THE EFFECTS OF DRUGS INTERACTING WITH OPIOID RECEPTORS ON ISCHAEMIC ARRHYTHMIAS IN ANAESTHETISED RATS.

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Naloxone has been shown to be antiarrhythmic in anaesthetised and conscious rats (Fagbemi et al, 1982). Indirect evidence suggests that this antiarrhythmic action may be due to antagonism of endogenously released opioids at specific receptors (Parratt and Sitsapesan, 1986). However, it is not known which opioid receptor subtypes are involved. In order to investigate this, the antiarrhythmic properties of a range of opioid antagonists with differing opioid receptor selectivities were investigated in anaesthetised rats subjected to coronary artery occlusion. The possibility that exogenous opioid agonists were arrhythmogenic was also investigated in the same model. The following drugs were used: Antagonists; naloxone ($\mu > k \simeq \delta$), Mr2266 and Mr1452 ($\mu \neq k > \delta$), M8008 ($\mu \simeq \delta >> k$, Smith, 1986). Agonists; U50-488H (k), leu-enkephalin (δ), diamorphine (μ).

Sprague-Dawley rats (250-350 g) were anaesthetised with pentobarbitone and artificially ventilated. Drugs or saline were administered intravenously 15 min before coronary artery occlusion. The severity of the arrhythmias was assessed in the 0-30 min post-occlusion period (Fagbemi et al, 1982).

Naloxone (0.5 mg/kg + 0.25 µg/kg/min) significantly reduced the total number of ventricular ectopic beats (VEB's) from 1078±305 to 213±70 ($P<0.05$) and the duration of ventricular tachycardia (VT) from 65±26 to 5±1 s ($P<0.05$). At this dose, naloxone is thought to antagonise δ and k receptors in addition to μ receptors. A lower dose of naloxone (50 µg/kg + 0.25 µg/kg/min), calculated to antagonise mainly μ receptors, had no significant effect.

Mr2266 (4 mg/kg) significantly reduced VEB's from 1111±238 to 247±74 ($P<0.01$) and the duration of VT from 69±23 to 7±3 s ($P<0.01$). Mr1452 (4 and 10 mg/kg) significantly reduced VEB's from 1123±177 in controls to 367±152 ($P<0.01$) and 362±159 ($P<0.01$) respectively. At 10 mg/kg the duration of VT was reduced from 66±15 to 8±5 s ($P<0.05$).

M8008 (1 mg/kg), diamorphine (0.05-0.5 mg/kg), leu-enkephalin (2-10 µg/kg/min) and U50-488H (0.1-1 mg/kg) were without effect on the arrhythmias resulting from coronary artery occlusion.

Antagonists which act preferentially at μ and k receptors were anti-arrhythmic in this model. M8008 which is twice as potent at δ receptors than at μ receptors (Smith, 1986) was without effect. Since naloxone was not antiarrhythmic at doses calculated to antagonise μ receptors only, it is tentatively suggested that k receptors may be important in mediating the antiarrhythmic actions of opiate receptor antagonists. The reason for the lack of effect of opioid agonists is not known; possibly the opioid receptors were already fully activated by endogenous ligands.

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CHARACTERIZATION OF CALCIUM ENTRY BLOCKERS IN ISOLATED GUINEA-PIG HEARTS; CARDIODEPRESSANT ACTIVITY OF FLUNARIZINE.

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So far, it has always been assumed that the diphenylalkylamines, in contrast with the classical calcium entry blockers nifedipine, verapamil and diltiazem, would not possess relevant cardiodepressant potency, neither in vitro nor in vivo (Holmes et al., 1984).

In the present study the effects on left ventricular pressure, heart rate and coronary flow of nifedipine, verapamil, diltiazem, bepridil, flunarizine and lidoflazine as examples of each subgroup of calcium entry blockers were compared in a quantitative manner in the Langendorff preparation of a guinea-pig heart. The left ventricular pressure was measured by means of an intraventricular balloon, filled with saline. The hearts were perfused with Tyrode-solution at 37° C and at a calcium concentration of 1.3 mM. The drugs were infused via a teflon catheter into the perfusate, directly before the heart in order to avoid drug adsorption to the glass wall. All calcium entry blockers displayed negative inotropic and negative chronotropic activity and increased the coronary flow. The rank orders of potency for the negative inotropic effect, negative chronotropic effect and increase in coronary flow, respectively, considerably differed for the investigated calcium entry blockers. For the reduction in contractile force the sequence: nifedipine > verapamil > bepridil > flunarizine > lidoflazine > diltiazem was found. For the negative chronotropic effect the rank order followed was: nifedipine > verapamil > lidoflazine > flunarizine > bepridil > diltiazem, and for the increase in coronary flow: nifedipine > lidoflazine > flunarizine > diltiazem > bepridil > verapamil.

In conclusion, the cardiodepressant action of the diphenylalkylamines, probably underestimated so far because of glass adsorption which prevents sufficient in vitro bath concentrations, can indeed be demonstrated in the present study. In this respect the diphenylalkylamines do not principally differ from the more classical calcium entry blockers. It should be realized, however, that during oral treatment of patients with flunarizine (5-10 mg daily) no relevant cardiovascular changes have ever been reported.

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CONTINUOUS 7-DAY INFUSION AND WITHDRAWAL OF VERAPAMIL AND DILTIAZEM IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

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Several authors report a hypersensitivity of human and rabbit aorta rings upon stimulation with noradrenaline after cessation of chronic treatment with verapamil (Nelson et al., 1984; Aceto et al., 1985). In order to study the antihypertensive effect and possible withdrawal symptoms of calcium entry blockers, verapamil (12 and 30 mg/kg/24 h i.v.) and diltiazem (4 and 12 mg/kg/24 h i.v.), respectively, have been infused continuously during 7 days in SHR. At day 8 the infusion was discontinued.

The lower dose of verapamil (12 mg/kg/24 h i.v.) did not affect the blood pressure or heart rate, the higher dose (30 mg/kg/24 h i.v.) caused a reduction of blood pressure and an initial fall in heart rate during the first days of infusion. Cessation of the infusions did not give rise to withdrawal symptoms. The blood pressure returned to pretreatment values not earlier than the second and third day after discontinuation of the infusion of the higher dose. Infusion and withdrawal of diltiazem in a dose of 4 mg/kg/24 h did not affect blood pressure or heart rate. Diltiazem in a dose of 12 mg/kg/24 h induced a slight, but significant fall in blood pressure, which returned to pre-infusion values 4-8 h after cessation of the infusion. Infusion and withdrawal of diltiazem in this dose did not affect the heart rate.

Verapamil effectively lowered blood pressure without signs of withdrawal symptoms in the rat. No overshoot of blood pressure or heart rate was observed. The difference in withdrawal effects between the results of in vitro experiments and our in vivo results in the rat may be explained by a long half-life of verapamil, due to its lipophilicity. This lipophilicity may also account for the slow return of the blood pressure to pretreatment values after the withdrawal of verapamil. Diltiazem showed a slight antihypertensive effect without the occurrence of withdrawal symptoms.

In conclusion, verapamil and to a lesser degree diltiazem give rise to a sustained reduction of blood pressure without a withdrawal syndrome in SHR.

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EFFECT OF α-ADRENOCEPTOR ANTAGONISTS AND CALCIUM ENTRY BLOCKING DRUGS ON VASOCONSTRICTION IN RAT PERFUSED HINDQUARTERS IN VITRO.

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The present study comprizes the characterization of a constant-pressure perfusion model of rat perfused hindquarters (RPH) with respect to α-adrenergic and K^+-induced vasoconstriction. In an earlier study, we reported that under the present conditions α-adrenergic vasoconstriction in this preparation is obviously mediated by the α_1-receptor subtype (Korstanje et al., 1985).

Male normotensive Wistar rats (240-350 g) were pithed and subsequently dissected. In order to minimize tissue damage caused by disruption of nutrients, an in situ preparation technique was developed. Preparation consisted of cannulation of abdominal aorta and vena cava inferior, performed between the ileac bifurcation and renal vessels, ileolumbar vessels being ligated. Subsequently, abdominal blood flow was interrupted to allow cannulation and the perfusion was started. Hindquarter flow was measured in the venous bed by means of a drop-counter device. Temperature was maintained at 37°C. Perfusion pressure was kept at 60 cm H_2O. Agonists were introduced via a rubber sleeve, proximal to the aorta cannula in a volume of 0.5 ml/kg. Perfusion fluid (PF) was gassed with carbogen and composed as follows: 118 mM Na^+; 5.9 K^+; 2.5 Ca^{++}; 1.6 Mg^{++}; 24.9 HCO_3^-; 1.2 $H_2PO_4^-$; 1.6 $So_4^=$; 11.1 glucose and 5% Dextran 70.000. Agonists were evaluated with respect to their potency and intrinsic activity in reducing hindlimb perfusion flow (% of initial value). For the α-adrenoceptor agonists cirazoline, methoxamine and St 587 (= 2-(chloro-5-trifluoromethylphenylimino)imidazolidine) E-max. amounted to 68 ± 4; 88 ± 4 and 20 ± 4%, respectively, whereas ED_{50}-values of 8.8 ± 3; 10 ± 5 and 30 ± 70 nmol/kg resulted, respectively ($\bar{x}$ ± SEM, n=5-9).

The effect of α-adrenoceptor antagonists on α-adrenergic vasoconstriction in RPH has been reported previously (Korstanje et al., 1985). Omission of calcium from PF did not significantly affect the potency or intrinsic activity of both methoxamine and cirazoline. Neither did 1 μM of the calcium entry blocking drug (CEB) gallopamil. In contrast, CEB's dose-dependently attenuated KCl-induced vasoconstriction in RPH. -Log IC_{50}-values (-log antagonist concentration to attenuate maximal response to KCl by 50%) of 6.7 ± 0.5; 5.8 ± 0.3; 6.0 ± 0.7 and 5.8 ± 0.6 (means ± SE, n=9-21) resulted for nifedipine, verapamil, bepridil and flunarizine, respectively. α-Adrenoceptor antagonists were inactive in this respect. These findings indicate that a neurogenic component in the contraction to KCl in RPH is lacking. It is concluded that the rat perfused hindquarters is a useful in vitro resistance-vessel model to study the effect of CEB and other vasodilators.

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MDL 72567: A DIHYDROPYRIDINE CALCIUM ANTAGONIST WITH REDUCED PROPENSITY TO CAUSE REFLEX TACHYCARDIA.

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Despite having high affinity for binding sites in the heart, dihydropyridine calcium antagonists show functional selectivity for vascular smooth muscle, causing vasodilatation and reflex tachycardia. We now describe a dihydropyridine derivative which exceptionally causes bradycardia in certain animal models. MDL 72567 is the 3-furoyl analogue of nifedipine and is the most hydrophilic dihydropyridine that we have tested.

In vitro, the compound was a potent calcium antagonist in K^+depolarized taenia caeci preparations from the guinea pig with a pA_2 of 8.8 ± 0.1 (Spedding 1982a). MDL 72567 was a more selective depressant of rate than developed tension in spontaneously beating rat atria when compared with nifedipine. In vivo, in pithed rats infused with angiotensin II (Spedding 1982b), nifedipine, nicardipine and PY 108-068 (10-3000 nmol/kg iv) lowered mean blood pressure without any effect on ECG intervals. In contrast, equivalent vasodilator effects of MDL 72567 (10-3000 nmol/kg iv) were associated with bradycardia and prolongation of PR intervals although, in contrast to verapamil, 2° heart block was not observed.

The effects of MDL 72567 and nifedipine (0.1-2 mg/kg) have been compared in conscious sino-aortic denervated (SAD)(Kreiger 1964) rats and sham operated controls, instrumented to record ECG, systemic and left ventricular blood pressure. In sham-operated rats both compounds produced equivalent falls in blood pressure. However, nifedipine caused a much greater reflex tachycardia which was accompanied by a negative inotropic effect. MDL 72567 induced an increase in myocardial contractility. In SAD rats, both drugs produced an enhanced fall in blood pressure accompanied by a negative inotropic effect. Nifedipine did not change heart rate in SAD rats; conversely MDL 72567 caused bradycardia.

MDL 72567 caused less reflex tachycardia than did nifedipine for a given fall in blood pressure in renal hypertensive dogs.

The above findings suggest that MDL 72567 might cause less reflex tachycardia in clinical use, than other dihydropyridines.

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NITRENDIPINE PROMOTES THE RELEASE OF CALCIUM FROM RAT HEART MITOCHONDRIA.

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During ischemia the myocardium undergoes several metabolic, functional and morphological changes. Major intracellular changes include the accumulation of high levels of calcium ion (Ca^{2+}) by cardiac mitochondria, resulting in mitochondrial swelling, impairment of oxidative phosphorylation, rapid depletion of tissue energy stores (ATP and creatine phosphate) and the loss of intracellular Ca^{2+} homeostasis (Regitz et al. 1984). Recent studies involving the Ca^{2+} antagonist nitrendipine have shown this dihydropyridine's ability to modify Ca^{2+} uptake by cardiac mitochondria (Baydoun et al, 1986), we now report its action on Ca^{2+} release.

Tightly coupled heart mitochondria were isolated from female Wistar rats by a modification of the method of Vercesi et al (1978). Ca^{2+} release was followed with the aid of a Corning Ca^{2+}- specific electrode coupled to a Pentracourt PM 10pH-meter and a BBC recorder (Model SE120) according to the method of McNamee et al (1985). The reaction medium contained 250mM sucrose, 5mM succinate, 2mM potassium dihydrogen phosphate and 5mM Tris-HCl(pH7.4.) Ca^{2+} uptake was initiated by the addition of rat cardiac mitochondria (2mg of protein) to the medium maintained at 37°C . When present nitrendipine (10-100μM) was added 10min after the addition of mitochondria. Protein was determined by the method of Gornall et al (1949).

