SYSTEMIC AND SPECIFIC REGIONAL HAEMODYNAMIC DIFFERENCES BETWEEN A NORMOTENSIVE AND HYPERTENSIVE STRAIN OF RAT.

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Systemic haemodynamic variations and changes in regional distribution of total cardiac output (CO) between strains of age matched CD derived and spontaneously hypertensive rats (SHR, Okamoto strain) were assessed. Of particular interest was the elucidation of any differential vascular responsivity which may exist between oestrogen-dependent organs.

Blood flow (BF) was quantitated in anaesthetised animals bilaterally ovariectomised 21 days prior to experimentation and in cycling sexually mature virgin female rats by the microsphere technique (15 μm NEN-TRAC) (Phaily and Senior 1978). BF was expressed using tissue wet weight as ml min^{-1} $100g^{-1}$. Significant differences were observed in: mean blood pressure (MBP) (SHR 205±10: CD 120±9 mmHg P<0.001; total body weight (cycling SHR 229±2: CD 291±3g P<0.001; ovariectomised SHR 249±1: CD 353±9g P<0.001) and in CO (SHR 90±4: CD 112±3 ml min^{-1} P<0.001). No significant difference in heart rate was noted. In both strains uterine BF varied during the oestrous cycle, being highest in pro-oestrous (P), lower in dioestrous (D) and lowest in the oestrous (O) phase. Only during P was flow significantly elevated in the CD strain (SHR P 66±12, D 29±6, O 10±3, CD, P 155±33***, D 32±2, O 23±5: P<0.001).

No cyclic alteration in uterine wet or dry weight was noted in either strain. The uterine vasculature of the ovariectomised CD strain appeared more responsive to oestrogen treatment than the SHR. 17β oestradiol (E_2, 0.5 μg/kg^{-1}) 3h induced hyperaemia was significantly higher in the CD (SHR 277±50: CD 536±102 P<0.001). However, unlike the hyperaemic response, the E_2-induced uterotrophic effect was similar in both strains. Like uterine BF ovarian flow followed a cyclic pattern; (SHR, P 534±61, D 339±65, O 174±57, CD, P 419±45, D 367±46, O 229±38 P<0.01), the peak in P was significantly higher in the SHR. Specific blood flow to several other organs was measured: kidneys, liver, stomach, adrenals, duodenum and spleen. Only in the spleen was a consistent alteration in flow observed between the two strains (SHR 102±9: CD 239±23 P<0.001). In 60% of the SHRs (N=75) there was no detectable difference in the proportion of microspheres lodged in the lungs of either strain (BF, SHR 43±9: CD 52±6). However, in 40% of SHRs (N=50) an increase in the proportion of lung entrappment was observed (BF, SHR 2080±232: CD 43±9 P<0.001), none of these animals are included in this study. 5% of the SHR colony were normotensive (NT) (MBP 125±10 mmHg). These NT littermates are considered the most appropriate control for the SHR strain. No differences were observed in the responses of ovariectomised NT or hypertensive SHRs to hormonal challenge by E_2.

Elevated peripheral resistance is deemed a usual haemodynamic occurence in most forms of essential hypertension. However, the SHR showed reduction in regional BF to the uterus and spleen only. Additionally, hypertension exhibited by the SHR is thought to be associated with a relatively normal CO, in this case CO was low. The increased pulmonary flow observed in some SHR may reflect an augmentation of cardiopulmonary shunting via arteriovenous anastomoses known to exist in rat lungs (Daly, 1958). This and other compensatory mechanisms for maintaining adequate regional perfusion may have been developed in the well established SHR colony used.

Phaily. S. & Senior, J. (1978) J. Reprod. Fert. 53, 91-97
Daly, I.B. (1958) O.J. Exp. Physiol. 43, 2-26

THE ADRENOCEPTORS OF THE YOUNG CHICK HEART.

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It has recently been reported that the noradrenergic receptors of the young chick heart differ from those of the mammalian heart (Tayo, 1984). It has been claimed that the inotropic responses to noradrenaline in this species are mediated via α-adrenoceptors while the chronotropic responses are mediated via a noradrenergic receptor that is blocked by neither propranolol nor phentolamine. This study was undertaken to examine further these atypical noradrenergic responses.

Right atria, left atria and right ventricular strips from young chicks (14-21 days old) were set up in a Krebs-bicarbonate solution gassed with 5% CO_2 in oxygen and maintained at 32C. Spontaneous rate of contraction of right atria and isometric developed tension of left atria and ventricular strips paced at 2Hz (threshold voltage ; 50%, 5 msec pulse-width) were recorded. Cumulative concentration-response curves to adrenergic agonists were constructed in the presence of desipramine (1 μM) and metanephrine (10 μM) to inhibit neuronal and extraneuronal uptake respectively. One concentration-response curve only was obtained from each tissue.

