg^{-1} to 24,20.5 and 18.3 mg kg^{-1}, respectively. GHBA in doses of 200 and 300 mg kg^{-1} exerted a similar effect. On the other hand, muscimol (up to 1 mg kg^{-1} i.p. or 50 ng i.c.v.) did not affect the protextive activity of PB. Conversely, only muscimol (1.5 mg kg^{-1} i.p. or 50 ng i.c.v.) caused a significant potentiation of the anti-convulsant action of DPH reducing its ED_{50} value from 18.5 mg kg^{-1} to 15.5 and 15.4 mg kg^{-1}, respectively. Both baclofen up to 15 mg kg^{-1} and GHBA up to 450 mg kg^{-1} had no significant influence on the protextive effect of DPH.

It is suggested that the anticonvulsant activity of DPH may be associated to a certain degree with $GABA_A$ receptor-mediated inhibition whilst that of PB seems to be dependent on $GABA_B$ receptor-mediated events. GHBA-induced enhancement of PB protextive activity may be related to specific high affinity binding sites for GHBA shown recently by Benavides et al. (1982).

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THE ANTICONVULSANT EFFECTS OF TAURINE, ITS DERIVATIVES AND GABA IN MICE

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Taurine - an amino acid which has been proposed to be an inhibitory neurotransmitter in the brain - is thought to possess antiepileptic actions (Huxtable, 1982). However, its actions on differently induced convulsions have not been systematically studied nor compared with those of GABA, which at present is best established among the cerebral amino acids as an inhibitory neurotransmitter in the brain.

Convulsions were induced in mice weighing 16-19 g by pentylenetetrazol (PTZ, 105 mg/kg s.c.), 3-mercaptopropionic acid (3MPA, 36.6 and 48.8 mg/kg s.c.), bicuculline (0.5 mg/kg i.v.), strychnine (1 and 1.25 mg/kg s.c.) and by minimal (12 mA) or maximal (50 mA) electroshock (0.2 s, 50 Hz). GABA either i.c.v. or i.p., taurine i.c.v. or i.p., homotaurine i.c.v., N-pivaloyltaurine i.p., and N-pivaloylhomotaurine i.p. were administered, each to 15-25 mice, 5-35 min before the convulsion-inducing drugs or electroshock. Control mice were given saline i.c.v. or i.p. Statistical significances were calculated by Chi-Square test.

In very large doses i.p. administered GABA penetrates into the brain of mice (Biswas & Carlsson, 1977). In our experiments i.p. administered GABA (60 mmol/kg) significantly reduced the occurrence of 3MPA-induced tonic convulsions (from 86 to 14%, $P<0.001$). I.p. GABA, however, clearly enhanced the convulsing effect of strychnine. I.c.v. administered GABA (1 µmol per mouse) decreased the PTZ-induced clonic convulsions (from 96 to 69%, $P<0.05$). Also i.c.v. taurine (0.6 and 1 µmol) as well as homotaurine (0.1 µmol but not 0.3 µmol) significantly decreased (from 90-100 to 61-68%, $P<0.05$) the percentage of mice with PTZ-induced clonic convulsions. Diazepam (0.25 mg/kg i.p.) potentiated the effect of 0.6 µmol taurine but not those of 1 µmol taurine or 0.1 µmol homotaurine. I.p. taurine (15 mmol/kg) did not significantly alter any of the convulsions studied.

The lipid soluble taurine derivative, N-pivaloyltaurine (15 mmol/kg) reduced the occurrence of both the PTZ-induced clonic (from 95 to 67%, $P<0.05$) and tonic (from 33 to 4%, $P<0.05$) convulsions. Diazepam pretreatment potentiated its effect so that only 5% (1/19; $P<0.001$) of mice had clonic convulsions. N-Pivaloyltaurine also antagonized the convulsive effects of bicuculline and 3MPA, and, in contrast to GABA, it antagonized the effect of strychnine. The corresponding homotaurine derivative, N-pivaloylhomotaurine, reduced the convulsions induced by PTZ, bicuculline and 3MPA about similarly as N-pivaloyltaurine did, but it potentiated strychnine convulsions. Both N-pivaloyl derivatives decreased the percentage of tonic convulsions induced by minimal electroshock from 40-50 to 0-6% ($P<0.01$).

The results suggest that taurine has anticonvulsive actions at about similar doses as GABA. The anticonvulsive spectrum of taurine but not that of homotaurine seems to differ from that of GABA.

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AUTORADIOGRAPHIC VISUALIZATION AND PHARMACOLOGICAL CHARACTERIZATION OF (^{3}H)-Ro 5-4864 BINDING IN THE CNS

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Ro 5-4864, the 4'-chloro derivative of diazepam, lacks the typical tranquillizing effects of this class of drugs. High affinity binding sites for ^{3}H-Ro 5-4864 occur in the CNS as well as in peripheral tissues, e.g. adrenal, kidney(Schoemaker et al.,1983).

The distribution of its binding sites in rat brain cryostat sections *in vitro* has now been studied autoradiographically. Binding was highest in the olfactory nerve and glomerular layers of the bulb, in the choroid plexus and most(not all) ependymal cells(Richards et al.,1982). Low densities were observed in all other brain regions including ependymal cells of circumventricular organs(median eminence and subcommissural organ) and in the rat and cat spinal cord where grey matter was labelled(uniformly) to a higher degree than white matter. A similar regional brain distribution occurred *in vivo* 5 min after an intravenous injection of the radiolabel.

The pharmacological specificity of these binding sites in the CNS was checked by co-incubating sections with molar concentrations of various nonradioactive compounds. The high density binding sites were decreased by 1μM Ro 5-4864 ≥ Ro 5-5115(7-deschloro-4'-chloro derivative of diazepam) > flunitrazepam > diazepam and ethyl-B-carboline-3-carboxylate. Only Ro 5-4864 and Ro 5-5115(50μM) were able to displace(maximally 30%) the low density binding sites in the CNS. The following compounds were inactive at both the high and low density binding sites: picrotoxin, melatonin, diphenylhydantoin(all 1μM) and meprobamate, baclofen, Ro 15-1788(the specific benzodiazepine antagonist)(50μM).

The substrate for Ro 5-4864 binding in the CNS could be glial cells since a high density of binding has been found on such cells in culture, on kainic acid-induced glioses and the glia-rich olfactory nerve layer in the bulb. The higher density of binding sites in the choroid plexus and ependyma than in some circumventricular organs suggests that the former may have a closer phylogenetic(glial?) ancestry when compared with the latter.

In conclusion, the distribution of ^{3}H-Ro 5-4864 binding sites in the CNS is atypical compared with those of benzodiazepine tranquillizers. At most a third of the low density sites are specific; these are of the low affinity type. Recent pharmacological investigations(Pieri et al.,1983) have shown that Ro 5-4864 has a completely different pharmacological profile to, for example, diazepam(no sedation, proconvulsive and convulsive). It remains to be seen whether these effects are mediated via specific receptors on glial cells.

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ELECTROPHYSIOLOGY OF MIDAZOLAM, A NOVEL BENZODIAZEPINE, AND Ro 147437, A BENZODIAZEPINE RECEPTOR ANTAGONIST, ON FROG SPINAL CORD

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Benzodiazepines can potentiate GABA-induced electrical responses of in vitro neurones (Choi et al 1977; Macdonald & Barker 1978; Nistri & Constanti, 1978) through an effect on specific receptors distinct from those which bind GABA (Simmonds 1980). In biochemical binding studies it is, however, difficult to observe this potentiation (Olsen 1982) which in the electrophysiological studies is often obtained with relatively high concentrations of benzodiazepines. We decided to re-examine this phenomenon using a new and more powerful watersoluble benzodiazepine (midazolam) and a specific benzodiazepine antagonist, Ro 147437. Our experimental model system was the in vitro spinal cord where GABA appears to be the transmitter of presynaptic inhibition (Nistri & Constanti, 1979).

Experiments were performed on in vitro slice preparations of the frog spinal cord as described by Nistri & Constanti (1978). Electrical DC recordings were obtained from dorsal root fibres following stimulation of adjacent ventral or dorsal roots. All experiments were conducted at 5-6°C and drugs were rapidly bath-applied as previously reported (Nistri 1981).

After 20-30 min application midazolam enhanced dorsal root potentials and GABA-induced depolarizations. In particular, the GABA dose-response curve was displaced upwards and to the left with consequent halving of GABA ED_{50} value (from 0.85 mM to 0.48 mM), but with no significant alteration in the maximal response amplitude. The midazolam ED_{50} value for GABA potentiation was 1 nM with a maximal enhancement noted at about 25 nM. Similar to the phenomenon seen with high doses of flurazepam (Nistri & Constanti, 1978), midazolam concentrations $\geq$ 100 nM antagonized GABA responses. Midazolam fully maintained its GABA enhancing properties in the presence of tetrodotoxin (3 μM) or nipecotic acid (1 mM): this indicates that the midazolam action was directly exerted on recorded cells and did not involve potentiation of GABA uptake and/or release. Glycine or glutamate depolarizations were not potentiated by 25 nM midazolam. Ro 147437 (IC_{50}=2 nM) antagonized midazolam-potentiated GABA responses without affecting control responses to GABA, glutamate or high K^+ solutions.

Our data thus show that very low doses of a benzodiazepine agent enhanced the action of GABA in a selective manner and that this effect was antagonized by Ro 147437. This supports the notion that, in our experimental system, the GABA neurotransmitter mechanisms are closely associated to benzodiazepine receptors which have a powerful modulatory role on the amino acid-evoked responses.

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EXCITATORY EFFECTS OF MICROIONTOPHORETICALLY APPLIED HISTAMINE IN THE RAT MEDULLA MAY BE MEDIATED VIA HISTAMINE H_2-RECEPTORS

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Histamine (HA) appears to have both excitatory and inhibitory actions on single neurones in the CNS, although it has been suggested that the excitatory actions occur predominantly in the hypothalamus (Haas & Wolf, 1977). We now report an excitatory action of microiontophoretically applied HA on single, spontaneously active neurones in the rat caudal medulla oblongata.

Male Wistar rats were anaesthetised with urethane (1.75g/kg,i.p.) and prepared for electrophysiological recording as described previously (Bradley & Dray, 1974). Conventional 5- or 7-barrelled micropipettes (tip diameter 4-9um) were used, the centre recording barrel containing 4M Na Cl. One barrel containing Pontamine Sky Blue (2.5% in Na acetate buffer pH 5.6) was used for current balancing and marking the position of cells, most of which were located in the nucleus reticularis gigantocellularis and adjacent reticular nuclei. The remaining barrels contained a combination of the following solutions : HA, 4-methylhistamine (4MH), 2-pyridylethylamine (2PEA), N-telemethylhistamine (TMH) (all at 0.2M,pH 4.5), acetylcholine (ACh) (0.3M,pH 4.5), ranitidine (0.1M, pH 4.5), atropine (0.1M, pH 4.5). Positive ejection currents between 0 and 80 nA were applied for 5 to 20 sec. except where specified.

Contrary to a previous report in which unanaesthetised decerebrate cats were used (Haas et al, 1973), HA was found to excite most neurones tested (107 cells excited, 7 not affected). The pharmacological specificity of this response was therefore investigated. The histamine H_2-receptor agonist 4MH produced a similar effect to HA on all neurones tested (24/24 excited), whereas the relatively selective histamine H_1-receptor agonist 2PEA caused only slight excitation of 5 neurones leaving 10 unaffected. The major metabolite of histamine in the brain, TMH, which is ineffective on peripheral H_1- and H_2-histamine receptors, produced no effect on 13 neurones and slight excitation on 6, at high ejection currents (60-100nA).

The effects of HA were completely antagonised by the H_2-receptor antagonist ranitidine (20-60nA, 2-20 min.) on 12 neurones, partially antagonised on 5 and unaffected on 4, while the effects of 4MH were completely antagonised on 6/6 neurones; responses to 2PEA and TMH were unaffected by this antagonist.

Ranitidine caused excitation of 20/21 neurones which was antagonised by atropine (6/6 cells) and appeared to be independant of H_2 antagonism. Acetylcholine also excited most neurones (85/90;5 unaffected); this excitation was potentiated both in magnitude and duration during and up to 5 min. after an ejection of ranitidine (19/19 neurones). The excitatory effect of iontophoretically applied ranitidine may therefore be mediated by an interaction with cholinergic mechanisms. This is consistent with the finding that contractions induced by ranitidine in the isolated lower oesophageal sphincter of the rat were antagonised by atropine (Bertaccini & Coruzzi, 1981).

In conclusion, histamine, when applied microiontophoretically to neurones in the rat medulla, produces an excitatory effect which appears to be mediated, at least in part, by histamine H_2-receptors.

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ACUTE AND CHRONIC EFFECTS OF THIP ON SEIZURE THRESHOLD

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4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) is a conformationally restricted analogue of γ-aminobutyric acid (GABA) with agonist properties at GABA receptors (Krogsgaard-Larsen et al, 1977) and which affords some protection against audiogenic seizures in susceptible mice (Meldrum & Horton, 1980). We have now investigated the activity of THIP against a variety of convulsant agents as well as some effects of chronically administered THIP. Male Sprague-Dawley derived rats were injected with various doses of THIP i.p. and at various times afterwards their seizure threshold to intravenous convulsants was measured by the method of Nutt et al (1980). THIP showed anticonvulsant activity against both bicuculline and penylenetetrazol in a dose-dependent fashion, although maximum effect was not achieved at 20 mg/kg THIP, which was the highest dose tested. The time course of the anticonvulsant effect of 10 mg/kg THIP against bicuculline showed maximal effect at 15 min and only a slight reduction at 1 h; by 24 h, however, seizure threshold had returned to normal. When tested 30 min after injection THIP (10 mg/kg) had anticonvulsant activity against pentylenetetrazol, bicuculline, and quipazine but not against strychnine (Table).