Previous studies confirmed that the addition of nitrendipine (20-100μM) prior to mitochondria produced a concentration-dependent inhibition of Ca^{2+} uptake rate, with the calculated IC_{50} value for this effect being 75.3 ± 2.1μM (n = 4). Conversely, in the present study introduction of nitrendipine (40-100μM), after the mitochondria had accumulated the Ca^{2+} load, induced a concentration-dependent stimulation of efflux with the rate of release increasing from 1.6 ± 0.05 to 114.2 ± 6.7 nmol Ca^{2+} min^{-1}mg of protein $^{-1}$ (n = 4); the EC_{50} value being calculated as 57.8 ± 1.3 μM (n = 4). Concentrations of nitrendipine between (10-30μM) failed to induce release of Ca^{2+}. Results also show that at 62.8 ± 1.7 μM nitrendipine (n = 4) the rate of Ca^{2+} influx equalled rate of Ca^{2+} efflux, at which point the influx rate was inhibited by 25.7 ± 2.2 % (n = 4) whilst efflux rate was stimulated by 68.4 ± 6.7% (n = 4). Replacement of nitrendipine by 25-100μM of the oxidised form (inactive) failed to inhibit uptake or induce release.

Data presented indicates that nitrendipine has the ability to alter intracellular Ca^{2+} homeostasis by a prime action on the Ca^{2+} efflux system associated with cardiac mitochondria. The action on release occurring at concentrations lower than those required to inhibit ATP synthesis.

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DILTIAZEM - RELATIONSHIP BETWEEN CONCENTRATIONS IN VIVO AND EFFECTS ON THE RAT UTERUS AND CARDIOVASCULAR SYSTEM.

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(+)-cis Diltiazem inhibited contractions of the rat uterus after bolus injection or infusion and prolonged gestation in the ovariectomized pregnant rat (Abel & Hollingsworth, 1985, 1986). The objectives of this study were to determine the kinetics and the relationship between serum concentrations of diltiazem and its effects on uterine contractions, b.p. and heart rate after bolus injection, 2 mg kg^{-1}, and during i.v. infusion, 100 µg kg^{-1} min^{-1}, in the ovariectomized anaesthetized non-pregnant rat. Serum concentrations of diltiazem and desacetyldiltiazem were determined by h.p.l.c. based on the methods of Verghese et al (1983) and Weins et al (1984).

The characteristics of the assay of diltiazem and desacetyldiltiazem respectively were: recovery from rat plasma - 93.2% (C.V. = 4.2%, n = 10), 100.6% (C.V.= 3.6%, n = 10); lower limit of sensitivity - 50 ng ml^{-1}, 25 ng ml^{-1}; intra-assay C.V. at 1000 ng ml^{-1} over 3 months - 5.2% (n = 3), 4.4% (n = 3); inter-assay C.V. at 250 ng ml^{-1} - 3.3% (n = 10), 4.5% (n = 10).

Blood samples (< 0.2 ml) were taken between 1 and 120 min after bolus injection. There was a biexponential decay of serum concentrations of diltiazem. Concentrations were 7.2 ± 1.2 µg ml^{-1} at 1 min, 2.1 ± 0.2 µg ml^{-1} at 5 min and 0.3 ± 0.07 µg ml^{-1} at 120 min. $T_{\frac{1}{2}\alpha}$ was approximately 1 min and $T_{\frac{1}{2}\beta}$ was 61.2 ± 13.0 min. Desacetyldiltiazem was only detected in a few serum samples at low concentrations. Reductions of 50% in integral of uterine contractions, 30% in mean b.p. and 25% in heart rate were observed at 5 min. Using all times there were significant correlations between the % changes in the 3 parameters and the log serum diltiazem concentrations. The IC50 for diltiazem was approximately 0.4 µg ml^{-1} for integral of uterine contractions and 0.7 µg ml^{-1} for b.p. and heart rate. Blood samples were taken between 5 and 180 min during infusion and between 1 and 80 min after cessation of infusion. C_{ss} of diltiazem of 2.2 ± 0.3 µg ml^{-1} were produced. Serum concentrations of desacetyldiltiazem, the major metabolite, of 2.2 ± 0.5 µg ml^{-1} were reached by 180 min in 6 out of 8 rats. Another metabolite, but no desacetyldiltiazem, was detected in 2 rats. Frequency of uterine contractions were initially reduced by 70% but subsequently increased to twice that of controls. Throughout there was a decline in amplitude of uterine contractions and a concomitant decrease in integral of 50% for the remainder of the infusion period. Mean b.p. was reduced by 30% and heart rate by 35% during the infusion. After cessation of infusion, diltiazem concentrations declined to 0.7 ± 0.1 µg ml^{-1} at 80 min but desacetyldiltiazem concentrations were still 1.9 ± 0.6 µg ml^{-1} at 80 min. There was a partial return in all 3 parameters towards control values by 80 min. Diltiazem was 3.2-fold as potent as desacetyldiltiazem as an inhibitor of spasms of isolated uteri to 40 mM K^+ (respective mean IC_{50} were 0.79 and 2.51 µg ml^{-1}, n = 4).

It has been shown that desacetyldiltiazem is a major, but not the only, serum metabolite of diltiazem in the rat. However, this metabolite does not significantly contribute to the effect of diltiazem on uterine contractions in vivo.

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5'-NUCLEOTIDASE AND NEUROMUSCULAR TRANSMISSION.

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Endogenous adenosine inhibits neuromuscular transmission (Sebastião & Ribeiro, 1985; Ribeiro & Sebastião, 1986). Since ATP is released at the neuromuscular junction (Silinsky, 1975), and when exogenously applied decreases transmitter output from motor nerve terminals (Ribeiro & Walker, 1975), it seemed of interest to know whether 5'-nucleotidase, the enzyme responsible for the final step in the hydrolysis of ATP into adenosine, would influence the inhibitory effect of ATP on neuromuscular transmission.

The experiments were carried out at room temperature (22 - 25^{o} C) on the isolated nerve-sartorius muscle preparation of the frog. The motor nerves were stimulated supramaximally with rectangular pulses of 10 μs duration applied once every 2 s . Intracellular techniques for recording evoked end-plate potentials (e.p.ps) were conventional (see e.g. Sebastião & Ribeiro, 1985). The normal bathing solution (pH 7) contained (mM): NaCl 117; KCl 2.5; Na_2HPO_4 1; NaH_2PO_4 1; $CaCl_2$ 1.8; $MgCl_2$ 1.2 . Muscle action potentials and twitches in response to nerve stimulation were prevented by adding tubocurarine (0.9 - 1.2 μM) to the bath.

5'-Nucleotidase (2.5 IU/ml) caused a small decrease (9 $\pm$ 1.0%, n=2) in the amplitude of e.p.ps . The 5'-nucleotidase inhibitor, α,β-methylene ADP (AOPCP, 50 μM), reversibly increased e.p.ps amplitude by 11 $\pm$ 1.2% (n=6, $P<0.05$). In the same experimental conditions, adenosine deaminase (2.5 IU/ml), an enzyme which inactivates adenosine, increased e.p.ps amplitude by 25 $\pm$ 4.3% (n=6, $P<0.05$). AOPCP (50 μM) reduced the inhibitory effect of ATP (1 - 25 μM), but not that of adenosine (10 μM), on e.p.ps amplitude. When applied in the presence of 5'-nucleotidase (2.5 IU/ml), AOPCP (50 μM) did not prevent the inhibitory effect of ATP (10 μM) on the amplitude of e.p.ps . The stable ATP analogue, α,β-methylene ATP (10 - 50 μM) was virtually ineffective on neuromuscular transmission, whereas β,γ-methylene ATP (1 - 50 μM), which can be hydrolysed into AMP/adenosine (Yount, 1975), mimicked the inhibitory effect of ATP on e.p.ps amplitude. This effect of β,γ-methylene ATP was also markedly attenuated by AOPCP (50 μM).

The results indicate that ATP has to be hydrolysed by 5'-nucleotidase in order to produce its inhibitory effect on neuromuscular transmission. The results also suggest that extracellular ATP is present at the neuromuscular junction, and contributes at least with 40 - 50% to the pool of endogenous adenosine which modulates neuromuscular transmission.

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THE EFFECTS OF BAY K 8644 ON THE GENERAL ANAESTHETIC POTENCIES OF ETHANOL AND ARGON.

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Calcium channel inhibitors, including dihydropyridines such as nitrendipine and nimodipine, have been shown to increase the general anaesthetic potency of several anaesthetic agents (Dolin & Little, 1986). We have now investigated the effects of BAY K 8644, a dihydropydine calcium channel activator, on the anaesthetic potencies of ethanol and argon. (The inert gas argon produces general anaesthesia at pressures greater than atmospheric.)

Male T.O. mice (24 - 30g) were used in all experiments. BAY K 8644 (Bayer U.K. Ltd.), dissolved in distilled water with one drop Tween 80 per 10 ml, was injected i.p. at doses of 1, 5 or 10 mg kg^{-1} 5 min prior to anaesthetic administration. Concurrently tested control groups received corresponding vehicle injections. Ethanol, 20% v/v in water, was administered i.p. and anaesthesia assessed at 5 min intervals until waking. For anaesthesia assessement each animal in turn was placed on its back; failure to regain an upright posture within 1 min was counted as anaesthesia. Rectal temperatures were maintained at 37 ± 0.5 °C by warming mat and overhead illumination. Argon (B.O.C.) was administered to mice in groups of four in a rotating cage inside a 20 L pressure chamber. A saturation time of 15 min was allowed at each pressure, then loss of righting reflex determined by rotating the cage. Each group of four mice included animals which had been given BAY K 8644 and those receiving vehicle only. Separate treated and control animals were used for temperature monitoring, maintained at 37 ± 0.5 °C. All assessements were made by an observer who was unaware of the prior treatment. Dose response curves to the anaesthetics were constructed for each 5 min interval. 'N' values were 10 for ethanol and 8 for argon; no animal was used for more than one experiment. Probit analysis was used to derive ED_{50} values, shown below:

Treatment	Dose	ED_{50}	95% fiducial limits
a) Ethanol anaesthesia (g kg^{-1}, at 20 min)			
Controls	vehicle	3.3	3.1 - 3.7
BAY K 8644	1 mg kg^{-1}	3.5 *	3.3 - 4.5
Controls	vehicle	3.1	2.8 - 3.4
BAY K 8644	5 mg kg^{-1}	2.6 **	2.3 - 2.9
BAY K 8644	10 mg kg^{-1}	2.8 *	2.5 - 3.1
b) Argon anaesthesia (atm, at 15 min)			
Controls	vehicle	16.5	15.9 - 17.1
BAY K 8644	1 mg kg^{-1}	17.6 *	16.9 - 18.4
Controls	vehicle	17.2	16.3 - 18.4
BAY K 8644	5 mg kg^{-1}	13.8 ***	12.9 - 14.6
BAY K 8644	10 mg kg^{-1}	15.4 **	14.4 - 16.5

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, χ^2 analysis (Ross, 1980)

A similar pattern was seen with both anaesthetic agents; BAY K 8644 at 1 mg kg^{-1} antagonised the anaesthesia but the higher doses caused a potentiation. The present results are compatible with the demonstrated partial agonist effects of BAY K 8644 at calcium channels, as our previous results showed that calcium channel inhibitors consistantly increased anaesthetic potency.

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ENHANCED EFFECT OF BAY K8644 ON INOSITOL PHOSPHOLIPID BREAKDOWN IN BRAIN SLICES FROM ETHANOL DEPENDENT RATS.

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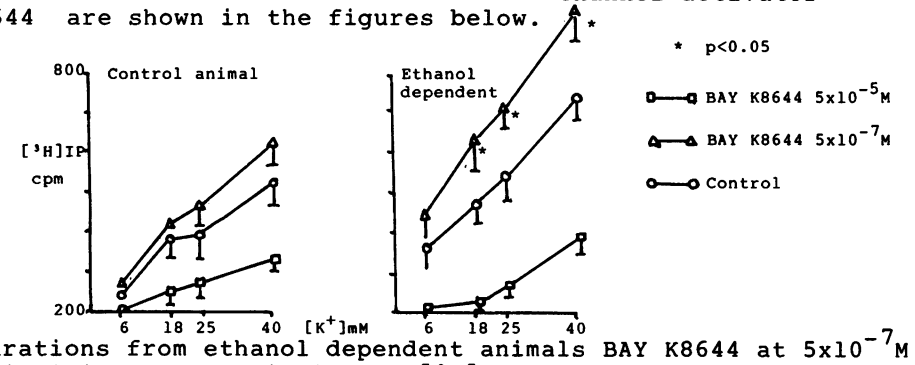
We have previously suggested that the ethanol withdrawal syndrome may represent a state of neuronal hypersensitivity to Ca^{++} which develops during chronic administration of the drug(1), and have further speculated that this increased Ca^{++} sensitivity is caused by increased inositol phospholipid breakdown in neuronal membranes (2). This possibility is strengthened by the observation that dihydropyridine Ca^{++} channel inhibitors can both inhibit the depolarisation-induced breakdown of inositol phospholipids(3) and prevent the ethanol withdrawal syndrome(4).

We have been investigating the ability of dihydropyridines to reduce or potentiate the enhanced inositol phospholipid breakdown which occurs in cortical slices from ethanol dependent rats by monitoring the accumulation of [^{3}H]-inositol phosphates ([^{3}H]-IP) after depolarisation with varying concentrations of K^+ in the presence of 10mM Li^+. Results for the Ca^{++} channel activator BAY K 8644 are shown in the figures below.



In preparations from ethanol dependent animals BAY K8644 at $5x10^{-7}$M caused significant potentiation of [^{3}H]-IP production at all depolarising concentrations of K^+ studied, whereas in preparations from control animals potentiation was only observed at 25 and 40mM K^+ and this was not significant. At $5x10^{-6}$M a smaller and more variable potentiation was observed and at $5x10^{-5}$M, BAY K8644 was frankly inhibitory.

These results suggest that in ethanol dependence, dihydropyridine sensitive Ca^{++} channels may have an enhanced functional role that could underly the alterations in Ca^{++} sensitivity that are observed as a response of the central nervous system to chronic ethanol administration. This has important implications for therapeutic intervention in alcohol tolerance, dependence and neurotoxicity.