Isoprenaline produced positive chronotropic and inotropic responses, right atrial rate increasing by 45.8 ± 9% [EC_{50} = 34.0 (10 - 484)nM] left atrial developed tension increasing by 256.2 ± 70% [EC_{50} = 274 (150 - 501)nM] and ventricular developed tension by 160.6 ± 40.7% [EC_{50} = 158 (40 - 626)nM]. In contrast, methoxamine produced only negative chronotropic responses in right atria and negative inotropic responses in ventricular tissues. Methoxamine did however produce positive inotropic responses in left atria, the developed tension increasing by 22.7 ± 5.2% in this tissue [EC_{50} = 0.51 (0.3 - 5.8)μM].

Cumulative concentration-response curves to noradrenaline were also obtained for the three cardiac tissues and the susceptability of these responses to blockade by phentolamine, prazosin and propranolol examined (Table 1).

Table 1: Effect of adrenergic antagonists on noradrenaline EC_{50} values (nM)

Antagonist	Right Atria	Left Atria	Ventricular Strip
none	39.6(11.2-141.4)	105(38.6-284)	123.3(29.0-521.6)
phentolamine(5μM)	18.8(4.0-37.0)	61.5(32.0-112.1)	207.3(100.6-433.8)
prazosin(10nM)	40.9(10.0-138.0)	168.4(47.2-310)	161.8(39.4-668.3)
propranolol(1μM)	2200(800-6200)**	6890(3380-14000)*	17000(6900-41500)***

*P<0.01 **P<0.005 ***P<0.001

Neither phentolamine (5μM) nor prazosin (10nm) had any significant effect on noradrenaline concentration-response curves in any of the tissues. The presence of propranolol (1μM) however, resulted in a rightward shift of noradrenaline concentration-response curves with a significant increase in the EC_{50} values in all three tissues (Table 1).

These results do not agree with those obtained by Tayo (1984), and indicate that both the chronotropic and inotropic responses to noradrenaline are mediated by β-adrenoceptors in the chick heart.

R.C.W. is a Bristol-Myers lecturer.

Tayo, F.M. (1984) Br. J. Pharmac. 81: 289-291

THE ADRENAL MEDULLA OBSCURES SYMPATHETIC INHIBITION BY SAR[1]-LEU[8]-AII AND THE ACE INHIBITOR CILAZAPRIL IN THE PITHED SH RAT.

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Since the first report of impairment of sympathetic responses by captopril in the pithed rat (Antonaccio & Kerwin, 1980), other angiotensin I-converting enzyme inhibitors (ACEI) have been examined for a similar effect. Cilazapril, 1mg.kg^{-1} i.v. (a potent non-thiol ACEI with antihypertensive activity; Natoff et al, 1985), did not inhibit the vasopressor responses to electrical stimulation of the sympathetic outflow (supramaximal voltage, 1-8 Hz, 0.5 ms pulse width, 15 s duration), or to post-junctional α-adrenoceptor stimulation with methoxamine (50 and 100μg.kg^{-1} i.v.; mainly α_1), and noradrenaline (0.25 and 0.5μg.kg^{-1} i.v.; mixed α_1 and α_2) in the pithed spontaneously hypertensive (SH) rat. Inhibition ($p<0.01$, n=5) of the responses to B-HT 920 (20 and 40μg.kg^{-1} i.v.; mainly α_2) was seen however. Infusion of Sar[1]-Leu[8] angiotensin II (Sar[1]-Leu[8]-AII) at 20μg.kg^{-1}.min^{-1} i.v., a dose which abolished the pressor effect to AII (1μg.kg^{-1} i.v.), also did not impair these sympathetic responses. When only the lumbar sympathetic outflow was stimulated, avoiding T5-T9 outflow to the adrenal medullae, cilazapril (1mg.kg^{-1} i.v.) inhibited the pressor responses ($p<0.05$, n=5). Similarly, following acute adrenal demedullation of the preparation, both cilazapril ($p<0.01$, n=5) and Sar[1]-Leu[8]-AII ($p<0.05$, n=6) consistently reduced the responses to electrical stimulation of the sympathetic outflow. Cilazapril further reduced the responses to sympathetic outflow stimulation previously inhibited by Sar[1]-Leu[8]-AII. While cilazapril also reduced the responses to the α-adrenoceptor agonists ($p<0.05$, n=5), Sar[1]-Leu[8]-AII did not influence their responses.

