THE EFFECT OF OMEPRAZOLE ON CYTOCHROME P-450-MEDIATED REACTIONS IN RAT LIVER.

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Omeprazole (5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulphinyl] -1H-benzimidazole) inhibits gastric acid secretion in animals and man (Larsson et al, 1985). Benzimidazole derivatives are known to inhibit hepatic microsomal mixed-function oxidase activity *in vitro* in rats (Murray et al, 1982) and to inhibit the metabolism of diazepam and phenytoin in man (Gugler and Jenssen, 1984, 1985). We have investigated the potential of omeprazole to interact with cytochrome P-450 by utilizing 3 model substrates:- ethylmorphine N-demethylation (EM) and ethoxycoumarin O-deethylation (ECOD) in rat liver 10,000 g supernatant and diazepam in cultured rat hepatocytes.

RESULTS

The Ki's of omeprazole for inhibition of EM and ECOD in 10,000 g supernatant were found to be 40 ± 27 μM and 76 ± 6 μM (mean ± S.D. in 4 rats). In comparison Ki values calculated for cimetidine were found to be 147 μM and 152 μM for inhibition of EM and ECOD respectively. The effects of omeprazole and cimetidine on diazepam (15 μM) clearance in cultured rat hepatocytes were compared; omeprazole (50 μM) inhibited diazepam clearance by 73% while 50 μM cimetidine only inhibited diazepam clearance by 50%.

The effects of repeated daily dosing of omeprazole to 6 rats (500 $mg.kg^{-1}$, 14 days p.o.) were assessed 24 h after the last dose in rat liver 10,000 g supernatant. Table 1 summarises the results obtained (mean ± S.D. from 6 rats).

TABLE 1

Parameter measured	vehicle	omeprazole
liver weight (g)	13.7 ± 1.6	15.9 ± 1.2*
cytochrome P-450 ($nmol.g\ liver^{-1}$)	22.8 ± 3.2	30.9 ± 3.8+
EM (1xKm)($nmol.min^{-1}.g\ liver^{-1}$)	167 ± 18	151 ± 11 *
EM (5xKm)($nmol.min^{-1}.g\ liver^{-1}$)	313 ± 18	257 ± 14 +
ECOD (1xKm)($nmol.min^{-1}.g\ liver^{-1}$)	62.1 ± 4.0	166 ± 11 +
ECOD (5xKm)($nmol.min^{-1}.g\ liver^{-1}$)	103 ± 4	234 ± 23 #

* $P<0.05$, + $P<0.01$, # $P<0.001$ compared with vehicle

CONCLUSION

Our results suggest that omeprazole interacts significantly with the mixed-function oxidase system *in vitro* and *in vivo* causing both inhibition and induction of cytochrome P-450. Comparisons with cimetidine suggest that omeprazole may be more potent than cimetidine in inhibiting cytochrome P-450.

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THE INFLUENCE OF ANAESTHETIC AGENTS ON THE PHARMACOKINETICS OF PARA-AMINOHIPPURIC ACID.

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As the extraction of para-aminohippuric acid (PAH) across the kidney approaches 90%, the body clearance of PAH can be used to assess renal blood flow. This is often utilized to evaluate renal function in experimental toxicology. Such assessments usually being made in animals under terminal anaesthesia.

The present study in male Wistar rats, (300±16g), has investigated the extent to which the pharmacokinetics of PAH are influenced by the anaesthetic agents used. For this, three anaesthetic regimens were selected:- fentanyl + fluanisone (0.08 + 2.5mg/kg i.p., 'Hypnorm') in combination with midazolam (1.5mg/kg i.p. 'Hypnovel'), urethane (1.75g/kg i.p.) and pentobarbitone sodium (67mg/kg i.p.).

^{3}H-labelled PAH, (50µmole, 2.5µCi), was injected via the left common jugular vein. Blood samples, (50µl), were obtained via the caudal vein with intravenous 0.9% saline replacement. Blood samples were solubilized and decolorized prior to liquid scintillation counting. Results from each animal were analysed according to a two-compartment model using a non-linear least squares regression programme, (See Table 1).

Table 1 — Pharmacokinetic Parameters (Mean±s.d.)

Anaesthetic Regimens	Clearance (mls/min)	Elimination Half-life (mins)	Volume of Distribution (mls)
Hypnorm/Hypnovel (n=5)	8.94±1.18	31±7	178±36
Urethane (n=5)	5.69±0.85*	56±10*	175±34
Pentobarbitone (n=4)	10.53±1.79	29±3	219±14

*P<0.05 - Duncans test.

The statistical analysis was performed using ANOVA and Duncans multiple range test. There were no significant differences between Hypnorm/Hypnovel and pentobarbitone anaesthesia for any of the pharmacokinetic parameters. However, the results clearly demonstrate a significant, (P<0.05), decrease in total body clearance of PAH and a prolongation of elimination half-life, without any significant alteration in the volume of distribution, for those animals induced with urethane anasthesia.

Urethane anaesthesia has previously been reported to significantly alter the pharmacokinetics of carboxyfluorescein (Woolfrey et al., 1985) and thiamine (Pipkin et al., 1982), compounds whose elimination like that of PAH is highly dependent on renal clearance.

It is probable that urethane-induced alterations in kinetic behavior are mediated through haemodynamic mechanisms. Previous reports (Van Der Meer et al., 1975) have observed a fall in systemic blood pressure and an increase in circulating catecholamines and corticosteroids.

Whatever the nature of the alteration in kinetic behaviour it is evident that care should be taken when assessing in vivo kinetics under urethane anaesthesia, especially for those compounds whose clearance is highly dependent on renal elimination.

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MECHANISMS OF P-AMINOHIPPURATE UPTAKE IN ISOLATED MEMBRANE VESICLES FROM THE DOG KIDNEY.

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In order to study the mechanisms of renal drug transport at a subcellular level a procedure was developed for the simultaneous isolation of brush border (BBMV) and basolateral membrane vesicles (BLMV) from the renal cortex of the Beagle dog. BLMV were prepared using a Percoll-gradient ultracentrifugation technique based on methods of Sacktor *et al.* (1981) and Van Heeswijk *et al.* (1984). BBMV were isolated using a $CaCl_2$-precipitation technique described by Beck & Sacktor (1978). The enzyme markers for BBMV, alkaline phosphatase, and for BLMV, Na-K-ATPase, were enriched from the crude homogenate 10- to 12-fold and 8- to 10-fold respectively. Cross contamination was minimal, as was contamination by marker enzymes for mitochondria, lysosomes and endoplasmic reticulum. Solute uptake in BBMV and BLMV was measured by rapid filtration. Both membrane preparations were evaluated functionally by measuring 25 µM D-[^{3}H]glucose uptake. BBMV showed a rapid sodium dependent D-glucose uptake resulting in a transient overshoot of radiolabeled solute uptake (265±24% of the equilibrium value). BLMV were practically free of Na^+ dependent D-glucose transport indicating that these membranes were almost completely free of the transport characteristics of BBMV.

The mechanisms of renal organic anion transport were studied by measuring the uptake of the model compound p-amino[^{3}H]hippurate (PAH). The uptake rate of 50 µM PAH was stimulated by a 100 mM Na^+-gradient in both BLMV (255±27%) and BBMV (240±20%), compared with a similar K^+-gradient. Interestingly, imposition of an inwardly directed H^+-gradient (pH extravesicular 6.0, intravesicular 7.4) could also stimulate PAH transport in BBMV (209±38%). Uptake of PAH showed the characteristics of carrier-mediated transport in that it was saturable and susceptible to inhibition by probenecid (5 mM). The apparent affinity constant for transport in BLMV was 0.8±0.3 mM and in BBMV 4.9±0.1 mM (Na^+ dependent) and 5.7±0.8 mM (H^+ dependent). Transport capacities were 0.8±0.1 (BLMV), 6.7±0.6 (BBMV,Na^+) and 7.9±0.6 (BBMV,H^+) nmoles/mg protein/15 sec. The coexistence of a Na^+- and H^+-coupled PAH transport was further investigated. Because BBMV are known to contain a Na^+-H^+ exchange system, an inwardly directed Na^+-gradient could lead to intravesicular alkalinization, thus indirectly stimulating the uptake of PAH. However, sodium dependent PAH uptake in BBMV was not decreased in presence of the Na^+-H^+ exchange inhibitor amiloride (5 mM). These results suggest a possible heterogeneity of the BBMV preparation or the carrier system with respect to organic anion transport.

In conclusion, the described isolation procedure yields pure and viable BBMV and BLMV. These preparations can be used to study renal transport of specific compounds at membrane level and to characterize the specificity of a transport system by interaction studies (Heijn *et al.*, 1986).

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ATP ANALOGUES AND THE GUINEA-PIG URINARY BLADDER: PHARMACOLOGICAL POTENCY IS RELATED TO RESISTANCE TO BREAKDOWN BY ECTOENZYMES.

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ATP induces contraction of the detrusor muscle of the guinea-pig isolated urinary bladder, by an action on P_2-purinoceptors. Ectonucleotidase enzymes present on the bladder preparation rapidly and sequentially dephosphorylate ATP to adenosine (Cusack & Hourani, 1984) and it has been suggested that the much higher potencies of certain ATP analogues are due to a possible resistance to dephosphorylation (Kasakov & Burnstock, 1983; Stone, 1985). Here, we have looked at the rates of dephosphorylation of a range of ATP analogues and compared them to their ability to induce contraction. Mucosa-free detrusor strips were incubated for 1 hr in solutions of the ATP analogues (100 μM) and aliquots were removed at intervals and assayed by h.p.l.c. on an ion-exchange column (Cusack & Hourani, 1984).

ATP was rapidly dephosphorylated, with a half-life of 15 min. Analogues of ATP with a substituent on the 2, 6 or 8 position of the purine ring were dephosphorylated to the corresponding ADP analogues at the same rate as ATP itself, and those whose pharmacology has been reported, 2-chloro-ATP and 2-methylthio-ATP, are equipotent with ATP at inducing contraction of the bladder (Burnstock et al., 1983).

Enantiomers of adenine nucleotides, in which the D-ribose moiety has been replaced by L-ribose, were used to test for stereoselectivity of the ecto-ATPase. L-ATP, 2-chloro-L-ATP and 2-methylthio-L-ATP were dephosphorylated at about the same rate as the natural enantiomers, showing that the ecto-ATPase is not stereoselective. The P_2-purinoceptor mediating contraction also shows no stereoselectivity towards these enantiomers (Burnstock et al., 1983).

The stereoselectivity of the ectoenzyme towards the triphosphate chain was tested by using the Rp and Sp diastereoisomers of the phosphorothioate analogues ATP-α-S and ATP-β-S. Removal of the terminal phosphate from ATP-α-S occurred at the same rate as removal from ATP and showed no stereoselectivity, while removal of the terminal phosphate from ATP-β-S was slower (half-life of 40 min) than from ATP, again without stereoselectivity. The P_2-purinoceptor on the bladder also shows no stereoselectivity towards any of the diastereoisomers of the phosphorothioates and while ATP-α-S is only equipotent with ATP at inducing contraction, ATP-β-S is more potent (Burnstock et al., 1984). ATP-γ-S was degraded more slowly (half-life of 40 min) than ATP and is also more potent at inducing contraction.

The methylene isosteres of ATP were resistant to dephosphorylation; α,β-methylene-ATP was very slowly degraded to α,β-methylene-ADP and no degradation of β,γ-methylene-ATP was detected. Both of these ATP analogues are more potent than ATP at inducing contraction (Kasakov & Burnstock, 1983, Cusack & Hourani, 1984).

These results support the suggestion that resistance to breakdown may enhance the potencies of adenine nucleotide analogues at inducing contraction of the guinea-pig urinary bladder. This is in contrast with our results for the taenia coli, where we found that for ATP analogues no apparent relationship exists between pharmacological potency and resistance to degradation (Welford et al., 1986).

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EFFECT OF CHRONIC TREATMENT WITH METHYL TESTOSTERONE OR METHENOLONE ON THE SENSITIVITY OF MICE TO MORPHINE.

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Exercise elevates plasma levels of β-endorphin and (met) enkephalin (Howlett et al 1984) and it is recognised that athletes take various anabolic/androgenic steroids in order to improve their physical response to exercise stress. We have examined the possible effects of chronic steroid administration on opioid receptors by determining the sensitivity to morphine of mice treated chronically with methyl testosterone or methenolone.

Male LACA mice were used in each of three experiments:- 1. Mice were injected weekly for four weeks with 0.4mg/kg i.m. methyl testosterone or methenolone; control mice received ethyl oleate vehicle. Three or four days after each injection the reaction time of each mouse was determined on a hot-plate (54°C) and this was repeated 15,30,60 and 120 min after the i.p. injection of 5,10 or 20mg/kg of morphine (5 mice per group). 2. Mice were injected weekly for six weeks with 1,5 or 10mg/kg i.m. of methyl testosterone or methenolone; control mice received ethyl oleate or saline (6 mice per group). The behavioural responses of these mice to 20mg/kg morphine were examined in an activity box and on a box-maze before and after the morphine injection. 3. Mice were injected daily for seven days with 0.5 or 10mg/kg i.p. methyl testosterone or methenolone; control mice received ethyl oleate (6 mice per group). Activity in the box-maze was determined before and after the administration of 20mg/kg morphine.

The results were as follows:- 1). Chronic treatment with methyl testosterone or methenolone did not alter the sensitivity of mice to a noxious stimulus (hot-plate). Tolerance to the antinociceptive action of morphine developed in all groups, but was significantly less severe in the methenolone group than in mice receiving methyl testosterone or ethyl oleate. 2). After six weekly injections of steroid the activity of mice receiving 1mg/kg methyl testosterone was significantly lower than that of the oleate treated animals but the higher doses of methyl testosterone and all doses of methenolone did not affect activity. Morphine stimulated the activity of oleate treated mice; however, no increase in activity was seen in mice treated with any of the doses of methyl testosterone or with the highest dose of methenolone. In the box-maze the only effect of morphine in control mice was to reduce the number of dips. However, in steroid treated mice some differential effects could be observed; in mice treated with methyl testosterone morphine reduced the number of rears whilst in methenolone treated mice morphine enhanced the number of crosses. 3). Similar changes in box-maze behaviour was observed in mice which had received daily doses of steroid. The most marked effects were the reduction in the number of dips in methyl testosterone treated mice receiving morphine and the increase in crosses induced by morphine in the methenolone treated group.

These results indicate that steroid treatment will alter the behavioural effects of morphine; further experiments are required to determine whether this action is limited to the anabolic or androgenic activity of the steroids.

S.F. is a Sports Council Science Scholarship Student.

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SPONTANEOUS MORPHINE WITHDRAWAL MODULATES α_2-ADRENOCEPTORS IN THE RAT BRAIN.

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The morphine abstinence syndrome appears to involve alpha-2 adrenoceptor-mediated mechanisms. Heroin addicts in abstinence have supersensitive platelet alpha-2 adrenoceptors and the severity of withdrawal correlates with the density of these receptors (García-Sevilla et al. 1985). This modulation of platelet alpha-2 adrenoceptors might reflect relevant adaptive changes of similar receptors in the brain. This study was designed to directly evaluate brain alpha-2 adrenoceptor changes in morphine-dependent rats during the spontaneous development of the abstinence syndrome. Groups of 6 male rats were made dependent on morphine (0.1-1.2 mg/ml in drinking water for 3 weeks) (Badawy et al. 1982) and after spontaneous withdrawal the severity of the abstinence syndrome (Blasig et al. 1973) and receptor densities were assessed. The specific binding of ^{3}H-clonidine to brain membranes (pooled from 6 rats) was used as a biochemical index to quantitate alpha-2 adrenoceptor density (Bmax).