M. Hudspith has a MRC Studentship. BAY K8644 was generously provided by Bayer (U.K).

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BEHAVIOURAL EFFECTS OF BAY K 8644 MEDIATED BY CENTRAL AND PERIPHERAL MECHANISMS IN THE RAT.

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The dihydropyridine Ca^{++} channel activator, Bay K 8644, has been shown to produce marked behavioural changes in the mouse following intraperitoneal (i.p.) injection (Bolger et al., 1985). These changes included ataxia, Straub tail, sedation, arched back and convulsions and were antagonized by nifedipine. We now describe the behavioural effects of BAY K 8644 in the rat and the results from experiments carried out to determine whether they are centrally or peripherally mediated.

Male Sprague Dawley rats (250-350g) were injected i.p. with BAY K 8644 and scored for various behaviours. Analgesia was measured using an Appelex DS 20 tail-flick apparatus. For intracerebroventricular (ICV) injections, cannulae were placed stereotaxically in the lateral ventricle 7 days prior to the experiment. Blood pressure and heart rate were measured using standard techniques in conscious rats via an arterial catheter located in the femoral artery.

Following i.p. injections, BAY K 8644, 1-4mg/kg, caused dose-related changes in behaviour which appeared after 20 min and were most marked 40-60 min after dosing. The most striking effect was the periodic muscle contraction causing elevation onto the hind limbs with curved back, opisthotonus and clenched paws. In addition there was reduced general activity, reduced reflex responses (pinna, corneal and limb flexion), Straub tail and analgesia. The effects of BAY K 8644, 1 mg/kg i.p., were blocked by nifedipine 10 mg/kg i.p. Following ICV injection of BAY K 8644, 5 and 20 µg/rat, the same characteristic muscle contraction with resulting body position was observed but activity and reflex responses were normal and analgesia was absent. The ICV administration of BAY K 8644 did not produce any cardiovascular changes in conscious rats.

The analgesic action of BAY K 8644 was further investigated to determine the mechanism and the possible involvement of the peripheral vasoconstrictor effects of BAY K 8644 in this response. Both BAY K 8644, 2 and 4 mg/kg i.p., and phenylephrine, 10 mg/kg i.p., a peripherally acting vasoconstrictor, caused analgesia. The analgesia was not affected by naloxone, 5 mg/kg i.p., whereas, the calcium-antagonists, nifedipine, 10 and 20 mg/kg i.p., and the dihydropyrrazolopyridine (MDL 72892), 10 mg/kg i.p., blocked the analgesia induced by both BAY K 8644, 2 mg/kg i.p., and phenylephrine, 10 mg/kg i.p. Neither antagonist *per se* had any effect on the tail-flick response. Using the same doses in conscious normotensive-rats, MDL 72892 antagonized the vasoconstriction and bradycardia produced by phenylephrine and BAY K 8644 ; the latter's effects were also blocked by nifedipine.

These results suggest that it is possible to distinguish central and peripherally mediated effects of BAY K 8644. The analgesic action of BAY K 8644 appears to be a peripheral effect which does not involve an opiate mechanism but Ca^{++} channels are implicated and the vasoconstrictor effects of BAY K 8644 may be involved. The central behavioural effects of BAY K 8644 are susceptible to blockade with nifedipine and may represent a consequence of Ca^{++} channel activation in the brain and subsequent effects on presynaptic mechanisms controlling neurotransmitter release (Spedding & Middlemiss, 1985 ; Bourson et al., 1986).

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CENTRAL BIOCHEMICAL AND BEHAVIOURAL EFFECTS OF BAY K 8644 IN THE RAT.

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There is growing interest in the role of voltage-dependent Ca^{++} channels in the control of centrally mediated events, such as, neurotransmitter release and its functional consequences (Spedding & Middlemiss, 1985). The Ca^{++} channel activator, BAY K 8644, causes marked behavioural changes in the mouse (Bolger et al., 1985) and rat (Bourson et al., 1986) which appear to be mediated via activation of Ca^{++} channels in the brain and may represent a functional consequence of effects on neurotransmitter release. The present communication describes the effects of Bay K 8644 on brain amine transmitters and their metabolites and on apomorphine-induced yawning. The latter is thought to be mediated by activation of presynaptic dopamine (DA) autoreceptors (Gower et al, 1984) which results in inhibition of DA release and reduction in DA metabolites (Bannon and Roth, 1983).

Male Sprague Dawley rats (250-350g) were injected with BAY K 8644, 2 and 4 mg/kg i.p., and the rats killed by decapitation 60 min later ; the various brain areas were dissected and frozen in liquid nitrogen. Amines were measured with HPLC and electrochemical detection (Wagner et al ; 1982). Yawning was induced by apomorphine, 0.08 mg/kg s.c., and the number of yawns elicited during the following 30 min period was counted. For intracerebroventricular (ICV) injections, cannulae were placed stereotaxically 7 days prior to the experiment.

Following injections of BAY K 8644, 2 and 4 mg/kg, the levels of 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5HIAA) were increased in the striatum and the cortex. There was no significant change in DA or 5-hydroxytryptamine concentrations. The increase in striatal and cortical DA metabolites induced by BAY K 8644, 4 mg/kg, was blocked by nifedipine 5 mg/kg i.p. Phenylephrine, 10 mg/kg i.p., which like BAY K 8644, produces marked vasoconstriction had no significant effect on brain amines. Apomorphine-induced yawning was blocked by BAY K 8644, 0.5 and 2.0 mg/kg i.p., and 5 and 20 µg/rat ICV and by haloperidol, 0.03 mg/kg s.c. The ICV injections of BAY K 8644 had no effects on blood pressure and heart rate in conscious rats. The blockade of apomorphine-induced yawning by BAY K 8644, 0.5 mg/kg, was antagonized by nifedipine, 5 and 10 mg/kg i.p. Nifedipine tended to induce yawning *per se* and significantly increased apomorphine-induced yawning. Apomorphine, 0.08 mg/kg s.c., reduced the concentration of DOPAC and HVA in the striatum, but not in the cortex, and this inhibitory effect of apomorphine was reversed by BAY K 8644, 0.5 and 2.0 mg/kg i.p.

These effects of BAY K 8644 are consistent with a central action of BAY K 8644 via activation of Ca^{++} channels. The functional interaction between BAY K 8644 and apomorphine on amine concentrations and yawning suggests a functional coupling between Ca^{++} channels and presynaptic mechanisms controlling neurotransmitter release. Furthermore, under specialized activation conditions *in vivo*, central Ca^{++} channel function is susceptible to blockade with calcium-antagonists.

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ANTIPARKINSONIAN ACTIVITY OF (+)-PHNO IN THE MPTP-TREATED MARMOSET FOLLOWING SUBCUTANEOUS INFUSION OR SKIN APPLICATION.

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Subcutaneous administration of the D-2 dopamine receptor agonist (+)-4-propyl-9-hydroxynapthoxazine ((+)-PHNO) reversed the persistent motor deficits observed in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated marmosets for between 90-120 min (Jenner et al, 1986). We now report on the potential anti-parkinsonian effects of (+)-PHNO using subcutaneous infusion with mini-osmotic pumps or by application to the skin in MPTP-treated marmosets.

The activity of MPTP-treated marmosets was monitored for an 8h (9.00-17.00h) period for two days prior to the start of the experiment. Animals were anaesthetised and then implanted subcutaneously with an osmotic minipump in the interscapular region primed to release 2 µg/kg (+)-PHNO every 90 min over the following 13 days. On recovery from anaesthesia animals exhibited normal activity and movements were well co-ordinated. Over the following 3 days, animals exhibited a high degree of activity during the light period but little activity during the dark period. Movements were those of the normal repetoire of the animals and stereotyped or dyskinetic movements were not observed. Over the following 10 days activity gradually declined but movement remained co-ordinated. The osmotic pumps were removed on day 12 and animals rapidly became akinetic. However, animals appeared more Parkinsonian than prior to the infusion and only returned to the pre-implantation state over the following 2 weeks.

In 3 MPTP-treated marmosets (+)-PHNO in a dose of 1 mg was applied to a $3cm^2$ area of abdominal skin. Within 30-90 min animals showed a normal repetoire of co-ordinated movements. Marked activity continued for a 3 day period and then declined but on day 5, animals were still more active than in the pre-administration state. No animal showed abnormal stereotyped movements.

Table 1: Cumulative movement counts following administration of (+)-PHNO

Subcutaneous infusion (2 µg/kg/90 min)		Movements in 8h (mean ± 1 SEM)	Application on the skin (1.0 mg/$3cm^2$)		
	Day			Day	
Pre-implantation period	-1	1039 ± 656	Pre-application period	-1	1449 ± 418
Implantation period	1	24416 ± 6094*	Application period	1	27350 ± 5224*
	3	27963 ± 8610*		2	33744 ± 6898*
	11	9379 ± 2253*+		3	27284 ± 7035*
				4	18170 ± 2651*
Post-implantation period	20	1092 ± 511♦		5	7453 ± 510*

* $P < 0.001$ compared to day (-1); ♦ $P < 0.01$ compared to day (11); n = 3-4
+ $P < 0.001$ compared to day (3).

(+)-PHNO is an effective anti-parkinsonian agent in the MPTP-treated marmoset by the transdermal route. A single application was active over several days. The drug is also effective by subcutaneous infusion but the results suggest the rapid development of tolerance.

Jenner, P. et al. (1986) BPS Meeting (Bath)

IS THE $GABA_B$ RECEPTOR PARTICIPATING IN THE GABA-BENZODIAZEPINE RELATIONSHIP?

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The anxiolytic effects of the benzodiazepines are closely related with the neurotransmitter GABA. This relationship has been proven to exist on receptor level and, although not unequivocally, in behavioural tests. Two different GABA receptors have been identified; the socalled GABA A and GABA B receptor. So far, the GABA receptor held responsible to bear the relationship with the benzodiazepine receptor, has proven to be the GABA A receptor.

However, we found that baclofen (Lioresal), a selective GABA B receptor agonist, does have an anxiolytic-like effect in a modified Geller-Seifter test using Wistar rats. More precisely; while baclofen is not effective in a conflict situation, a dose dependent enhancement of extinction of conflict behaviour was found. This effect was evoked in a dose range between 1 and 3 mg/kg, where no sedation or ataxia is present. Naturally, the benzodiazepines have an anti-conflict effect and, inherent to this, an increase of extinction of conflict behaviour can be seen in our experiments.

In an effort to investigate the relationship between the GABA B and benzodiazepine receptor, we investigated the effect of baclofen on [^{3}H]-diazepam binding. These experiments were performed on washed and unwashed crude synaptic membranes of Wistar rats. The effects of pretreatment of the rats with baclofen (1.5-20 mg/kg, injected i.p. 30 min. before decapitation) and addition of baclofen (1-10 μM) to the synaptic membranes of naieve rats were studied. Pretreatment with baclofen in vivo caused a downregulation of Bmax (18 %) in unwashed cortical and cerebellar membranes whereas no significant change was found in the washed membranes. To rule out the influence of GABA A receptors in the assay, bicuculline (100 μM) was included in half of the binding assays. Under these circumstances, downregulation of Bmax in the unwashed cerebellar and cortical tissues persisted (18 and 9 % resp.), although no significance was reached in the latter tissue. On the contrary, a significant Bmax upregulation (13 %) was found in the washed cerebellar membranes, whereas no clear influence of baclofen could be detected in the washed cortical membranes. Preliminary results of in vitro experiments indicate that addition of baclofen (10 μM) to washed cortical membranes causes a 10 % upregulation of Bmax.

In conclusion, the selective GABA B receptor agonist baclofen was shown to have an effect on Bmax of cortical and cerebellar benzodiazepine receptors. The cerebellar tissue was more sensitive to the baclofen influence than the cortical tissue. The different distribution and properties of the type 1 and type 2 benzodiazepine receptors in the cortex and cerebellum may be the cause of this observed difference.

INVESTIGATION OF THE NEUROTOXICITY OF THE GABA-TRANSAMINASE INHIBITOR, VIGABATRIN IN RATS.

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Current hypotheses on the underlying neurochemical abnormalities in epilepsy postulate that there is an inbalance between the inhibitory and excitatory neurotransmitter systems and that augmentation of the inhibitory mechanisms may prove a useful approach to the development of new drugs for epilepsy (Meldrum 1975). Vigabatrin (γ-vinyl GABA) is a specific enzyme-activated irreversible inhibitor of GABA-transaminase, the enzyme responsible for the breakdown of the major inhibitory neurotransmitter, GABA in neurones and glia. Vigabatrin raises brain GABA concentrations in animals (Jung et al. 1977) and protects against seizures in various experimental models of epilepsy (Schechter & Grove 1980, Meldrum & Horton 1978). Recent controlled trials of vigabatrin in patients with epilepsy have shown promising results (Rimmer & Richens 1984; Gram et al 1985). However, pre-clinical toxicity tests have shown that vacuolation of the white matter (intramyelinic oedema) of the central nervous system occurs with high doses of this compound given chronically to rat, mouse and dog, but not in monkey (Centre de Recherche Merrell International report). This does not appear to be associated with any neuronal damage and the significance of the changes is unknown. We thus decided to investigate the neurotoxicity of vigabatrin further.

16 MRC hooded rats were treated with one of two GABA-T inhibitors, ethanolamine-o-sulphate (EOS) 300mg/kg daily or vigabatrin 300mg/kg daily via their drinking water. These doses given chronically had been shown in preliminary studies to cause similar inhibition of GABA-T (vigabatrin $\bar{x}$ = 26.6 ± 3.0% of controls, n = 5, EOS $\bar{x}$ 23.2 ± 4.6% of controls, n = 14), and had elevated brain GABA levels to a similar degree (vigabatrin $\bar{x}$ = 303 ± 65% n = 5, EOS = 314 ± 39% n = 14). A control group of 8 rats was also followed.