Convulsant	Seizure threshold (mg of convulsant/kg) Control	THIP	P (2-tailed t-test)
Pentylenetetrazol	36.00 ± 2.00 (3)	47.00 ± 5.00 (3)	<0.05
Bicuculline	0.34 ± 0.03 (3)	0.53 ± 0.07 (3)	<0.025
Quipazine	47.00 ± 5.00 (5)	61.00 ± 8.00 (6)	<0.01
Strychnine	0.80 ± 0.14 (3)	0.83 ± 0.06 (3)	N.S.

Each value is the mean ± S.D. with the number of animals in brackets

Twenty-four hours after the last of 10 once-daily injections of THIP (10 mg/kg) the seizure threshold to bicuculline was slightly elevated (controls 0.27 ± 0.03 mg/kg, mean ± S.D., n = 7; chronic THIP 0.31 ± 0.03 mg/kg, n = 6, P <0.025, 2-tailed t-test). This may reflect an alteration in GABA receptor number or a rebound increase in endogenous GABA activity following chronic administration of a GABA agonist. The acute experiments suggest that THIP, in addition to its GABA agonist activity, may possess some 5-HT antagonist properties, although we cannot exclude the possibility that quipazine may act indirectly through the GABA system.

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INTRACEREBRAL CHOLINOMIMETICS PRODUCE SEIZURE-RELATED BRAIN DAMAGE IN RATS

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The functional role of acetylcholine (ACh) and muscarinic cholinergic receptors is strongly implicated in the etiology of human epilepsy as well as in experimental models of seizures. Intracerebroventricular and intrahippocampal administration of muscarinic cholinergic agonists in rats produces convulsive activity, which can be behaviourally and electrographically attributed to limbic seizures. Electrophysiological studies indicate that ACh exerts both direct muscarinic excitatory effect on hippocampal pyramidal cells and rapid, powerful, muscarinic inhibitory effect upon excitatory and inhibitory afferents to pyramidal neurons (Valentino & Dingledine, 1981). These results raise the possibility that endogenous muscarinic cholinergic mechanisms in the hippocampus may contribute to certain epileptiform phenomena in limbic structures including their neuropathological consequences.

Adult male Wistar rats weighing 220-240g were stereotaxically implanted with permanent, stainless-steel guide cannulae under pentobarbitone anaesthesia (50 mg/kg ip). For EEG recordings, bipolar twisted electrodes were positioned stereotaxically in ipsilateral hippocampus, amygdala, septum and contralateral hippocampus. Surface recordings were made from jeweler screws placed over the occipital cortex. Five days after surgery, unilateral injections of bethanechol (BCh) (12.5-50 µg, pH 7.4) or saline were made into the hippocampal CA 3 subfield, dorsal striatum, substantia nigra or lateral brain ventricle (icv) in a volume of 0.5 µl over a period of 3 min. For histological evaluation rats were sacrificed 12 hs after microinjection.

Shortly after intrahippocampal injection of BCh (50 µg) an increase in the frequency of the hippocampal theta rhythm ipsi- and contralaterally to the injected site and high frequency rhythms in the other affected structures were observed. By 5-10 min after injection, EEG showed spiking activity of high frequency initially restricted to the injected hippocampus with rapid propagation to the lateral septum, amygdala, contralateral hippocampus and neocortex. Examination of frontal forebrain sections revealed disseminated, apparently seizure-related, pattern of brain damage. The pattering of distant damage after intrahippocampal, intrastriatal, intranigral or icv injections of BCh (50 µg) varied in intensity and location, but most frequently involved the piriform cortex, olfactory tubercle, entorhinal cortex, subiculum, amygdaloid complex, temporoparietal cortex and hypothalamic nuclei. Neuropathological changes were occasionally observed in the lateral septum and thalamus. Intrastriatal and intranigral microinjections of BCh did not result in any tissue pathology at the injection sites.

ACh is naturally present in the hippocampal formation as the neurotransmitter of the septohippocampal pathway. Since the convulsant activity of cholinomimetics has been previously demonstrated and since we describe here a brain damaging action of BCh which is closely related to that observed in human epileptics, the possible involvement of ACh and septohippocampal pathways in epileptic brain damage should be considered. Wood et al. (1979) attributed the convulsions after intrahippocampal kainic acid to the elevation of the hippocampal ACh content. In this regard, it is important to ask whether cholinergic mechanisms contribute to brain damaging effects of this neurotoxin. Further work is also necessary to establish whether the sustained stimulation of septohippocampal pathway can account for the development of brain damage. These studies may have crucial importance for refining the mechanisms of epileptic brain damage in man.

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THE CONVULSANTS, 3-MERCAPTOPROPIONIC ACID AND DMCM ENHANCE (^{3}H)-D-ASPARTATE RELEASE FROM RAT CORTEX

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2-Amino-7-Phosphonoheptanoic acid, which is a potent antagonist of excitation due to the aspartate preferring agonist, N-Methyl-D-Aspartate (Evans et al 1982), blocks the convulsant action of 3-Mercaptopropionic acid (3-MP) and methyl 6, 7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), suggesting that 3-MP's and DMCM's convulsant actions are mediated by aspartate (Czuczwar & Meldrum, 1982). We have tested this directly, by studying the effect of 3-MP and DMCM on the release of preloaded ^{3}H-D-aspartate from rat cortical slices in vitro.

The methods used to study the release of previously accumulated radio-labelled neurotransmitter from small slices of rat brain were those of Kerwin & Pycock (1979).

Potassium chloride (25mM) markedly stimulated the efflux of ^{3}H-D-aspartate from slices of rat parietal cortex and this effect of potassium was markedly reduced in a calcium free medium. Potassium chloride (25mM) also markedly stimulated the release of ^{3}H-D-aspartate in a double stimulation protocol when added for 4 minute, 6 (S1) and 22 (S2) minutes after the start of collection. The ratio of the magnitude of the two evoked releases (S2/S1) was 0.67 $\pm$ 0.2 (n = 8). 3-MP when present during the second stimulation period, potentiated the potassium evoked release of radioactivity giving S2/S1 ratios of 50 μM : 0.83 $\pm$ 0.21; 100 μM : 1.17 $\pm$ 0.32; $p < 0.05$ from control and 200 μM : 1.18 $\pm$ 0.05; $p < 0.05$ from control. None of these doses of 3-MP had any effect on the spontaneous efflux of ^{3}H-D-aspartate.

DMCM, 10 & 100 μM, produced a marked stimulation of both the spontaneous and evoked (S2) release of radioactivity. This alteration in spontaneous levels in the second part of the DMCM containing experiments precluded any comparison of S2/S1 ratios. However, when DMCM was present the rate of release was significantly different ($p < 0.05$) from parallel controls at all points on the efflux curve.

This evidence greatly strengthens the possibility that 3-MP and DMCM produce their convulsant actions at least in part via the release of the putative excitatory transmitter aspartate and is in contrast to the hitherto accepted convulsant mechanism attributed to 3-MP (GABA synthesis inhibition).

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ANXIOGENIC ACTIONS OF PICROTOXIN AND PENTYLENETETRAZOLE: REVERSAL BY CHLORDIAZEPOXIDE

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Not all drugs that act at the benzodiazepine receptors are anxiolytic and anti-convulsant. It has recently been shown that β-carboline-3-carboxylate ethyl ester and the triazoloquinoline, CGS 8216, are anxiogenic (File et al, 1982; File & Lister, 1983) and proconvulsant (Cowen et al, 1981; File, 1983). The benzodiazepine receptors form part of a supramolecular complex, including GABA and picrotoxin binding sites, and both picrotoxin and pentylenetetrazole are thought to cause convulsions through their action at the picrotoxin binding site (Olsen & Leeb-Lundberg, 1981). The purpose of the present study was to determine whether sub-convulsant doses of these two drugs were anxiogenic.

The drugs were assessed in the social interaction test of anxiety. In this test, a specific decrease in the time spent in social interaction by pairs of male rats, that is not secondary to a decrease in motor activity, indicates an anxiogenic effect. The drugs were dissolved in deionised water and injected i.p. in a volume of 2 ml/kg. In Experiment 1, chlordiazepoxide (CDP, 5 mg/kg, n = 7 pairs) and picrotoxin (2 and 4 mg/kg, n = 7 pairs/grp) were injected 30 min before test. In Experiment 2, CDP (5 mg/kg, n = 8 pairs) was injected 30 min and pentylenetetrazole (PTZ, 5, 10 and 20 mg/kg, n = 8 pairs/grp) 5 min before test. The test lasted 7.5 min, during which the frequency and duration of social interaction was scored 'blind' by two independent observers. The rats were tested between 06.30 and 11.30 h, in an order randomised for drug treatment.

Picrotoxin (2 and 4 mg/kg) significantly ($p<.0001$) reduced active social interaction in a dose-related manner, and the reduction reached significance at the 1% level even for the 2 mg/kg dose. Motor activity was unchanged at this lower dose, but at 4 mg/kg it was significantly reduced ($p<.01$). The anxiogenic effect of picrotoxin (4 mg/kg) was significantly reversed by CDP (5 mg/kg, $p<.001$), as was the motor reduction ($p<.05$).

Although pentylenetetrazole (5 mg/kg) had little effect on social interaction, PTZ (10-20 mg/kg) produced a dose related decrease in social interaction which was significant for the 20 mg/kg dose ($p<0.001$). This reduction was partially reversed by CDP (5 mg/kg, $p<0.01$). PTZ (20 mg/kg) however also reduced locomotor activity ($p<0.001$), and although this may have been secondary to the reduction in social interaction, an unambiguous interpretation of an anxiogenic effect is not possible.

The clear anxiogenic action of picrotoxin and the possible anxiogenic action of PTZ illustrate that this effect can be mediated by a site other than the benzodiazepine receptor.

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BEHAVIOURAL STUDIES WITH ETHYLCHOLINE MUSTARD AZIRIDINIUM (ECMA)

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ECMA, an irreversible inhibitor of choline transport (ChT) (Rylett & Colhoun, 1980), has been promoted as a means of producing a central cholinergic 'lesion' which may usefully model senile dementia of the Alzheimer type. Thus, 65 nmol ECMA markedly reduced ACh concentrations in mouse cortex and hippocampus which was maximal 7 days after icv injection (Fisher et al, 1982). Hemicholinium-3 (HC-3) given icv in mice impairs passive avoidance learning (Caulfield et al, 1981); we have therefore studied the effects of ECMA in this test.

Male CD-1 mice (20-24g on dosing) were given ECMA (in 5µl Krebs solution) 7 days before training. The ECMA was prepared from the mustard precursor (acetate derivative, Jackson & Hirst, 1972). NMR analysis of the precursor produced peaks consistent with predicted structure. Cyclization (cf Fisher et al, 1982), 82 ± 1.5% theoretical value by thiosulphate titration, yielded an aziridinium preparation with the following properties: 50% hydrolysis in 4h (pH 7.4, 37°C); IC_{50} against sodium-dependent ChT after 5 min preincubation (37°C) with mouse cortex prisms was about 0.5µM (IC_{50} for HC-3 40nM); reversal of inhibition by tissue wash (3x, 300 vols. total), following incubation with $10xIC_{50}$, was complete for HC-3 and undetectable for ECMA.

Doses of ECMA $\geq$ 1.5nmol produced the delayed signs of toxicity (weight loss, abnormal posture, hyperexcitability), observed after 65 nmol ECMA by Fisher et al (1982) and 5nmol of our preparation killed 20-30% of the mice within 7 days. 50nmol killed all of the mice within 60 minutes. A behaviourally effective dose of HC-3 (2.2nmol; Caulfield et al, 1981) given concommitantly did not reduce the toxicity of ECMA. In the passive avoidance task, 11 out of 20 vehicle-injected controls avoided entry into the dark compartment for >120s, on retrial 24h post-training. Mice given a sub-toxic dose of ECMA (0.5nmol) 7 days pre-trial also learned the task; median retrial avoidance latency was 75s, with 8 out of 20 avoiding for >120s. Passive avoidance learning was therefore not significantly affected by ECMA, in contrast with the impairment seen after HC-3 (Caulfield et al, 1981).

It therefore appears that clear effects of ECMA on passive avoidance learning in mice cannot be seen at sub-toxic doses. Toxicity may be a consequence of generalised central cholinergic lesions, although the lack of effect of HC-3 on ECMA toxicity does not accord with this. Thus, it seems unlikely that ECMA-treated animals can be used as models of the behavioural disorders in senile dementia of the Alzheimer type.

We are grateful to Dr. F. Binns of Salford Fine Chemicals for synthesizing ECMA precursor.

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INTERACTION OF Ro 15-1788 WITH THE BENZODIAZEPINE RECEPTOR

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Ro 15-1788 is an imidazobenzodiazepine which potently displaces benzodiazepines from their CNS binding sites and which is reported to antagonise their pharmacological effects (Hunkeler et al, 1981).