The chronic administration of morphine did not alter the density of brain alpha-2 adrenoceptors, except in the striatum where it decreased (29±1%; n=6; $P<0.05$). Brain alpha-2 adrenoceptor densities followed peculiar time courses after spontaneous withdrawal. In the hypothalamus, receptor density (Bmax=139±12 fmol/mgP; n=6) began to increase 3-6 h after withdrawal (11±1% - 32±3%; n=6; $P<0.05$), increased progressively by 9-15 h (36±3% - 44±5%; n=6; $P<0.05$), peaked around 24 h (53±2%; n=2; $P<0.001$) and gradually returned to baseline values by 48 h (33±2%; n=3; $P<0.05$) and 72 h (6±0.4%; n=3). A less marked modulation was obtained in cortex, brainstem and striatum. The severity of the abstinence syndrome (control score: 0.89±0.15; n=4) followed a similar time course. The withdrawal reaction increased by 3-6 h (1.8-2.1 fold; n=6; $P<0.05$), increased further by 9-15 h (3.1-3.5 fold; n=7; $P<0.01$), peaked at 24 h (4.4 fold; n=3; $P<0.001$) and gradually returned to control values by 48 h (2.5 fold; n=3; $P<0.05$) and 72 h (1.3 fold; n=3). There was a significant correlation ($r=0.564$; $P<0.01$) between the severity of the abstinence syndrome (behavioural ratings) and the density of alpha-2 adrenoceptors (Bmax for ^{3}H-clonidine) in the hypothalamus. The severity of withdrawal did not correlate with the densities of alpha-2 adrenoceptors in cortex ($r=0.134$), brainstem ($r=-0.031$) and striatum ($r=0.220$).

It is concluded that modulation of hypothalamic alpha-2 adrenoceptor density is a relevant biochemical process in the development of the morphine withdrawal syndrome. The adaptive process of this inhibitory receptor might reflect contraregulatory changes which would tend to overcome the increased release of noradrenaline associated with the opiate withdrawal reaction.

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LACK OF ANTIAVERSIVE EFFECT OF μ- AND κ-OPIOID AGONISTS IN CONDITIONED TASTE AVERSION IN THE RAT.

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It has been suggested that the mechanism of conditioned taste aversion (CTA) in the rat is similar to that of emesis (Coil et al, 1978); the CTA test in the rat could therefore be suitable as a screening test for antiemetic drugs (Landauer et al, 1985). In order to investigate this possibility, we have now studied whether μ- and K-opioid agonists have an antiaversive effect in the rat, as we have shown in the dog that these agonists antagonize apomorphine-induced emesis (Blancquaert et al, submitted). In preliminary experiments, we observed that morphine (3 mg/kg i.p.) antagonizes CTA induced by apomorphine (Blancquaert et al, 1985); we have now extended this CTA study.

The method of Cairnie and Leach (1982) was followed to induce CTA except that, instead of gamma-irradiation, apomorphine (30 mg/kg i.p.), lithium chloride (70 mg/kg i.p.) or copper sulphate (100 mg/kg intragastrically) were used as CTA-inducing stimuli. The μ-agonists morphine (1, 3, 10 and 30 mg/kg i.p.), fentanyl (0.003, 0.01, 0.03 and 0.1 mg/kg i.p.) and methadone (1 and 3 mg/kg i.p.), and the K-agonists bremazocine (0.001, 0.01, 0.1 and 1 mg/kg i.p.) and ethylketocyclazocine (EKC, 0.001, 0.01, 0.1 and 1 mg/kg i.p.) were tested for their antiaversive effect against CTA induced by the different stimuli (n = 6 for each possible combination). Data obtained after pretreatment with an opioid were compared to those in the aversive control by the Mann-Whitney U-test ($p < 0.05$).

Apomorphine-induced CTA was prevented by the μ-agonist morphine, but only in a dose of 3 mg/kg. The K-agonist EKC prevented apomorphine-induced CTA in a dose of 0.1 mg/kg, while bremazocine 1 mg/kg potentiated it. Morphine 1 mg/kg, fentanyl 0.1 mg/kg, methadone 3 mg/kg, bremazocine 0.001 mg/kg and EKC 1 mg/kg also antagonized apomorphine-induced CTA but significance was never reached. The development of lithium chloride-induced CTA was not prevented by any of the opioids tested; it was potentiated by bremazocine (0.1 and 1 mg/kg). Copper sulphate-induced CTA was antagonized by at least one dose of each agonist studied, but this never reached significance. In most cases, the doses of opioid agonists showing antagonism of copper sulphate-induced CTA did not correspond to those antagonizing apomorphine-induced CTA.

The variability of the results, together with the lack of dose-dependency of the observed antiaversive effects and the lack of parallellism of the observed antiaversive effects against the 3 aversive stimuli, illustrate that an antiaversive effect in the rat is not a common feature of μ- and K-opioid agonists. This suggests that the CTA test in the rat is unsuitable as a screening test for potential antiemetic drugs.

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THE TOPOGRAPHY OF BEHAVIOURS PROMOTED BY A SELECTIVE D-2 DOPAMINE RECEPTOR AGONIST IN COMBINATION WITH A SELECTIVE D-1 AGONIST.

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One unexpected action of blocking D-1 dopamine receptors with the selective antagonist SCH 23390 in the intact animal is to attenuate the expression of stereotyped behaviour induced by the selective D-2 agonist RU 24213 (Pugh et al, 1985). A corollary of this effect might be that stimulating D-1 receptors would potentiate the expression of such a response. We have previously noted that the selective D-1 agonist SK&F 38393 can indeed potentiate stereotyped behaviour induced by RU 24213 (Mashurano & Waddington, 1986), and we have now investigated in more detail the nature of this effect.

Male Sprague-Dawley rats were challenged s.c. either with RU 24213 or SK&F 38393, or given RU 24213 10 min after SK&F 38393. They were subsequently assessed at 10 min intervals using a stereotypy rating scale combined with a behavioural check list (Pugh et al, 1985; Mashurano & Waddington, 1986).

Drugs		Stereotypy score	Sn + L	Fixated Sn	Li/Gn
RU 24213	2.5	1.8±0.2	4/10	0/10	0/10
	15.0	2.7±0.2	5/6	0/6	0/6
	50.0	3.0±0.0	7/7	0/7	0/7
SK&F 38393	40.0	0.9±0.2	0/8	0/8	0/8
SK&F 40.0 + RU	2.5	3.4±0.3*	8/8*	5/8*	2/8

Means ± s.e.Mean; *, $p < 0.05$ vs. RU 24213 2.5 mg/kg given alone

2.5 - 50.0 mg/kg RU 24213 induced stereotyped sniffing and locomotion (Sn + L), but failed to induce fixated sniffing or licking/gnawing (Li/Gn) even at the highest dose; 40.0 mg/kg SK&F 38393 + 2.5 mg/kg RU 24213 resulted in significantly higher stereotypy scores at 30 min and a significantly greater overall prevalence of stereotyped sniffing and locomotion than either drug alone; fixated sniffing and licking/gnawing were only evident in the combination group (Table).

Despite the administration even of near toxic doses, the selective D-2 agonist RU 24213 failed to induce fixated stereotyped sniffing and perioral stereotypies. Conversely, the selective D-1 agonist SK&F 38393, which fails to induce stereotypy, was able to change the topography of response to a 20-fold lower dose of RU 24213 to include these features. Stimulation of both D-1 and D-2 receptors appears to be required for their manifestation, emphasising the important regulatory role of D-1 activity in the expression of D-2-stimulated behaviours (Waddington et al, 1986).

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PRESERVATION OF MESOLIMBIC DOPAMINE FUNCTION MAY EXPLAIN BEHAVIOURAL RECOVERY IN THE MPTP-TREATED MARMOSET.

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Treatment of marmosets with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces an initial severe Parkinsonian state but the gross motor deficits disappear over the following weeks (Jenner et al, 1985). However, MPTP treatment is associated with a permanent loss of nigro-striatal dopamine neurons. We now examine alterations in mesolimbic dopamine function following MPTP treatment.

In the first experiment common marmosets (282-340g) were treated with MPTP (1-4 mg/kg ip) for 3-7 days. All animals were grossly akinetic and rigid at 10 days following MPTP treatment. Over the following 3-4 month period animals recovered such that they showed fewer less co-ordinated movements than control marmosets. Determination of caudate nucleus, putamen and nucleus accumbens, dopamine, HVA and DOPAC concentrations at 10 days showed a dramatic decrease in these parameters in all brain regions (Table 1). At 3-4 months, there was still a profound decrease in dopamine, HVA and DOPAC concentrations in the caudate nucleus and putamen although there was some recovery compared to 10 days. However, in the nucleus accumbens at 3-4 months dopamine, HVA and DOPAC concentration had recovered substantially compared to 10 days.

In the second experiment, common marmosets (233-298g) received either MPTP (2-4 mg/kg ip for 6 days) alone or were pretreated with the monoamine oxidase B inhibitor MDL 72145 (0.5 or 2.0 mg/kg ip for 2 days) and then received MDL 72145 (0.5 or 2.0 mg/kg ip) plus MPTP (2-6 mg/kg ip) for 6 days. MDL 72145 administration was continued for a further 2 days. MPTP alone caused marked motor deficits which persisted. Animals treated with MDL 72145 (0.5 or 2.0 mg/kg) plus MPTP showed less behavioural change. One month following MPTP administration alone there was a marked fall in both caudate nucleus and nucleus accumbens dopamine, HVA and DOPAC content. Levels of dopamine, HVA and DOPAC in animals pretreated with MDL 72145 (2.0 mg/kg) were normal in both brain areas. However, despite the behavioural recovery, in animals receiving MDL 72145 (0.5 mg/kg) plus MPTP, only a partial preservation of dopamine, HVA and DOPAC was observed in the caudate nucleus whereas substantial protection occurred in nucleus accumbens.

Table 1: Alterations in dopamine, HVA and DOPAC caused by MPTP treatment

	Caudate nucleus		Putamen		Nucleus accumbens	
	Controls	MPTP	Controls	MPTP	Controls	MPTP
10 days						
Dopamine	14.3±1.5	0.22±0.09*	13.0±0.2	0.17±0.06*	10.4±1.1	0.82±0.40*
HVA	8.1±1.1	0*	10.8±1.0	0.01±0.01*	7.7±2.1	0.17±0.12*
DOPAC	1.8±0.3	0.18±0.02	1.8±0.2	0.16±0.03*	1.4±0.2	0.04±0.03*
3-4 months						
Dopamine	16.7±2.6	1.8 ±0.9*	15.2±1.6	2.8 ±1.4*+	10.1±0.8	7.4 ±0.8*+
HVA	10.0±1.8	2.2 ±0.9*+	6.1±0.8	1.6 ±1.1*	8.3±0.4	4.6 ±1.5+
DOPAC	1.1±0.2	0.20±0.08*	1.0±0.3	0.20±0.08*	2.3±0.3	1.2 ±0.2+

* $P < 0.05$ compared to controls; +$P < 0.05$ compared to 10 days; n = 3-7

Behavioural recovery of marmosets from the motor deficits induced by MPTP appears linked to reversal of initial losses of mesolimbic dopamine function.

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A RADIOLIGAND BINDING ASSAY TO DETERMINE β-ADRENOCEPTOR SELECTIVITY OF COMPOUNDS IN THE CENTRAL NERVOUS SYSTEM.

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Peripheral tissues are most commonly used to assess the β-adrenoceptor subtype selectivity of compounds. Here we describe a ligand binding assay which allows the β_1 & β_2 adrenoceptor selectivity of such compounds to be assessed in the CNS.

A crude mitochondrial/synaptosomal fraction was prepared from rat cortex by homogenisation in 0.32M sucrose/1mM EDTA followed by centrifugation at 20,000g. The pellet was extensively washed (4x) in 50mM TRIS HCl buffer, pH 7.8, containing 0.5mM EDTA. The assay consisted of membranes (50µg protein), (-)-[^{125}I] pindolol over a range 10-650pM, usually 150pM, 150µM phentolamine, 300µM GTP and 20mM TRIS HCl buffer, pH 7.8, containing 1mM EDTA and 10mM $MgCl_2$. (-)Isoprenaline (200µM) was used to define non-specific binding. The assay was incubated for 20 min at 37°C and terminated by rapid filtration through GF/C filters using a Brandel M-24R Cell Harvester. The extensive washing and addition of GTP and Mg^{++} ensured that the binding sites were in a low affinity state.

Using this method a binding site with a Kd of 169pM ± 36 and Bmax 52fmoles/mg ± 9 (n=7) was observed in the cortex. Scatchard analysis of the data showed a straight line fit indicating that the ligand had recognised only one class of binding site.

The highly selective β-adrenoceptor antagonist CGP 20712A (100nM) (Dooley & Bittiger, 1984) was shown to occupy 98% of the β_1 receptors and only 2% of the β_2 receptors when included in the assay. These conditions therefore convert a heterogenous population of beta adrenergic binding sites to an essentially homogenous population of β_2 sites. The reverse was achieved using the β_2 selective antagonist ICI 118-551 (O'Donnell & Wanstall, 1980) (30nM) which was shown to occupy 80% of the β_2 receptors and only 8% of the β_1 subtype.

It was therefore possible to offer β-adrenoceptor compounds binding to (a) total, (b) β_1 or (c) β_2 adrenoceptors. The observed shifts in the resulting displacement curves gives a measure of an unknown compound's selectivity in the cerebral cortex.

Using this method we have shown zinterol and salmefamol to be β_2 selective whereas practolol, prenalterol and corwin have been shown to be β_1 selective. More surprisingly, however, (±) clenbuterol (O'Donnell, 1976) which has been shown to exhibit β_2 adrenoceptor selectivity in peripheral tissues was shown to be non-selective in rat cerebral cortex.

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CORTICAL $GABA_B$ BINDING IS UNALTERED FOLLOWING CHRONIC ORAL ADMINISTRATION OF DESMETHYLIMIPRAMINE AND ZIMELIDINE IN THE RAT.

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Recent studies have shown that chronic administration of antidepressant drugs influence γ-aminobutyric acid (GABA) receptors. $GABA_A$ and benzodiazepine binding sites are reduced following chronic administration of various antidepressants to rats (Suzdak & Gianutsos, 1985; Suranyi-Cadotte et al, 1984), while $GABA_B$ sites are markedly increased (Pilc & Lloyd, 1984; Lloyd et al, 1985).