After 6 months all rats were in good condition apart from 1 rat in the EOS group who succumbed to a coincidental tumour. The rats given vigabatrin failed to gain weight as rapidly as the others (final weight = 83% of control and EOS rats). The animals were sacrificed and the brains examined histologically. Both treatment groups showed vacuolation in the white matter, particularly in the cerebellum, but the changes were much more severe in the vigabatrin treated rats. None of the control rats showed any significant degree of vacuolation.

Thus brain vacuolation was a consequence of chronic treatment with both drugs. Although equivalent changes in GABA metabolism occurred with both EOS and vigabatrin, the degree of vacuolation differed markedly. It is proposed that the neuropathological changes are unlikely to be a direct consequence of GABA-T inhibition, but occur by some other, as yet unknown mechanism.

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PURINE MEDIATION OF SYNAPTIC INHIBITION BY Ro 15-1788 ON RAT HIPPOCAMPAL SLICES.

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The benzodiazepine 'antagonists' such as Ro 15-1788 have been shown to have a variety of behavioural effects which may be unrelated to their interaction with benzodiazepine binding sites (File & Pellow, 1986). It was recently reported by King et al. (1985) that Ro 15-1788 would also suppress paired pulse inhibition in the hippocampal slice and experiments were therefore carried out to determine whether this compound had any inhibitory effect on excitatory pathways in this in vitro preparation.

Slices of hippocampus from male rats were cut at a thickness of 500μm and maintained in bicarbonate buffer (mM: NaCl 115, KCl 2.0, KH_2PO_4 2.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 10) in a water saturated atmosphere of 5% CO_2 in oxygen. Slices were transferred when required to a 1ml perfusion chamber at 30^0C. Drugs were added into the superfusion fluid. CA1 pyramidal cells were activated antidromically from the alveus or orthodromically from the stratum radiatum by a concentric bipolar electrode and constant current pulses of 50-500μA at 0.1Hz.

Adenosine and Ro 15-1788 both produced a depression of orthodromic potentials at concentrations having little effect on antidromic spikes. The ED50 for adenosine was 25±5μM (n=18), while at 50μM, Ro 15-1788 produced a reduction of 48±7.6 percent (n=5) of the population spike size.

The adenosine antagonists 8-phenyltheophylline (10μM) or 8-(p-sulphophenyl)-theophylline (50μM) antagonised the effects of both adenosine and Ro 15-1788, the latter producing a reduction of only 11.2±2.1 percent of the evoked population spike in the presence of 8-phenyltheophylline. Adenosine deaminase 5U/ml also reduced the effects of adenosine and Ro 15-1788, the spike depression by the latter being cut from 44±6.8 percent to 18±4.3 (n=5).

These results suggest that the inhibitory effects of Ro 15-1788 on orthodromically evoked population spikes may be mediated via purines. This in turn may result from the fact that Ro 15-1788 has been shown to inhibit the uptake of adenosine into brain slices and synaptosomes (Morgan et al., 1983), an effect which may result in the accumulation of extracellular adenosine within the brain.

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TEMPERATURE DEPENDENCE OF SUBSTRATE INTERACTION WITH [^{3}H]-MAZINDOL BINDING TO THE NORADRENALINE TRANSPORTER.

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It is well established that the binding of [^{3}H]desipramine is closely related to the neuronal noradrenergic transporter in various tissues. However, whereas drug potency for inhibition of ^{3}H-desipramine binding at 25°C is significantly correlated with that for inhibition of noradrenaline uptake, substrates for the noradrenergic transporter are virtually inactive as inhibitors of [^{3}H]desipramine binding (Raisman et al. 1982). To reexamine this apparent paradox, we studied the binding of [^{3}H]mazindol (MZD), a newly available, high affinity ligand for the noradrenergic transporter (Javitch et al., 1984), in the rat salivary gland.

The salivary glands from male Sprague-Dawley rats (150-200 g) were homogenized in 50 volumes of 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. The homogenate was filtered over gauze and washed thrice by centrifugation at 4°C (45000 xg, 10 min). The final pellet was taken up into 50 mM Tris HCl (120 mM NaCl, 5 mM KCl) buffer to an original tissue concentration of 150 mg/ml, and a 100 µl aliquot of this homogenate was incubated with ^{3}H-MZD (New England Nuclear, spec. act. 19.6 Ci/mmol) in a final volume of 1 ml for either 30 min at 25°C or 120 min at 0°C. The incubation was terminated by the addition of 3 ml ice-cold buffer and immediate filtration over 0.05 % polyethenimine pretreated Whatman GF/F filters, which were then washed four times with 3 ml buffer. Nonspecific binding, defined using 100 µM nisoxetine, represented 10-20 %.

At 25°C [^{3}H]MZD binds with high affinity (Kd : 1.65 nM) to a single class of binding sites in the salivary gland (Bmax : 270 fmol/mg protein). Whereas the noradrenaline uptake inhibitors nisoxetine, oxaprotiline and maprotiline inhibit [^{3}H]MZD determined at 2nM binding (pIC_{50} = -log IC_{50} (M) : 7.83 $\pm$ 0.28, 7.47 $\pm$ 0.09 and 7.00 $\pm$ 0.02), the transporter substrates (-) noradrenaline, (-) adrenaline and metaraminol are virtually inactive (pIC_{50} = 4.00 $\pm$ 0.03, 3.96 $\pm$ 0.01 and 4.30 $\pm$ 0.12). When [^{3}H]MZD binding is studied at 0°C, the inhibitory potency of nisoxetine and maprotiline equals that observed at 25°C (pIC_{50} = 7.93 $\pm$ 0.16 and 7.00 $\pm$ 0.09) whereas oxaprotiline is slightly less active (pIC_{50} = 6.69 $\pm$ 0.21). However, metaraminol, (-) noradrenaline and (-) adrenaline were 85, 50 and 4.5 times more active as an inhibitor of [^{3}H]MZD binding at 0°C than at 25°C (pIC_{50} : metaraminol, 6.22 $\pm$ 0.09 ; (-) noradrenaline, 5.71 $\pm$ 0.24 ; (-) adrenaline, 4.60 $\pm$ 0.01). Similar results were obtained using ^{3}H-desipramine as a radioligand for the noradrenergic transporter in the salivary gland or using either of these radioligands to label the transporter in the rat vas deferens.

The present study indicates that the affinity of uptake blockers for inhibition of ^{3}H-MZD binding to the noradrenergic transporter in the rat salivary gland is relatively independent of the incubation temperature. In contrast, substrates for the noradrenergic transporter possess significantly higher affinity for inhibition of [^{3}H]MZD binding at 0°C than at 25°C. The underlying mechanism of substrate temperature-sensitivity in its affinity for the [^{3}H]MZD recognition site and its relevance to the mechanisms of inhibition of noradrenaline transport remain to be elucidated.

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POTENTIATION BY ANGIOTENSIN II OF [^{3}H]-NORADRENALINE RELEASE FROM THE CORTEX BUT NOT THE LOCUS COERULEUS OF THE RAT.

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Angiotensin II (ANGII) has been shown to potentiate noradrenaline release from the hypothalamus (Garcia-Sevilla et al., 1979) and from postganglionic sympathetic fibres (Henderson & Hughes, 1974; Cline, 1985). We have examined the effects of ANGII on [^{3}H]-noradrenaline release ([^{3}H]-NA) from two brain regions, the locus coeruleus and parietal cortex. The locus coeruleus comprises a dense cluster of noradrenergic cells which may give rise to axon collaterals mediating local feedback within the nucleus itself (Aghajanian et al., 1977). The parietal cortex receives its noradrenergic input from the locus coeruleus.

400 µm thick slices of locus coeruleus or parietal cortex were superfused at 37°C with a bicarbonate buffered Krebs solution containing [^{3}H]-NA (50 nM for locus coeruleus; 25 nM for cortex; 30-45 Ci.mmol^{-1}) for 30 min and then washed for 90 min with Krebs containing pargyline (10 µM) and desmethylimipramine (300 nM). [^{3}H]-NA release was evoked by raising the potassium concentration of the superfusate from 2.5 to 22.5 mM for 2 min. Two exposures to potassium (S_1 and S_2) were made at an interval of 20 min. Drugs were applied before and during the second exposure to potassium.

The potassium-evoked release of [^{3}H]-NA from both the locus coeruleus and cortex was abolished by either tetrodotoxin (1 µM) or by removal of calcium ions from the superfusate. ANGII (10 - 1000 nM) did not significantly alter the basal efflux of [^{3}H]-NA from either the locus coeruleus or cortex. Although ANGII (3 - 100 nM) did not alter the potassium-evoked release of [^{3}H]-NA from the locus coeruleus it did potentiate the potassium-evoked release from the cortex in a concentration-dependent manner, Table 1. This potentiation of [^{3}H]-NA release by ANGII in the cortex was antagonised by the ANGII antagonist saralasin (SAR: 0.1 - 3 µM) in a concentration-dependent manner.

Table 1 Release of [^{3}H]-NA from cortex slices expressed as the S_2/S_1 ratio.

ANGII (nM)	S_2/S_1	(n)	ANGII (nM) + SAR (nM)	S_2/S_1	(n)
0	0.60±0.04	(7)	30 100	0.93±0.07	(4)
3	0.84±0.11	(4)	30 300	0.82±0.12	(4)
30	1.01±0.09	(4)	30 3000	0.66±0.04	(4)

Values given are mean ± SEM

It is surprising that ANGII potentiated [^{3}H]-NA release from the cortical projections of locus coeruleus neurones but not from the locus coeruleus itself. The function of ANGII binding sites within the locus coeruleus observed by autoradiographic techniques (Harding et al., 1981) remains to be elucidated.

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UPTAKE OF [^{11}C] - QUINIDINE IN DOG BRAIN STUDIED BY POSITRON EMISSION TOMOGRAPHY (PET)

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The concentrations of a drug in the brain after acute systemic administration vary according to the mode of administration and a number of still poorly defined variables. In ongoing studies we use PET to try to unravel some of the intricate pharmacokinetic mechanisms involved. As part of our search for pharmacological probes suitable for these studies, we assessed the uptake of ^{11}C-labeled quinidine in dog brain.

(^{11}C)-Quinidine (1 mg/kg; 11 - 87 mCi; Van Haver et al., 1985) was injected in the femoral vein, the internal carotid artery or the vertebral artery in dogs anaesthetized with pentobarbital. Using the NEURO ECAT (Ortec) PET-system, 25 consecutive scans of a single transaxial slice of the head were performed in a period of 85 min starting at end-injection time; in additional experiments PET-scans of the thorax were also obtained. Radioactivity in blood collected from the femoral artery was measured in a gamma counter. Concentration-time curves were calculated for different regions of interest in the tissues and for blood. Results are expressed as normalized concentrations by dividing radioactivity/cm^3 by the radioactivity administered per gram of body weight (Hartvig et al., 1984).

The distribution of radioactivity after bolus injection of (^{11}C)-quinidine in the femoral vein (n = 10) was characterized by a rapid decay of blood activity with only limited uptake into the brain (maximum normalized concentration 0.42 $\pm$ 0.09; mean $\pm$ SD); radioactivity in arterial femoral blood was at all times higher than in the brain. Uptake in muscle tissue of the head (maximum normalized concentration 1.01 $\pm$ 0.45) was consistently higher than in the brain ($p < 0.005$); PET-scans of the thorax revealed a high activity in the heart as compared to the brain. The uptake of (^{11}C)-quinidine into the brain was low when compared with the uptake, under similar experimental conditions, of (^{11}C)-antipyrine, a compound that crosses the blood brain barrier easily and is not bound to either plasma or tissues (maximum normalized concentration 2.41 $\pm$ 0.50; normalized concentration after 80 min 1.09 $\pm$ 0.07; n = 10) (Agon et al., 1985).
A temporary increase in the availability of (^{11}C)-quinidine in the cerebral circulation had no marked effect on the brain uptake; after bolus injection in the internal carotid artery (n = 2) or the vertebral artery (n = 4), maximum normalized brain concentrations were similar to those observed after i.v. injection.

The results of the present study confirm and extend the scarce literature data indicating that in dogs the passage of quinidine through the blood brain barrier is limited; they suggest that PET with (^{11}C)-quinidine can be a useful tool for the study of factors altering the permeability of the blood brain barrier.

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[^{3}H]-SPIPERONE BINDING IN MOUSE STRIATAL MEMBRANES FOLLOWING CHRONIC PRETREATMENT WITH SCH 23390 AND HALOPERIDOL.

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The ability of haloperidol and other neuroleptic agents to produce behavioural and biochemical supersensitivity is a well documented phenomenon (Tarsy and Baldessarini,1974; Burt et al,1977). Chronic treatment with dopamine D-2 antagonists induces an up-regulation of D-2 receptors by upto 40% (Mackenzie and Zigmond,1985), and it has been suggested that this may be related to the development of tardive dyskinesia following long term neuroleptic treatment in man (Burt et al,1977). With the recent introduction of reportedly selective D-1 antagonists, in particular SCH 23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol)(Iorio et al,1983) the question of drug induced antipsychotic activity without attendant D-2 supersensitivity has been raised. The present study set out to determine the effect chronic administration of haloperidol and matched doses of SCH 23390 would have upon striatal D-2 binding sites.

Male GB1 mice (25-30g commencing weight) received either haloperidol (10mg/kg), SCH 23390 (10mg/kg) or vehicle, injected intraperitoneally daily for a period of 14 days. Three days after cessation of chronic drug treatment animals were sacrificed. The striata were removed and homogenized in 40 volumes ice-cold TRIS buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$ and 1mM $MgCl_2$. Following centrifugation the resuspended pellets were frozen under liquid nitrogen. Total binding was determined by incubating the tissue with ^{3}H-spiperone (final concentration 0.1-10nM), while non-specific binding was determined in the presence of 1uM (+)-butaclamol.