Equilibrium binding studies of classical 1,4-benzodiazepines are consistent with a simple bimolecular interaction of these compounds with their recognition sites. However, kinetic studies indicate that their binding is more complex: the dissociation of [^{3}H]-flunitrazepam is biphasic and exhibits negative cooperativity (Doble et al, 1982). Similar kinetic studies using [^{3}H]-Ro 15-1788 have been carried out to determine whether the same phenomena are observed for this antagonist.

Well-washed rat cerebellar membranes were incubated with the radioligand at 4°C. The final concentration of the radioligand was 0.5 nM and of the membranes was 1:800 (w/v). Tris citrate buffer, 0.1 M, pH 7.1 was used throughout. After 1 h, triplicate 1 ml aliquots were removed and filtered through Whatman GF/B filters followed by 3 x 5 ml washes with ice-cold buffer to determine total equilibrium binding. Dissociation was initiated by the addition of 10 µM diazepam, 2 µM Ro 15-1788 or 2 µM βCCE and 1 ml aliquots were removed and filtered as above to measure total binding at various time intervals. The displacing ligand was present from the beginning of a parallel incubation used to define non-specific binding.

The dissociation of specifically bound [^{3}H]-Ro 15-1788 from the cerebellum was monophasic (k_{-1} = 5.03 ± 0.08 x 10^{-2} min^{-1}) whereas the dissociation of [^{3}H]-flunitrazepam was biphasic; 70% from a fast compartment (k_{-1} = 12.83 ± 1.87 x 10^{-2} min^{-1}) and the remainder from a slow compartment (k_{-1} = 1.35 ± 0.51 x 10^{-2} min^{-1}). These results were from computer-assisted fitting of exponentials to representative experiments initiated by diazepam. The dissociation of [^{3}H]-Ro 15-1788 was monophasic and took place at a similar rate irrespective of whether diazepam, Ro 15-1788 or βCCE were used to initiate dissociation.

The monophasic dissociation of Ro 15-1788, in contrast to the biphasic dissociation of flunitrazepam, may reflect the inability of this antagonist to induce a conformational change in the receptor.

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DIFFERENCES IN EEG SEIZURES INDUCED BY INTRAVENTRICULAR INJECTION OF FOLIC AND KAINIC ACIDS IN HALOTHANE ANAESTHETIZED RATS

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Observations that 5-methyltetrahydrofolate and kainic acid (KA) share a common binding site in a rat cerebellar preparation (Ruck et al, 1980) and that folic acid (FA) and KA have some common neurotoxic properties (Olney et al, 1981) have led to speculation that both convulsants may act through similar mechanisms. To further examine the convulsant actions of FA and KA we have compared their effects on the electroencephelogram (EEG) with the known convulsants, bicuculline, picrotoxin, penicillin and N-methyl-D-aspartate (NMDA), following intracerebroventricular (ICV) injection.

Following induction of anaesthesia with halothane, rats (Wistar, male, 220-250g) were placed in a stereotaxic frame and anaesthesia (1% halothane/oxygen mixture, administered by face mask) maintained throughout the experiment. Epidural screw electrodes for cortical EEG recording were inserted into the skull over the left and right frontal and parietal cortices. Hippocampal EEG was recorded via a concentric bipolar electrode placed in the right dorsal hippocampus (4.0 mm post., 3.0 mm lat. to bregma, at a depth of 2.0-2.5 mm from the dura). The convulsants, or control fluid were injected in a dose volume of 5 µl into the left lateral ventricle (0.9 mm post., 1.5 lat. to bregma, at a depth of 5.0 mm from the skull surface). EEG was recorded for at least 15 min pre-drug and 0.5-3.0h post-drug.

Our studies revealed that the gross EEG seizure activity induced by the convulsants was of two qualitatively distinct types:

a) bicuculline (> 0.7 nmol), picrotoxin (> 0.08 nmol), penicillin (> 150 nmol) and FA (> 5.0 nmol) induced discrete high amplitude hippocampal spiking with an onset of 1-4 min. Higher doses of these compounds produced polyspikes and cortical spiking, synchronous with hippocampal spiking.

b) KA (> 0.1 nmol) and NMDA (> 5.0 nmol) initially induced fast, high amplitude spiking and spindle or paroxysmal bursts from the hippocampus usually accompanied by a reduction in cortical EEG. Following a period of post-ictal depression the hippocampal EEG seizure activity continued with alternating periods of post-ictal depression. Spread of seizure activity to the cortex was only observed after very high doses of KA (> 20.0 nmol) and was not observed after NMDA (up to 100 nmol). Control animals injected with vehicle displayed no overt EEG changes. Thus, FA, in contrast to KA, resembled the disinhibitory convulsants penicillin, bicuculline and picrotoxin which reduce GABA mediated inhibition (Woodbury, D.M., 1980). The effects of FA in this study are consistent with previous findings showing a similarity between the actions of picrotoxin and FA (Hill and Miller, 1974). The effects of the excitants KA and NMDA were similar, suggesting that FA and KA initiate seizure activity by different mechanisms.

Our conclusions agree with other studies on FA and KA using microiontophoresis (Evans et al, 1982) and epileptic kindling (Miller et al, 1982). Additionally, since halothane, unlike barbiturate anaesthesia (unpublished observations), did not mask EEG seizures, this technique may be of further value in the study of convulsants.

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MULTIPLE BENZODIAZEPINE BINDING SITES IN RAT PITUITARY

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Evidence is accumulating that GABA may play a role in the control of hormone release from the anterior pituitary gland (Racagni et al 1982), and a dense innervation of the neurointermediate lobe has been demonstrated immunohistochemically (Vincent et al 1982). It is possible that benzodiazepines may modify GABA-ergic responses in the pituitary as has been reported in the central nervous system, where high affinity binding sites are present. The presence of such sites in the pituitary has not been demonstrated.

Pituitaries were removed from male Wistar rats (150-200 g), dissected into anterior (AP) and neurointermediate (NI) lobes, and frozen at -20°C. Homogenates were prepared in 50 volumes of 25 mM KH_2PO_4 buffer (pH 7.1) from pooled tissue samples using a teflon/glass homogeniser. Samples were centrifuged at 48,000 g for 10 minutes and the membrane pellet washed a further three times. Radioligand binding assays were carried out using {^{3}H} flunitrazepam ({^{3}H} FNM,1nM) on membrane samples of 250 µg protein (AP) and 50 µg protein (NI) in duplicate, in a total assay volume of 2 ml. After incubation at 0°C for 90 min samples were filtered through GF/B filters, and washed three times with 5 ml of ice cold buffer.

Specific binding (displaced by 10 µM diazepam) was detected in both areas: 28.6 fmol/mg protein in AP and 101 fmol/mg protein in NI. The contribution of 'central-' and 'peripheral-type' benzodiazepine binding sites was analysed by utilising the selective affinities of clonazepam and Ro5-4864 for these sites. In brain and kidney membranes respectively, these compounds showed IC_{50}'s of 0.86 nM and 5,400 nM (clonazepam) and 80,000 nM and 2.0 nM (Ro5-4864), with Hill Coefficients close to unity. In AP and NI both of these compounds produced extended displacement curves, (n_H <<1) showing distinct plateau regions, and which could be resolved into two clear components by Hofstee plots. Central-type binding was defined as that displaced by 200 nM clonazepam: a concentration at which there is ≈ 100% displacement from central-type and negligible displacement from peripheral-type sites. This accounted for 27% (7.7 fmol/mg protein) of total specific binding in AP and 34% (34.2 fmol/mg protein) in NI; a ratio NI:AP of 4.4:1. Peripheral-type (clonazepam-insensitive) binding was present at 20.9 fmol/mg in AP and 66.4 fmol/mg in NI; a ratio NI:AP of 3.2:1 (n=5).

Scatchard analysis of {^{3}H} FNM binding (0.1-500 nM) to whole-pituitary membrane preparations showed a biphasic curve. 200 nM clonazepam resolved this into two linear components: clonazepam-sensitive, K_D 1.6 ± 0.2 nM, B_{max} 22.5 ± 2.8 fmol/mg protein; clonazepam-insensitive, K_D 69 ± 6 nM, B_{max} 1.54 ± 0.06 pmol/mg protein, (n=3).

GABA has been shown to facilitate {^{3}H} benzodiazepine binding to 'central-' but not 'peripheral-type' receptors (Marangos et al 1982). 100 µM GABA increased specific binding of {^{3}H} FNM to whole pituitary washed in 50 mM Tris citrate (pH 7.1). Clonazepam-sensitive binding was increased by 80% from 8.4 ± 1.1 to 15.0 ± 1.3 fmol/mg protein ($P < 0.05$), while clonazepam-insensitive binding was not significantly altered (17.0 ± 1.3 to 14.7 ± 0.5 fmol/mg protein) n=4.

These results demonstrate the presence of a small population of central type benzodiazepine binding sites in the rat pituitary, predominantly in the neurointermediate lobe, which are intimately associated with GABA recognition sites.

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ENHANCED SEROTONERGIC BEHAVIOUR IN BARBITURATE DEPENDENT MICE

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Various authors have suggested an involvement of the serotonergic system in the development of barbiturate tolerance and dependence: 5-HT depletion has been shown to delay the development of barbiturate tolerance (Lyness & Mycek, 1980) whereas administration of the 5-HT precursor L-Tryptophan accelerates tolerance development (Lê, et al., 1979). We have previously reported no change in 5-HT turnover in chronic barbital treatment and a reduction during withdrawal (Dawbarn et al., 1982), although Nabeshima & Ho (1981) observed decreased 5-HT synthesis in pentobarbital tolerant mice and no change in withdrawal. We have therefore looked at changes in behavioural responses to serotonergic drugs and in vitro [^{3}H]-5-HT receptor binding in barbital dependent and withdrawn mice.

Female LACG mice were fed powdered food containing milled barbital in a schedule known to induce tolerance and dependence, for a total of one week. Groups of ten naive, dependent and withdrawn mice were used for behavioural testing after administration of the following drugs: 5-HTP (100mg/kg s.c.) preceded by carbidopa (25mg/kg i.p.) 30 minutes beforehand; fenfluramine (10mg/kg i.p.), or quipazine (5mg/kg i.p.). Optimal doses and times of behavioural assessment were established in pilot tests. Animals were placed in perspex boxes immediately after drug injection and serotonergic behaviours were scored in an all-or-none fashion 30 minutes post quipazine and fenfluramine or one hour post 5-HTP administration. The following behaviours were scored: Tremor, sniffing, reciprocal forepaw treading, head bobbing and shaking, lateral head weaving, rearing and mouthing. [^{3}H]-5-HT binding was assayed in crude membrane fractions prepared from forebrains of naive and dependent female mice according to the method of Nelson, et al., (1978) with some modifications. The receptor binding assay of Bennett & Snyder (1976) was used, with several modifications.

Barbital dependent mice showed behavioural supersensitivity to the three drugs tested. After 5-HTP administration controls showed sniffing and tremor with occasional head movements, in barbital dependent mice these effects were intensified and rearing was seen in 6/10 animals. Fenfluramine adminstration caused sniffing in control animals and head movements in half of these whereas 9/10 barbital dependent mice showed head movements and the incidence of rearing was increased. Quipazine produced forepaw treading and tremor in control animals; rearing, mouthing and head movements occured in addition to these effects in barbital dependent mice. There was no significant difference in serotonergic behaviour of naive and barbiturate withdrawn mice after any of the drugs tested at 48 hours post withdrawal. We found no change in [^{3}H]-5-HT binding to membrane fractions of whole forebrain of barbital dependent compared to naive mice.

The results of behavioural tests suggest an increase in serotonergic sensitivity in barbital dependent mice, which may be obscured in withdrawal by behavioural changes associated with the withdrawal syndrome. We are currently looking for possible changes in receptor binding in discrete brain areas of dependent animals.

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SEROTONERGIC MODULATION OF STRIATAL DOPAMINE RELEASE

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Binding studies have revealed the presence of two types of 5-hydroxytryptamine (5HT) binding site in brain (Peroutka and Snyder, 1979). However, the models available to test the function of these sites are imperfect (Middlemiss, 1982). 5HT agonists have been reported to decrease the release of radioactivity from striatal slices preloaded with [^{3}H]-dopamine ([^{3}H]-DA) (Ennis et al, 1980). This study further evaluates the use of this system as a functional test for 5HT receptors.

Striata were dissected from two, 200-300 g male Wistar rats and chopped into 250 x 250 μm prisms using a McIllwain tissue chopper. The slices were then incubated for 20 min at 37°C in 5 ml Krebs-Henseleit buffer containing 0.2 mM ascorbic acid, 1 μm pargyline, 2 mg/ml glucose and 10^{-7}M [^{3}H]-DA (specific activity 46 Ci/mmol).

The resulting tissue suspension was gently centrifuged and washed 3 times with 5 ml Krebs-Henseleit and resuspended in 0.5 ml Krebs, 50 μl aliquots of the suspension were placed in each of eight 250 μl superfusion chambers. The tissue was superfused with Krebs-Henseleit with glucose (continuously gassed with 5% CO_2 in O_2) at a flow rate of 0.4 ml/min for 20 min before fractions were collected. Stimulated release was induced by the addition of 25 mM KCl during the 5th and 15th 4 min collection periods; test drugs were added at the beginning of the 10th collection period. The stimulated efflux of radioactivity is expressed as the percentage increase above baseline of the 3 samples collected directly after the depolarizing stimulus. Results are given as the ratio of the second and first stimuli (S_2/S_1).