We now report the effects of chronic administration of desmethylimipramine (DMI) and zimelidine (ZIM) on $GABA_B$ binding sites in rat cortex. Drugs were administered orally to mimic clinical usage. Male Wistar rats (200g starting weight) were dosed twice daily (between 8-10am and 4-7pm) for 21 days with DMI or ZIM (1.25 and 5 mg/kg, p.o.) or given a single dose of drug. Twenty four hours after the final dose, rats were killed, cerebral cortices dissected and membranes prepared (Bowery et al, 1983). Membranes were stored in 20 volumes Tris-HCl, pH7.4 at -20°C prior to assay. $GABA_B$ binding was performed essentially as Bowery et al, 1983 (Tris-HCl pH7.4 containing 2mM $CaCl_2$ and 40μM isoguvacine, 1nM ^{3}HGABA and 5-150nM unlabelled GABA, approx. 0.4mg of membrane protein). Specific binding was defined with 10^{-4}M (±) baclofen. The maximal number of binding sites (Bmax) and equilibrium dissociation constants (K_D) were determined in single cortices by non-linear regression analysis. Saturation analysis of $GABA_B$ binding sites was well-fitted to a single binding site in all groups.

Neither acute or chronic administration of DMI or ZIM significantly altered the K_D or Bmax of $GABA_B$ binding sites (Table 1).

Table 1: Saturation analysis of $GABA_B$ binding sites

TREATMENT GROUPS	DMI		ZIM	
	K_D	Bmax	K_D	Bmax
Vehicle-treated controls (8)	26±3	1.29±0.05	21±2	1.30±0.05
1.25mg.kg^{-1} single dose (4)	30±7	1.15±0.05	34±9	1.20±0.13
1.25mg.kg^{-1} x 2/day for 21 days (8)	19±1	1.25±0.07	18±1	1.25±0.06
5mg.kg^{-1} single dose (4)	30±9	1.24±0.07	22±2	1.24±0.11
5mg.kg^{-1} x 2/day for 21 days (8)	20±1	1.27±0.06	21±2	1.25±0.07

K_D = nM, Bmax = p mole/mg protein. Results are expressed as mean ± S.E.M. for the number of animals shown in parentheses.

The present results differ from the marked increase in $GABA_B$ binding sites reported by Pilc & Lloyd, 1984 and Lloyd et al, 1985. While methodological factors (oral v s.c. infusion, whole v frontal cortex, rats killed 24h v 72h post drug) may in part explain these differences, our results suggest that up-regulation of $GABA_B$ binding is a less robust effect of chronic antidepressants than previously reported.

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ACTIVATION OF "5-HT_1-LIKE" RECEPTORS STIMULATES WAKEFULNESS.

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Several reports indicate that functional activation or inhibition of serotonergic (5-HT) system stimulates sleep or wakefulness, respectively, However, other studies provide evidence which is inconsistent with this simplified model of 5-HT regulation of sleep (Morgane and Stern, 1974). Recent discovery of multiple central 5-HT binding sites (Peroutka and Snyder, 1979), might clarify the divergent results published with regard to the role of 5-HT system in vigilance. In order to examine the functional relevance of central 5-HT_1-like receptors in modulation of sleep-waking pattern, we used the following putative 5-HT_1 agonists: 5-CT-5-carboxamidotryptamine (5-HT_{1A}=5-HT_{1B}), BEA 1654-N (3-acetyl-aminophenyl) piperazine hydrochloride (5-HT_{1A} > 5-HT_{1B}), 8-OH-DPAT-8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (5-HT_{1A} >> 5-HT_{1B}) and RU 24969-5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl) -indole succinate (5-HT_{1B} > 5-HT_{1A}).

Male Wistar rats weighing 250-300 g were implanted under anaesthesia with electrodes for cortical EEG and for electromyogram of neck muscles. In some rats intraventricular cannula was implanted. After surgery the rats were allowed a week for recovery. Sleep polygraphic recording started at 8 a.m. and it was scored visually in three stages: wakefulness (W), slow wave sleep (SWS) and rapid eye movements sleep (REMS). The total time of recording (TT) was 6 h. Fifteen different sleep parameters were evaluated.

5-CT (2.5-10 µg, i.c.v.) prolonged a sleep latency (56±10 min after 5 µg of 5-CT, compared with 30±4 min in the control rats), while total SWS was only slightly affected. However, REMS and corresponding parameters such as REMS/TT and REMS/total sleep time were significantly reduced (67% and 77%, respectively).
BEA 1654 (1-5 mg, s.c.) affected sleep only in the first hour after drug administration. In this period only the highest dose of BEA 1654 induced a slight increase of W (18%) and total abolition of REMS.
8-OH-DPAT (0.5-2 mg/kg, s.c.) increased W and latencies of SWS and REMS in a dose-dependent manner. In order to examine an involvement of catecholaminergic system, 8-OH-DPAT was administered in reserpinized rats (2.5 mg/kg, i.p., 18 h prior). In these rats, the wakefulness induced by 8-OH-DPAT was further potentiated, reaching 100% of TT. This might indicate that arousal induced by 8-OH-DPAT is not mediated by activation of catecholaminergic system.
RU 24969 (0.25-2 mg/kg, s.c.) induced a dose-dependent increase of W and corresponding decrease of SWS. After administration of the highest dose of RU-24969 only a wakefulness was observed during total recording time. REMS was completely abolished with all drug concentrations within the indicated dose-range.

In conclusion, our results indicate that activation of "5-HT_1-like" receptors is associated with increase of wakefulness and corresponding decrease of SWS and particularly, REMS. The most intensive arousal effect was observed after administration of RU 24969, which has a preferential affinity for 5-HT_{1B} binding sites. It is conceivable that 5-HT_{1B}-like receptors located presynaptically are involved in the physiological regulation of the sleep-waking cycles, by causing a functional deficiency of 5-HT system, due to an inhibition of transmitter release and/or synthesis.

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ANTIDEPRESSANT DRUGS AND ECS ATTENUATE THE HYPOTHERMIA AND BEHAVIOURAL SYNDROME FOLLOWING 8-OH-DPAT ADMINISTRATION TO RATS.

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Repeated administration to mice of antidepressant drugs and electroconvulsive shock (ECS) attenuates the hypothermic response to the selective 5-HT_{1A} agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) (Goodwin et al, 1985a). In the mouse this hypothermic response occurs in the absence of a behavioural change and appears to be mediated by 5-HT_{1A} receptors located presynaptically (Goodwin et al, 1985b). In the rat however 8-OH-DPAT induces both a hypothermic response and the appearance of the serotonin behavioural syndrome (Tricklebank et al, 1984, Goodwin & Green, 1985). The effect of ECS and antidepressant drugs on these changes induced in rats by 8-OH-DPAT has now been examined.

Rats were administered ECS (125v, 1s) under halothane anaesthesia on 5 occasions over 10 days; controls received halothane alone. Drug treated animals received daily intraperitoneal injections of zimeldine (20 mg/kg), desipramine (20 mg/kg), tranylcypromine (20 mg/kg) or flurazepam (10 mg/kg); controls received vehicle (0.9% saline). The hypothermic response was examined after administration of 8-OH-DPAT (0.1 mg/kg s.c.), a dose which induces few behavioural changes. A higher dose of 8-OH-DPAT (0.75 mg/kg s.c.) was employed to produce the behavioural syndrome, individual components of which were scored as absent (0), present (1), definite (2) or severe (3) at 10, 20 and 30 min after injection. The scores for head weaving, forepaw treading, flattened body posture and hind limb abduction were combined for each time-point to give a total behavioural score; this score and the temperature fall seen after 30 min are given as mean $\pm$ S.D.

Following repeated ECS, the hypothermic response to 8-OH-DPAT (0.1 mg/kg s.c.) became attenuated. The effect was maximal 21 days after the final ECS; controls $-1.0 \pm 0.1^{0}C$; ECS treated $-0.2 \pm 0.1^{0}C$ ($p < 0.05$). Repeated administration of zimeldine, desipramine and tranylcypromine for 14 days similarly attenuated the response; flurazepam was without effect. Only zimeldine significantly attenuated the response 24 hrs following a single injection.

Following repeated but not a single ECS, the serotonin syndrome produced by 8-OH-DPAT (0.75 mg/kg) was significantly attenuated; controls, 18 ± 4; ECS, 9 ± 4 ($p < 0.05$). However the spontaneous locomotor activity of the ECS treated group was increased. Repeated administration of zimeldine, desipramine and tranylcypromine, also produced an attenuation of the serotonin syndrome. Single injections and repeated administration of flurazepam, in contrast, were without effect.

The present results broadly confirm the attenuation of the hypothermic response to 8-OH-DPAT already described in the mouse (Goodwin et al 1985a). In addition, however, and most strikingly, there is an attenuation of the behavioural syndrome produced by 8-OH-DPAT in the rat. It appears therefore that several anti-depressant treatments including ECS, but not the anxiolytic flurazepam attenuate 5-HT_{1A} function in two quite different modalities.

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HIGH AFFINITY INHIBITION BY ERGOTAMINE OF [^{3}H]-8-OH-DPAT BINDING TO HIPPOCAMPAL $5HT_{1A}$ RECEPTORS.

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8-Hydroxy-2-(di-N-propylamine)tetralin (8-OH-DPAT) is a potent and selective agonist at the serotonin $5HT_{1A}$ receptor and has been proven useful in characterizing the functional effects associated with this 5HT receptor subtype (Tricklebank, 1985). Moreover, [^{3}H]-8-OH-DPAT can be used as a radioligand to label with high affinity the postsynaptic $5HT_{1A}$ receptor in the rat hippocampus (Gozlan et al., 1983). Although [^{3}H]-8-OH-DPAT binding sites are also present on 5HT nerve endings in the rat striatum, their pharmacology differs from the $5HT_{1A}$ receptor (Gozlan et al., 1983). In the present study, we examined the effects of the ergot alkaloid ergotamine and its analogs on [^{3}H]-8-OH-DPAT binding to rat hippocampal and striatal membranes.

[^{3}H]-8-OH-DPAT (C.E.A., Saclay, France ; spec. act. 85 Ci/mmol) binding was determined essentially as described by Gozlan et al. (1983). The hippocampus or striatum of male Sprague-Dawley rats (160 - 200 g) was homogenized in 50 mM Tris HCl buffer (pH 7.4 ; 100 mg tissue/ml). Membranes were washed thrice by centrifugation at 4°C (48,000 x g, 10 min), resuspended to the same tissue concentration in buffer and preincubated for 10 min at 37°C. Membranes (100 µl) were then incubated with [^{3}H]-8-OH-DPAT in 1 ml Tris HCl buffer containing 10 µM pargyline for 10 min at 37°C, harvested by filtration over Whatman GF/B filters and washed using three 5 ml aliquots of ice-cold buffer. Specific [^{3}H]-8-OH-DPAT binding (70-80 %) was defined using 10 µM 5HT.

In the rat hippocampus, [^{3}H]-8-OH-DPAT binds with high affinity (Kd = 2.24 $\pm$ 0.35 nM) to a single class of binding sites (Bmax = 541 + 43 fmol/mg protein, n = 5). Specific [^{3}H]-8-OH-DPAT binding is sensitive to inhibition by 5HT (IC_{50} = 5.7 nM), and spiperone (IC_{50} = 23 nM), when measured at a radioligand concentration of 1 nM. [^{3}H]-8-OH-DPAT binding is stereospecifically inhibited by ifenprodil and its stereoisomers ; (-)-erythro-ifenprodil shows an IC_{50} value of 150 nM, whereas its (+)-isomer inhibits binding with an IC_{50} of 2200 nM. The corresponding (+)-threo-isomer has an IC_{50} of 6065 nM. Ergotamine is among the most potent drugs to inhibit hippocampal [^{3}H]-8-OH-DPAT binding (IC_{50} = 0.48 $\pm$ 0.06 nM). Dihydroergotamine shows similar affinity (IC_{50} = 0.53 $\pm$ 0.13 nM), whereas dihydroergocornine, dihydroergocristine and dihydroergocryptine are slightly less potent (IC_{50} = 3.0 $\pm$ 1.35 ; 4.3 $\pm$ 1.3 ; 7.3 $\pm$ 1.5 nM, respectively).

Ergotamine inhibition of striatal [^{3}H]-8-OH-DPAT binding (1.0 nM) is biphasic, with 47 $\pm$ 1 % of the total specific binding being inhibited with an IC_{50} of 1.54 $\pm$ 0.26 nM. The remainder of the specific binding of [^{3}H]-8-OH-DPAT is not affected by ergotamine at concentrations less than 100 µM, thus providing evidence for the heterogeneity of [^{3}H]-8-OH-DPAT binding in this tissue.

The present study shows that the ergot alkaloids are among the most potent inhibitors of [^{3}H]-8-OH-DPAT binding to $5HT_{1A}$ receptor in the rat hippocampus, with ergotamine and dihydroergotamine having an affinity in the subnanomolar range. Although it remains to be firmly established whether these drugs act as agonists or antagonists at this receptor, their activity at the $5HT_{1A}$ receptor must be taken into account in the interpretation of their pharmacological effects. The stereospecific interaction of ifenprodil with [^{3}H]-8-OH-DPAT binding may offer a useful tool for the functional characterization of this recognition site. Finally, the present study indicates that ergotamine may be useful in the characterization of the heterogenous population of striatal [^{3}H]-8-OH-DPAT binding sites.

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ADMINISTRATION OF LITHIUM TO HEALTHY VOLUNTEERS DECREASES [^{3}H]-5HT UPTAKE WITHOUT MODIFYING [^{3}H]-IMI BINDING IN BLOOD PLATELETS.

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Lithium salts are extensively used in the treatment and prophylaxis of manic-depressive illness, but the mechanism by which lithium exerts its effect is not yet fully clarified. The involvement of serotonin (5HT) in the pathophysiology of manic-depressive illness is well established, and there is now convincing evidence that in blood platelets of depressive patients, 5HT uptake is significantly decreased when compared with healthy volunteers (Born et al., 1980 ; Coppen et al., 1980). After chronic treatment with lithium (for 3 months or more), the uptake and storage of 5HT by platelets returns to normal values, while a short-term lithium treatment (2-3 weeks) decreases the maximal 5HT uptake values in platelets from bipolar patients (Meltzer et al., 1983). On the other hand, chronic lithium administration ($\leq$ 55 days) does not affect ex vivo 5HT uptake in rat blood platelets (Genefke, 1972). Therefore, we decided to study the effects of lithium administration on the 5HT transporter of blood platelets from healthy volunteers, by measuring both [^{3}H]-5HT uptake and [^{3}H]-IMI binding in the same subjects (Poirier et al., 1984).

Six volunteers (three male and three female, 24 to 40 years old), in good health and giving informed consent, took lithium orally at 9.00 a.m. and 9.00 p.m. for three weeks achieving plasma levels of 0.5 meq/l. Blood was taken at 9.00 a.m. on day 1 (baseline), day 10, day 21 (12 hr after the last dose), and subsequently once a week during 4 weeks. The experimental procedures are described elsewhere (Poirier et al., 1984).

At day 1, the parameters for [^{3}H]-5HT uptake were the following : Km = 0.79 $\pm$ 0.13 μM ; Vmax = 32.8 + 9.8 pmoles/10^8 platelets/min (n=6). At day 10, the Km values were not modified (Km = 0.77 $\pm$ 0.28 μM, n=4), while the Vmax values of [^{3}H]-5HT uptake were decreased by 44 % (Vmax = 18.5 $\pm$ 3.8 pmoles/10^8 platelets/min, n=4). The decrease in Vmax of [^{3}H]-5HT uptake persisted at day 21 (Vmax = 23.6 $\pm$ 4.8 pmoles/10^8 platelets/min, n=4), as well as after one week of washout (Vmax = 15.5 $\pm$ 3.4 pmoles/10^8 platelets/min, n=4, at day 29). In two out of six volunteers for whom the uptake was measured after four weeks of washout, the decrease of Vmax of [^{3}H]-5HT uptake was still present (Vmax = 19.0 and 15.7 pmoles/10^8 platelets/min respectively at day 50). The Km values were not modified.