These findings demonstrate that exclusion of the adrenal medulla is necessary to reveal the inhibitory effect of Sar[1]-Leu[8]-AII and cilazapril on sympathomimetically-mediated actions in the SHR. Cilazapril may act post-junctionally as it inhibits both stimulus responses and those to post-junctional α-adrenoceptor agonists, while Sar[1]-Leu[8]-AII, which has no effect on the responses to post-junctional α-agonists, acts prejunctionally only.

Antonaccio, M.J. & Kerwin, L. (1980) Europ.J.Pharmac. 68, 202-209.
Natoff, I.L., Nixon, J.S., Francis, R.J., Klevans, L.R., Brewster, M., Budd, J., Patel, A.T., Wenger, J. & Worth, E. (1985) J.Cardiovasc.Pharmac. 7, 569-580.

INVESTIGATION OF THE RECEPTORS MEDIATING IN VIVO VASODILATOR RESPONSES TO A.T.P. AND ADENOSINE.

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ATP and adenosine can both evoke vasodilation in vivo. It is not clear, however, whether ATP acts via an "ATP-receptor" or at an adenosine receptor after enzymic degradation to the nucleoside. We have investigated this question by examining the effects of an adenosine receptor antagonist (8-phenyltheophylline, 8PT) and of two putative ATP receptor antagonists (α-β-methylene ATP and antazoline) on vasodilator responses evoked by ATP and adenosine in the dog hind-limb.

Beagle dogs (12-18 kg) were anaesthetised with sodium pentobarbitone (30-40 mg/kg, i.v.) and artificially ventilated. Blood gases and pH were monitored and maintained within normal limits. One hind-limb was surgically denervated and perfused at constant flow with the animals blood via the femoral artery. Hind-limb perfusion pressure was measured and matched to systemic pressure by adjustment of flow. Adenosine, ATP and isoprenaline were administered in saline by close intra-arterial bolus injections.

Isoprenaline (1-300 ng), ATP (0.1-30 μg) and adenosine (3-100 μg) evoked dose-related decreases in hind-limb perfusion pressure. The maximum vasodilator response evoked by ATP and by adenosine were similar in magnitude. ATP was significantly ($p<0.02$) more potent than adenosine (log ED_{50} = 0.34±0.11 μg and 1.13±0.14 μg, n=6 respectively). Dose-response curves to ATP, adenosine and isoprenaline were generated during i.v. infusion of 8PT (69 μg/kg/min) or its vehicle (polyethylene glycol, 0.1M NaOH, 50:50 vol/vol at 0.013-0.015 ml/kg/min) for 1.5h. 8PT infusion caused a significant increase in hind limb perfusion pressure (from 128±5 to 163±4 mmHg, $p>0.01$). 8PT displaced the adenosine dose-response curve to the right in a parallel manner (log dose ratio = 1.93±0.21, n=6) whereas responses to ATP and isoprenaline were not significantly altered (log dose-ratio = -0.02±0.22 and 0.18±0.09 respectively, n=6). α-β methylene ATP (5 mg/kg i.a.) evoked a large transient decrease in hind-limb perfusion pressure, blood pressure and heart rate. Responses to both ATP and to adenosine were slightly decreased after the response to α-β methylene ATP had subsided (n=3). Antazoline (5 mg/kg i.v.) had no significant effect on hind-limb perfusion pressure and caused a small variable decrease in responses evoked by both ATP and adenosine (n=4).

The selective antagonist effect of 8PT against adenosine but not ATP responses, together with the higher potency of the nucleotide indicates that ATP does not act via an adenosine receptor in this preparation. Doses of antazoline and of α-β methylene ATP that are known to block in vivo responses to ATP (Shimada and Stitt, 1984; Duval et al, 1985) did not selectively inhibit ATP responses in the dog hind-limb. This indicates that either a different type of ATP receptor is present in this preparation or that antazoline and α-β methylene ATP are not receptor antagonists.

Shimada, S.G. and Stitt, J.T. (1984) Br.J.Pharmac. 83, 577-589.
Duval, N., Hicks, P.E. and Langer, S.Z. (1984) Eur.J.Pharmac. 110, 373-377.

URINARY ENZYME EXCRETION AND THE DETECTION OF DRUG-INDUCED RENAL DAMAGE IN THE RAT.

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Urinary enzyme excretion has been used as a non-invasive, early indicator of drug-induced renal damage. The present study demonstrates a) the transient nature of abnormal urinary enzyme excretion, even with the progression of morphological damage, and b) the difficulty in assessing the source of enzymuria (i.e. extra- or intra-renal).