Addition of 25 mM KCl caused up to a 10-fold increase in efflux of radioactivity. The magnitude of this stimulation and of the absolute amount of radioactivity released was variable. However, the S_2/S_1 ratio was less variable (0.81-1.29 with a mean of 1.01, n=48). Addition of 10^{-5}M or 10^{-6}M 5HT to the superfusion medium for the second half of the experiment results in a large increase in basal release of radioactivity and an inconsistent effect on the S_2/S_1 ratio. 10^{-5}M but not 10^{-6}M 5-methoxytryptamine (5-MT) reduced the K^+ stimulated release of radioactivity (S_2-S_1 ratios of 0.61 and 1.05 respectively, n=12). The reduction in radioactivity release induced by 5-MT was not reversed by either metergoline or methysergide at 10^{-6}M. Superfusion of the slices with 10^{-5}M or 10^{-6}M apomorphine substantially reduced the K^+ stimulated efflux of radioactivity (S_2/S_1 ratios of 0.135 and 0.085 respectively, n=8) an effect which was reversed by haloperidol (10^{-7}M).

Overall, these results suggest that further evaluation of this system is needed before it can be used as a model for 5HT receptors. However, it may be possible to study 5-HT receptors in other brain regions using neurotransmitter release techniques.

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COMBINED β-PHENYLETHYLAMINE AND NALOXONE ADMINISTRATION INDUCES INTENSE BEHAVIOURAL STIMULATION IN THE RAT

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Naloxone (NAL), an opiate antagonist, blocks the increase in locomotor activity produced by moderate doses of (+)-amphetamine in rats (Holtzman, 1974). On the other hand, low doses of dopamine agonists diminish operant rates of responding, and this inhibitory effect is potentiated by NAL (Harris et al, 1977). Recently, behavioural analysis in rats and mice has demonstrated that, at least in part, the effects of β-phenylethylamine (PEA) are dopamine-mediated (Dourish, 1981, 1982). The aim of the present study was to determine the nature of a possible PEA-NAL interaction affecting unconditioned behaviour in the naive rat.

Subjects were 120 adult, male, Sprague-Dawley rats, weighing 200-250 g. Each rat was injected twice prior to the 30 min test. For the first injection, the animals were allocated to 4 groups (n=36), and were treated with 0, 1.0, 3.0 or 10.0 mg/kg naloxone HCl respectively by S.C. route, 15 min before testing. Within each group, animals were divided amongst 6 groups (n=6 each), and were treated with 0, 6.25, 12.5, 25.0 or 50.0 mg/kg PEA HCl respectively by i.p. injection, immediately before testing. Isotonic saline served as vehicle. Testing was conducted in individual perspex cages positioned in automatic activity recording devices (Opto Varimex Minor), controlled by a microprocessor/microcomputer system. The system generated total horizontal activity (HA), ambulatory (A), and vertical activity (VA) scores. Behavioural components were also observed and rated by two observers according to categories previously defined (Dourish, 1981, 1982).

At the highest dose (50 mg/kg), PEA induced a stereotyped behavioural syndrome, characterized by sniffing, headweaving, forepaw padding, hyperreactivity and splayed hindlimbs. PEA alone did not significantly affect HA, A or VA scores. Similarly, NAL alone did not significantly affect these measures. However, under certain dose combinations, PEA administered in conjunction with NAL produced significant enhancement of HA and A scores. VA scores remained unaffected. Thus, over the first 15 min of the test period, PEA (12.5-50.0 mg/kg) in combination with 10 mg/kg NAL produced substantial increases in HA and A. Other behavioural effects produced by high dose PEA treatment (hyperreactivity, splayed hindlimbs) were attenuated by NAL.

Whilst (+)-amphetamine-induced hypermotility is blocked by NAL, the present data show that PEA and NAL interact to produce striking increases in HA and A. The recent discovery of specific binding sites for PEA in the brain (Hauger et al, 1982), suggests that the combination of PEA action at such sites with antagonist action at opiate receptors may be responsible for intense behavioural activation.

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ANTAGONISM OF THE CONVULSANT EFFECT OF β-PHENYLETHYLAMINE BY BENZODIAZEPINES IN MICE

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Preliminary observations in our laboratory indicated that at high doses β-phenylethylamine (PEA) reliably induced convulsions in mice. The present study was designed to examine further the convulsant properties of PEA and to assess the anticonvulsant effects of some benzodiazepines in this model.

Male Swiss Webster mice (20-28g) were injected i.p. with PEA HCl at doses of 100, 150 or 200 mg/kg (n=10 per group) and placed immediately in individual perspex observation chambers for a 20 min test. Two observers recorded the number of mice which showed convulsions, the latency to first convulsion and the latency to loss of the righting reflex. Chlordiazepoxide (CDP) at doses of 5, 10 or 20 mg/kg diazepam (D) at doses of 2.5, 5 or 10 mg/kg or their vehicle were injected i.p. 15 min prior to PEA (200 mg/kg) in an attempt to antagonise PEA's convulsant effect. PEA at doses of 150 (7 out of 10 animals) or 200 mg/kg (9 out of 10 animals) induced convulsions in mice. A 100 mg/kg dose of PEA did not produce convulsions. The latency to the first convulsion was approximately 11-12 min and the latency to loss of the righting reflex was approximately 13 min. Before and after showing convulsions PEA-treated animals displayed a stereotyped hyperactivity syndrome which was dominated by abortive grooming as previously described (Dourish, 1982a). CDP and D antagonised PEA-induced convulsions in a dose-dependent fashion. CDP (5 mg/kg) had no effect on convulsions. Similarly, CDP (10 mg/kg) did not reduce the number of animals showing convulsions but tended to increase the latency measures. However, a 20 mg/kg dose of CDP completely abolished convulsions in all mice tested. Similarly, 5 or 10 mg/kg of D antagonised PEA's convulsant effects. Interestingly, the mice which were protected against convulsions by CDP or D pretreatment displayed intense gnawing, biting and licking directed at the floor and wall of the chamber. These effects have been described previously following PEA in combination with propranolol or with serotonin antagonists, and have been attributed to a resulting potentiation of PEA's effects on dopaminergic mechanisms (Dourish, 1982b).

The present data confirmed the convulsant potency of PEA in mice when administered in large doses. Benzodiazepine pretreatment successfully abolished the convulsant effect, and produced behaviour which was characteristic of intense dopaminergic activation. Since convulsant and anticonvulsant properties of drugs have been linked to central GABAergic transmission (Meldrum, 1979), the present data imply a GABA antagonistic effect of PEA when given in large doses.

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ARE VASCULAR 'D' AND '5-HT_2' RECEPTORS FOR 5-HYDROXYTRYPTAMINE THE SAME?

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It has been suggested that the 5-HT_2 binding sites, characterised in rat brain using radiolabelled ligand binding, are the same as the 5-HT_2 receptors of rat isolated vasculature at which the novel antihypertensive agent, ketanserin, is a potent antagonist (Van Nueten et al., 1981). Humphrey et al. (1982) have suggested that this 5-HT_2 receptor is similar to the so-called classical "D" receptor, identified in vascular smooth muscle, and which is blocked potently and competitively by the 5-HT antagonists, methysergide and cyproheptadine. The suggestion has been examined further in the isolated rat caudal artery, purported to contain 5-HT_2 receptors (Van Nueten et al., 1981), and the isolated rat aorta, claimed to contain "D" receptors (Forster and Whalley, 1982). The results were compared with data obtained in the rabbit aorta, another preparation containing vascular "D" receptors (Humphrey et al., 1982; see Apperley et al., 1980).

Middle caudal arteries and thoracic aortae were removed from male Wistar rats (200-350g), anaesthetized with sodium pentobarbitone (90 $mg.kg^{-1}$ i.p.) or killed by cervical dislocation. Four cylindrical segments from either vessel were prepared for the recording of isometric contractions using a method similar to that of Edvinsson et al (1974). pA_2 values for the 5-HT antagonists (Table 1) were then determined as described previously (Apperley et al., 1976).

Table 1 : pA_2 values (30 min) for antagonists against 5-HT-induced contractions of isolated vascular preparations.

	Isolated rat caudal artery		Isolated rat aorta		Isolated rabbit aorta*	
	pA_2	Slope	pA_2	Slope	pA_2	Slope
Ketanserin	8.8 (8.4-9.2)	1.0 (0.6-1.3)	8.4 (7.8-9.0)	0.9 (0.8-1.1)	8.7 (8.4-9.0)	0.9 (0.8-1.0)
Methysergide	10.0 (9.5-10.5)	0.7 (0.5-0.8)	7.7 (7.6-7.8)	**	8.5 (7.9-9.1)	0.9 (0.7-1.1)
Cyproheptadine	8.7 (8.5-9.0)	1.3 (1.0-1.6)	9.0 (8.6-9.3)	0.8 (0.6-0.9)	8.7 (8.4-9.1)	0.9 (0.7-1.1)

Each value is the mean (95% confidence limits) of 4-8 separate estimates.

*Data from Humphrey et al (1982)

**Antagonism was not always concentration-dependent.

The data suggests that the recently described "5-HT_2" receptor is similar to the classical "D" receptor. However ketanserin alone of the antagonists acted competitively in every preparation.

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FURTHER EVIDENCE FOR THE HETEROGENEITY OF VASCULAR RECEPTORS FOR 5-HYDROXYTRYPTAMINE

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Based upon the differences in rank-orders of agonistic potencies of a series of tryptamine analogues (Feniuk et al., 1981) and the antagonistic activity of the 5-HT 'D' receptor antagonist, methysergide (Apperley et al., 1980), we have previously suggested that the 5-HT receptor mediating contraction in the rabbit isolated aorta and dog saphenous vein differ. We now present evidence, using the selective 5-HT_2-ligands, ketanserin and spiroperidol which supports our original contention.

Spiral vascular strips of rabbit isolated aorta and dog saphenous vein were prepared as previously described for measurement of isometric tension changes and the antagonistic potencies of ketanserin (3.0×10^{-9} - 3.0×10^{-7} mol/l) and spiroperidol (3.0×10^{-9} - 3.0×10^{-7} mol/l) determined by calculating their pA_2 values, using 5-HT or the α-adrenoceptor agonist methoxamine as agonists. The pA_2 values were calculated according to the method of Arunlakshana & Schild (1959). The results are summarised in Table 1.

Table 1: Antagonistic potencies of ketanserin and spiroperidol against 5-HT induced contractions of dog saphenous vein and rabbit isolated aorta.

		RABBIT AORTA		DOG SAPHENOUS VEIN
		5-HT	Methoxamine	5-HT
Ketanserin	pA_2	8.67 (8.38 - 8.95)	7.80 (7.32 - 8.27)	< 6
	slope	0.94 (0.84 - 1.03)	1.05 (0.64 - 1.46)	
Spiroperidol	pA_2	8.64 (8.34 - 8.95)	7.76 (7.34 - 8.18)	< 6
	slope	1.15 (1.01 - 1.28)	1.14 (0.81 - 1.46)	

pA_2 values calculated according to Arunlakshana & Schild (1959). Values are means (95% confidence limits) from at least 4 experiments.

Ketanserin and spiroperidol were potent competitive antagonists of 5-HT in the rabbit aorta and weaker competitive antagonists of methoxamine. In contrast neither compound antagonised the contractile effect of 5-HT in the dog saphenous vein.

The results from this study provide confirmatory evidence that the 5-HT receptor mediating contractions in the rabbit aorta and dog saphenous vein differ. Furthermore the high affinity of ketanserin and spiroperidol at 5-HT_2 binding sites (Leysen et al., 1982) and at vascular 'D' receptors in the rabbit aorta (see Apperley et al., 1980)suggest that both may be similar.

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CHRONIC ANTIDEPRESSANT TREATMENT, HORMONAL MANIPULATION AND CORTICAL SEROTONIN S_2 RECEPTORS

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Chronic tricyclic antidepressants cause a down-regulation of serotonin (S_2) receptors in rat frontal cortex (Peroutka & Snyder, 1980). The loss following imipramine treatment was reported to be prevented by ovariectomy (Kendall et al., 1981). Similarly the up-regulation of central dopamine receptors by long term haloperidol has been reported to be prevented by hypophysectomy (Hruska et al., 1980). Therefore, we have investigated the effect of chronic administration of several psychotropic drugs on S_2 receptors of frontal cortex of ovariectomised, hypophysectomised and sham-operated rats.

From each of these groups, rats (180-220g) were injected intraperitoneally with a tricyclic antidepressant, imipramine ($10mgkg^{-1}$), an atypical antidepressant, mianserin ($10mgkg^{-1}$), a psychostimulant, methylphenidate ($5mgkg^{-1}$), a specific serotonin uptake inhibitor, citalopram ($10mgkg^{-1}$) or vehicle, daily for twenty one days. Forty eight hours after the last injection animals were killed by decapitation and frontal cortices removed. A mixed microsomal - mitochondrial preparation was made (Peroutka & Snyder, 1979) and K_d and B_{max} values for (^3H) spiperone binding were measured from full saturation analyses according to the method of Withy et al. (1981) (specific binding defined using 1μM LSD.)