In [^{3}H]-IMI binding studies, the Bmax values at day 10 were not significantly different from baseline (Bmax = 640 $\pm$ 100 fmoles/mg prot., n=4 at day 1 ; Bmax = 702 $\pm$ 171 fmoles/mg prot. at day 10). Similarly, the Kd values were not affected by treatment. It is concluded that short-term lithium administration to healthy volunteers decreases the Vmax values of [^{3}H]-5HT uptake in platelets, without affecting the parameters of [^{3}H]-IMI binding. These results are in agreement with the report of a decrease of Vmax values for [^{3}H]-5HT uptake in platelets of bipolar patients after 2-3 weeks of treatment with lithium (Meltzer et al., 1983). The dissociation between the effects of lithium on 5HT uptake and IMI binding confirms the hypothesis that the imipramine receptor and 5HT uptake, although closely associated, can be modulated independently. However, we cannot exclude the possibility that chronic lithium treatment may affect [^{3}H]-IMI binding in platelets with a time course different from that observed for 5HT uptake.

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STUDIES WITH 5-CARBOXAMIDOTRYPTAMINE AND KETANSERIN ON RAT BRAIN-STEM NEURONES.

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At least two distinct populations of 5HT binding sites have been identified in rat brain, termed 5HT1 and 5HT2 (Peroutka and Snyder, 1979). Microiontophoretic application of 5HT has both excitatory and depressant effects on the firing rate of medullary brainstem neurones in the rat. In this study we have examined the effects of the 5HT2 antagonist ketanserin, the alpha 1 adrenoceptor antagonist prazosin and the 5HT1 agonist 5-carboxamidotryptamine (5CT) in order to investigate further the nature of the receptors involved in the responses to 5HT.

Experiments were performed on single spontaneously active neurones in the medullary brainstem of the rat anaesthetised with halothane. Recording sites were marked by the ejection of pontamine sky blue dye.

The effects of ketanserin and prazosin were examined on cells giving consistent excitatory responses to 5HT and noradrenaline. Ketanserin (0.3 mg/kg i.v., n=5) reversibly attenuated the excitatory response to 5HT but had little effect on the excitatory response to noradrenaline. Conversely, prazosin (0.8 mg/kg i.v., n=5) reversibly attenuated the excitatory response to noradrenaline but had little effect on the excitatory response to 5HT. Iontophoretic application of ketanserin also selectively and reversibly blocked the excitatory response to 5HT (n=7). The effects of 5CT were examined on 22 cells giving a consistent excitatory response to 5HT. 5CT evoked no response on 19 cells strongly excited by 5HT and had only weak excitatory effects on the remaining 3 cells.

Iontophoretic application of ketanserin failed to attenuate the depressant responses to 5HT on any of the 5 cells studied. This is in agreement with a previous report that ketanserin in doses up to 2 mg/kg i.v. does not attenuate the depressant response to 5HT (Davies et al. 1985). The effects of 5CT were examined on 9 cells giving consistent depressant responses to 5HT. 5CT evoked marked and prolonged depressant responses on all 9 cells. These responses were not accompanied by any reduction in spike amplitudes or changes in spike duration. When both compounds were applied with identical ejecting currents 5CT was observed to evoke responses up to 10 times larger than those to 5HT.

These results suggest that the excitatory effects of 5HT on medullary brainstem neurones are mediated by a 5HT2-like receptor. The marked depressant effects of 5CT suggest that the depressant responses to 5HT may involve a 5HT1-like receptor. Davies and Roberts (1985) reported that 8-OH-DPAT also has marked depressant effects on the firing rate of brainstem neurones. This taken together with the effects of 5CT indicates the involvement of the 5HT1A subtype in the depressant response to 5HT.

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EFFECTS OF SELECTIVE DESTRUCTION OF HIPPOCAMPAL 5-HT INPUT ON L-TRYPTOPHAN UPTAKE AND BINDING.

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Previously we have demonstrated the existence of L-tryptophan binding sites in cerebral cortex with properties very similar to those of the L-tryptophan carrier present in brain tissue (Balfour and Benwell 1985). The purpose of the present study was to examine this relationship further.

Male Sprague Dawley rats weighing approximately 200g were used. The serotonergic input to the hippocampus was lesioned selectively with 5,7-dihydroxytryptamine (5,7-DHT) using the method of Quik and Azmitia (1983). Control rats received the vehicle (0.02% ascorbic acid in saline). The animals were killed 8-10 days later. The hippocampus, hypothalamus and cerebral cortex were dissected from the rest of the brain and the uptake of L-tryptophan and 5-hydroxytryptamine (5-HT) by synaptosomes measured as described previously (Benwell and Balfour, 1982). L-Tryptophan binding was determined using membranes prepared from the P2 tissue fraction according to the method of Balfour and Benwell (1985).

The Effect of Lesioning The Fornix Fimbria and Cingulum Bundle with 5,7-DHT on 5-HT and L-Tryptophan Uptake and L-Tryptophan Binding

Treatment Group	Brain Region	5-HT Uptake (fmoles/mg/min)	L-Tryptophan (pmoles/mg/min)	L-Tryptophan Binding (pmoles/mg)
Controls	Hippocampus	214.0±38.0(3)	3.18±0.25(3)	2.81±0.29(3)
Lesioned	Hippocampus	66.8±22.0(5)**	2.13±0.19(5)**	1.81±0.11(5)**
Controls	Hypothalamus	302.0±21.0(3)	2.42±0.14(3)	1.16±0.14(3)
Lesioned	Hypothalamus	273.0±48.0(4)	2.30±0.21(3)	1.24±0.13(5)
Controls	Cortex	220.0±28.0(3)	2.71±0.05(3)	1.19±0.16(3)
Lesioned	Cortex	212.0±26.4(5)	2.47±0.16(3)	1.29±0.04(5)

Results are the means±SEM of the number of observations shown in parentheses. The initial concentrations of ^{3}H-5-HT and ^{3}H-L-tryptophan were 10 and 90nM respectively. **$p<0.01$ significantly different compared with controls.

The lesion caused a highly significant reduction ($p<0.01$) in 5-HT uptake by hippocampal synaptosomes but had no effect on uptake by hypothalamic and cortical synaptosomes. The lesion also caused a significant ($p<0.01$) and selective reduction in L-tryptophan uptake by hippocampal synaptosomes which was associated with a comparable decrease ($p<0.01$) in L-tryptophan binding to P2 membranes from this region. Similar changes ($p<0.01$) were observed with tissues prepared from lesioned rats incubated with 5μM ^{3}H-L-tryptophan. The results suggest that L-tryptophan binding to membranes prepared from the P2 fraction appears to represent binding to L-tryptophan uptake sites present in CNS tissue and may provide a useful method of assessing L-tryptophan uptake capacity in circumstances which preclude the preparation of viable synaptosomes or tissue slices.

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ALTERED 5-HT SENSITIVITY AND SYNAPTIC MORPHOLOGY IN RAT CNS INDUCED BY LONG-TERM EXPOSURE TO CONTINUOUS LIGHT.

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Exposure to long-term continuous light results in free-running circadian behaviour which in some animals may develop into arrhythmicity (Pittenrigh & Daan, 1976; Mason, 1984; 1986). Continuous light exposure is also reported to alter synaptic morphology in the suprachiasmatic nuclei (SCN) of the rat (Guldner & Ingham, 1979). Electrophysiological studies have demonstrated that rats exposed to continuous light show altered neuronal sensitivity to iontophoresed 5-HT (Mason, 1984; 1986). The present study was undertaken to determine if altered neuronal sensitivity to 5-HT is accompanied by changes in synaptic morphology in the hippocampus and SCN in the same animal.

Male pigmented hooded Long-Evans rats were kept on either a 12 hr light:12 hr dark (LD, n=12) lighting cycle (lights on 08.00-20.00 hrs GMT) or under continuous light (LL, n=12, 150-200 lux) for 73-118 days. Extracellular recordings were made from hippocampal pyramidal cells of rats anaesthetised with urethane (1.3-1.5 g/kg, i.p.). Effects of iontophoresed 5-hydroxytryptamine creatinine sulphate (5-HT: 20 mM, pH 4.0) and gamma-aminobutyric acid (GABA: 20 mM, pH 4.0) were observed on acetylcholine (20 mM, pH 4.0) evoked discharge. Neuronal sensitivity was evaluated from the iontophoretic charge (ejection current (nA) x time (s)) required to obtain a 50% reduction in firing rate (I.T50); a low I.T50 product reflects a high sensitivity. Three LD and four light exposed rats, following electrophysiological analysis of neuronal sensitivity, were fixed by perfusion for electron microscopic analysis of postsynaptic density (Guldner & Ingham, 1979).

Recordings from hippocampal neurones of rats exposed to continuous lighting showed either (i) an increased sensitivity to iontophoresed 5-HT (I.T50: 3.8±0.7 nC, n=19 cells, 8 rats; mean ±s.e., $p<0.001$) or (ii) a subsensitivity to 5-HT (I.T50: 1545±339 nC, n=13 cells, 4 rats, $p<0.001$) when compared with rats kept under LD conditions (I.T50 LD: 126.5±11.4, n=40 cells). Exposure of rats to continuous light had little effect on the sensitivity of hippocampal pyramidal neurones to iontophoresed GABA (I.T50 LD: 317±10 nC, n=40 cells; I.T50 LL: 293±15 nC, n=45 cells).

Electron microscopic analysis of postsynaptic density was made in the SCN and in the hippocampus. For the SCN of continuous light exposed rats there was a reduction in the mean postsynaptic thickness (20.4±1.02 nm, mean ±s.e., n=200 profiles/4 rats, $p<0.001$) compared with rats kept in LD conditions (25.7±0.96 nm, n=150 profiles/3 rats). In contrast there was no significant difference in the distribution of hippocampal axo-somatic postsynaptic densities of rats exposed to continuous light (14.31±0.57 nm, n=150 profiles) compared with LD conditions (13.68±0.54 nm, n=150 profiles, $p<0.5$).

This shift in SCN synaptic profiles towards those with reduced postsynaptic density was found only in those rats, exposed to continuous light, whose hippocampal neurones exhibited an increased sensitivity to iontophoresed 5-HT.

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SUBTYPES OF THE 5-HT$_1$ RECEPTOR REGULATE [^{3}H]5-HT AND ENDOGENOUS GLUTAMATE RELEASE IN RAT CEREBELLUM.

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Evidence is accumulating for different 5-HT receptor subtypes in the CNS. In particular, the existence of multiple 5-HT$_1$ binding sites, termed 5-HT$_{1A}$, 5-HT$_{1B}$ (Pedigo et al., 1981) and 5-HT$_{1C}$ (Pazos et al., 1984) has been proposed. Functional studies are required to verify whether the multiplicity of 5-HT binding sites reflects the presence in the CNS of multiple receptors. In the present work two 5-HT$_1$ receptor subtypes regulating transmitter release have been identified and characterized pharmacologically in rat cerebellum.

The release of [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) and that of endogenous glutamate (GLU) were studied using rat cerebellum synaptosomes in superfusion (Raiteri et al., 1974). Synaptosomes were incubated 15 min at 37^o C either with 0.06 µM [^{3}H]5-HT or without radioactive label (experiments of endogenous GLU release) and depolarized with 15 mM KCl. Fractions were collected and counted for radioactivity or analyzed for GLU content by HPLC with fluorescent detection.

The release of GLU was potently inhibited by 5-HT (pEC$_{30}$ = 9.77). The 5-HT action was antagonized by the non-selective 5-HT antagonist methiothepin , but not by the 5-HT$_2$ antagonists ketanserin, methysergide or cinanserin. Exogenous 5-HT also inhibited [^{3}H]5-HT release (pEC$_{30}$ = 8.73) and methiothepin, but not the 5-HT$_2$ antagonists counteracted 5-HT . The receptors involved (5-HT autoreceptors on 5-HT terminals and heteroreceptors on GLU terminals) were activated by the 5-HT$_1$ agonist RU 24969. The 5-HT$_{1A}$ selective agonist 8-OH-DPAT activated the presynaptic heteroreceptors (pEC$_{30}$ = 7.98) but was ineffective at the autoreceptors. On the contrary, (-)propranolol, used as a 5-HT$_1$ antagonist, shifted to the right the concentration-response curve of 5-HT at the auto- but not at the heteroreceptors. Spiperone, used as a 5-HT$_{1A}$ antagonist, was ineffective at both receptors.

It is concluded that: 1) GLU terminals in rat cerebellum possess 5-HT receptors mediating inhibition of GLU release; 2) 5-HT autoreceptors exist also in rat cerebellum; 3) both 5-HT auto- and heteroreceptors belong to the 5-HT$_1$ type; however, while the autoreceptors could be classified as 5-HT$_{1B}$ (Middlemiss, 1984), the heteroreceptors do not conform easily to criteria defining the three known subtypes of the 5-HT$_1$ binding sites.

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EFFECTS OF CATECHOLAMINE DEPLETION ON WET-DOG SHAKES PRODUCED BY INTRATHECAL ADMINISTRATION OF A TRH ANALOGUE (CG 3509).

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In conscious rats, prazosin partially antagonises the wet-dog shake (WDS) behaviour produced by intrathecal (i.t.) injection of the TRH analogue, CG 3509 (Bennett et al., 1986), suggesting catecholaminergic pathways may be involved in this response. We have investigated the effects of pretreatment with α-methyl-p-tyrosine (α-MPT) to reduce catecholamine levels, and N-chloroethyl-N-ethyl-2-bromobenzylamine (DSP4) to selectively destroy noradrenergic neurones (Jonsson et al., 1981), on the CG 3509-induced WDS in rats.

Male Wistar rats were cannulated intrathecally under sodium methohexitone (60 $mgkg^{-1}$ i.p.) anaesthesia so that the cannula tip was at the thoraco-lumbar junction (Bennett et al., 1986). WDS were counted, number min^{-1} for 30 min post i.t. injection, in the following groups of rats. One group (n=6) were given i.t. CG 3509 (2 μg) 11 and 15 days after surgery, ~16 h after saline (0.9% i.p.) or α-MPT (300 $mgkg^{-1}$) respectively and the responses compared to control saline injections given 7 days after surgery. In another two groups (n=5) DSP4 (300 μg i.t. 1 day after surgery or 350 μg i.t. 1 and 3 days) was given 1 h after fluoxetine (5 $mgkg^{-1}$ i.p.) followed by CG 3509 (2 μg i.t.) 7 days later and compared with rats (n=6) given vehicle (day 1 i.t.) and CG 3509 (2 μg i.t.) 7 days later. At the end of the DSP4 study noradrenaline and dopamine levels in the thoracic cord and brain stem were assayed using HPLC with electrochemical detection. α-MPT-induced catecholamine depletion was determined in a separate group of rats 16 h after saline (n=5) or 300 $mgkg^{-1}$ α-MPT (n=7) i.p. Values are given as mean±s.e.mean and statistical analysis used the Student's t-test.