Table 1 ^{3}H-Spiperone binding following chronic dopamine antagonist treatment

Drug	B_{max} (fmol/mg protein)	K_D (nM)	n
Saline	88.8±9.7	0.50±0.10	5
Haloperidol	124.0±9.5	0.45±0.10	5
SCH 23390	106.2±10.8	0.49±0.10	5

The number of D-2 binding sites (B_{max}) was significantly enhanced ($p<0.05$) by chronic haloperidol treatment. However, chronic pretreatment with SCH 23390 produced only a marginal change in B_{max}. This could possibly be explained by a lack of specificity of the antagonist at the dose level used in this study, which would correlate with the previously reported non-selectivity of this compound in in vivo studies (Pugh et al,1985).

The results suggest that in the dose regimen used in this study, SCH 23390 appears not to produce a significant up-regulation of striatal D-2 dopamine receptors, and as such may have potential as a long-term neuroleptic agent.

We gratefully acknowledge gifts of SCH 23390 (Schering) and (+)-butaclamol (Dr.K. O'Boyle, Dublin).

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EFFECT OF SYMPATHETIC NERVE STIMULATION ON THE RELEASE OF BETAXOLOL FROM THE HEART OF THE ANAESTHETIZED DOG.

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The β_1-adrenoreceptor antagonist betaxolol (BTXL) is accumulated by slices of rat atria from which it can be released, together with noradrenaline (NA), by electrical stimulation (Petruzzo et al. 1986). This effect, which has also been observed in vivo with the non-selective antagonist propranolol (Daniell et al. 1979), may possibly explain the persistence of the hypotensive effects of these drugs after withdrawal.
Here, we report the effects of postganglionic stimulation (4Hz, 3 min.) on the release of BTXL and NA into the coronary sinus of dogs pretreated for 7 days with 1 mg/kg/day s.c. of BTXL. BTXL was determined by a new highly sensitive gas chromatographic-mass spectrometric method, and NA by HPLC. The effects of BTXL pretreatment were also explored at the level of the positive chronotropic responses induced by accelerans nerve stimulation (0.25-4Hz), and by injections of isoprenaline (30-300 ng/kg i.v.). Blood samples were taken simultaneously from the aorta and coronary sinus (CS) before , during and after stimulation of the accelerans nerve. Coronary blood flow (CBF) was monitored by an electromagnetic transducer placed on the circumflex coronary artery.

TABLE BTXL concentrations in blood from CS during accelerans nerve stimulation.(% of control ± S.E.M ; N = 5).

Before stimulation	During stimulation			After stimulation	
2 min	1 min	2 min	3 min	5 min	15 min
97.0 ± 1.5	94.3 ± 4.0	103.5 ± 5.9	104.6 ± 6.7	97.4 ± 3.5	98.4 ± 3.9

The BTXL blood concentration 30 minutes before stimulation was 8.4±2.0 (s.e.m.) ng/ml (range 2.2-10.6 ng/ml) and it was taken as control value.

Concentrations in CS blood were not significantly increased during sympathetic nerve stimulation (Friedman's test), and levels were similarly unchanged in aortic blood. In contrast, NA concentrations in the CS were increased 6-fold during stimulation, while the CBF was increased by 23%. BTXL (1 mg/kg/day s.c. for 7 days) antagonized similarly the positive chronotropic effects induced by isoprenaline and by electrical stimulation, indicating that the neuronally mediated tachycardia was not preferentially antagonised under these conditions.
In conclusion, although BTXL is accumulated in the heart of the dog during administration for 1 week, its release is not detectable under our experimental conditions. These results do not exclude the possibility that BTXL is released exclusively from the atria, as demonstrated in the rat (Petruzzo et al, 1986; Arbilla et al, 1986). However, release from the atria, even if it does occur, does not seem to increase the β-receptor blocking effects of BTXL. This is probably due to the concomitant stimulation-induced release of NA, which competes effectively for the same receptors.

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EFFECTS OF ANTIDEPRESSANT AND NEUROLEPTIC DRUGS ON THE QUANTITATIVELY ANALYZED ELECTROENCEPHALOGRAM OF THE RAT.

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The study of drug effects on animal EEG has been hampered by a lack of control of the vigilance state of the animal. Changes of the vigilance state are parallelled by profound EEG changes. Small drug effects are lost in this spontaneously changing EEG. Thus relatively high doses have to be given (Yamamoto, 1985). We have developed a system to stabilize the vigilance state of the rat as good as possible (Krijzer et al, 1983). Based on this vigilance controlling system we present the effects of 3 antidepressants and 3 neuroleptics.

Aim of this study was to look for systematic differences between antidepressants and neuroleptics.
EEG was differentially recorded from the frontal and parietal cortex of freely moving rats. During an experiment the rat was placed in a drum within a sound attenuated Faraday cage. During the EEG-recording period the drum turned very slowly (0.2 rpm) to control the vigilance state. The rats were given clinically effective antidepressant and neuroleptic drugs as well as saline. Drug dosages and rats were randomized according to a Greek-Latin square. EEG was recorded the 6 min immediately before drug injection and at 30, 60 and 90 min after drug. During each of these vigilance controled EEG recording periods, 128 EEG samples of 2.56 sec. each were recorded and the power spectra calculated. From these 128 power spectra a mean power spectrum was calculated: one before and 3 after drug administration. Power spectral changes after drug or saline were expressed as percentage of the pre-drug power value. Changes were calculated for a 2 Hz wide frequency band which moved over the powerspectra. These percentage changes were used as input for an analysis of variance. This resulted in 3 t-profiles per drug dose: one for each time after treatment.

A clear dose and time relationship can be seen for all the drugs tested. The results of the antidepressant chlorimipramine 2, 4 and 10, mianserin 2, 4 and 10 and iprindol 4, 10, 20 and 40 mg/kg i.p. are shown as well as the results from the neuroleptics haloperidol 0.05, 0.1 and 0.2, thioridazine 1, 2 and 4 and clozapine 1, 2, 4 and 8 mg/kg i.p. By averaging the t-values per time for each drug dosage a drug profile is calculated for the aforementioned drugs. Based on the drug profile CMI decreases the power significantly below 4.3 Hz, from 7.5 to 9 Hz and from 40 to 100 Hz. Mianserin decreases the power significantly from 0 to 2 Hz, from 8 to 10 Hz and from 55 to 100 Hz and increases the power nonsignificantly from 4 to 8 Hz and from 20 to 40 Hz. Haloperidol induces a significant power increase from 12 to 23 Hz and a significant power decrease from 27 to 100 Hz. Thioridazine induces a significant power increase from 3.5 to 7 Hz and from 11 to about 31 Hz as well as a power decrease from about 55 to 100 Hz. Clozapine induces a significant power increase from about 2 to 32 Hz and a significant power decrease from 40 to 100 Hz.
Iprindol decreases the power significantly below 20 Hz and from 55 to 100 Hz and increases the power from 30 to 40 Hz.

Based on this limited number of drugs it may be stated that there are systematic differences between antidepressants and neuroleptics.

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636P

TYPICAL PROFILES FOR THE ANTIDEPRESSANT AND NEUROLEPTIC DRUG CLASS BASED ON QUANTITATIVELY ANALYZED CORTICAL EEG OF THE RAT.

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Based on human EEG studies typical profiles for amongst others the antidepressant and neuroleptic drugs have been obtained (Itil, 1982). Drug induced EEG changes in animals however have never resulted in comparable profiles that could be used for the classification of drugs. Drug-induced cortical EEG changes of a number of antidepressants and neuroleptics are shown and a profile for each drug class is calculated. Aim of this study was whether it might be possible to construct an antidepressant and neuroleptic profile which should differ clearly from each other.

Based on power spectral analysis from cortical EEG of the rat a number of drug profiles have been calculated (Krijzer, 1986). By averaging the drug profiles of clinically effective antidepressant and neuroleptic drugs two sets of reference profiles have been calculated. Each set consists of 3 profiles, resp. 30, 60 and 90 min after treatment. The following drugs and doses were used for the antidepressant profile: imipramine 2 and 4, amitriptyline 1, 2 and 4, iprindol 4, 10, 20 and 40, chlorimipramine 2, 4 and 10, mianserin 2, 4 and 10, nomifensine 10, bupropion 4 and 10, zimeldine 4, 10 and 20, fluvoxamine 2, 4 and 10 and clovoxamine 4 and 10 mg/kg. For the neuroleptic profile the following drugs were used: chlorpromazine 0.2, 0.4 and 0.8, haloperidol 0.05, 0.1 and 0.2, sulpiride 20 and 40, clozapine 1, 2, 4 and 8, thioridazine 1,2 and 4, mezilamine 1, 2 and 4 and trifluperazine 0.5, 1 and 2 mg/kg. All drugs were given intraperitoneally except nomifensine which has been given orally suspended in tragacanth.
The antidepressant and neuroleptic profiles differ very clearly. The neuroleptic profile is characterized by a significant power increase from 0 to 7 Hz and from 11 to 30 Hz as well as by a power decrease around 8 Hz and above 30 Hz. The antidepressant profile is characterized by an overall power decrease from 0 to 100 Hz except for a small frequency band around 6 Hz. Very typical for the antidepressant profile seems to be the power decrease from 8 to 14 Hz. By applying a Wilcoxon with the individual antidepressant and neuroleptic drugprofiles as input the significantly different frequency band between both class profiles has been determined: 0.4 - 25 Hz.
This frequency band can be used to discriminate between antidepressant and neuroleptic drugs.

There is a clear systematic difference between the antidepressant and neuroleptic profile. Therefore, the cortical EEG of the rat can be used for classification of antidepressant and neuroleptic drugs.
As the antidepressants included in this profile belong to pharmacologically different drugs it may be hypothesized that the typical antidepressant EEG effect seems to be independent from the pharmacological profile. This does not hold true for the neuroleptic profile which may be a dopaminolytic profile.

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ARE 5-HT_{1A} BINDING SITES RELEVANT FOR THE ANTIHYPERTENSIVE EFFECTS OF DU 29373?

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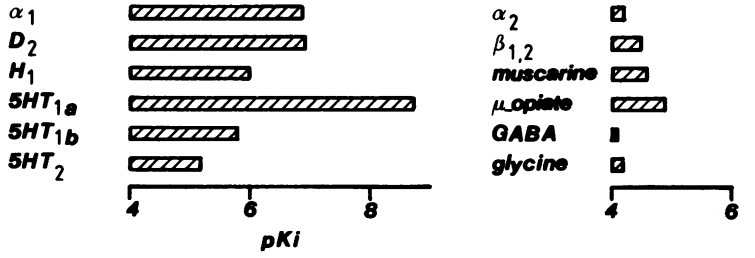
DU 29373, flesinoxan.HCl, is a potent, longacting, orally active antihypertensive compound. Most probably, it has a central site of action (Calis et al., 1986). In rats the compound is not converted to metabolites which affect blood pressure. In the search for its pharmacological mode of action the affinity of DU 29373 for different receptors in rat brain homogenates was determined using well defined binding assays.



As shown in Figure 1, DU 29373 has a pharmacologically significant affinity for only α_1, D_2 and $5HT_{1A}$ receptors, and is a very selective $5HT_{1A}$ ligand in (^{3}H)8-OH-DPAT binding. To estimate the in vivo relevance of these binding data, the effects of oral DU 29373 on tyrosine hydroxylase (TH) in rats was measured as an indicator for D_2-affinity. DU 29373 yielded an ED_{150} of 22 mg/kg on TH increase, a dose far above (about 20 times) that needed to lower blood pressure significantly in SH rats. Similarly, α_1-antagonism was not observed in cats even at doses producing maximal blood pressure reductions. Thus, the only pharmacologically relevant affinity sofar observed is that for $5HT_{1A}$ receptors.

DU 29373 shares affinity for $5HT_{1A}$ receptors with drugs like 8-OH-DPAT(DPAT), buspirone (Bus) and TVX-Q-7821(TVX). However, in vivo these drugs behaved quite differently. TVX and Bus (10 mg/kg p.o.) had no effects on blood pressure and heart rate in SH rats whereas DPAT (2.5 mg/kg s.c.) decreased heart rate strongly and blood pressure moderately. DU 29373 (1 mg/kg p.o. and higher) mainly decreased blood pressure and heart rate marginally (Calis et al., 1986, unpublished observations). Moreover, in rats the behavioural effects of these drugs are very different. DU 29373, Bus and TVX showed no overt behavioural effects, but DPAT potently induced activation and stereotypy at relevant cardiovascular doses. DU 29373 appears to be the most selective and potent of the drugs in terms of affinity for the $5HT_{1A}$ receptor subtype, and this may partially explain the specific pharmacological action of the drug. However, we have no knowledge of the intrinsic activity of the molecule at this site.

In conclusion, it is tempting to speculate that the antihypertensive properties of DU 29373 are related to the affinity of the molecule for the $5HT_{1A}$ receptor. Further studies are needed to elucidate the process which translates this pharmacological property into the cardiovascular response.

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INVOLVEMENT OF CENTRAL SEROTONERGIC PATHWAYS IN NEFOPAM-INDUCED ANTINOCICEPTION.

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The mechanism of the analgesic action of nefopam is not known, but it is a central acting nonnarcotic analgesic (Piercey & Schroder, 1981). As central serotonergic pathways are implicated in nociception and analgesia we wanted to investigate possible involvement of these pathways in nefopam-induced analgesia.

Male albino NMRI mice (25-35g) were used. Nefopam dissolved in saline was administered i.p. in a dose of 15 mg/kg (10ml/kg) 15 min before testing, control groups received saline only. Two different nociceptive assays were used, the formalin test (Hunskaar et al., 1985) and the increasing temperature hot plate test. In the formalin test 20 ul of a 1 % solution of formalin in saline was injected into the dorsal surface of the right hindpaw. The amount of time the animal spent licking the injected paw was recorded during the first 2 min after injection. In the increasing temperature hot plate test, the mouse was placed on the plate at a temperature of 43 ^{o}C and then the temperature was increased at a rate of approximately 2.5 ^{o}C/min. The temperature when the first hind paw lick occurred was recorded. Lesions of the ascending serotonergic pathways were made by systemic administration of p-chloroamphetamine (PCA) (Hunskaar et al., 1986) and 5-hydroxytryptamine (5-HT) depletion in all serotonergic systems was obtained by using the tryptophan hydroxylase inhibitor p-chlorophenylalanine (PCPA).The lesions were biochemically verified by measuring synaptosomal uptake of [^{14}C]-5-HT (PCA) and the depletion of 5-HT was verified by HPLC measurement of the 5-HT levels in cortex and spinal cord (PCPA).