No significant differences in the K_d and B_{max} values were obtained between the operated and sham-operated animals for imipramine or any other of the treatments. Therefore, all the values for each drug treatment irrespective of operation were combined and the average K_d and B_{max} values were calculated (Table 1). Only the B_{max} values for imipramine and mianserin treated rats were significantly lower than the control values. There were no significant changes in the K_d values indicating that the changes in receptor number were not due to residual drug.

Table 1 Binding parameters of S_2 receptors after various treatments

Treatment	B_{max} (fmoles mg^{-1})	K_d (nM)
Distilled water	244.8 ± 12.9	0.36 ± 0.03
Methylphenidate	243.1 ± 12.5	0.36 ± 0.02
Imipramine	157.7* ± 9.1	0.32 ± 0.03
Mianserin	101.0* ± 8.1	0.48 ± 0.12
Citalopram	211.8 ± 20.9	0.44 ± 0.06

*$P<0.05$; values ± s.e. mean

The results show that only the chronic treatment of rats with imipramine and mianserin down-regulated S_2 cortical receptors in the rat. There was no S_2-receptor loss after citalopram or methylphenidate showing that raising the local concentration of either serotonin or dopamine alone is not a sufficient stimulus for down-regulation. The changes observed were not affected by the hormonal manipulations tested.

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PHENYLBIGUANIDE MIMICS THE EFFECTS OF 5-HYDROXYTRYPTAMINE ON THE RAT ISOLATED VAGUS NERVE AND SUPERIOR CERVICAL GANGLION

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Fastier et al (1959) reported that phenylbiguanide (PB) activated sensory structures that responded to 5-hydroxytryptamine (5HT), but was devoid of 5HT-mimetic effects on either rat blood vessels or stomach strip. 5HT-induced depolarisations of the rat isolated vagus nerve (VN) and superior cervical ganglion (SCG) are distinct from 5HT effects on many smooth muscle preparations in their resistance to blockade by methysergide, although metoclopramide behaves as a competitive antagonist of 5HT on both neuronal tissues (Ireland et al., 1983). It was therefore of interest to investigate the possibility that PB may interact with the 5HT receptor present on the VN and SCG.

Male lister hooded rats (270-330g) were anaesthetised with chloral hydrate (300mg/kg i.p.), and the VN or SCG were excised and desheathed. Agonist-induced depolarisations were recorded extracellularly from VN or SCG preparations mounted in 2-compartment perspex baths (Ireland et al. 1982).

On the VN, PB, $3x10^{-7}$-$3x10^{-5}$M produced rapid depolarisations of similar latency to 5HT-induced responses. The equipotent molar ratio (EPMR) (Drug/5HT) for PB on the VN ranged from 1.4 to 1.6. The maximum response evoked by PB was approximately 79-81% of the 5HT maximum. Metoclopramide, $1x10^{-6}$-$x10^{-4}$M, caused parallel rightwards shifts of the PB dose- response curve. Analysis of the antagonism by the method of Arunlakshana and Schild (1959), yielded a straight line of gradient 0.92 and a pA_2 of 6.48. Corresponding values for the antagonism of 5HT were 0.98 and 6.60 respectively (Ireland et al., 1983). Neither slope was significantly different from unity ($p>0.05$). It was found that in addition to DMPP and GABA (Ireland et al., 1982) (-)-noradrenaline, $3x10^{-7}$-$3x10^{-4}$M depolarised the VN. The effects of these agonists were virtually abolished by hexamethonium, $3x10^{-4}$M, bicuculline, $1x10^{-5}$M, and phentolamine, $1x10^{-6}$M, respectively. However, at these concentrations these antagonists failed to affect the dose response curves to either 5HT or PB or modify the antagonism of 5HT and PB by metoclopramide.

On the SCG, PB, $1x10^{-6}$-$3x10^{-4}$M, induced rapid, dose-related depolarisations that closely resembled those produced by 5HT. The EPMR values for PB on the SCG were between 0.92 and 1.24. The maximum response to PB was 89-97% of the tissue 5HT maximum. Metoclopramide, $3x10^{-6}$-$1x10^{-4}$M, produced parallel rightwards shifts of the PB dose-response curve. A plot of log (dose ratio-1) against log of the antagonist concentration, was a straight line with a slope of 0.83. The pA_2 was 5.58. Values previously obtained with metoclopramide against 5HT on the SCG were 0.82 and 5.74 respectively (Ireland et al., 1983). Neither gradient was significantly less than unity ($p >0.05$). PB and 5HT-induced depolarisations of the SCG were not antagonised by hexamethonium, $3x10^{-4}$M, picrotoxin, $3x10^{-5}$M, (-) propranolol, $1x10^{-7}$M, and atropine, $1x10^{-5}$M. These antagonists also failed to modify the antagonism of 5HT or PB produced by metoclopramide.

The results clearly demonstrate that on both the rat VN and SCG, there is a close similarity between the depolarising actions of PB and 5HT. It is suggested that the effects of 5HT and PB may be mediated through the same 5HT receptor subtype.

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HAEMODYNAMIC EFFECTS OF CIRAZOLINE IN DOGS

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Cirazoline is an imidazoline derivative which is being developed as a nasal vasoconstrictor. This compound is a strong postjunctional vascular α_1-adrenoceptor agonist with prejunctional α_2-adrenoceptor antagonist properties (Cavero et al., 1982). At the latter receptors, it may behave as a partial agonist (see Cavero et al., 1982: discussion section). This communication describes the haemodynamic effects of cirazoline in comparison to phenylephrine.

Dogs weighing 14-18 kg were anaesthetized with pentobarbitone (35 mg/kg i.v.) and placed under artificial respiration. They were prepared for the measurement of mean aortic pressure (MAP: mmHg), cardiac output (CO: l/min/10 kg), total peripheral vascular resistance (TPR: mmHg/l/min/10 kg), heart rate (beats/min), femoral (FBF: ml/min/10 kg) renal (RBF: ml/min/10 Kg) and superior mesenteric artery (MBF; ml/min/10 kg) blood flows, left ventricular end diastolic pressure (LVEDP: mmHg) and the natural logarithm (ln) of $dLVP_{50}/dt$ (sec^{-1}), a left ventricle contractility parameter. Cirazoline (0.3, 1.0 and 3.0 µg/kg i.v.) and phenylephrine (1.0, 3.0 and 10.0 µg/kg, i.v.) were given as bolus injections. In 2 animals the effects of a 15 min infusion of cirazoline (1.0 µg/kg/min) were also studied.

I.v. administration of cirazoline and phenylephrine produced profound dose-related changes in most of the measured hemodynamic parameters as reported in the following table where the base-line mean values (C) and their percent changes (% Δ) are given. An asterisk indicates a significant response ($p<0.05$: paired t-test). The effects of these bolus injections lasted between 3 and 5 min. However, they persisted when the cirazoline was given by infusion.

	Cirazoline (n=6)				Phenylephrine (n=6)	
Parameter	1.0 µg/kg i.v.		3.0 µg/kg i.v.		10.0 µg/kg i.v.	
	C	% Δ	C	% Δ	C	% Δ
MAP	113.8 ± 3.4	21*	119.2± 1.8	45*	117.0± 3.7	25*
CO	1.6± 0.1	-31*	1.5± 0.2	-58*	1.4± 0.2	-30*
TPR	75.2± 7.8	82*	83.0± 9.4	291*	96.1±14.7	89*
HR	158.3±12.9	-10*	156.0±13.2	-23*	159.0±14.9	-7*
MBF	270.5±63.7	-37*	266.7±62.2	-71*	288.8±76.2	-39*
RBF	218.2±46.0	-39*	217.0±46.2	-84*	216.0±44.8	-32*
FBF	90.8±17.6	-20*	95.5±18.7	-40*	101.3±23.5	-24*
LVEDP	6.6± 0.8	57*	7.0± 0.8	172*	7.4± 0.9	59*
$\ln(dLVP_{50}/dt)$	38.7± 3.5	-7	38.3± 3.1	-12	37.7± 3.2	-7

These results indicate that cirazoline produces profound haemodynamic changes which are compatible with a stimulation of postjunctional α-adrenoceptors. These effects should be taken into account when studies unrelated to its main pharmacological property are carried out under in vivo conditions since certain responses may be influenced by the haemodynamic state of the animal.

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EFFECTS OF CIRAZOLINE ON NASAL CAVITY PRESSURE MEASURED BY PLETHYSMOGRAPHY IN ANAESTHETIZED CATS

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Cirazoline is an imidazoline derivative which is presently being developed as a nasal vasoconstrictor. Its pharmacological profile indicates that the compound is primarily a postjunctional α_1-adrenoceptor agonist and, in addition, it can block and stimulate prejunctional α_2-adrenoceptors (Cavero et al., 1982: see discussion section). The aim of this paper is to show that the potential therapeutic action of cirazoline occurs in cats at doses which are several times below those that were found to produce effects which are apparently compatible with a stimulation of cardiac prejunctional α_2-adrenoceptors (Duval & Langer, this meeting).

Cats weighing 2-3 kg were anaesthetized with pentobarbitone (35 mg/kg i.v.). The trachea was cannulated and artificial respiration performed throughout the experimental procedure. Blood pressure was measured from a cannulated carotid artery and drugs were administered by the i.v. route. Nasal cavity pressure was measured with a plethysmographic method (Loux, 1970). The effects of i.v. cirazoline (0.5, 1.0 and 2.0 µg/kg), phenylephrine (0.5, 1.0, 2.0 µg/kg) and adrenaline (0.5 µg/kg i.v.) were studied on carotid artery blood pressure and nasal cavity pressure. It should be noted that an enlargement of the nasal cavity (e.g. as produced by vasoconstrictor agents) manifests itself as a fall in intranasal pressure.

The table reports the maximal effects of cirazoline, phenylephrine and adrenaline on nasal cavity pressure (NCP) and on the changes (Δ) in carotid artery systolic pressure (SBP) from control (C) values in a group of 4 cats.

Compound	Dose (µg/kg i.v.)	NCP (cm H_2O)	SBP (mmHg)	
			C	Δ
Cirazoline	0.5	-3.3 ± 0.4	168 ± 7	+28 ± 1
	1.0	-5.0 ± 0.5	164 ± 8	+52 ± 4
	2.0	-5.6 ± 0.7	166 ± 4	+57 ± 1
Phenylephrine	0.5	-2.0 ± 0.4	166 ± 8	+20 ± 4
	1.0	-2.7 ± 0.3	164 ± 7	+25 ± 3
	2.0	-3.1 ± 0.5	168 ± 4	+32 ± 5
Adrenaline	0.5	-4.6 ± 0.3	166 ± 7	+22 ± 1

These results indicate that cirazoline is a potent nasal vasoconstrictor and hypertensive agent in intact anaesthetized cats.

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MODIFICATION OF RESPONSES TO MONOAMINES BY SOME CARBONYL REAGENTS IN THE RAT ANOCOCCYGEUS MUSCLE

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The rat anococcygeus muscle has been shown to contain more than one amine oxidising activity. Mitochondrial monoamine oxidase (MAO), which may exist in two forms known as A and B, is found predominantly as MAO-A and another semicarbazide sensitive, clorgyline-resistant amine oxidase (CRAO) has been detected (Callingham, 1981). Unlike MAO, CRAO is sensitive to inhibition by a variety of carbonyl reagents which include semicarbazide, benserazide and hydralazine (Coquil et al. 1973; Lyles & Callingham, 1982). Not only are the observed specific activities of these enzymes dependent upon the particular substrate and its concentration, but the enzymes themselves are located differently in the cell with MAO on the mitochondria and CRAO on the cell membrane (Wibo et al. 1980).

The possible pharmacological importance of the CRAO activity was examined by measuring the effect of selective enzyme inhibitors on the contractile responses of the rat anococcygeus muscle to appropriate monoamines. Pairs of rat anococcygeus muscles from male Wistar rats (250-300g) were suspended under 1g tension in 10 ml organ baths containing Krebs-Henseleit solution at 37°C gassed with O_2 and CO_2 and contractile responses to a variety of amines measured isometrically. (-)Noradrenaline (NA), adrenaline (AD) and dopamine (DA) were left in contact with the tissue for 30 sec before washout while indirectly acting sympathomimetic amines, tyramine (TYR), β-phenethylamine (PEA), isoamylamine (IAA) and benzylamine (BZ) were left for 1.5 min. Responses to amines were compared before and after exposure to hydralazine (5×10^{-6}M), benserazide (5×10^{-5}M) and semicarbazide (10^{-3}M) which selectively inhibit CRAO. The technique of oil immersion on washout of drug (Kalsner & Nickerson, 1969) was employed to compare rates of disposition of amine before and after exposure to inhibitors.

Directly acting amines (-)-NA (3 μM), (-)-AD (1 μM) and DA (10 μM) produced responses that were largely unaltered by these inhibitors, both in height and duration, in either Krebs or mineral oil. Responses to TYR (10 μM) were not significantly prolonged by the inhibitors in Krebs solution and only by about 10% when the Krebs solution was replaced by mineral oil at the peak of contraction. Responses to PEA (30 μM), IAA (300 μM) and BZ (1 mM) were potentiated in height and duration by inhibitors on washout with Krebs and more particularly with oil. Benserazide increased responses to PEA, IAA and BZ in both height and duration resulting in a 3-fold increase in the areas under the response curves. In oil, the potentiated responses were maintained as plateau contractions for longer than 10 min. Semicarbazide also caused 3-fold potentiations of PEA, IAA and BZ responses, and plateau contractions to these amines in oil. Hydralazine increased the area of the response curves to IAA, PEA and BZ about 4-fold and also produced plateau contractions in oil.