Compared with saline i.t. (1±0), CG 3509 (140±11) produced a significant increase ($P<0.001$) in WDS, but this effect was significantly attenuated ($P<0.001$) by α-MPT pretreatment (48±5). The control levels of noradrenaline and dopamine in the thoracic cord (169.3±13.0 and 24.4±1.4 ngg^{-1} wet weight, respectively) were both significantly reduced ($P<0.001$) by 80 to 92% after α-MPT. In contrast, compared with control rats (114±17), neither DSP4 at 300 μg (132±19) nor 2x350 μg (130±18) produced any significant alteration in the CG 3509-induced WDS behaviour. Following DSP4 dopamine levels were unaltered in both thoracic cord and brain stem but thoracic noradrenaline content was significantly reduced in a dose-dependent manner by 300 μg (-30%, $P<0.02$) and 2x350 μg DSP4 (-71%, $P<0.001$).

The attenuation of the CG 3509 response and decreased catecholamine levels after α-MPT is consistent with a catecholamine involvement in the spinal effects of CG 3509. Although the reduction in spinal cord noradrenaline after 2x350 μg DSP4 and α-MPT were similar, the lack of effect of DSP4 on the CG 3509 response could be due to the development of postsynaptic adrenoceptor supersensitivity (Benkirane et al., 1985). Alternatively the effects of i.p. α-MPT on spinal dopaminergic or extraspinal catecholaminergic neurones, not affected by i.t. DSP4, could be responsible for the differences observed.

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UNUSUAL BEHAVIOURAL AND BINDING EFFECTS AFTER CO-ADMINISTRATION OF CLENBUTEROL AND CHLORDIAZEPOXIDE IN MICE.

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We have previously used chlordiazepoxide to induce 'rebound depression' (inactivity following a surge of hyperacticity) in rats introduced to unfamiliar environments (Davies & Steinberg, submitted). Clenbuterol, a centrally active direct β-receptor agonist (Ross, 1980), has been proposed as an anti-depressant in man, though its effects on the behaviour of laboratory rodents are equivocal. We now report that a single dose of clenbuterol followed by chlordiazepoxide has remarkable behavioural effects in mice.

129SV adult experimentally naive male mice were tested for 5 minutes in an open-topped wooden Y-shaped runway under the influence of either clenbuterol 5 mg/kg or chlordiazepoxide 5 mg/kg, given i.p. at 30 and 20 mins respectively beforehand, or the combination of these two drugs or of saline.

The greatest behavioural changes occurred with the clenbuterol/chlordiazepoxide combination; most of these animals displayed pronounced and sustained backward walking ('retropulsion'), an effect which was rarely seen with clenbuterol and chlordiazepoxide when given separately, and not at all with saline. All drug treatments induced bouts of immobility in these mice, and the incidence of this was greatest when the drugs were given in combination (Table 1).

Table 1 Incidence of two kinds of mouse behaviour induced by clenbuterol and/or chlordiazepoxide

	Immobile	B/W	n
Saline	0	0	9
Clen 5	4	2	9
CDZP 5	6	1	9
Mix	8	8	10

Incidence of backward walking (B/W) was greatest in clenbuterol/chlordiazepoxide combination group (Mix): 8/10 mice walked backwards ($P<0.001$, binomial test). All 8 mice also showed bouts of immobililty.

To examine whether the drug combination caused changes in β-adrenoceptors in the brains of these animals, we measured 3H-dihydroalprenolol binding to β-binding sites in the cerebral cortex. However, there were no significant binding changes with any of the drug treatments. Chlordiazepoxide, unlike the inverse benzodiazepine agonists, appears to have no effects on β-adrenoceptors (Little et al, 1985).

Retropulsion has been reported before and is widely thought to involve serotonergic neurones in the CNS (Da Prada et al, 1982). Whether the behavioural effects of clenbuterol and chlordiazepoxide in combination are also mediated by serotonergic neurones remains to be seen.

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A STUDY OF THE EXTRANEURONAL O-METHYLATING SYSTEM FOR CATECHOLAMINES IN THE RAT CEREBRAL CORTEX.

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Although the extraneuronal O-methylation of catecholamines in many isolated peripheral preparations is well established (Trendelenburg 1980), there have been few studies of this system in the brain (Hendley et al., 1971). We have, therefore, studied the uptake and O-methylation by catechol-O-methyltransferase (COMT) of (±)^{3}H-Isoprenaline (^{3}H-ISO) in isolated slices of the rat cerebral cortex.

0.25 mm thick slices of the cerebral cortex, prepared by a McIlwaim tissue chopper, were obtained from male Wistar rats (240-300g). Following a 30 min pre-incubation period in 2 ml physiological salt solution (PSS), maintained at 37°C and gassed with 95% O_2 5% CO_2, slices were exposed to 50 nmol/l ^{3}H-ISO for a further 30 min and then washed briefly in ice-cold PSS. The O-methylated metabolite of ISO (^{3}H-OMI) content was determined in both incubation medium and slice as described by Bönisch (1978). With the exception of phenoxybenzamine, which was washed from the slices prior to incubation, all drugs were included in both the pre-incubation and the incubation media.

When exposed to 50 nmol/l ^{3}H-ISO, cerebral cortex slices produced 328.8±16.0 pmol/g/30 min (n=6) ^{3}H-OMI and accumulated 68.9±4.6 pmol/g ^{3}H-ISO. Neither effects were influenced by the uptake$_1$ inhibitor desipramine (300 nmol/l). 30 umol/l U-0521, an inhibitor of COMT (Trendelenburg 1980), abolished the formation of ^{3}H-OMI and this was associated with an increase in the accumulation of ^{3}H-ISO in the slice to 207.4±7.1 pmol/g (n=12).

OMI caused a concentration-related reduction of the accumulation of ^{3}H-ISO in U-0521 (30 umol/l)-treated slices with a maximum reduction of 54.8±1.1% (n=12) at 600 umol/l. This was termed the "OMI-sensitive component" of ^{3}H-ISO accumulation. The uptake$_2$ inhibitors phenoxybenzamine and corticosterone and the beta-adrenoceptor antagonist propranolol caused a concentration-related inhibition of the "OMI-sensitive component" of ^{3}H-ISO accumulation in U-0512-treated slices. Although the effective concentrations of OMI and corticosterone (IC_{50} ~ 30 umol/l) are higher than those observed at uptake$_2$ in the perfused isolated rat heart (IC_{50} ~ 2 umol/l - Trendelenburg 1980), they are comparable to those observed in slices of the rat submaxillary gland (Major et al., 1978). However, the pronounced inhibitory effects of propranolol remains to be explained. (see: Hendley et al ., 1971).

In conclusion, we have provided direct evidence for the existence of an extraneuronal O-methylating system in the rat cerebral cortex. The uptake component of this system has many pharmacological similarities with uptake$_2$ described in many peripheral preparations.

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EFFECTS OF METHIOTHEPIN ON THE POTASSIUM-EVOKED RELEASE OF [^{3}H]-NORADRENALINE IN RAT PINEAL GLANDS.

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The rat pineal gland contains high levels of 5-hydroxytryptamine (5HT) localized both in the pinealocytes and in sympathetic nerve fibers innervating the gland (Jaim-Etcheverry and Zieher, 1971). There is increasing evidence for the existence of presynaptic inhibitory 5HT receptors localized on sympathetic nerves in the periphery, probably of the $5HT_1$ subtype (Göthert et al., 1986). We therefore examined whether noradrenergic nerve endings of the rat pineal gland possess presynaptic release-modulating 5HT receptors, which could subserve a physiological role for the interaction between 5HT and noradrenaline (NA) in the pineal.

Male rats (180 - 200 g) were killed by decapitation and their pineal gland removed immediately. The endogenous NA stores were labelled in vitro by incubating the pineals with 0.33 µM [^{3}H]-(±)-NA for 45 min in Krebs' solution at 37°C. The pineal glands were transferred to superfusion chambers and superfused with Krebs solution at a rate of 1 ml/min. Two periods of stimulation (50 mM K^+ for 4 min) were applied 60 min (S_1) and 104 min (S_2) after the beginning of superfusion. Drugs were added 20 min before S_2. Results are expressed as the ratios of fractional release obtained between the second and the first periods of stimulation.

When the presynaptic 5HT receptor antagonist methiothepin (0.01 - 1 µM) was added to the medium 20 min before S_2, it increased in a concentration-dependent manner the K^+-evoked release of [^{3}H]-NA from rat pineal glands (S_2/S_1 = 2.52 ± 0.21, n = 6 for 1 µM methiothepin, $p < 0.001$, when compared with the control ratio S_2/S_1 = 0.87 ± 0.05, n = 7). Under these conditions, the 5HT agonists lysergic acid diethylamide (LSD, 0.01 - 1 µM) or 5-methoxytryptamine (0.1 - 10 µM) failed to modify significantly either the K^+-evoked release of [^{3}H]-NA or the spontaneous outflow of radioactivity. Exposure to 5HT (0.1 - 10 µM) did not affect the K^+-evoked release of [^{3}H]-NA, while the spontaneous outflow of radioactivity was significantly increased by 1 and 10 µM 5HT. When the endogenous stores of 5HT were depleted by pretreating rats with para-chlorophenylalanine (PCPA, 300 mg/kg i.p., 48 h before experiments), a 82 % decrease in pineal 5HT levels was obtained, as measured by ED-HPLC (control = 138 ± 45 ng 5HT/pineal, n = 4 ; PCPA = 25 ± 4 ng/pineal, n = 6, $p < 0.005$). Pretreatment with PCPA increased by 90 % the endogenous levels of NA (control = 3.9 ± 1.6 ng NA/pineal, n = 4 ; PCPA = 7.5 ± 1.0 ng NA/pineal, n = 6). In PCPA-treated rat pineal glands, the K^+-evoked release of [^{3}H]-NA was not modified when compared with control rats. Exposure to methiothepin increased the K^+-evoked release of [^{3}H]-NA to a similar extent in PCPA-treated rats when compared with controls (S_2/S_1 = 2.85 ± 0.30, n = 3 and S_2/S_1 = 2.52 ± 0.21, n = 6 in the presence of 1 µM methiothepin for PCPA and control rats, respectively). As observed in control rats, 5HT, LSD and 5-methoxytryptamine failed to modify the release of [^{3}H]-NA in PCPA-treated rat pineal glands.

Uptake experiments were carried out by incubating normal pineal glands with 0.33 µM [^{3}H]-(±) NA for 10 min, at 37°C or 0°C. Cocaine 10 µM completely inhibited the specific uptake of [^{3}H]-NA into rat pineal glands. In the presence of 1 µM methiothepin, [^{3}H] -NA uptake was also inhibited by 90 %.

Our results indicate that presynaptic 5HT receptors are not present on noradrenergic nerve endings of the rat pineal gland. The increasing effect of methiothepin on the K^+-evoked release of [^{3}H]-NA in normal and PCPA-treated rat pineal glands is likely to be due to the inhibition by this drug of [^{3}H]-NA uptake.

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DOPAMINERGIC MODULATION OF D-[^{3}H]-ASPARTATE AND [^{14}C]-ACETYLCHOLINE RELEASE FROM RAT STRIATAL SLICES.

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Dopaminergic binding sites in striatum are associated with nigrostriatal dopaminergic, corticostriatal glutamatergic neurons and cholinergic and GABA-ergic interneurons (Hall et al., 1983). Conflicting evidence exists for the functional importance of the dopamine receptors associated with the corticostriatal glutamatergic projections (Stoof et al., 1982; Mitchell & Dogett, 1980).
We studied the dopaminergic modulation of D-^{3}H-aspartic acid and ^{14}C-acetylcholine release using a double label approach. Striatal slices (dimensions: 2 x 0.3 x 0.3 mm) were stimulated twice with 12.5, 18.75 or 25 mM KCl for 2 min. With 18.75 mM KCl stimulation 0.68% D-^{3}H-aspartic acid and 10.73% ^{14}C-acetylcholine was released. The release of D-^{3}H-aspartic acid was partially dependent on the presence of Ca, in particular at high KCl concentrations, whereas the release of ^{14}C-acetylcholine was almost completely Ca-dependent. Pergolide (0.01-10 μM) dose-dependently inhibited D-^{3}H aspartic acid release up to 55% reduction at 10 μM, whereas the D-1 agonist SKF 38393 increased the release insignificantly (20%). Sulpiride (0.01-10 μM) had no effect but haloperidol (10 μM or more) severely reduced D-^{3}H-aspartic acid release up to 65% reduction at 10 μM. Studied at 10 μM neither amisulpiride nor SCH 23390 had any effect, but chlorpromazine (-30%) and clozapine (-36%) reduced D-^{3}H-aspartic acid release. The inhibitory effect of 10 μM pergolide was dose-dependently antagonized by 0.1-10 μM amisulpiride and sulpiride. No antagonism was observed with similar concentrations of haloperidol, chlorpromazine and clozapine, while 1 and 10 μM SCH 23390 showed weak antagonism. Pergolide also inhibited ^{14}C-acetylcholine release up to 55% reduction at 10 μM, while SKF 38393 was inactive. Sulpiride (0.01-10 μM) dose dependently increased the release of ^{14}C-acetylcholine up to 25% increase at 10 μM. Haloperidol had a biphasic effect with facilitation at 1-100 nM. Amisulpiride, chlorpromazine and SCH 23390 also significantly increased ^{14}C-acetylcholine release. The inhibitory effect of 3 μM pergolide was fully and dose-dependently antagonized with 0.03-1 μM haloperidol and 0.3-10 μM sulpiride. The effect of 10 μM pergolide was completely and dose-dependently antagonized by 0.1-10 μM amisulpiride and less so by haloperidol, whereas chlorpromazine, clozapine, SCH 23390 and sulpiride were much weaker or not active at all. Reserpine pretreatment of the animals had no effect on the ability of sulpiride or pergolide to modulate D-^{3}H-aspartic acid release. It reduced the ability of haloperidol to elevate ^{14}C-acetylcholine release but the effect of sulpiride was unaffected.
This study demonstrates that the dopamine agonist pergolide reduces the release of D-^{3}H-aspartic acid and ^{14}C-acetylcholine release. The antagonism studies are in agreement with a D-2 modulation of ^{14}C-acetylcholine release. The antagonism by sulpiride and amisulpiride of the pergolide induced inhibition of D-^{3}H-aspartic acid implicates the involvement of a D-2 receptor. The inability of haloperidol to antagonize this effect is probably due to its own strong depressant effect. An endogenous modulation of D-^{3}H-aspartic acid release has not been found, but these dopamine receptors may contribute to the modulation of striatal functions by dopamine agonists.

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MK-801, A NOVEL, ORALLY ACTIVE ANTICONVULSANT, IS A POTENT, NON-COMPETITIVE N-METHYL-D-ASPARTATE-RECEPTOR ANTAGONIST.

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Recently, there has been considerable interest in the anticonvulsant and neuroprotective properties of antagonists for excitatory amino acid receptors, particularly those selective for the N-methyl-D-aspartate (NMDA) sub-type (Schwarcz & Meldrum; 1985). However, the limited ability of the available competitive NMDA-receptor antagonists to penetrate the central nervous system restricts their therapeutic potential. In this communication we report that MK-801, a novel, orally active anticonvulsant (Clineschmidt et al; 1982), with a previously undetermined mechanism of action, is a potent and selective NMDA antagonist, which acts in a non-competitive and agonist dependent manner.