PCPA-induced depletion of 5-HT did not significantly change the control responses in the formalin test (saline: 55$\pm$5 s; PCPA: 60$\pm$4 s, P>0.4). PCPA pretreatment significantly reduced the effect of nefopam (saline pretreated: 15$\pm$3 s; PCPA pretreated: 33$\pm$3 s, P<0.001). PCA-induced lesions of the ascending serotonergic pathways did not alter control responses in the formalin test (saline: 91$\pm$11 s; PCA: 82$\pm$7 s, P>0.5) or the increasing temperature hot plate test (saline: 46.9$\pm$0.09 ^{o}C; PCA: 47.0$\pm$0.26 ^{o}C, P>0.7). In contrast to the findings from the PCPA treated groups, nefopam-induced analgesia was not affected by PCA pretreatment (saline pretreated: 34$\pm$9 s; PCA pretreated: 28$\pm$9 s, P> 0.6 (formalin test); saline pretreated: 48.8$\pm$0.25 ^{o}C; PCA pretreated: 49.2$\pm$0.36 ^{o}C, P>0.3 (the increasing temperature hot plate test))(N=6-9 in each group).

In conclusion, the data suggest that the descending serotonergic system is involved in nefopam-induced antinociception.

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ANALGESIC EFFECTS OF NEFOPAM AT SPINAL AND SUPRASPINAL SITES.

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It is well documented that nefopam possesses analgesic activity in humans (Heel et al., 1980). Nefopam is chemically unrelated to other analgesics, does not affect prostaglandin biosynthesis, and does not bind to opiate receptors. Previously it has been shown that nefopam is effective when administered intracerebrally to mice, but is reported to be ineffective after intrathecal injections (Piercey and Schroeder, 1981). The aim of the present study was to examine the analgesic activity of (±)nefopam (the racemic compound) and the two enantiomers, (+) and (-)nefopam, after intraperitoneal (i.p), intracerebroventricular (i.c.v.) and intrathecal (i.th.) administration.

Male albino NMRI mice were used. The animals were tested on a 55^{0}C hot-plate 15 min after i.p. (n=7) injections (0.6-20 mg/kg) and 10 min after i.c.v. (n=9) or i.th. (n=8-9) injections (5-20 ug/mouse). Response criterion was licking of a hindpaw. In addition tail-flick testing was performed 15 min after i.p. (n=9-11) injection of 20 mg/kg of (±)nefopam in intact and spinally transected animals.

(±)Nefopam demonstrated clear analgesic activity in the hot-plate test after i.p. administration. A dose of 10 mg/kg prolonged the latencies by 90% as compared to controls. (+)Nefopam was more potent than both (-) and (±)nefopam.

Intracerebroventricular injection of 5 ug (±)nefopam (0.15 mg/kg) prolonged the latencies by 140% and thus had a stronger effect than 10 mg/kg injected i.p. (+)Nefopam had significantly stronger effect than (-)nefopam. (±)Nefopam also prolonged response latencies when administered i.th. Latencies were 160% longer than in controls after 20 ug (0.6 mg/kg). (+)Nefopam had stronger effect than (-)nefopam also after i.th. injection.

(±)Nefopam significantly prolonged the tail-flick latencies in intact animals (30% longer than controls), but had no effect in the mice with spinal cord transections.

The present study has demonstrated analgesic activity of nefopam in the hot-plate test not only after i.c.v., but also after i.th. administration in the mouse. It thus seems that nefopam has both a supraspinal and a spinal site of action. The failure of nefopam to prolong the tail-flick latencies in spinalized animals may indicate that the analgesic activity of nefopam in the spinal cord is produced by activation of descending pain-modulating systems. Furthermore, the (+) enantiomer of nefopam is more potent than the (-) enantiomer both after systemic and central administration.

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EFFECTS OF EXCITATORY AMINO ACID ANTAGONISTS ON CATECHOL-INDUCED SEIZURES.

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The role of the excitatory amino acid antagonists in epilepsy is currently of interest and these agents are known to be effective anticonvulsants against a number of seizure models (Jones et al., 1984). This work investigates the effects of a number of excitatory amino acid antagonists on sensory evoked catechol-induced convulsions.

Experiments were performed on female albino rats (Sheffield strain, weight range 190-210g) anaesthetised with urethane 1.2-1.4 g/kg i.p.) into which catechol was infused intravenously. (2.5 mg/kg/min). Electromyographic activity was recorded from Flexor carpii and evoked by electrically stimulating sensory afferents at the wrist (10-20 V; 20 s width; 0.17 Hz). Typically this evoked muscle response consists of three temporally distinct components, which are totally absent in the non-catechol treated anaesthetised animal and which are the result of catechol-induced activation of three separate reflex pathways: a propriospinal reflex (M1), a long-loop reflex involving the sensorimotor cortex (M2) and a cerebellar reflex (M3). Excitatory amino acid antagonists were administered either i.c.v. or i.p. and their effects assessed by measuring the probability of occurrence of each of these components in a pre-drug control period (10-15 min) and subsequent post-drug period (usually 40-50 min). Results are expressed as the mean percentage difference in probability (S.D. of mean) and shown in the table below.

Drug	Dose µmol/kg i.c.v.	Mean % Difference ± s.d. M1	M2	M3
2-amino 7-phosphono valearic acid	1.2	-	-53.7 ± 17.4**	-57.2 ± 30.7*
Gamma-D-Glutamyl glycine	2.0	-	-34.2 ± 19.3*	-59.2 ± 18.5**
cis 2,3 piperidine dicarboxylic acid	1.4	-	-66.0 ± 10.0**	-65.0 ± 11.2**
2-amino 7-phosphono heptanoic acid	230 (i.p.)	-	-29.6 ± 10.6**	-22.1 ± 13.6*

** $P < 0.001$; * $P < 0.01$ Student paired t-test: n = 4 to 6

All of the excitatory amino acid antagonists significantly reduced the probability of occurrence of M2 and M3 but had no effect on M1, indicating a supraspinal site of action. These results suggest that excitation at excitatory amino acid receptors (particularly the N-methyl-D aspartate receptor) is important in sensory evoked catechol-induced seizures, and is consistent with reports that catechol selectively increases the K^+-evoked release of aspartate from slices of rat cortex (Minchin and Pearson, 1981; Collins and Dewhurst, 1986).

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RADIOLABELLED AND ENDOGENOUS DOPAMINE RELEASE FROM STRIATAL SLICES: EFFECT OF ELECTRICAL STIMULATION AND REGULATION BY D_2 RECEPTORS.

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The regulation of catecholamine release from brain slices by presynaptic receptors is usually studied by monitoring the release of radioactivity after prelabelling the tissue with radiolabelled amine. However, there is little direct evidence that the release of radiolabel provides an accurate quantitative assessment of endogenous transmitter release; indeed, we have shown recently that labelled and endogenous dopamine (DA) release can be affected quite differently by various releasing stimuli (Herdon et al. 1985). We have now made direct comparisons between the release of labelled and endogenous DA in response to electrical field stimulation, and compared how their spontaneous and electrically-evoked release are regulated by D_2 autoreceptors.

Rat striatal slices were prepared, prelabelled with ^{3}H-DA and superfused as described previously (Herdon et al. 1985). GBR 12921 (1 μM) was present throughout all superfusions to inhibit DA reuptake without releasing DA (Herdon & Nahorski, 1986). Endogenous DA in superfusate fractions was measured by HPLC with electrochemical detection and ^{3}H-DA present in fractions collected from the detector cell was quantified by scintillation counting. Electrical field stimulation was performed using monophasic rectangular pulses (3Hz, 2 msec, 24 mA) for 2 min. Two stimulations (S_1 & S_2) were applied 45 min. apart; spontaneous release was measured before each stimulation (Sp_1 & Sp_2). Drugs were added 20 min. before the second stimulation.

The ratio between the amounts of DA released by the two stimulations (S_2/S_1) was much lower for ^{3}H-DA (0.54 ± 0.10) than for endogenous DA (0.83 ± 0.11); however, the ratios of spontaneous release (Sp_2/Sp_1) were more similar (endogenous 1.16 ± 0.07; ^{3}H-DA 0.89 ± 0.07). Pergolide (1 μM) reduced both spontaneous and electrically-evoked release of both endogenous and ^{3}H-DA by 20-30%, whilst sulpiride (1 μM) increased spontaneous release of both endogenous and ^{3}H-DA by 40-80% and electrically-evoked release by up to 200%. Sulpiride-induced increases in release were greatly reduced by simultaneous addition of 1 μM pergolide.

These results confirm our previous findings (Nahorski & Strupish, 1985; Herdon & Nahorski, 1986) that both spontaneous and depolarisation-induced endogenous DA release can be regulated by D_2 autoreceptors. They also show that, despite differences in response to releasing stimuli between ^{3}H-DA and endogenous DA (Herdon et al. 1985), their regulation by autoreceptors appears similar. Of particular interest is the finding that spontaneous ^{3}H-DA release can be modulated by autoreceptors, since this is not generally observed in studies on labelled release (e.g. Lehmann et al. 1983), probably because only total ^{3}H (of which only ca. 20% is ^{3}H-DA) is usually assayed. However, it must be emphasised that release of either endogenous or ^{3}H-DA from prelabelled tissue may be different to release from non-prelabelled tissue, since the prelabelling process itself appears to alter the intracellular source from which subsequent release occurs (Herdon et al. 1985).

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THE PROFILE OF ACTION OF SULPIRIDE AS AN ANTI-SCHIZOPHRENIC AGENT.

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Sulpiride is an antipsychotic agent which has been reported to have additional effects to reduce anxiety, depression and cause an alerting or 'disinhibitory' effect in man. The present study investigates and compares the potential for such actions in animal models.

Neuroleptic action was assessed as ability to antagonise locomotor hyperactivity induced by dopamine infused into rat nucleus accumbens (see Costall et al, for experimental details), an alerting action by an ability to enhance spontaneous activity of the rat (measured using photocell cages) during the period 20.00-22.00h and an antidepressant effect was measured in mice in the Porsolt test. The antianxiety effect was assessed in mice using a two compartment white and black box where mice normally show an aversive response to exploration (eg rearing and line crossings) in the white area, an effect antagonised by anxiolytic agents (see Crawley and Goodwin 1980). At least 6 animals were used for each treatment, data being analysed using analysis of variance and Dunnett's 't' test. Sulpiride (2.5-10 mg/kg i.p. 3 times daily) administered throughout a 13 day period of dopamine infusion antagonised the hyperactivity response from maximum counts of 184-238/60 min to 43-88 counts/60 min ($P<0.001$). In the assessment of the alerting potential, the control level of spontaneous activity (90-110 counts/30 min) was enhanced by sulpiride (2.5-20 mg/kg) the response demonstrating a bell shaped curve, achieving a maximum increase of 80% ($P<0.001$) to be followed by a depression of activity at higher doses. In the Porsolt test, sulpiride (0.1-1.0 mg/kg i.p.) caused a reduction in the immobility by up to 55% ($P<0.001$), the response declining over the range 1 to 10 mg/kg to return to control levels. Amitriptyline (5-20 mg/kg i.p.) showed a similar spectrum of activity. In the anxiety testing box, sulpiride (0.5-5.0 mg/kg i.p.) increased rearing and line crossing behaviour in the white 'aversive' area by approximately 2 fold ($P<0.001$), with corresponding decreases in behavioural responding in the black area. Diazepam (0.1-5.0 mg/kg i.p.) had an identical spectrum of action.

Thus sulpiride was shown to be 5 to 10 times more potent in the Porsolt test of despair than in the other rodent models to increase alertness, antagonise anxiety or to inhibit dopamine-induced effects, sulpiride having a comparable potency in the latter three tests. The data would support the clinical hypotheses that at the doses required to secure the antipsychotic action, sulpiride may exert alerting, antidepressant or anxiolytic effects.

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ACUTE CLOBAZAM ADMINISTRATION INDUCES ANTICONVULSANT TOLERANCE TO N-DESMETHYCLOBAZAM IN MICE.

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Tolerance to the anticonvulsant effect of clobazam develops very rapidly in mice (Gent et al., 1985). The principal metabolite of clobazam, N-desmethylclobazam (NDMC), is also an effective anticonvulsant in this species but induces a lesser degree of tolerance when administered alone (Feely et al., 1985). In order to investigate further this potentially important difference we have studied the relationship between anticonvulsant activity and plasma benzodiazepine concentration following acute doses of clobazam and NDMC.

Adult male mice (Tuck No 1), 25-35g in weight, were randomised into groups of 5. Seven groups were given a single dose of clobazam (5mg/kg; s.c.), four groups received NDMC (5mg/kg; s.c.). In each study an extra group of mice, acting as controls, received an equivalent volume (2.5ml/kg) of the benzodiazepine vehicle (Haigh et al., 1984). Between 0.25 and 8h after clobazam administration and 1, 3, 6 and 9h after NDMC administration, groups of mice were tested for anticonvulsant activity using an i.v. infusion of pentylenetetrazole (PTZ; Haigh et al., 1984). Control mice were tested 1h after vehicle injection. Blood samples were taken from all benzodiazepine treated animals immediately after their convulsive tests and plasma concentrations of clobazam and NDMC were assayed by GLC and HPLC respectively (Haigh et al., 1984).