These results may indicate that inhibition of CRAO activity, which was confirmed by enzyme assay in homogenates of rat anococcygeus muscle, is capable of prolonging the contractions produced by PEA, IAA and BZ particularly under conditions where these drugs are maintained in the vicinity of the cell membrane.

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MONOAMINE OXIDASE ACTIVITIES IN RAT MESENTERIC ARTERIES AND VEINS

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Many mammalian tissues contain two forms of monoamine oxidase, MAO-A and MAO-B (see Fowler et al, 1978) while some may also contain another monoamine oxidase activity inhibited by carbonyl reagents but not by acetylenic MAO inhibitors such as clorgyline and pargyline. This clorgyline-resistant amine oxidase (CRAO), first identified in rat aorta by Coquil et al. (1973) has been shown to be concentrated in the cell membrane of vascular smooth muscle (Wibo et al, 1980) and of brown adipose tissue (Barrand & Callingham, 1982), where it appears to be able to deaminate primary monoamines.

In the light of the possible importance of these enzymes in deaminating not only endogenous but also ingested monoamines, a preliminary study has been made of their activities towards suitable substrates in the mesenteric blood vessels of the rat.

Mesenteric arteries and veins were dissected from male Wistar rats by the method of Kwang et al. (1981), to remove almost all contaminating fat and connective tissue. The vessels were homogenized separately (1:20 w/v) in 1 mM potassium phosphate buffer, pH 7.8. The supernatant fractions following centrifugation at 600g for 10 min were used for assay of MAO and CRAO activities with [^{3}H]-tyramine (TYR), [^{14}C]-benzylamine (BZ) and [^{14}C]-β-phenethylamine (PEA) as substrates.

Use of the selective MAO inhibitor clorgyline, showed that deamination of BZ (0.01 - 1.0 mM) in both arteries and veins was almost entirely by CRAO. Even at 1.0 mM BZ no significant MAO activity could be found. About half the deamination of TYR (0.01 - 1.0 mM) was brought about by CRAO while the relative proportions of MAO-A and MAO-B depended upon substrate concentration. In the arteries deamination of 0.01 mM TYR by MAO was due to MAO-B while at 1.0 mM 70% of the MAO activity was MAO-A. In the veins some MAO-A activity was seen at 0.01 mM TYR but it only reached about 40% at 1.0 mM. With PEA as substrate both MAO-A and MAO-B could be detected but more than 70% of its deamination was by CRAO.

Double reciprocal analysis produced single component regressions for all three substrates over the relevant concentration range for each enzyme. No significant differences in Km values between arteries and veins were seen, either for MAO or CRAO but detailed analysis of MAO-A and MAO-B was not attempted. Mixed substrate experiments showed that TYR and PEA competed with BZ for deamination by CRAO yielding Ki values similar to their Km values for this enzyme. It was also found that 5-hydroxytryptamine which is not a substrate for CRAO in mesenteric blood vessels caused mixed inhibition of BZ deamination, with a Ki value from the slope replot of 0.19 mM.

Homogenates of mesenteric blood vessels, possess MAO-A, MAO-B and CRAO activities whose relative importance depends upon the particular substrate and its concentration. The activity of CRAO is possibly sufficient for it to be an important factor in the deamination of circulating primary monoamines in this vascular bed without the need for an uptake system across the cell.

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HISTOCHEMICAL LOCALISATION OF CLORGYLINE-RESISTANT AMINE OXIDASE AND ITS EXTRACTION FROM CELL MEMBRANES

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A clorgyline resistant amine oxidase (CRAO), distinct from the flavin dependent monoamine oxidase (MAO), and more closely resembling the copper- and pyridoxal phosphate-dependent amine oxidases of plasma and connective tissue, has been identified in brown adipose tissue of the rat. From its subcellular distribution it appears that the enzyme is largely membrane bound, most probably to the outer cell membrane (Barrand & Callingham, 1982). Further investigation of this enzyme has now been undertaken.

Visualisation of enzyme activity in interscapular brown adipose tissue of male Wistar rats at the ultrastructural level has been attempted using a method adapted from that of Hanker et al, (1973) for monoamine oxidase. Positive staining in mitochondria was achieved by this method using the MAO substrate tryptamine. Staining along the edge of the brown fat cells, but not around the endothelial cells of blood capillaries was observed when the CRAO substrate benzylamine (BZ), or tyramine and in some cases tryptamine were used. Staining was largely absent when substrate was omitted, and reduced when the tissues were preincubated with hydralazine (an inhibitor of CRAO activity). Whether this staining actually represents sites of CRAO activity is not yet clear but certainly such a location fits with the available biochemical evidence.

Solubilisation of the enzyme has also been attempted. Mild treatments e.g. altering ionic strength, EDTA treatment, that are known to remove loosely bound proteins from membranes (Coleman, 1973) were ineffective in releasing CRAO, indicating that attachment to the membrane may involve some hydrophobic component. Lipid extraction with butanone released the enzyme into the solvent, with some loss of activity. In the presence of the non-ionic detergent Triton X-100 at concentrations between 0.02% and 2%, no significant loss of CRAO activity was seen, unlike with MAO activity (Fowler et al, 1980), and at a Triton/protein ratio of 0.3-0.4 the enzyme became released from the membrane. Gel filtration of the solubilised enzyme through an Ultrogel AcA 34 column in the presence of 0.1% Triton resulted in a band of CRAO activity (recovery about 70-80%) within the gel corresponding to a MW between 160,000-180,000. No alteration in Km for BZ was noted after this treatment. Removal of Triton from the enzyme either by dialysis or by ammonium sulphate precipitation followed by desalting through a Sephadex G-25 column resulted in the enzyme no longer being delayed within the gel but appearing instead in the void volume.

A 15-fold purification of the enzyme was achieved by selective solubilisation with Triton and gel filtration. This preparation gave a single band of protein on SDS gel electrophoresis. CRAO-specific radioactive ligand binding is however necessary to prove that this band comprises the soluble CRAO itself. It would appear that this approach yields a soluble fraction of CRAO that does not differ significantly in its kinetic properties from CRAO bound to the cell membrane and is thus a suitable preparation for detailed biochemical study.

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MONOAMINE OXIDASE ACTIVITIES OF RAT ISOLATED VENTRICULAR MUSCLE CELLS

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Selective effects of inhibitors on the metabolism of monoamines by rat whole heart homogenates have indicated the existence of three amine oxidase activities in this organ. These include mitochondrial monoamine oxidase (MAO) A and B, and also an enzyme sensitive to inhibition by carbonyl reagents (e.g. semicarbazide) but resistant to the MAO inhibitor clorgyline (Lyles and Callingham, 1975). The latter enzyme, of unknown physiological importance, is further distinguishable from MAO by virtue of a much lower K_m (around 5 μM) for benzylamine (BZ) metabolism, and it is consequently responsible for almost all rat heart oxidation of BZ when very low (e.g. 1 μM) assay concentrations of amine are employed (Clarke et al, 1982). The heart contains various constituents, including muscle and other cells, as potential sources of these enzymes. Their possible localization in isolated rat ventricular myocytes is examined here.

Ventricles from male Wistar rats (250 - 320g) were homogenized individually in 1mM potassium phosphate pH 7.8. Homogenates were centrifuged at 800g for 10 min and supernatants used for enzyme assays. Myocytes were obtained from pooled ventricles of 3-4 rats by collagenase dispersal of minced tissue, filtration of separated cells through nylon mesh, and selective isolation of muscle cells by centrifugation through dense media containing bovine serum albumin (Glick et al, 1974; Powell et al, 1980). Production of highly enriched myocyte fractions by these methods was monitored by light microscopy. Cells were washed, resuspended and homogenized in 1mM potassium phosphate in order to yield corresponding supernatants to those above for comparative enzyme assays.

Specific deaminating activities (nmol/h/mg protein) of homogenates from ventricular tissue (n = 6 rats) and myocytes (n = 5 preparations), respectively towards the substrates ^{3}H-5-hydroxytryptamine (5-HT) and ^{14}C-BZ were: 5-HT (at 1mM), 220 $\pm$ 20 and 275 $\pm$ 79; BZ (at 1mM), 7.1 $\pm$ 0.8 and 4.9 $\pm$ 0.6; BZ (at 1μM), 1.12 $\pm$ 0.05 and 0.67 $\pm$ 0.10.

Inhibition of amine metabolism by varying concentrations of clorgyline was studied in some homogenates. Similar inhibition patterns in cell (n = 3) and tissue (n = 5) homogenates were obtained for each substrate. Clorgyline (10^{-7}M) completely inhibited 5-HT metabolism, indicating only MAO-A activity towards this substrate. Biphasic inhibition curves with clorgyline (10^{-10} to 10^{-3} M) showed a proportional contribution to 1mM BZ metabolism of around 50% (MAO-A) and 30% (MAO-B). The residual activity (20%) was not inhibited by 10^{-3}M clorgyline. With 1μM BZ, the clorgyline-resistant activity represented around 90% of total deamination. In addition, the clorgyline-resistant proportions of BZ metabolism (at 1μM or 1mM) corresponded to those proportions found to be sensitive to inhibition by 10^{-3}M semicarbazide.

In conclusion, these results indicate the presence of MAO-A and -B and also the clorgyline-resistant, semicarbazide-sensitive amine oxidase in isolated ventricular muscle cells. The activities and properties of these enzymes appear to be similar to those of the whole ventricular tissue from which the myocytes are derived.

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CIRAZOLINE (LD3098) STIMULATES PRESYNAPTIC α_2-ADRENOCEPTORS IN THE CAT'S HEART

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The importance of species differences in studying the effects of drugs acting at presynaptic, release modulating receptors has been stressed recently (Langer, 1980). Cirazoline is a potent α_1-adrenoceptor agonist which has the unique property of blocking α_2-adrenoceptors (Cavero et al., 1982). The stimulation of presynaptic α_2-adrenoceptors by cirazoline was reported in the perfused cat spleen (Dubocovich et al., 1980) but not in tissues from the rat or the dog (Cavero et al., 1982). Since these apparently divergent results may simply reflect species differences we decided to study the effects of cirazoline and clonidine on the α_2-adrenoceptors that modulate the release of noradrenaline in the cat's heart.

Female cats weighing 2.6 - 3 kg were anaesthetized with pentobarbital (45 mg/kg i.p.), artificially respired and bivagotomised. Carotid blood pressure and heart rate were recorded. The effects of cirazoline on α_2-receptors of sympathetic nerves was assessed using the procedure of Dubocovich et al. (1980). The ansa subclavia was stimulated for 45 sec. periods at 0.1 - 0.25 - 0.5 and 1 Hz, using supramaximal voltage and a 1 ms pulse width. When stable responses had been obtained, prazosin was administered (300 µg/kg i.v.) to block α_1-adrenoceptors. Ten minutes later the frequency response curve to accelerans nerve stimulation was repeated and in a series of control experiments the positive chronotropic responses to accelerans nerve stimulation remained constant throughout the experiment.

The resting heart rate was 139 $\pm$ 8 b/min (n=4) in the group receiving cirazoline and 153 $\pm$ 4 b/min in the group receiving clonidine. There were no significant changes in basal heart rate throughout the experiment in both experimental groups. As shown in Table 1, both cirazoline and clonidine inhibited the neurally induced tachycardia in a dose-dependent manner.

Table 1 : Effects of cirazoline and clonidine on the tachycardia elicited by accelerans nerve stimulation in the cat

Dose µg/kg i.v.	% INHIBITION OF NEURAL TACHYCARDIA							
	CIRAZOLINE n=4				CLONIDINE n=3			
	0.1 Hz	0.25 Hz	0.5 Hz	1 Hz	0.1 Hz	0.25 Hz	0.5 Hz	1 Hz
10	26 ± 5	13 ± 4	11 ± 3	8 ± 1	46 ± 7	28 ± 8	12 ± 2	4 ± 2
30	35 ± 11	20 ± 5	14 ± 2	10 ± 4	73 ± 3	60 ± 5	32 ± 2	12 ± 4
100	54 ± 9	37 ± 10	26 ± 3	16 ± 3	87 ± 2	72 ± 6	52 ± 7	14 ± 3
300	88 ± 8	66 ± 7	46 ± 10	28 ± 9	89 ± 3	81 ± 5	62 ± 8	23 ± 3

The inhibition by clonidine was more frequency dependent than that obtained by cirazoline and clonidine was approximately 3 times more potent than cirazoline (Table 1). These inhibitory effects of cirazoline and clonidine were antagonized by the selective α_2-adrenoceptor antagonist RX 781094 at 300 µg/kg, i.v. (Chapleo et a., 1981).

It is concluded that cirazoline, a potent α_1-adrenoceptor agonist, has presynaptic α_2-adrenoceptor agonist properties in the cat.

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ABSORPTION OF OESTRONE, OESTRONE GLUCURONIDE AND OESTRONE SULPHATE FROM RAT GUT IN SITU

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The in vivo absorption of estrone (E_1), estrone glucuronide (E_1G), and estrone sulphate (E_1S) from the rat intestine has been studied using a closed loop technique based on the method of Doluisio et al., (1969). Following anaesthesia with urethane, the small intestine was isolated and cannulated at the duodenal and ileal ends with glass cannulae connected to a three-way tap and 20 ml syringe. The bile duct was cannulated. The entire length of intestine was cleared of particulate matter by perfusing with a physiological buffer (pH 6.5) at 37°C.