Responses to excitatory amino acids were recorded from slices of rat cortex using a greased gap technique similar to that described by Harrison & Simmonds (1985). Approximately 1mm wide cortical wedges (0.5mm thick) were mounted in a two compartment bath with the ventral margin of the cortical tissue traversing a greased slot which separated the chambers, so that the cortical tissue lay almost entirely within one compartment and the white matter entirely within the other. The chamber containing the cortical tissue (vol = 0.3ml) was continuously gravity perfused with oxygenated, Mg^{2+}-free artificial cerebrospinal fluid (aCSF), containing tetrodotoxin (0.1μM), at a rate of 1.5 - 2ml min^{-1}. Excitatory amino acid agonists (3 - 40μM) were applied for one minute periods with an intervening period of not less than 10 min.

Continuous perfusion of slices with MK-801 produced a potent blockade of depolarising responses to NMDA. The threshold concentration for this effect was approximately 75nM, which resulted in a flattening of the concentration-response relationship. This antagonism was dependent upon the addition of the agonist (NMDA), rather than the length of time the tissue was in contact with MK-801, and thus the block increased progressively with additions of increasing concentrations of NMDA. This effect was highly selective for NMDA as MK-801 in the highest concentrations tested had no effect on responses to quisqualate (30μM MK-801) or kainate (1μM MK-801). In Mg^{2+}-free aCSF and in the absence of tetrodotoxin, spontaneous depolarising potentials were present in the majority of slices (Harrison & Simmonds; 1985) and this epileptiform activity was abolished by MK-801 at concentrations which blocked NMDA responses.

These results suggest that MK-801 is a potent, selective and non-competitive NMDA antagonist and provide an explanation for its anticonvulsant properties.

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THE IDENTIFICATION OF A NOVEL BINDING SITE FOR THE ANTICONVULSANT, MK-801, IN RAT BRAIN MEMBRANES.

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MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate) is a potent and orally-active anticonvulsant having both anxiolytic and sympathomimetic properties (Clineschmidt et al, 1982). In an attempt to establish the mechanism of action of MK-801 it was found that this compound had little affinity in binding assays which label receptors for catecholamines and γ-aminobutyric acid/benzodiazepine (Clineschmidt et al 1982). We have investigated this question by studying the binding properties of tritium labelled MK-801.

All binding studies were carried out in brain membrane preparations obtained from Sprague-Dawley rats (200 - 300g). The P_2 fraction was suspended in a Krebs buffer, incubated at 23°C for 60 min and assayed by rapid filtration through GF/B filters. In rat cortical membranes, 5nM [^{3}H]-MK-801 (22.5 Ci/mmol) labelled a site with 80% displaceable binding as defined by 100μM unlabelled MK-801. This binding was fully reversible and saturable and was abolished by pre-heating the membranes at 80°C for 10 min. Scatchard analysis of the saturation data indicated a monophasic plot with apparent affinity (Kd) and site density (Bmax) values (Mean ± S.E.M., n=6) of 37.2 ± 2.7nM and 0.825 ± 0.102pmol/mg of protein respectively. This binding of [^{3}H]-MK-801 showed regional specificity with the hippocampus exhibiting the highest density, followed by the cortex, striatum and medulla-pons, while the cerebellum failed to show significant binding. In addition to showing stereoselectivity, this site also possessed an unusual pharmacological specificity (Table I).

Table I Displacement of [^{3}H]-MK-801 (5nM) binding in rat cortical membranes

	Ki(Mean ± S.E.M.,nM)	n
MK-801	31±2	10
(-)MK-801	212±25	5
Phencyclidine	875±38	9
(±)Ketamine	4916±528	5
(±)SKF10,047	7522±721	7

The pharmacological relevance of this MK-801 binding site was enforced by the demonstration that MK-801 and its (-)isomer resembled the other active compounds above in showing potent and selective blockade of electrophysiological responses of N-methyl-D-aspartate (NMDA) (Kemp et al 1986, Anis et al 1983). Indeed, there was an excellent correlation (r=0.99) between the potency of these compounds in displacing [^{3}H]-MK-801 binding and blocking NMDA responses.

These results suggest that the anticonvulsive action of MK-801 results from its binding with high affinity to sites associated with NMDA receptors.

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D-AMINOPHOSPHONOVALERATE SELECTIVELY ANTAGONIZES N-METHYL-D-ASPARTATE INDUCED CURRENTS IN VOLTAGE CLAMPED MOTONEURONES IN VITRO.

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N-methyl-D-aspartate (NMDA) is a potent neuronal excitant acting on specific excitatory amino acid receptors widely distributed throughout the central nervous system (Watkins and Evans, 1981). Recent intracellular work on the frog spinal cord has shown that D-aminophosphonovalerate (D-APV) selectively antagonizes NMDA evoked excitations compared to quisqualate (Corradetti et al., 1985). In the present study we have used single microelectrode voltage clamping to demonstrate directly antagonism by D-APV of the inward slow currents underlying NMDA induced motoneuronal depolarizations.

Recordings were made from motoneurones of the frog (Rana temporaria) spinal cord parasagittal slice superfused with magnesium-free oxygenated Ringer at 7°C (Corradetti et al., 1985). 2 M CsCl or 3 M KCl filled microelectrodes were used to voltage clamp motoneurones near their resting membrane potential (the mean of which for 24 cells was -70 $\pm$ 2mV). Glutamate (1-2 mM), quisqualate (15-30 µM) or NMDA (15-30 µM) induced slow inward currents (mean $\pm$ s.e.m.) of 223 $\pm$ 29 pA (n = 24), 223 $\pm$ 64 pA (n = 12) and 227 $\pm$ 34 pA (n = 8) respectively. The time course of decay for quisqualate and NMDA currents were longer than those for glutamate: 139 $\pm$ 23s and 212 $\pm$ 51s versus 76 $\pm$ 12 ms. In the presence of 10 µM D-APV NMDA currents were reduced by 91% while quisqualate currents remained unaltered and glutamate currents were antagonized by 42%.

These data are the first demonstration in the spinal cord of the selective antagonism by D-APV of the inwardly directed current responsible for NMDA motoneuronal depolarizations. Since glutamate currents were also antagonized, it appears that glutamate is a mixed agonist with actions at both NMDA and quisqualate receptors (Mayer and Westbook, 1984).

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INFLUENCE OF CARDIOPULMONARY RESUSCITATION ON THE PLASMA CONCENTRATIONS OF THE CALCIUM ENTRY BLOCKER NIMODIPINE IN THE DOG.

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The calcium entry blocker nimodipine is of potential clinical interest in the prevention of neurologic damage in patients resuscitated from cardiac arrest (Dearden, 1985). Since it distributes rapidly and extensively to tissues and is highly extracted in the liver, severe circulatory failure can be expected to alter its pharmacokinetics as described for lidocaine (Benowitz,1984). We therefore investigated in the dog the influence of cardiopulmonary resuscitation on the plasma concentrations of nimodipine. Antipyrine was also studied as a model substance with a small volume of distribution and a low hepatic extraction.

In mechanically ventilated dogs (5.5-18 kg) anesthetized with ketamine and paralyzed with gallamine, nimodipine (18 µg/kg over 3 min followed by 0.55 µg/kg/min, n=6) or antipyrine (21 mg/kg over 3 min followed by 0.2 mg/kg/min, n=6) was infused into the left femoral vein throughout the experiment. After sixty min of infusion, ventricular fibrillation was induced with a transthoracic current (60 Hz; 20-40 V AC); after 3 min of complete cardiopulmonary arrest, mechanical ventilation (100% O_2) and external cardiac massage (either manually or with a mechanical thumper) were instituted. Blood samples were drawn from the jugular vein or the pulmonary artery and analyzed with gas liquid chromatography.

Table 1 Plasma concentrations of nimodipine and antipyrine 15 min and 1 min before cardiac arrest and between the 8th-13th min of cardiopulmonary resuscitation (CPR)

	Nimodipine (ng/ml)			Antipyrine (µg/ml)		
	Before cardiac arrest		During CPR	Before cardiac arrest		During CPR
	15 min	1 min		15 min	1 min	
(1)	19.3	22.5	44.2	13.3	13.8	17.9
(2)	14.4	13.0	35.0	10.7	10.4	16.2
(3)	6.6	5.9	33.3	18.9	21.2	27.4
(4)	23.1	17.8	49.5	11.3	10.8	13.6
(5)	12.0	14.2	35.3	10.3	13.2	18.2
(6)	14.1	15.0	64.8	32.8	23.2	65.0
mean	14.9	14.7	37.0	16.2	15.4	26.4
s.e.mean	2.3	2.2	8.2	3.6	2.2	8.0

The present data show that there is a marked increase (226 ± 53%) in the venous plasma concentrations of nimodipine during cardiopulmonary resuscitation presumably because of a slowing of distribution and a decrease of hepatic elimination under these conditions (Benowitz,1984). The increase with antipyrine (50 ± 18%) tends to be smaller possibly because its distribution is restricted to body water and because its hepatic elimination is not flow-dependent. Further experiments are necessary to assess the relative importance of changes in distribution and elimination during cardiopulmonary resuscitation.

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THE EFFECT OF AMLODIPINE, NIFEDIPINE AND DILTIAZEM ON Ca^{++} CONTRACTIONS OF CANINE CORONARY ARTERY RINGS.

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The object of this study was to compare the effects of amlodipine, a new Ca^{2+}-channel inhibitor, nifedipine and diltiazem on receptor-operated Ca^{2+}-channels (ROC) and potential operated channels (POC) of dog coronary artery.

Rings (2-3 mm long) of the left circumflex coronary artery from greyhounds were suspended on parallel wire hooks at a resting tension of 1.5 g and the contraction recorded isometrically. The physiological salt solution was a Ca-free HEPES buffer containing (mmol/L) NaCl 144; glucose 11, HEPES 5, KCl 5.8, $MgCL_2$ 1.2, EGTA 2 and propranolol 1 uM gassed with 5% CO_2 in O_2 and maintained at 37°C. After 30 minutes the EGTA concentration was reduced to 0.1 mM and the tissues were left to equilibrate for a further 60 minutes. Each agonist was added for fifteen minutes and cumulative concentration curves to $CaCl_2$ were produced either in the presence or absence of Ca^{2+}-channel inhibitors. The equilibrium time for nifedipine and diltiazem was 1 hr and for amlodipine 3 hours. The Ca^{2+}-channel inhibitors produced parallel rightward shifts of the Ca^{2+}-concentration curves; the one exception was that amlodipine significantly reduced the maximum response to adrenaline. Table 1 shows the EC_{50} values calculated from the Ca^{2+}-concentration response curves for Ca^{2+} in control tissues in the presence of each of the respective agonists and the equipotent molar ratios obtained in the presence of nifedipine, diltiazem and amlodipine.

Table 1. Effects of nifedipine, amlodipine and diltiazem on Ca curves in dog coronary artery rings (n=6-9)

	K^+ 40 mM	Adrenaline 1 uM	5HT 5 uM	U46619 5 nM
EC_{50} for Ca (mM)	0.043±0.008	0.61±0.08	14.6± 4.9	0.69±0.04
Nifedipine EMR (0.01 uM)	23	2.3	1.3	2.3
Amlodipine EMR (0.01 uM)	1.5	8.5	1.0	5.6
Diltiazem EMR	39	2.7	1.7	2.7

These results support the view (C. Cauvin et al.) that nifedipine and diltiazem are more effective inhibitors of POC than ROC. In contrast, amlodipine appears to show little discrimination between the two types of channel. Therefore, although nifedipine and amlodipine are of similar chemical structures these results suggest that they may have different pharmacological profiles.

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INHIBITION OF THE EFFECTS OF ENDOTHELIAL-DERIVED RELAXANT FACTOR (EDRF) IN AORTA BY PALMITYL CARNITINE.

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Palmityl carnitine is a lipid metabolite which accumulates in ischaemic conditions and which can directly activate Ca^{2+} channels in smooth muscle (Mir & Spedding, 1986). The contractile effects of the dihydropyridine Ca^{2+} channel activator, Bay k 8644, in rat aorta are reduced in the presence of the endothelium by a tonic release of EDRF (Spedding et al, 1986); we have tested the effects of palmityl carnitine in the presence and absence of the endothelium.

Rings of rat aorta were set up in Tyrode solution at 35^0C and gassed with 95% O_2: 5% CO_2. In the presence of a mild (12 mM K^+) depolarising stimulus Bay k 8644 (10 nM - 1 µM) contracted rat aorta and the effects were markedly reduced in the presence of the endothelium, as reported previously (Spedding et al, 1986). Palmityl carnitine (1 - 1000 µM) also contracted the aorta in a concentration- dependent manner, but there was no difference between rubbed preparations and preparations in which the endothelium was intact ($p > 0.1$; $n = 6$). The influence of the Ca^{2+} channel activators on endothelium-dependent relaxations induced by acetylcholine was therefore investigated. Acetylcholine (1 µM)- induced relaxations of contractions of rat aorta evoked by phenylephrine (1 µM) were abolished by palmityl carnitine (10 - 100 µM) although Bay k 8644 (1 µM) had no effect. Acetylcholine-induced relaxations were rapidly (<30 sec) reversed by palmityl carnitine (10 µM) which did not reverse the effects of other relaxant agents (milrinone, 10 µM). Palmitic acid (10 µM) and carnitine (10 µM) did not affect acetylcholine-induced relaxations. Palmityl carnitine (100 µM for 20 min) did not change the morphology of the endothelium as assessed by the silver staining technique ($AgNO_3$ 10% for 20 min).

Palmityl carnitine (10 - 100 µM) and Bay k 8644 (1 µM) shifted concentration-response curves to Ca^{2+} to the left in K^+ (40 mM)-depolarised ileum preparations from the guinea-pig. However, whereas Bay k 8644 shifted concentration-response curves to histamine (0.001 - 3 µM) and carbachol (0.01 - 3 µM) to the left in ilea maintained in low K^+ (5 mM), palmityl carnitine shifted the curves to the right, even in the presence of Bay k 8644 (1 µM).

These findings confirm that palmityl carnitine may increase sensitivity to Ca^{2+} in K^+-depolarised tissues (Mir & Spedding, 1986), but the substance may also have several other effects. As acyl carnitines are produced in ischaemic states they may be important modulators of cellular metabolism.

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VASCULAR RECEPTORS FOR ATRIAL NATRIURETIC PEPTIDE IN THE RAT, EFFECT OF HYPERTENSION ON THE BINDING CHARACTERISTICS AND BIOLOGICAL RESPONSE.

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Atrial natriuretic peptide (ANP) is a 28-amino acid peptide isolated from the mammalian atria endowed with powerful diuretic, natriuretic, vasorelaxant and aldosterone-inhibitory properties. This peptide may be involved in the regulation of salt and water balance as well as of vascular tone. We have recently demonstrated the presence of vascular and adrenal receptors for ANP in the rat (Schiffrin et al., 1985). We therefore decided to investigate the regulation of binding sites and biological response to ANP in blood vessels in different models of hypertension in the rat.