15 min after clobazam administration the minimal convulsant dose (MCD) of PTZ was 95.3 + 3.7mg/kg (mean $\pm$ s.e. mean), but it had returned to control values (38.5 $\pm$ 0.3mg/kg) after 6h. During this time plasma clobazam levels fell rapidly from around 550ng/ml, becoming undetectable 2-4h after the dose. In contrast, the concentration of metabolite increased for the first hour of the experiment reaching peak levels of about 1600ng/ml, then declined only slowly during the next 7h, so that concentrations of 1200ng/ml were still present when no anticonvulsant activity remained. The increase in MCD of PTZ produced by NDMC when given alone was of smaller magnitude but longer duration than that with clobazam. 1h after NDMC the MCD of PTZ ws 53.8 $\pm$ 3.0mg/kg compared with control values of 32.1 $\pm$ 0.9mg/kg. This effect decreased very slowly during the next 8h and was still measurable 9h after the dose; plasma levels of NDMC declined from approximately 1400 to 1000ng/ml.

The anticonvulsant effect produced in mice by a dose of clobazam was observed for sometime after plasma concentrations of clobazam had become negligible, emphasising the importance of NDMC. (A close correlation between plasma and brain concentrations has been reported previously with this compound (Caccia et al., 1980).) During this time the levels of NDMC did not change significantly even though the MCD of PTZ returned to control values. One explanation for such an anomaly might be the development of acute tolerance; this phenomenon has already been documented in humans receiving clorazepate, when sedation decreased despite the maintenance of plasma desmethyldiazepam levels (Greenblatt et al., 1979). When the metabolite was given alone, the MCD of PTZ was increased by levels of NDMC (~1200ng/ml) which had been without effect after clobazam administration. This suggests that the development of acute tolerance requires the presence of clobazam itself and supports the hypothesis that benzodiazepines differ in their tolerance - inducing potential.

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TRYPTAMINE MIMICS THE EXCITATORY EFFECT OF 5-HYDROXYTRYPTAMINE IN THE RAT HIPPOCAMPAL SLICE.

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The endogenous amines 5-HT and tryptamine are known to produce similar effects in the central nervous system but there is growing evidence that the two compounds may be acting through separate mechanisms (Jones, 1982). Selective binding sites for these compounds, which possibly correspond to separate receptors are present in many parts of the CNS, including the hippocampus (Cascio & Kellar, 1983). Here 5-HT has both excitatory and inhibitory effects. Recently the receptor mediating the latter effect has been found to be similar to the 5-HT 1A binding site whereas the excitatory effect of 5-HT has not yet been classified (Beck et al. 1985; Rowan & Anwyl 1985, 1986). As a development of this work the present study compared the effects of 5-HT and tryptamine on hippocampal pyramidal cells in vitro and examined the effects of some 5-HT receptor antagonists.

Transverse hippocampal slices (450 μ thick) from male albino Wistar rats (200-250g) were used according to the method of Rowan and Anwyl (1985). The slices were superfused with modified Kreb's solution at 35°C. The stratum radiatum was stimulated at a frequency of 0.05 Hz and an extracellular population spike which was 50% of the maximum amplitude was recorded in the pyramidal cell layer of the CA1 region. Agonists and antagonists were applied via the perfusion medium for 10 min and at least 1h respectively.

5-HT produced an initial increase followed by a maintained decrease in the amplitude of the population spike in a concentration dependent manner. The inhibitory effect of 10^{-5}M 5-HT was greatly reduced from 81 ± 8% inhibition to 17 ± 9% (n = 5) when the slices were pretreated with the 5-HT 1A antagonist spiperone (10^{-6}M). This appeared to unmask the excitatory effect of 5-HT which increased from 22 ± 11% facilitation to 103 ± 20%. Tryptamine had purely excitatory effect on the pyramidal cells which was rapidly reversible on washout. It produced a concentration dependent increase in the amplitude of the population spike in the range of 10^{-5} to 5 x 10^{-4}M. This was approximately five times less potent than 5-HT when measured in the presence of 10^{-6}M spiperone. There was a significant positive correlation between the excitatory effect of tryptamine (5 x 10^{-5}M)&that produced by 5-HT (10^{-5}M), when experiments in which 5-HT also gave an inhibitory effect, were excluded (r^2 = 0.83, $P < .05$, n = 7).

Pretreatment of slices with the classical 5-HT antagonist methysergide (10^{-5}M) or the selective 5-HT2 antagonist ketanserin (10^{-5}M) or the selective 5-HT M-receptor antagonist ICS 205-930 (10^{-6}M) did not significantly affect the excitatory response to either 5 x 10^{-5}M tryptamine or 10^{-5}M 5-HT.

The evidence from the present experiments indicates that 5-HT and tryptamine may share similar mechanisms with regard to their excitatory effects on hippocampal pyramidal cells in that their effects were significantly correlated. Whether or not the responses are mediated through a common 5-HT or tryptamine receptor remains to be determined. The insensitivity of the effects of both agents to the antagonists implies that the receptor does not correspond to either M-receptors or 5-HT1 or 5-HT2 binding sites.

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ACUTE MILD STRESS CAUSES α_2-ADRENOCEPTOR UPREGULATION IN RAT CEREBRAL CORTEX.

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We have shown previously that after repeated saline injections (once-daily for 14 days, but not for 5 days) α2- and β-adrenoceptor binding in the cerebral cortex of injected animals is significantly less than in unhandled controls; these receptor changes are thought to underlie adaptation to stress (Stanford et al., 1984). In contrast, the activity of synaptosomal tyrosine hydroxylase of the injected animals is significantly greater than in controls. Similar differences in enzyme activity have recently been found when 14 saline injections are given over a period of 3.5 days (4 times daily; Anderson et al., 1985). Our first aim was to show whether binding changes could also be detected after this shorter period of stress and, secondly to distinguish whether differences in binding represent downregulation of receptors in stress-adapted animals or upregulation in the controls, being handled for the first time immediately before death. This was done by comparison of α2-adrenoceptor binding in the cerebral cortex of animals killed in the animal house (taken individually from their cages to an adjacent room) with that in animals given the acute stress of moving to the laboratory immediately before death. Saline injections were administered at 2.5 hr intervals between 9.15 am and 4.45 pm; all injected animals with their paired controls were killed 24 hrs after the last injection. Controls received the minimum disturbance required for maintenance. α2-adrenoceptor binding in the cerebral cortex used 3H-clonidine (conc. range: 0.2-2.8nM) as the radioligand and phentolamine (10μM) as displacing ligand. Binding data were analysed using the 'ligand' computer program (Munson & Rodbard, 1980).

	Kd (nM)	%	Bmax (pMoles/g.prot)	%
Control	2.05 ± 0.27		50 ± 5	
Moved Control	2.37 ± 0.66**	126 ± 21	63 ± 6*	127 ± 10
Saline	1.81 ± 0.26	93 ± 13	53 ± 7	106 ± 12
Moved Saline	2.02 ± 0.41	106 ± 22	48 ± 6+	96 ± 1

* $p<0.05$ cf control + $p<0.05$ cf moved control ** $p<0.05$ cf saline. (matched pair t-test) n = 6.

After 3.5 days the number of α2-adrenoceptor binding sites is significantly less in moved saline-injected animals than in the moved controls. These findings contrast with those of our earlier study where animals receiving only one injection per day showed no changes by 5 days. However, there were no differences in binding when the two unmoved groups were compared. Since binding in the unmoved controls was significantly less than in moved controls, these data suggest that the acute stress of moving causes a rapid upregulation of α2-adrenoceptors and that this rise is prevented by previous experience of handling and saline injection. These findings are relevant to current theories of the role of adrenoceptor changes in the actions of drugs used to treat anxiety and depression, clinical conditions associated with stress.

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5-HT_2 RECEPTOR ANTAGONISTS SHOW ANXIOLYTIC-LIKE ACTIVITY IN THE X-MAZE.

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Although many studies have indicated a role for 5-HT in anxiety (eg Gardner, 1985), the precise mechanism of its involvement is unclear. The present study is being undertaken to evaluate the effects of 5-HT receptor ligands in the elevated X-maze. This maze has opposite open and enclosed arms, total entries measure exploratory activity while the degree of preference for enclosed arms is taken as an indicator of anxiety (Handley & Mithani, 1984). The model discriminates anxiolytic and anxiogenic agents from inactive compounds (Handley & Mithani, 1984; Pellow et al, 1985).

Maze exploration was determined in groups of 6 male hooded rats (200-250 g) over a 10 minute period 30 min after pretreatment with drug or vehicle (.9% NaCl) ip.

Dose-response curves were prepared for each drug. The non-selective 5-HT receptor agonist 5-methoxy-N,N-dimethyl tryptamine (5-MeODMT) was significantly anxiogenic over the dose-range 0.25-2.5 mg/kg. In contrast, ketanserin, ritanserin and R-56,413, which are selective antagonists for the 5-HT_2 receptor (Colpaert et al, 1985) showed potent anxiolytic-like activity over a similar dose range. Table 1 illustrates the effects of doses causing an approximate 50% increase (anxiolytic) or decrease (anxiogenic) in open/total entry ratio.

Unlike diazepam, 5HT_2 receptor antagonists show weak or negligible activity in operant conflict models of anxiety (Colpaert et al, 1985; Gardner, 1986). We have also observed this lack of activity in operant conflict in the strain of rats used here. This suggests that the two types of model might be measuring different aspects of anxiety, only one of which is sensitive to 5-HT_2 receptor antagonists.

Table 1: No of entries in 10 min + sem (% paired saline control)

	dose	total	p	open/total	p<
saline*		11.6+ .9		0.31+.01	
diazepam	2.5	10.4+2.1	(73)ns	0.48+.02 (148)	.01
ritanserin	0.05	12.2+2.8	(108)ns	0.42+.02 (145)	.01
ketanserin	0.5	8.8+1.0	(118)ns	0.39+.02 (155)	.01
R-56413	0.5	7.0+1.1	(71)ns	0.43+.03 (133)	.05
5-MeODMT	0.5	18.5+2.5	(127)ns	0.20+.03 (54)	.01

* mean of all control groups

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RITANSERIN REDUCES MORPHINE, AND CLONIDINE, WITHDRAWAL TICS.

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Although head-twitches and body shakes (rodent tics) are usually associated with 5-HT agonists and precursors (Green and Heal 1985), they in fact occur following a wide variety of drugs and treatments. In most cases these are blocked by 5-HT antagonists such as methysergide and cyproheptadine, but there are certain exceptions. Among these is the head-twitch occurring in mice following precipitated morphine abstinence, which was not antagonised by cyproheptadine (Bednarczyk and Vetulani 1978). There may thus be two separate mechanisms for tic induction, only one of which is 5-HT related. Since there is now evidence that 5HT agonist tics are due to an effect at the $5HT_2$ receptor subtype (Tricklebank, 1985), we decided to re-examine morphine abstinence tics using the potent and specific $5HT_2$ receptor antagonist ritanserin (Leysen, 1981). Clonidine withdrawal has also been reported to induce tics in rodents (Aceto and Harris, 1981), and reduces morphine withdrawal signs in man. Table 1 shows that tics induced by both morphine and clonidine withdrawal were reduced by ritanserin.

Table 1: Effect of ritanserin 0.25 mg/kg or gum-acacia/saline vehicle on tic rate (± sem) in morphine or clonidine withdrawn mice.

A:	vehicle	ritanserin	n/group	F(AxB)	df	P
B: morphine	5.30±.37	2.60±.37	10	9.39	1/18	.01
saline	1.10±.23	.80±.37				
B: clonidine	4.25±.49	2.38±.42	11	6.21	1/21	.05
tap water	.75±.18	.50±.15				

Using MF1 mice, morphine was injected ip 2x daily, doubling the dose each time to 300 mg/kg on injection 2, day 4; naloxone 10 mg/kg ip was given to all groups on day 5, 10 min before counting tics. Clonidine was given in drinking water for 6 days (actual consumption 4.4-4.8 mg/kg/day) and tics counted 22 h after withdrawal. Tics were counted for 20 min; rates on withdrawal of either drug were low but consistently 5-fold above the spontaneous rate. Ritanserin significantly reduced withdrawal tics but the reduction in the spontaneous rate was not significant. It therefore appears likely that $5HT_2$ receptors are involved in the tics which follow both morphine and clonidine withdrawal.

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5-HYDROXYTRYPTAMINE AGONISTS: NEUROCHEMICAL PROFILE AND EFFECTS ON AGGRESSIVE BEHAVIOUR.

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It is generally accepted that serotonin plays an important role in mediating aggressive behaviour. Electrical or chemical lesions of the 5HT neuronal pathways may result in increased aggressive behaviour in rats*. Moreover, it has been suggested that enhancement of 5HT neurotransmission results in inhibition of aggressive behaviour*. In order to further investigate the involvement of serotonin in agonistic behaviour, we studied the effects of a number of serotonin agonists on aggression and the neurochemical properties of these agonists.
The affinity for different serotonin receptors (S_{1A}: ^{3}H-DPAT binding; S_{1B}: ^{3}H-5HT + DPAT binding: S_2: ^{3}H-spiroperidol binding) was determined for a number of agonists. The most selective S_{1A} agonist is 8-OH DPAT, whereas trifluoromethylphenylpiperazine (TFMPP) is most selective for S_{1B} binding sites.
The K^+-stimulated release of ^{3}H-serotonin from cortex slices was used to establish the activity of these compounds on the serotonin autoreceptor in vitro. Quipazine and 8-OHDPAT were inactive in this model, whereas the other agonists induced a pronounced inhibition of serotonin release. TFMPP appeared to be a partial agonist on this receptor.
The effects of these compounds on serotonin turnover in vivo was studied by measuring 5-HTP levels in different brain areas after inhibition of aromatic amino acid decarboxylation. All compounds, except quipazine, lowered 5 HTP levels. Also 8-OHDPAT was very active in lowering serotonin turnover in vivo, in spite of its lack to activate presynaptic serotonin receptors in vitro.
The effects of these serotonin agonists was studied in different paradigms for aggressive behaviour (isolation aggression in mice, maternal aggression and muricide in rats).