Rats fasted overnight were divided into 9 groups and [^{3}H]E_1 (10 nM, 1 µM, 10 µM; 0.5 µCi/ml in pH 6.5 phosphate buffered saline, PBS : ethanol, 9:1 v/v; (10 ml), [^{3}H]E_1G (200 nM, 10 µM, 100 µM; 0.5 µCi/ml in PBS; 10 ml) and [^{3}H]E_1S (10 nM, 10 µM, 100 µM; 0.5 µCi/ml; 10 ml) pumped into the intestinal lumen. [^{14}C] Polyethylene glycol 4000 (0.1 µCi/ml of perfusate) was used as a non-absorbable marker. Aliquots (50 µl) were removed from the two syringes alternately at 5 min intervals for 40 min (E_1) and 10 min intervals for 1h (E_1G and E_1S), and the radioactive content determined. Radioactivity was also determined in aliquots of bile.

The absorption of E_1 was very rapid and the absorption curve followed a biexponential profile. The half life of the first phase ($t1/2\alpha$) which approximated to the time required for the drug concentration to decrease to half in the lumen, was 4.7 ± 0.3 (mean ± S.D.; 10 nM), 4.7 ± 0.7 (1 µM) and 4.5 ± 0.4 min (10 µM). The half life of the second phase ($t1/2\beta$) was approximately 27 min for each concentration. In all experiments more than 90% of the dose was absorbed in 40 min. The peak drug concentration appeared in bile between 15 and 20 min.

The absorption profiles of the conjugated steroids were monoexponential and the time required for the original drug concentration to decrease to half calculated by regression analysis to be as follows: E_1G, 159 ± 6 (200 nM), 229 ± 58 (10 µM), 299 ± 59 min (100 µM); E_1S, 215 ± 14 (10 nM), 200 ± 40 (10 µM), 157 ± 17 min (100 µM). The percentage loss of E_1G from the lumen in 60 min was 24% (200 nM), 19% (10 µM) and 15% (100 µM) and of E_1S, 18% (10 nM), 22% (10 µM) and 25% (100 µM).

The results of this study indicate that not only are E_1G and E_1S poorly absorbed in comparison with E_1 but also there is some intact absorption of the conjugates. The biphasic decline in E_1 is consistent with a three compartment absorption model with accumulation in the tissue compartment.

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α-RECEPTORS ASSOCIATED WITH FLUID ABSORPTION IN RAT JEJUNUM AND ILEUM

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There is evidence that the splanchnic sympathetic nervous system is involved in the control of fluid and ion absorption by the small intestine. Splanchnic nerve stimulation (Brunsson et al. 1979) or exogenous noradrenaline, both in vivo (Levens et al. 1979) and in vitro (Field & McColl 1973), all enhance absorption. These responses appear to be through α-adrenoceptors since phentolamine but not propanolol will partially reverse the effects of noradrenaline. This study was undertaken to further characterise the receptor system involved in the control of absorption by rat small intestine.

The everted sac technique of Wilson & Wiseman (1954) was used. Fluid absorption was measured in sacs of proximal jejunum and distal ileum which were incubated in the presence or absence of 10^{-3}M noradrenaline and varying concentrations of α-adrenoceptor antagonists. Results are expressed as the IC_{50} which is the concentration of antagonist necessary to produce a 50% inhibition of the maximum response to noradrenaline.

The IC_{50} values in the jejunum were indoramin, 100nM; prazosin, 180nM; phentolamine, 4μM; WB4101 (2-([2',6'-dimethyoxy]-phenoxyethylamino) methylbenzodioxane), 19μM; yohimbine, 70μM; rauwolscine, 260μM. It would appear that α_1-adrenoceptor antagonists inhibit jejunal fluid absorption at low concentrations whereas α_2-adrenoceptor antagonists are only effective at concentrations which are likely to produce non-specific actions. These results are consistent with the conclusion that, unlike secretion in the rat jejunum which is an α_2-mediated process (Nakaki et al. 1982), noradrenaline-stimulated absorption by this area is an α_1-mediated event.

The terminal ileum gives a response to noradrenaline which is similar to that observed in the jejunum. However, the effects of α-adrenoceptor antagonists are very different. Phentolamine was the most effective antagonist with an IC_{50} of 150nM. Prazosin and indoramin showed only partial inhibition (40% and 60% respectively) at concentrations of 100μM to 1mM. Yohimbine and rauwolscine were effective over a similar concentration range to that defined in the jejunum (IC_{50} 400μM and 63μM respectively). However the potency of prazosin was increased (IC_{50} 155nM) in the presence of 1μM yohimbine. Similarly, the potency of yohimbine was also increased (IC_{50} 20nM) in the presence of 100μM prazosin.

These results are consistent with the view that noradrenaline-stimulated fluid absorption is mediated by an α_1 adrenoceptor in the jejunum while in the ileum both α_1 and α_2 adrenoceptors are involved and, in this area of the intestine, inhibition of one receptor is compensated by the activity of the alternative receptor.

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ANTAGONIST ACTIONS OF SOME NOVEL BENZOQUINOLIZINES AT 5-HYDROXY-TRYPTAMINE RECEPTORS AND α_2-ADRENOCEPTORS

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The alpha$_2$ adrenoceptor antagonism of closely related benzoquinolizines has been reported by Lattimer et al (1982). In further studies of these compounds their antagonism of 5-hydroxytryptamine (5-HT) has been compared with that of some yohimbine stereoisomers. It has been observed by Weitzell et al, (1979) that the potency order of the yohimbine stereoisomers as alpha$_2$ antagonists closely resembled that as 5-HT antagonists reported by Lambert et al (1978). It was of interest to investigate if a similar relationship was true for the chemically dissimilar benzoquinolizine alpha$_2$ adrenoceptor antagonists.

The rat isolated ileum was used to assess 5-HT antagonism, Sakai et al (1979). Dose-response curves to single doses of 5-HT were obtained using isometric recording. Each tissue was exposed to three concentrations of antagonist, with an equilibrium time of 30 min. at each concentration. Between 3 and 6 determinations were made with each compound. Data were analysed by the method of Arunlakshana and Schild (1959).

Table 1. Antagonism at 5-HT receptors and alpha$_2$-adrenoceptors.

Drug	Alpha$_2$			5-HT		
(Wy No)	pA$_2$	(95% limits)	Slope	pA$_2$	(95% limits)	Slope
26703	8.46	(8.17-8.94)	0.89	7.25	(6.67-8.77)	1.02
26392	8.08	(7.84-8.43)	1.13	5.96	(5.69-6.39)	1.10
25309	7.81	(7.52-8.32)	1.08	5.21	(4.88-6.33)	1.07
24965	6.27	(5.98-6.98)	0.68	<5.0		
Yohimbine	7.58	(7.44-7.70)	0.83	7.89	(7.44-8.84)	0.64
Rauwolsince	7.65	(7.51-7.81)	1.10	7.38	(7.19-7.72)	0.84
Corynanthine	5.49	(5.33-5.65)	-	5.40	(5.22-5.48)	1.20

The present results together with those of Lattimer et al (1982) suggest that, with the exception of Wy 24965, these benzoquinolizines display competitive antagonist activity at both alpha$_2$-adrenoceptors and 5-HT receptors in these test systems, their potency at 5-HT receptors being substantially less than that at the alpha$_2$-site.

The 5-HT antagonist potency of yohimbine is much greater than that of the benzoquinolizines with comparable alpha$_2$ antagonist potency.

The similarity in potency at the two receptor sites amongst the yohimbine isomers noted by Weitzell et al (1979) and confirmed in our present study (with the exception that yohimbine appeared a more potent 5-HT antagonist than rauwolscine) also appears to be the case within this group of benzoquinolizines. Thus, for both alpha$_2$-adrenoceptors and 5-HT receptors, the potency order is:- Wy 26703 > Wy 26392 > Wy 25309 > Wy 26965.

Arunlakshana, O. and Schild, H.O. (1959) Br. J. Pharmac. Chemother. 14 48-58.
Lambert, G.A; et al (1978) Eur. J. Pharmacol. 49 39-48.
Lattimer, N; et al (1982) Br. J. Pharmac. 75 (2) 154.
Sakai, K; et al (1979) Br. J. Pharmac. 66 405-408.
Weitzell, R; et al (1979) Naunyn-Schmiedeberg's Arch. Pharmac. 308 127-136.

EFFECTS OF WY 26392, WY 26703, RX 781094 AND YOHIMBINE ON NORADRENALINE OVERFLOW IN THE RABBIT ISOLATED PULMONARY ARTERY

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Noradrenaline (NA)release from noradrenergic nerve endings is regulated by a negative feedback mechanism triggered by the neurotransmitter itself through activation of presynaptic $alpha_2$-adrenoceptors (Langer, 1977). The selective $alpha_2$-adrenoceptor antagonist yohimbine blocks this feedback mechanism evoking an increase in stimulation-induced transmitter overflow (Starke et al, 1975). Previous studies have shown that the novel compounds Wy 26392, Wy 26703 and RX 781094 are selective antagonists at $alpha_2$-adrenoceptor sites (Lattimer et al, 1982; Chapleo et al, 1981). The effects of these compounds on stimulation evoked release of ^{3}H-NA have been evaluated in comparison with yohimbine.

The method was based on that of Starke et al (1974) using spiral strips of rabbit pulmonary arteries. Preparations were incubated (30 min) in Krebs solution containing $1.5\text{-}2\times10^{-6}$M (7,8-^{3}H)-NA together with ascorbic acid (10^{-4}M) and ethylenediaminetetracetic acid (EDTA, 3×10^{-5}M). The tissues were then superfused with Krebs solution containing ascorbic acid, EDTA, corticosterone (4×10^{-5}M) desipramine (5×10^{-7}M) and propranolol (4×10^{-6}M). The tissue tension was recorded isometrically. Each strip was stimulated twelve times (S_1-S_{12}) with trains of 300 pulses at 2Hz and 1ms pulse duration at supramaximal voltage. Stimulations were 24min apart. The superfusate was collected in 3 min fractions and ^{3}H measured by scintillation spectrometry. Increasing concentrations of compounds were included in the perfusion fluid 12 min before S_5, S_7, S_9 and S_{11}. Drug effects were calculated by comparing responses during S_4 with those during S_6,S_8, S_{10} and S_{12}.

Wy 26392, Wy 26703,RX 781094 and yohimbine evoked concentration-dependent increases in tritium overflow. The potency order was yohimbine > Wy 26392 > Wy 26703 > RX 781094, the concentrations evoking a 50% increase in overflow being 8×10^{-8}M, 4.2×10^{-7}M, 5.4×10^{-7}M and 1.9×10^{-6}M respectively. The response curve for yohimbine was bell-shaped, the peak increase in overflow occurring at a concentration of 3×10^{-7}M (+114%). In contrast to yohimbine, both Wy 26703 and Wy 26392 increased ^{3}H-NA overflow over the concentration range 10^{-7} to 3×10^{-5}M with no evidence of a bell shaped curve. The maximum increases in release were seen at 3×10^{-5}M Wy 26392 (+178%) and at 10^{-5} and 3×10^{-5}M Wy 26703 (+151%). The maximum increase in release for RX 781094 was at 10^{-5}M (+85%).

All compounds significantly inhibited tissue contractions ($alpha_1$) the concentrations evoking a 50% inhibition being 4.2×10^{-6}M (yohimbine), 8.5×10^{-6}M (Wy 26392) 1.05×10^{-5}M (RX 781094) and 1.4×10^{-5}M (Wy 26703).

The results with Wy 26392, Wy 26703, RX 781094 and yohimbine support the view that these compounds have $alpha_2$-adrenoceptor antagonist actions, enhancing release of ^{3}H-NA at concentrations which do not reduce the stimulation evoked contractions. The potency order for ^{3}H-NA release of yohimbine > Wy 26392 > Wy 26703 > RX 781094 is surprising since we have previously determined the $alpha_2$-adrenoceptor pA_2 values on the rat vas deferens as 7.58, 8.08, 8.46 and 8.04 for yohimbine, Wy 26392, Wy 26703 and RX 781094 respectively (Lattimer et al 1982 and unpublished observations).

Chapleo, C.B. et al (1981) Br. J. Pharmac. 74, 842P
Langer, S.Z. (1977) Br. J. Pharmac. 60, 481-497
Lattimer, N. et al (1982) Br. J. Pharmac. 75, 154P
Starke, K. et al (1974)Naunyn-Schmiedeberg's Arch. Pharmacol. 285, 133-150
Starke, K. et al (1975) Eur. J. Pharmacol. 34, 385-388

CHANGES IN α_1- AND α_2-ADRENOCEPTOR SENSITIVITY TO AGONISTS AFTER CHRONIC ADMINISTRATION OF DESMETHYLIMIPRAMINE OR YOHIMBINE

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Chronic antidepressant treatment has been shown to reduce the sensitivity of presynaptic α_2-adrenoceptors (Crews & Smith 1978) and to reduce the binding of $\{^3H\}$-clonidine to cortical membranes (Smith et al 1981). In contrast, chronic administration of yohimbine or desmethylimipramine has been reported to increase binding of $\{^3H\}$-clonidine in the cortex (Johnson et al, 1980).