Receptor for ANP were studied in membrane preparations of rat mesenteric artery and aorta, as previously described (Schiffrin et al., 1985) in competition experiments with 50 µg of membrane protein per tube. Biological experiments were performed of helical strips of thoracic aorta or rings of mesenteric artery of the rats, suspended in 15-ml bath filled with oxygenated Krebs bicarbonate solution. C-R curves to ANP or analogues were measured on precontracted vessels (noradrenaline or phenylephrine EC_{90}) after having measured a C-R curve to the adrenergic agonist.

ANP receptors on the mesenteric artery and aorta appeared to be identical in the binding and biological assays of both tissues since the order of potency of analogs of ANP was identical on the four assay systems (ANP, 99-126 ≥ 101-126 ≥ 103-126 = 103-125 > 103-123). Sodium depletion or moderate sodium loading did not induce any changes in ANP binding sites of the mesenteric artery or the response of the precontracted aorta strips. Sodium loading in the presence of reduced renal mass (unilateral nephrectomy) resulted in a down-regulation of vascular ANP receptor (from 202 ± 25 to 100 ± 22 fmol/mg protein, $p < 0.001$). In 1-K, 1-C renal hypertensive rats the vascular ANP receptors were decreased (from 147 ± 32 to 79 ± 26 fmol/mg protein, $p < 0.05$), while tended to be lower in 2-K, 1-C renal hypertensive rats (from 151 ± 15 to 125 ± 19 fmol/mg protein, N.S.). The precontracted aorta strips of both types of renal hypertensive rats were less sensitive to ANP ($p < 0.05$). Deoxycorticosterone treatment in the absence or in the presence (DOCA-salt hypertension) of uninephrectomy induced a decrease in the number of binding sites for ANP (from 255 ± 60 to respectively 92 ± 21 and 60 ± 13 fmol/mg protein, $p < 0.01$) as well as a decrease in the biological response in precontracted aorta strips ($p < 0.05$).

From these results, it is concluded that ANP binding sites appeared to be identical in the aorta or the superior mesenteric artery. ANP binding sites as well as its biological response in precontracted aorta strips are decreased in conditions associated with volume expansion, e.g. salt loading in the presence of uninephrectomy; treatment with DOCA; one-kidney, one-clip and DOCA hypertension. Furthermore, hypertension per se does not appear to result in down-regulation of vascular ANP receptors.

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THE ACTION OF CALCITONIN GENE RELATED PEPTIDE ON BOVINE ENDOTHELIAL CELLS.

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Human calcitonin gene related peptide (hCGRP) is a potent vasodilator both in vivo and in vitro, the latter having been shown to be dependent on intact endothelium (Hughes et al, 1985). We have examined the response of endothelial cells to selected vasodilatory peptides in terms of their capacity to release prostacyclin, and activate adenylate cyclase.

Bovine aortic endothelial cells, AG 4762, (NIA Aging Cell Repository, USA) were cultured to passage 16-17, and then sub-cultured on microbeads as previously described for superfusion studies (Gordon & Martin, 1983). Over 11 experiments, bradykinin caused release of 6-oxo-$PGF_{1\alpha}$, with half maximal stimulation at 100 nM bradykinin. 1 μM bradykinin caused release of 208.2 ± 160.3 pg 6-oxo-$PGF_{1\alpha}$/ml/10^6 cells, measured by radioimmunoassay of the superperfusate. hCGRP, vasoactive intestinal peptide (VIP) and human calcitonin caused no stimulation of prostacyclin production.

Adenylate cyclase activity was measured in membrane preparations of AG 4762 cells by the method of Salomon et al (1974). hCGRP produced a dose dependent increase in adenylate cyclase activity from a basal level of 20.7 ± 2.5 pmol cAMP/min/mg protein to a maximum of 134.4 ± 52.9 pmol cAMP/min/mg protein (n = 3, mean ± S.E.M.). The K_{act} value was 944 ± 215 x 10 nM. Isoprenaline also caused adenylate cyclase activation, the basal level of 28 pmol cAMP/min/mg protein rising to 106.27 cAMP/min/mg protein at 10 μM with a K_{act} value of 700 nM. In other experiments the hCGRP-dependent increase in adenylate cyclase activity was shown to require the presence of GTP. No enzyme activation was observed with VIP, substance P, bradykinin, or with human or salmon calcitonin.

The activation of adenylate cyclase by hCGRP in bovine endothelial cells is not coupled to prostacyclin release. Conversely, prostacyclin release stimulated by bradykinin is independent of adenylate cyclase activation. The lack of cross-reactivity in these experiments between hCGRP and human or salmon calcitonin, (which have 19% and 30% sequence homology with hCGRP respectively), suggests the presence of a specific receptor for CGRP. The K_{act} value for adenylate cyclase activation by CGRP is greater than predicted for such a potent vasodilator, but this may be a result of using hCGRP with bovine cells. We are currently examining the effect of hCGRP on cultured human umbilical vein endothelial cells.

In summary, cyclic AMP appears to be a mediator of hCGRP action on cultured bovine endothelial cells.

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EFFECT OF A VASOPRESSIN V_1 ANTAGONIST ON THE BP RESPONSE TO ELECTRICAL STIMULATION OF THE MEDULLARY A1 REGION.

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Low frequency electrical stimulation (<20 Hz) of the A1 region of the caudal ventrolateral medulla (CVL) causes a fall in mean arterial blood pressure (MAP) whereas an increase in MAP occurs at higher frequencies (Imaizumi et al., 1985). Noradrenergic neurones in this region innervate the hypothalamic supraoptic and paraventricular nuclei which synthesize vasopressin (AVP) (Sawchenko & Swanson, 1982) and may regulate AVP secretion. The present study sought to determine a possible role for AVP in the MAP response to electrical stimulation of the A1 region using a vasopressin V_1 antagonist.

Male Wistar rats (280-320 g) under halothane anaesthesia were cannulated in the left carotid artery for continuous MAP measurement and stereotaxically cannulated in the third ventricle for continuous collection of CSF. Three CSF samples (approximately 10 µl each) were collected during a 45 min control period followed by 1 ml of blood (replaced with 1 ml saline) via the carotid cannula. A concentric needle electrode (diameter 100 µm) was implanted stereotaxically into the A1 region of the CVL and stimulated by square wave pulse of 1 ms duration, 2 V at 10 or 50 Hz for 10 s every 5 min. Following three stimulations during the control period, the V_1 antagonist $d(CH_2)_5Tyr(Me)AVP$ (10 μgkg^{-1}) or saline were administered i.v. and the three stimulations repeated. One CSF sample was taken after each period of stimulation. The experiment was then repeated in acutely adrenalectomised rats. After the experiments, rats were killed by decapitation and a final plasma sample taken. AVP was measured by radioimmunoassay in the CSF and plasma samples following extraction in acetic acid or florisil respectively. Values are expressed as mean±s.e. mean (n=6) and compared using student's t-test.

Stimulation of the A1 region at 10 Hz caused an immediate decrease in MAP (-21±0.5 mmHg) which was unaffected by the V_1 antagonist or by adrenalectomy. However the recovery of MAP following stimulation was significantly delayed by the V_1 antagonist ($P<0.05$). Stimulation of the A1 region at 50 Hz caused an immediate increase in MAP (27.9±3.3 mmHg) followed approximately 20 s later by a smaller secondary rise in MAP (7.9±0.9 mmHg). The initial pressor response was unaffected by the V_1 antagonist or by adrenalectomy, however the V_1 antagonist significantly reduced the amplitude of the secondary response to 2.6±0.9 mmHg ($P<0.05$), and adrenalectomy prolonged its duration.

Chemical sympathectomy was produced in a separate group of rats (n=6) by systemic administration of 6-OH dopamine (100 $mgkg^{-1}$; i.v.) 36-42 h before the experiment. In these rats A1 stimulation at 50 Hz did not produce an initial pressor response however the secondary response was present and significantly reduced by the V_1 antagonist to 1.8±0.9 mmHg ($P<0.01$).

A1 stimulation at 50 Hz elevated plasma AVP, from prestimulation levels of 8.6±1.3 pg/ml to 72.0±9.8 pg/ml and decreased the CSF levels although this did not reach significance.

These results suggest that AVP is secreted following electrical stimulation of the A1 region and that this peptide may play a role in eliciting the secondary rise in MAP seen following stimulation at 50 Hz. Whether this effect is mediated via noradrenergic neurones remains to be determined.

J.V.J. is an SERC CASE student in association with ICI Pharmaceuticals.

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BENEFICIAL EFFECTS OF ALLOPURINOL ON SURVIVAL AND INFARCT SIZE FOLLOWING CORONARY ARTERY LIGATION IN THE RAT.

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Evidence is accumulating that oxygen free radicals contribute to ischemia induced cell damage. Allopurinol, a xanthine oxidase inhibitor, has been shown to reduce the extent of tissue injury following reperfusion of severely ischemic myocardium (Peterson et al., 1985). However, it is unknown if allopurinol can modify the ultimate tissue damage occurring as a result of permanent cessation of coronary blood flow. The purpose of this study was therefore to evaluate the effects of allopurinol on survival and infarct size following coronary artery ligation (CAL) in the rat.

The study was performed on male Sprague-Dawley rats weighing 240-325 g. Myocardial infarction was induced by ligation of the left coronary artery immediately distal to its origin. Animals were randomly assigned to control and allopurinol treated groups. Allopurinol was administered at the dose of 30 mg/kg orally 18 hours prior to CAL, at 20 mg/kg i.v. 15 min prior to surgery and at 30 mg/kg orally 3 hours after CAL. Animals were sacrificed at 6 and 24 hours post CAL, their hearts were removed, sliced into 2.5 mm thick slices, stained in triphenyl tetrazolium chloride solution and photographed. Infarct size was measured by planimetry and evaluated as percent of the left ventricular volume. Infarct size was also evaluated in rats which died between 3 and 6 hours post-ligation.

During the first 6 hours after CAL only 6 out of 39 animals died in the allopurinol treated group compared to 15 out of 39 in control ($p<.05$). There was no difference in survival frequency between the two groups from 6 to 24 hours post CAL. Although the infarct size evaluated up to six hours after CAL was virtually the same in both control and allopurinol treated groups, at 24 hours after CAL the infarct size in untreated animals was significantly larger than in allopurinol treated animals ($p<.05$).

Thus, the inhibition by allopurinol of infarct size extension between 6 and 24 hours post CAL indicates that allopurinol may reduce free radical mediated damage to the zone adjacent to the nonperfused area and, in turn, prevent extension beyond the original tissue damage. Yet, because the inhibition of this extension does not coincide with the period of augmented survival, the effects of allopurinol on survival during development of myocardial infarction do not appear to be associated with infarct size limitation. Instead, antiarrhythmic properties of allopurinol (Wexler and McMurtry, 1981) may be responsible for increased survival during the early (up to 6 hrs post CAL) developmental phase of myocardial infarction.

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THE TACHYCARDIAC RESPONSE TO 5-HYDROXYTRYPTAMINE IN THE SPINAL GUINEA-PIG .

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5-Hydroxytryptamine (5-HT) elicits tachycardia via "5-HT_1-like", 5-HT_2 or 5-HT_3 ('M') receptors in, respectively, the cat (Saxena *et al.*, 1985; Connor *et al.*, 1986), rat (Saxena & Lawang, 1985) or rabbit (Fozard, 1984). However, in the guinea-pig the mechanism of tachycardiac action of 5-HT is not clear. Trendelenburg (1960) suggested the involvement of both a direct (lysergide-sensitive) and an indirect (morphine, cocaine and dichloroisoprenaline-sensitive, but reserpine-insensitive) mechanisms. Recently, a direct β_1-adrenoceptor stimulation has been denied (Walter *et al.*, 1984) or invoked (Eglen *et al.*, 1985). We have now analysed the tachycardiac effect of 5-HT in guinea-pigs (albino x Dunkin-Hartley) which were spinalized under halothane, N_2O anaesthesia and artificially ventilated. In separate groups (n=6-10) effects of cumulative doses of 0.9% NaCl (0.5, 1.5 and 4.5 ml), methiothepin, ketanserin, MDL 72222 and atenolol (each 0.5, 1.5 and 4.5 mg/kg), propranolol (0.1, 0.3 and 1 mg/kg), indalpine (2, 5 and 10 $mg.kg^{-1}$) and reserpine (3 $mg.kg^{-1}$ on 2 days) were studied against tachycardia elicited by 5-HT and isoprenaline. As shown in Table 1, the tachycardiac responses to 5-HT were not affected by antagonists of the three types of 5-HT receptors, but were attenuated by β-adrenoceptor antagonists and the 5-HT-uptake inhibitor, indalpine. In addition, in the reserpine-treated animals, the responses to 5-HT were not dose-dependent and showed rapid tachyphylaxis. We conclude that the 5-HT-induced tachycardia in the guinea-pig is mainly due to a release of catecholamines by a mechanism similar, though perhaps not identical, to that of tyramine.

Table 1. Tachycardiac response (mean±SEM beats/min) to 5-HT or isoprenaline.

	5-Hydrxytryptamine (μg/kg)								Isoprenaline (0.1 μg/kg)	
	15		30		60		120			
	B	A	B	A	B	A	B	A	B	A
0.9% NaCl	4±2	3±2	16±5	11±5	26±5	24±5	29±4	30±5	17±4	22±6
Methiothepin	4±1	1±1	12±3	10±4	22±6	17±5	25±8	18±7	16±5	12±5
Ketanserin	16±6	8±5	27±5	21±7	37±4	38±3	37±7	44±4	33±5	30±3
MDL 72222	7±3	2±2	11±2	10±3	20±2	18±3	24±3	20±2	26±9	24±7
Propranolol	10±2	0±0*	17±4	5±2*	26±5	14±6*	37±6	12±5*	52±5	6±4*
Atenolol	10±3	2±1*	24±6	5±3*	36±7	8±3*	38±7	9±2*	30±6	7±4*
Indalpine	11±3	1±1*	34±8	16±8*	48±7	25±11*	54±7	25±9*	43±6	28±9
Reserpine	20±3	1±1*	18±4	2±2*	16±4	3±2*	13±3	1±1*	54±11	54±11

B and A represent, respectively, responses *before* and *after* treatment (in case of reserpine, 1st and 2nd set of doses). Data with the highest dose used is presented, except methiothepin (1.5 mg/kg) where the highest dose affected isoprenaline response. *, $p<0.05$ vs respective control response; analysis of variance followed by Duncan's new multiple range test.

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FURTHER DIFFERENCES BETWEEN 5-HT RECEPTORS OF ATRIUM AND VENTRICLE IN CAT HEART.