Table 1 Neurochemical profile of serotonin agonists and effects on aggressive behaviour (ED_{50} in mg/kg).

	binding (pKi)			release	5HTP	isol.aggr.	muric.	mat.aggr.
	S1A	S1B	S2	pD_2	ED_{50}(ip)	ED_{50}(or)	LED (ip)	LED (ip)
8-OHDPAT	8.8	7.2	4.4	< 5.0	0.2	0.3	>5.0	0.1
TFMPP	6.4	7.8	6.1	7.7	1.9	0.2	0.5	0.5
Quipazine	5.8	6.8	6.0	< 5.0	>10.0	5.0	4.0	2.5
RU 24969	8.3	8.1	6.1	8.0	1.0	0.7	1.0	0.5
5MeODMT	8.2	7.3	-	5.5	-	-	1.0	-

Based upon the observed effects, it is obvious that manipulation of the serotonin system affects aggressive behaviour in rodents differentially. However the specificity of the behavioural effects of the serotonin agonists with different neurochemical profiles awaits further investigations.

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THE EFFECT OF MEPTAZINOL ON CARBACHOL-INDUCED PHOSPHOINOSITIDE BREAKDOWN IN RAT BRAIN SLICES.

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The antinociceptive effect of meptazinol may be due, in part, to direct or indirect actions on cholinergic mechanisms in addition to its properties as an opioid mixed agonist-antagonist (Bill et al., 1983). We have shown previously, using ligand-binding studies in rat brain membrane preparations, that meptazinol has the characteristics of a muscarinic antagonist (Brennan and Briggs, 1983). The present study has examined the effect of meptazinol on muscarinic receptor-coupled phosphoinositide breakdown.

Male Wistar rats (250-300g) were stunned and decapitated, the brains rapidly removed and dissected over ice. Cross-chopped slices of cerebral cortex (350μm) were cut with a McIlwain tissue chopper and preincubated for 1 hour at 37°C in Krebs Ringer bicarbonate buffer with 10mM glucose, equilibrated under $95\%O_2/5\%CO_2$ to a pH of 7.4. The slices were then incubated for a further 75 min in buffer containing 5mMLiCl and approximately 0.3μM[^{3}H] inositol. After the incorporation of [^{3}H] inositol, aliquots of the slices were incubated for a further 15 min with or without added antagonist drugs, and then for a final 45 min with or without added agonist drugs (Brown et al. 1984). The subsequent extraction and assay of total labelled water-soluble inositol phosphates was according to the method of Berridge et al. (1982).

Stimulation of phosphoinositide breakdown by carbachol was inhibited by atropine in a dose-dependent manner; Schild plots gave a mean pA_2 of 8.69 (SEM 0.04, n=4) with a slope not differing significantly from unity. Increasing concentrations of meptazinol also caused a progressive shift to the right in the carbachol dose-response curve, with a mean pA_2 of 4.44 (SEM 0.06, n=4) and a Schild plot slope not differing significantly from unity. The Ki values from these experiments (atropine 2.04×10^{-9}M, meptazinol 3.65×10^{-5}M) are similar to those previously obtained in ligand-binding studies: 1.48×10^{-10} and 1.56×10^{-5} respectively (Brennan and Briggs, 1983). Experiments with atropine and morphine alone showed that neither caused a significant increase in phosphoioposititide breakdown above basal levels. Concentrations of meptazinol above 10^{-4}M induced a modest phosphoinositide response, the maximal effect being one sixth of that evoked by carbachol.

Brown et al. (1984) have shown that acetylcholine alone is virtually inactive, whereas physostigmine alone produces a small stimulation, in the phosphoinositide breakdown system which we have used. Our results are consistent with meptazinol acting as a competitive inhibitor at muscarinic receptors. We would suggest that it may thus induce acetylcholine release by presynaptic mechanisms, and that the ability of meptazinol to inhibit acetylcholinesterase recently demonstrated by Ennis et al. (1986) would protect such endogenous acetylcholine. These complex actions would explain the potential for meptazinol to produce analgesia by both opioid and cholinergic effects.

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CHOLINE CARRIER ACTIVITY IN RAT CORPUS STRIATUM, HIPPOCAMPUS AND CEREBRAL CORTEX.

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Sodium-dependent choline uptake (SDCU) and choline acetyltransferase (ChAT) activities in rat corpus striatum are 3-5 times those in hippocampus and cerebral cortex, yet striatal cholinergic neurones are relatively unbranched and apparently in no greater concentration (1%) than in other areas of brain. Ethylcholine mustard aziridinium (ECMA) an irreversible inhibitor of SDCU is transported by HC-3 sensitive carriers into cortical synaptosomes, where it strongly inhibits ChAT. Phenoxybenzamine (PB) also an inhibitor of SDCU is not transported in this way and has greatly limited access to intrasynaptosomal ChAT (Pedder & Prince, 1984). We have therefore compared the effects of these aziridiniums in fine slices from rat cortex, hippocampus and striatum.

RESULTS Regardless of area sampled (0.1 x 0.1 x 1 mm slices) 3 μM ECMA or 100 μM PB (5 min reactions) caused inhibition of SDCU not reversed by washing (5% v/v x 3, 0°C). Inhibition by PB was identical in the three areas (38-43%). ECMA was more potent, and more so in the striatum (74% inhibition) than in the other two areas (57,63%). Throughout, Na-independent uptake was unaffected.

AZIRIDINIUM-INHIBITION OF Na-DEPENDENT CHOLINE UPTAKE*

		n	STRIATUM	HIPPOCAMPUS	CORTEX
ECMA,	% control,	10	26.4 (1.2)	36.9 (2.5)	42.8 (5.9)
	% inhibition,	6	73.6	63.1	57.2
PB,	% control	10	62.0 (2.5)	58.3 (8.4)	57.5 (7.2)
	% inhibition	6	38.0	41.7	42.5
Δ ECMA			35.6	21.4	14.7

(*3H-choline 1μM, 1μCi; 4 min, 10 mg tissue, 37°C; brackets SE of mean %)

DISCUSSION (1) There is no evidence that aziridinium-inhibition of striatal SDCU differs in characteristics from that in cortex and hippocampus, ie irreversible throughout. (2) Consistent with Curti & Marchbanks (1984) however, rates of inhibition by ECMA are greater where choline uptake is at faster rates, ie striatum > hippocampus ~ cortex. (3) The concentration of cholinergic nerve terminals in these three areas therefore could well be similar, with striatal choline carrier activity per nerve terminal greater than in the other areas. (4) However, inhibition of SDCU by PB, predominantly from the outer surface of synaptosomes, is identical in the three areas, ie not related to rates of choline uptake. (5) Inhibition by ECMA over and above (Δ ECMA) that by PB is in proportion to SDCU, ie striatum > hippocampus ~ cortex. (6) Thus, greater accumulation of ECMA in individual striatal synaptosomes leads to greater inhibition of the carriers from the inner face of the synaptosomes. This observation requires that choline uptake activity per striatal nerve terminal is greater than comparable values in the other areas.

Curti & Marchbanks (1984) J Membrane Biol, 82, 259
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DETECTION OF ACETYLCHOLINE RELEASED FROM GUINEA-PIG ILEUM, USING AN ADAPTED CASCADE SYSTEM.

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Despite the fact that biological assay methods are still extensively used to estimate acetylcholine (ACH) release from tissues, a number of experimental set-ups used have shown some drawbacks, concerning the sensitivity and specificity of the test (Paton, 1957; Hutchinson et al., 1976; Cowie et al., 1978).

We therefore adapted and modified the cascade bioassay system, described by Vane (1964), to measure the release of ACH from whole guinea-pig ileum. A segment of the latter (± 100 mg) was mounted in an isolated organ bath (5 ml), to which the cholinesterase inhibitor prostigmine (5×10^{-8} g/ml) was added. The assay tissues consisted of two guinea-pig ileum longitudinal muscle strips, continuously superfused with Krebs (5 ml/min). One of the strips was blocked for the action of ACH by hyoscine (1×10^{-6} g/ml). In order to prevent contractions of the assay tissues by prostigmine, morphine (1×10^{-5} g/ml) was added to the superfusion fluid. ACH was allowed to accumulate during resting conditions and during electrical coaxial stimulation (40 mA, 0.2 Hz, 1 msec). After 30 min, the bathing fluid was automatically pumped over the cascade assay organs and contraction of the second (hyoscine) strip was subtracted from the contraction given by the first strip. The netto contractions were compared with those of standard concentrations of ACH superfused over the assay organs. Release of ACH was also measured after pre-incubation of the guinea-pig ileum with indomethacin (1×10^{-6} g/ml) and morphine (1×10^{-6} g/ml) for 90 minutes. Results are shown in Table 1.

Table 1 : Release of ACH during 30 min from whole guinea-pig ileum expressed as ng ± SEM/g wet weight of tissue (number of experiments evaluated).

DRUGS		INDOMETHACIN 1×10^{-6} g/ml	MORPHINE 1×10^{-6} g/ml
RESTING CONDITIONS	353 ± 97 (12)	219 ± 37 (14)	159 ± 24 (16)
STIMULATION	839 ± 208 (12)	312 ± 52 (13)	348 ± 71 (14)

Electrical stimulation significantly increased the release of ACH ($P < 0.05$; Wilcoxon) which was largely prevented by addition of indomethacin and morphine ($P < 0.05$). The latter drugs did not affect the basal release of ACH in resting conditions.

It can be concluded from this series of experiments that our bioassay cascade system is a sensitive and fast method to measure ACH as a biologically active agent.

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PARASYMPATHETIC DENERVATION OF THE RAT PAROTID GLAND: AN INVESTIGATION OF MUSCARINIC RECEPTORS USING [^{3}H]-N-METHYLSCOPOLAMINE.

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The rat parotid gland provides an excellent model for the study of denervation supersensitivity in the parasympathetic system, since it is one of the few effector tissues in which complete postsynaptic parasympathectomy (Px) can be performed. Two to three weeks following the avulsion of this nerve, supersensitivity to cholinergic, α-adrenergic and peptidergic agonist stimulation develops, as judged by a decrease in the threshold dose of drug required to evoke secretion and an increase in the amount of saliva secreted in response to supraliminal doses of drugs (Ekstrom, 1980; Ekstrom & Wahlestedt, 1982). Several different cellular mechanisms have been proposed to be involved in the postsynaptic supersensitivity described above including changes in receptor number and/or affinity for agonists and alterations in the metabolism of second messengers. The present study has focussed on the first possibility by investigating the binding of [^{3}H]-N-methyl-scopolamine ([^{3}H]-NMS) to the rat parotid gland membranes.

Unilateral parasympathetic postganglionic denervation of the rat parotid gland was performed in pentobarbitone anaesthetised rats by means of the avulsion of the auriculo-temporal nerve, each animal providing a control and a denervated gland. Three weeks following surgery parotid glands were removed, weighed and the tissue homogenised in Hepes 20 mM buffer at pH 7.4. After a slow speed spin the supernatant was centrifuged at 40,000 g for 20 min. The pellet was lysed by resuspension in distilled water and respun at 40,000 for 25 min. The final pellet was resuspended in the original buffer. Assays were performed in triplicate at 30 °C for 25 min and bound radioactivity separated by centrifugation at 40,000 g for 5 min. The specific binding was determined in the presence of 10 uM atropine. The specific binding of [^{3}H]-NMS (0.3 nM) reached equilibrium within 10 min, represented 70-80% of the total binding and was found to be linear with membrane protein range 0.1 - 1.1 mg protein/assay. Binding was optimal at pH 7.4 and temperature of 30 °C. [^{3}H]-NMS binding was of high affinity and specificity with a Kd of 0.4 ± 0.05 nM and a Bmax of 138 ± 9.56 fmol/mg protein.

(Px) caused a decrease, compared with the contralateral control, of ∿ 40% in the Bmax of [^{3}H]-NMS expressed as fmol/gland, 3 weeks following surgery ($P < 0.05$, $n = 3$) (Px) also caused a significant 67% decrease in the mean IC_{50} value obtained for carbachol displacement of specific [^{3}H]-NMS binding (control, 276 ± 41 μM and Px, 91 ± 16 μM respectively, $P < 0.05$, $n = 3$) suggesting an increase in the affinity for carbachol for the receptor. An explanation for this observation is that Px causes an increase in the affinity of the muscarinic receptor but how this is coupled to supersensitivity of the tissue requires further investigation, especially the possibility of an altered receptor-signal transduction mechanism between the receptor and phospholipid turnover/Ca^{++} mobilisation.

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THE INFLUENCE OF HAEMOPHILUS INFLUENZAE VACCINATION ON RAT CHOLINERGIC MUSCARINIC PULMONARY RECEPTOR CHARACTERISTICS.

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Previously it has been demonstrated that Haemophilus influenzae (HI) vaccination of experimental animals induced changes compatible with a dysregulation in the cholinergic system, such as enhanced plasma cholinesterase activity and lung cGMP levels (Terpstra et al., 1979) and an enhanced contractibility of guinea pig tracheal spirals to carbachol (Schreurs et al., 1980). Linking the more physiological studies to receptor characteristics, the effects of HI were assessed on rat pulmonary cholinergic muscarinic receptors characterized by radioligand binding studies and in vitro studies on isolated tracheal spirals.

Using ^{3}H-quinuclidinyl benzilate in a radioligand binding assay, no differences in binding characteristics were found (B_{max}: 139.3 $\pm$ 30.1 vs. 195.8 $\pm$ 127.5 fmol/mg protein, controls n=6 and vaccinated rats n=7 resp.; K_d: 111.8 $\pm$ 20.1 vs. 94.0 $\pm$ 10.3 pmol/l resp.); bethanechol inhibition characteristics in vaccinated and control animals did not differ and also no Gpp(NH)p influence on agonist inhibition could be observed.
On the other hand muscarinic receptor function in tracheal spirals appeared to be changed: an increased sensitivity to carbachol was found.

Therefore the effects of HI vaccination do not seem to be the result of an impairment of the muscarinic cholinergic receptors themselves, but due to changes in processing the signal evoked by receptorbinding.

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