The present study investigates the effects of chronic desmethylimipramine (DMI) or yohimbine treatment, on the response of α_2-adrenoceptors in the rat cerebral cortex and vas deferens to clonidine and of α_1-adrenoceptors in the anococcygeus muscle to methoxamine.

Rats (250-300g) were dosed twice daily with DMI (10mg/kg po), yohimbine (1mg/kg po) or distilled water (1ml/kg po) for 14 days. The animals were killed 24h after receiving the last dose and the vasa deferentia, anococcygeus and cerebral cortices removed. Cumulative dose-response curves to clonidine were obtained on the electrically stimulated vas deferens (0.1Hz, 1 msec, 90 mA) and cumulative dose-response curves to methoxamine were obtained on the anococcygeus muscle. Dose-response curves to clonidine were obtained on K^+-evoked tritium release from cortical slices preloaded with $\{^3H\}$-NA.

Preparation	Controls	EC_{50} Yohimbine	DMI
Vas deferens (Clonidine)	$4.4 \pm 0.7 \times 10^{-9}$ (12)	$3.7 \pm 0.9 \times 10^{-9}$ (6)	$6.9 \pm 1.1 \times 10^{-9}$ (8)*
Cortex (Clonidine)	$3.9 \pm 1.0 \times 10^{-8}$ (9)	$8.5 \pm 1.8 \times 10^{-9}$ (7)**	$2.0 \pm 0.4 \times 10^{-7}$ (6)***
Anococcygeus (methoxamine)	$9.1 \pm 0.8 \times 10^{-8}$ (14)	$9.6 \pm 1.9 \times 10^{-8}$ (7)	$1.4 \pm 0.2 \times 10^{-7}$ (8)**

Figures in brackets refer to n, *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$.

Yohimbine had no significant effect on the EC_{50} for clonidine on the electrically stimulated vas deferens or on the response of the anococcygeus to methoxamine. The sensitivity of the α_2-adrenoceptors to clonidine in the cerebral cortex was significantly increased. DMI significantly decreased the sensitivity of the cerebral cortex to clonidine and of the anococcygeus muscle to methoxamine. There was a slight decrease in the sensitivity of the vas deferens to clonidine.

These results show that chronic DMI treatment can produce a functional subsensitivity of both central and peripheral α_1 and α_2-adrenoceptors. In contrast chronic treatment with yohimbine had no effect on the peripheral α_1 and α_2-adrenoceptors studied but increased the sensitivity of the central α_2-adrenoceptors located on noradrenergic nerve terminals in the cerebral cortex.

Crews F.T and Smith C.B. (1978). Science 202, 322-324.
Johnson R.W. et al (1980). Eur. J. Pharmacol. 67 123-127
Smith C.B. et al (1981). Brain Res. 210 413-418.

2-ALKYL ANALOGUES OF RX781094: POTENT, SELECTIVE ANTAGONISTS AT PERIPHERAL α_2-ADRENOCEPTORS

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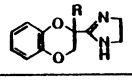
RX 781094 is a selective $\alpha_2$adrenoceptor antagonist both in the periphery (Chapleo et al., 1981) and in the CNS (Dettmar et al., 1981). The present communication describes the selectivity profiles of RX 781094 and a number of 2-alkyl analogues of RX 781094 in isolated tissues and pithed rats.

The prejunctional (α_2) and postjunctional (α_1)-adrenoceptor antagonist properties of the compounds were assessed in vitro by determining pA_2 values against UK 14,304 and noradrenaline in the electrically-stimulated (0.1Hz) rat vas deferens and anococcygeus muscle respectively. Experimental conditions were as described previously (Doxey et al., 1977) except that the Krebs solution contained cocaine (0.9 µM), corticosterone (40 µM) and propranolol (0.1 µM). Male Sprague-Dawley normotensive rats (300-350g) were pithed, vagotomised and diastolic blood pressure (DBP) and heart rate (HR) recorded. Drugs were injected into a femoral vein. Rats were pretreated with atropine (1.0 mg/kg) and tubocurarine (1.0 mg/kg). Prejunctional α_2-adrenoceptor antagonist potency was determined in rats in which the resting HR was elevated by 90-110 beats/min by continuously stimulating (0.1-0.3Hz, 0.5ms, 60V) the spinal cord (T1-T5). Separate groups of rats (n=6-8) were used to determine the cumulative i.v. dose of UK 14,304 required to reduce the tachycardia by 50% (ED_{50}) following either saline or a selected dose of one of the analogues. Postjunctional activity was assessed by constructing dose-pressor response curves to the α_1-adrenoceptor agonist cirazoline. The doses of cirazoline required to elevate DBP by 50 mmHg (ED_{50}) were determined in saline and drug treated rats. The dose-ratio (DR) shifts produced by the antagonists against UK 14,304 and cirazoline were calculated from the ED_{50} values. The antagonist doses required to produce DR of 2 were determined. The results obtained are shown in Table 1.

Table 1. Antagonist potencies and selectivities of RX 781094 and analogues.

COMPOUND	STRUCTURE	RAT ISOLATED TISSUES			PITHED RATS		
		VAS DEFERENS pA_2 (α_2) vs UK 14304	ANOCOCCYGEUS pA_2 (α_1) vs NORADRENALINE	RATIO α_2/α_1	HR (α_2) Dose for DR2 vs UK14304 µM/kg	DBP (α_1) Dose for DR2 vs CIRAZOLINE µM/kg	RATIO α_2/α_1
RX 781094	H	8·33 ± 0·04	6·00 ± 0·10	214	0·10	6·6	66
RX 801079	$-CH_3$	8·12 ± 0·07	5·55 ± 0·10	371	0·31	38·9	125
RX 811033	$-CH_2CH_3$	8·71 ± 0·04	6·06 ± 0·06	447	0·06	8·0	133
RX 811054	$-CH_2CH_2CH_3$	8·94 ± 0·04	6·42 ± 0·05	331	0·04	3·1	77
RX 811005	$-C<^{CH_2}_{CH_3}$	8·00 ± 0·05	4·82 ± 0·14	1514	0·30	30·4	101

All compounds possessed selectivity for peripheral α_2-adrenoceptors which was equal to or greater than that of RX 781094. RX 811033 and RX 811054 were more potent α_2-adrenoceptor antagonists than RX 781094. Similar profiles for these compounds were found in the CNS (Gadie et al, 1983).

Chapleo, C.B. et al (1981). Br.J.Pharmac. 74, 842P
Dettmar, P.W. et al (1981). Br.J.Pharmac. 74, 843-844P
Doxey, J.C. et al (1977). Br.J.Pharmac. 60, 91-96.
Gadie, B. et al (1983). This meeting.

2-ALKYL ANALOGUES OF RX 781094: POTENT, SELECTIVE ANTAGONISTS AT CENTRAL α_2-ADRENOCEPTORS

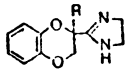
Gadie, B., Lane, A. C., McCarthy, P. S., Tulloch, I. F. and Walter, D. S. Pharmacology Department, Reckitt and Colman, Hull, HU8 7DS.

The selectivity and potency of the benzodioxan RX 781094 as an antagonist at α_2-adrenoceptors have previously been demonstrated in a variety of tests (Chapleo et al., 1981; Dettmar et al., 1981). A number of 2-alkyl analogues of RX 781094 have now been examined using two methods of assessment for activity at central α_2-adrenoceptors.

Central α_1/α_2 selectivities were determined in competition binding studies in rat neocortical membranes using ^{3}H-781094 (α_2) and ^{3}H-prazosin (α_1), according to previously described techniques (Doxey et al., 1982). Central antagonist potency after i.v. administration was assessed by the ability to reverse guanoxabenz (0.3 mg/kg, i.v.)-induced mydriasis in the anaesthetised rat (Berridge et al, 1982). To determine oral potency, conscious, fasted rats were pretreated with various antagonist doses 30 min before induction of anaesthesia (sodium pentobarbitone, 60 mg/kg i.p.); 15 mins later the degree of antagonism of the mydriatic effect of the ED_{99} dose (0.3 mg/kg, i.v.) of guanoxabenz was measured.

All the 2-substituted benzodioxan analogues were found to possess a greater central α_2-selectivity than either RX 781094 or yohimbine (Table 1). The high affinities of the 2-ethyl and 2-n-propyl analogues observed in vitro were reflected in their marked i.v. and oral potencies.

Table 1 Antagonist potencies at central α_2-adrenoceptors

Antagonist	 R	Rat mydriasis test AD_{50} (μmol/kg) i.v.	Rat mydriasis test AD_{50} (μmol/kg) p.o.	Cortical binding α_2 Ki(nM)	Cortical binding α_1 Ki(nM)	α_1/α_2
RX781094	-H	0.18±0.05	16.6±3.4	3.1±0.4	91±3	29
RX801079	$-CH_3$	0.25±0.06	17.7±3.8	4.4±0.4	449±90	102
RX811033	$-CH_2CH_3$	0.06±0.006	2.9±0.5	1.0±0.1	174±29	172
RX811054	$-CH_2CH_2CH_3$	0.12±0.004	2.0±0.3	1.0±0.1	107±31	108
RX811005	$-C<^{CH_2}_{CH_3}$	0.54±0.04	24.2±3.4	16.3±1.8	1794±185	110
Yohimbine		2.20±0.04	15.3±1.5	40.0±5.5	230±16	6

Values are mean ± s.e.m.

These data show that both the selectivity and antagonist potency of the benzodioxan RX 781094 at central α_2-adrenoceptors can be markedly enhanced by alkyl substitution in the 2-position. The central pharmacological profiles of these analogues are thus consistent with the results obtained in peripheral tissues (Doxey et al, 1983).

Berridge, T. L. et al (1982) Br.J.Pharmac. 75, 49P
Chapleo, C. B. et al (1981) Br.J.Pharmac. 74, 842P.
Dettmar, P. W. et al (1981) Br.J.Pharmac. 74, 843P.
Doxey, J. C. et al (1982) Br.J.Pharmac.(in Press).
Doxey et al.(1983). This meeting.

BINDING CHARACTERISTICS OF (^{3}H)-RX 781094 ON HUMAN INTACT PLATELETS

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RX 781094 is a novel α-adrenoceptor antagonist (Chapleo, Doxey, Myers and Roach, 1981) which selectively binds to α_2-adrenoceptors in rat cerebral cortex membranes (Howlett, Taylor and Walter, 1982). Human platelet α-adrenoceptors are predominantly of the α_2-type and show a similar binding profile to those in rat brain. We have therefore investigated the binding characteristics of [^{3}H]-RX 781094 on intact human platelets for comparison both with the studies in rat brain and with previous studies on the human platelet.

[^{3}H]-RX 781094 (specific activity 16.6 Ci/mmol) was a gift from Dr. J. Doxey (Reckitt and Colman Ltd., Hull, U.K.).

Venous blood taken from healthy male volunteers (age 23-35) was anticoagulated with 1% EDTA and platelet-rich plasma prepared by centrifugation at 180g for 15 min at 20°C. The platelets were separated from the plasma by further centrifugation (1200g for 7.5 min at 10°C) and resuspended in the incubation medium comprising 0.1% EDTA, 150 mM NaCl, pH 7.5. Preliminary studies indicated that non-specific binding was significantly greater, and specific binding therefore proportionately a smaller fraction of total binding, at 37°C than at 2°C. All subsequent incubations were therefore carried out at 2°C. Aliquots of resuspended platelets were incubated with [^{3}H]-RX 781094 (range 5-60 nM) for 20 min then centrifuged at 6500g for 1 min to terminate incubation. Specific binding was identified as that inhibited by 5 μM phentolamine and constituted 50% total binding at 14 nM.

Both association and dissociation of [^{3}H]-RX 781094 occurred rapidly with a half-time of less than one minute in each case. Consequently equilibrium was established within 15 min at 7.5 nM even at 2°C. Specific binding was saturable and Scatchard analysis indicated a single binding site with affinity K_d=38.9 ± 3.5 nM and capacity B_{max}=64 ± 7 fmol/10^8 platelet (mean ± s.e.m., n=4). Simultaneous assay of [^{3}H]-yohimbine binding in these four subjects indicated no significant difference in capacity (B_{max}=54 ± 4 fmol/10^8 platelet) but a significantly higher affinity (K_d=5.18 ± 0.66 nM; $p<0.001$). The rank order of potency for inhibition of [^{3}H]-RX 781094 followed the expected pattern for an α_2-adrenoceptor (yohimbine>RX 781094>phentolamine>methysergide>prazosin>mepyramine>haloperidol>propranolol). A similar pattern of inhibition was observed for the corresponding biogenic amine agonists, and the active (-)stereoisomers of adrenaline and noradrenaline were more than 20-fold more potent inhibitors than the (+)stereoisomers.

These data indicate that the binding capacity of [^{3}H]-RX 781094 on intact human platelets is similar to that identified by [^{3}H]-yohimbine. The affinity of [^{3}H]-RX 781094 on the intact human platelet is considerably lower than that in the rat cerebral cortex. Furthermore the affinity of RX 781094 is less than that of yohimbine in the platelet whereas in the rat brain it has been reported to be higher than that of yohimbine. Further studies are needed to identify the cause of this apparent difference.

Chapleo, Doxey, Myers and Roach Br. J. Pharmac. (1981) 74, 842P
Howlett,Taylor and Walter Br. J. Pharmac. (1982) 76, 294P