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It has previously been reported that 5-hydroxytryptamine (5-HT) receptors of kitten atrium and ventricle have different properties (Kaumann 1983; 1985). 5-HT increases sinoatrial rate and left atrial contractile force through receptors blocked by phenoxybenzamine (PBA) (insurmountable) and by methysergide (surmountable). The affinity of 5-HT for atrial receptors was low as estimated from receptor occlusion with PBA, and the 5-HT_2 receptor antagonist ketanserin did not block the effects of 5-HT. 5-HT also enhances contractile force of right ventricular papillary muscle but the effect is not antagonised by PBA, methysergide or ketanserin. These observations led to the proposal that atrial and ventricular receptors are distinct and are neither 5-HT_1 nor 5-HT_2. Recently Saxena et al. (1985) concluded that sinoatrial receptors of cat resemble 5-HT_1 because 5-carboxamide tryptamine (5-CT), a 5-HT analogue with high affinity for 5-HT_1 receptors ($K_D \sim 2$ nM) of brain (Engel et al., 1983), causes tachycardia at low concentrations. Hence, the hypothesis was examined here that atrial receptors are 5-HT_1 by estimating the affinity of 5-CT with PBA. Concentration-effect curves for 5-CT were determined on spontaneously beating right atria and paced left atria (0.5 Hz) of kitten at 32.5°C in modified Krebs solution containing 0.2 mM ascorbate and 6 µM cocaine. 5-CT caused positive chronotropic effects in right atria and increased contractile force in left atria but not in papillary muscles (paced at 0.2 Hz). 20 µM 5-CT did not antagonise the positive inotropic effects of 5-HT in papillary muscles. PBA (50-300 nM for 5-10 min followed by wash out) antagonised in an irreversible and partially insurmountable way the atrial effects of 5-CT. The equilibrium dissociation constant for 5-CT was calculated from (Kaumann et al. 1982):

$$K_A = (EC50_2 - EC50_1.E_{max})/ (1- E_{max})$$

$EC50_1$ and $EC50_2$ are EC50 values for 5-CT before and after the PBA treatment; E_{max} is the maximum effect after PBA, expressed as a fraction of the maximum effect of 5-CT before PBA. The $EC50_1$'s for 5-CT were ($\bar{x}$ ± SD, -log M) 8.0±0.3 on 5 right atria and 7.6±0.4 on 8 left atria. The K_A's for 5-CT were ($\bar{x}$ ± SD, -log M) 7.3±0.3 on 4 right atria and 7.1±0.2 on 6 left atria. The affinity of 5-CT estimated for atrial receptors is 20 to 30 times lower than for brain 5-HT_1 receptors.

Conclusions. 1. 5-HT receptors of atrium and ventricle are distinct. 2. Atrial 5-HT receptors exhibit some receptor reserve for 5-CT and 5-HT (Kaumann 1983 and 1985). 3. Atrial 5-HT receptors are D-receptors which are different from 5-HT_1 and 5-HT_2.

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MEDIATION OF 5-HYDROXYTRYPTAMINE (5-HT)-INDUCED CONTRACTION OF THE RAT URINARY BLADDER VIA 5-HT_2 AND 5-HT_3 RECEPTORS.

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Recently we showed that the biphasic response (initial spike followed by a longer-lasting contraction) of the cat urinary bladder elicited by 5-HT is mediated by neurotropic 5-HT_3 ('M') and musculotropic 5-HT_2 receptors, respectively (Saxena *et al.*, 1985). In the rat, 5-HT induces a monophasic contraction of the urinary bladder but a selective agonist of "5-HT_1-like" receptors, 5-carboxamidotryptamine, is ineffective (Saxena & Lawang, 1985). However, since morphine as well as methysergide (Vanov, 1965), ketanserin or cyproheptadine (Saxena & Lawang, 1985) only partially block the 5-HT-induced contraction of the rat urinary bladder, it seems likely that two types of 5-HT receptors are involved in this species also. Using the selective antagonists of 5-HT_2 (ketanserin) and 5-HT_3 (MDL 72222; Fozard, 1984) receptors, administered sequentially after one another, we have investigated if indeed such is the case.

Rats (about 300 g body weight, male or female) were anaesthetized with pentobarbital sodium (100 mg/kg, i.p.) and artificially ventilated after tracheal cannulation. Pressures in the aorta and urinary bladder were recorded with P23Dc Statham transducers on a Grass polygraph. The effects of i.v. injections of 0.9% NaCl (saline), MDL 72222 and ketanserin tartrate were studied on the urinary bladder contractions elicited by 5-HT and 1,1-dimethyl-4-phenyl piperazinium iodide (DMPP), injected into the descending aorta. The results summarized in Table 1 show that MDL 72222 decreased the response to 5-HT only after the animals had been treated with ketanserin. On the other hand, ketanserin antagonized the

Table 1. Increase in Urinary Bladder Pressure (mmHg, Mean±SEM) by 5-HT or DMPP.

	5-HT (30 μg/kg, i.a.)			DMPP (100 μg/kg, i.a.)		
	Control	Interv. 1	Interv. 2	Control	Interv. 1	Interv. 2
Group 1	30±4	25±3	20±2* **	22±4	23±3	20±2
Group 2	28±5	36±5	2±1* **	20±5	13±2	7±1* **
Group 3	28±3	12±1*	2±1* **	15±3	7±1*	5±1*

Interv., Intervention. Intervention 1 and 2 in the three groups (n=6 each) were: 1 ml saline twice, Group 1; MDL 72222 followed by ketanserin (each 0.3 mg/kg, i.v.), Group 2; and ketanserin followed by MDL 72222 (each 0.3 mg/kg, i.v.), Group 3. *, $p<0.05$ vs control; **, $p<0.05$ vs intervention 1; both analysis of variance followed by Duncan's new multiple range test.

responses to both 5-HT and DMPP irrespective of treatment with MDL 72222. Although we do not know the nature of the interaction between ketanserin and DMPP, it is concluded that both 5-HT_3 and 5-HT_2 receptors - the latter to a greater extent - mediate the 5-HT-induced rat urinary bladder contraction. The presence of 5-HT_3 receptors was confirmed by bladder contractions observed with 2-methyl-5-HT (100 μg/kg, i.a.) in two additional rats.

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ENDOTHELIAL DESQUAMATION AFFECTS THE TRANSFER OF SEROTONIN FROM PLATELETS INTO THE BLOOD VESSEL WALL.

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Attempts are made to link cardiovascular diseases such as hypertension and vasospasm to an exaggerated liberation of serotonin (5-hydroxytryptamine, 5HT) from aggregating blood platelets (Vanhoutte & Cohen, 1983). Therefore we investigated if 5HT released by dog platelets could penetrate autologous blood vessels. Mongrel dogs were anaesthetized with sodium pentobarbitone. Venous blood was collected on 5.8 mM EDTA, platelet rich plasma was prepared and incubated with 0.1µM ^{3}H-5HT (28 Ci/mmol). The platelets were washed once and resuspended in Ca^{2+}-free Krebs' with 0.2 % gelatin. The platelet suspension (PS) contained 10^8 platelets/ml with 3.1 ± 0.6 x 10^6 dpm/ml, of which less than 2 % was free. Eight rings without endothelium were prepared from one of the saphenous veins (3 mm wide, 9.5 ± 0.4 mg, n = 56). The segments were added to 1 ml PS, which was stirred with or without 0.1 unit thrombin (Thr) in the presence or absence of 30 nM prostacyclin (PGI_2). After 10 min the radioactivity and the 5HT content (HPLC with electrochemical detection) of the Krebs' were measured; the vessel segments were removed, washed 24 times with 5 ml Krebs' and their ^{3}H-content was determined. The remaining 4 segments were incubated in Krebs' with or without PGI_2 from which the platelets had been removed by centrifugation one min after injection of saline or thrombin.

The platelets gradually released some radioactivity (41 ± 6%) and 5HT (1.8 ± 0.5 nmol after 10 min) when stirred with vessel segments. In response to Thr, 77 ± 3 % of the radioactivity was released within 1 min, and 90 ± 2 % or 4.0 ± 0.4 nmol 5HT after 10 min. PGI_2 significantly suppressed the release of 5HT and radioactivity in both situations. The specific activity of the free 5HT at the end of the aggregation was identical for the eight treatments. Some ^{3}H (900-8000 dpm/mg tissue) remained associated with the vessel after extensive washing, and this was proportional to the radioactivity released by the platelets. The same tendency was observed when the platelets were removed before addition of the vessel rings, but 50% less radioactivity accumulated. Previous experiments with soluble 5HT indicated that 70 % of this radioactivity is present in the adrenergic nerve terminals of the vessel (Verbeuren et al. 1983).

We then investigated whether platelet-derived 5HT could accumulate in the vessel when it was presented exclusively to the intima and whether endothelium affected the 5HT accumulation. To this end the PS was perfused through the lumen of saphenous vein segments (4-6 cm length) with or without endothelium, in the presence or the absence of Thr. In all tissues, an accumulation of radioactivity was detected, which was enhanced about ten fold by Thr, and was significantly less in the tissues with endothelium (25 to 50 % of that noted in the absence of endothelium). These experiments illustrate that platelet-derived 5HT can enter the vascular wall, especially at sites of endothelial denudation or damage; it may then be taken up by the adrenergic nerves or activate smooth muscle cells, directly or after release as cotransmitter (Verbeuren et al., 1983). Since experiments with canine left circumflex coronary arteries yielded similar results, its release as cotransmitter could predispose to coronary vasospasm since 5HT constricts coronary arteries in contrast to noradrenaline.

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COMPARISON OF [^{3}H]-NORADRENALINE - AND [^{3}H]-SEROTONIN-RELEASE IN DOG BASILAR ARTERIES: INFLUENCE OF RAUBASINE AND DIHYDROERGOCRISTINE.

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The rauwolfia serpentina alkaloid raubasine (RA) and the ergot alkaloid dihydroergocristine (DHEC) are used in combination (14:1 molar ratio, Iskédyl^R-P.F. Médicament) for the treatment of symptoms associated with mental ageing (Briley, 1985). In the central and peripheral nervous systems both RA and DHEC are active at alpha$_1$- and alpha$_2$-adrenoceptors while DHEC also interacts at dopamine and serotonin receptors (Briley, 1985; Roquebert and Demichel 1984; Roquebert et al. 1984). We recently illustrated that canine basilar arteries can accumulate and release serotonin (5HT) (Verbeuren et al. 1983). The present study was designed to compare the accumulation and the release of ^{3}H-noradrenaline (NA) and ^{3}H-5HT in basilar arteries of the dog and to investigate the influence of RA and DHEC and their combination as used in Iskédyl^R on the release of both neurotransmitters.

Helical strips of the basilar arteries were incubated (120 min) with 0.3 µM of either ^{3}-NA or ^{3}H-5HT and subsequently mounted for superfusion in order to measure the efflux of ^{3}H from the tissues. After labeling and initial washout (120 min) the arteries incubated with ^{3}H-5HT contained only 30 % of the ^{3}H-content detected in the tissues labeled with ^{3}H-NA (Table 1); the basal fractional ^{3}H-release was higher in the arteries labeled with ^{3}H-5HT (Table 1). These results suggest that ^{3}H-5HT leaks easier from its storage places than ^{3}H-NA. Electrical stimulation (2 Hz) evoked an overflow of ^{3}H in tissues labeled with ^{3}H-NA and ^{3}H-5HT; this 2 Hz-induced overflow, expressed as fractional release, was equal in both groups of preparations (Table 1) illustrating that in the arteries, at the time of stimulation, a similar portion of ^{3}H-NA and ^{3}H-5HT is liberated by the impulses.

Table 1 : Accumulation and release of ^{3}H-NA and ^{3}H-5HT in dog basilar arteries

	^{3}H-NA	^{3}H-5HT
^{3}H-content (DPM/mg)	526000 ± 67000	146000 ± 18000
Basal efflux (%/4 min)	0.25 ± 0.02	0.77 ± 0.13
Electrical stimulation, 2 Hz (%/4 min)	2.08 ± 0.35	2.27 ± 0.57

At the highest concentration used (25 µM), RA increased the spontaneous ^{3}H-release; at lower concentrations (0.75 to 7.5 µM) the compound increased the 2 Hz-induced ^{3}H-overflow. DHEC (0.054 to 1.8 µM) did not affect the basal ^{3}H-efflux but reduced the 2 Hz-evoked ^{3}H-overflow. High concentrations of the 14:1 mixtures of RA and DHEC augmented the spontaneous ^{3}H-efflux and decreased the stimulation-induced ^{3}H-overflow. The substances exerted similar effects on the arteries labeled with ^{3}H-NA and ^{3}H-5HT except that DHEC decreased the release of ^{3}H-5HT more than that of ^{3}H-NA (to respectively 66 % and 85 % of control). Although the available evidence suggests that ^{3}H-5HT accumulates into the adrenergic nerves of the dog basilar artery (Verbeuren et al. 1983), the present data illustrate some quantitative differences between the accumulation and the release of both transmitters. Our studies also show the profound effects of RA, DHEC and their combination on the release of the two neurotransmitters.

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SURMOUNTABLE ANTAGONISM BY KETANSERIN OF 5-HYDROXYTRYPTAMINE-INDUCED CONTRACTIONS IN DOG BASILAR ARTERY.

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5-Hydroxytryptamine (5-HT) causes contractions of dog basilar artery which are reported to be blocked insurmountably by a variety of antagonists including ketanserin (Müller-Schweinitzer & Engel, 1983). Peroutka et al. (1983) proposed an involvement of 5-HT_1 receptors, but Müller-Schweinitzer & Engel, based on a correlation between IC_{50}'s and binding K_D's of antagonists, suggested involvement of 5-HT_2 receptors. However, IC_{50}'s are not meaningful because they depend on 5-HT concentration. If 5-HT_2 receptors are involved, a competitive antagonist of 5-HT_2 receptors (i.e. ketanserin) should block the effects of 5-HT in surmountable manner. To investigate this we determined concentration-effect curves for 5-HT in the absence and presence of ketanserin (incubated for 2 h). Up to mM concentrations of 5-HT were used in the presence of ketanserin. The experiments were carried out at 32.5°C on helical strips of dog basilar artery. The endothelium was rubbed off. The strips were studied in a modified Krebs solution containing 0.2 mM ascorbate and 6 µM cocaine. The strips were stretched to 4 mN. The EC_{50} (-log M) for 5-HT was ($\bar{x}$ ±SEM) 7.1±0.1 (n=19). In the presence of ketanserin the concentration-effect curve for 5-HT became biphasic with both a high and low-sensitivity component. In the presence of ketanserin 5-HT caused about 80% of the maximum effect observed in the absence of ketanserin. Concentration-ratios (CR) derived from EC_{50}'s for the low-sensitivity component and from the EC_{50}'s of 5-HT in the absence of ketanserin (B) were used to estimate the dissociation equilibrium constant $K_B = [B]/(CR-1)$. The data in Table 1 are $\bar{x}$ ±SEM.

Table 1 Characteristics of the antagonism of 5-HT effects by ketanserin

nM		High sensitivity component		Low sensitivity component		-log M
Ketanserin	n	maximum (%)	pEC_{50}	maximum (%)	pEC_{50}	K_B
100	11	38 ± 4	7.9 ± 0.1	79 ± 6	4.6 ± 0.1	9.5 ± 0.1
1000	8	22 ± 2	7.6 ± 0.1	82 ± 6	3.7 ± 0.1	9.4 ± 0.1

The estimated K_B values agree with the affinity of ketanserin found for 5-HT_2 receptors of rat brain (Leysen et al., 1982) and pulmonary and coronary artery of calf (Frenken & Kaumann, 1984).
Conclusions 1. 5-HT contracts dog basilar artery in part through 5-HT_2 receptors. 2. Low concentrations of 5-HT also contract the basilar artery via receptors that are distinct from 5-HT_2 (i.e. ketanserin-resistant). 3. These properties are not restricted to the basilar artery because they resemble those observed in dog coronary artery (Frenken and Kaumann, 1985). 4. The incomplete surmountability with 5-HT of the ketanserin blockade is not yet understood.

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