Male Wistar rats (220±28g) received a single s.c. injection of normal saline (NS), potassium dichromate (Pd) or mercuric chloride (Hg) (doses in table). From 54 hrs post-dose, the animals were placed in metabolism cages (1 per cage) to collect an 18h (overnight) urine sample. To minimize faecal and bacterial contamination the animals were fitted with anal cups (Ryer et al.1971), and urine collection vessels were kept at 1-2°C. The urine was dialysed for 3h prior to enzyme analysis. Neither Pd nor Hg were found to have any effect on the enzyme kinetics of the assays used. Protein determination was performed using the TCA/Ponceau S method (Pesce et al. 1973) which is not affected by interfering substances such as glucose and urea. No significant differences in urinary flow rates were observed between any of the treatments. Histopathological examination was performed on the tissue at 72h post dose.

Table 1: Urinary enzyme excretion and the detection of drug-induced renal damage in the rat

Treatments	Tot.Protein (mg/24)	GT	AP	LAP (units/24 hrs)	LDH	AST	NAG (units/hr)
NS 1ml/kg	5.11 ±1.51	0.44 ±0.05	1.52 ±0.19	0.11 ±0.02	0.22 ±0.08	0.04 ±0.02	143 ±23
Pd 20mg/kg	39.14** ±3.13	0.53 ±0.09	2.63 ±0.26	0.11 ±0.03	7.93** ±0.14	2.50** ±0.41	1361** ±69
Hg 2mg/kg	16.04** ±2.49	1.11 ±0.22	1.63 ±0.58	-	1.42** ±0.47	0.34** ±0.10	565** ±98

Results Arithmetic mean ± SEM *P<0.05; **P<0.01 Dunnets test
NAG (N-acetyl-β-D-Glucosaminidase) units = nmols 4-methylumbelliferone released/60 min incubation

For Pd and Hg treatments, the excretion of the brush border enzymes γglutamyl transferase (γGt), alkaline phosphatase (AP) and leucine aminopeptidase (LAP) were not significantly different from control values despite morphological and functional evidence of progressive proximal tubule necrosis with loss of brush border membranes. Pd treated animals had significantly (P<0.01) elevated plasma bilirubin LDH (Lactate dehydrogenase) and AST (Aspartate aminotransferase) levels. Elevated urinary excretion of LDH and AST may result from hepatic damage with increased glomerular permeability or from renal tissue with the elevation in plasma LDH and AST secondary to renal damage (Raab 1972). The fall off in enzyme activity with time despite persistent histochemical and histological evidence of continued cellular damage has been previously reported (Cottrell et al. 1976). Indeed, Stroo et al (1977) observed a return in maltase and AP to control levels within 4h of a single Pd injection. Nevertheless, enzyme excretion is particularly useful as a toxicological screening or early diagnostic technique but it requires a broad selection of enzyme measurements and the absence of elevated enzymuria requires further functional tests to be performed before renal involvement can be dismissed. The use of tissue specific isoenzymes will increase the value of the technique.

Ryer, F.H., Walker, D.W. (1971) Lab.An.Sci.21, 6, 942-943
Pesce, M.A., Strande, C.S. (1973) Clin.Chem. 19, 11, 1265-67
Raab, W.P. (1972) Clin.Chem. 18, 1, 5-25
Stroo, W.E., Hook, J.B. (1977) Tox.App.Pharm. 39, 423-34
Cottrell, R.C. et al. (1976) Fd.Cosmet.toxicol. 14, 593-98

THE EFFECT OF MIGLYOL 812 OIL ON THE GASTROINTESTINAL ABSORPTION OF PROPRANOLOL

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Whilst it has been shown that lipids can modify the oral bioavailability of highly lipophilic drugs, relatively little is known about their effect on more hydrophilic compounds. In the present study the gastrointestinal absorption of propranolol was compared following oral administration to rats in aqueous systems and a Miglyol 812 (fractionated coconut oil) oil-in-water (o/w) emulsion.

Solutions of propranolol spiked with [^{3}H]-propranolol were prepared either as a Miglyol 812 (50%) o/w emulsion stabilised with 6% Tween 80, in water containing 6% Tween 80, or in water alone. Male Wistar rats (180-220 g) were fasted overnight with water ad libitum, and then orally dosed with 0.5 ml of each formulation (10 $mgkg^{-1}$, 100 $\mu Cikg^{-1}$). For intravenous (i.v.) administration, 0.1 ml of propranolol solution in isotonic phosphate buffered saline, pH 7.4, was administered as a bolus injection via a cannula previously inserted in the jugular vein (1-2 $mgkg^{-1}$, 50 $\mu Cikg^{-1}$). Blood samples were taken from the tail vein at various times after dosing, 0.1 M sodium hydroxide added, and then propranolol quantified by liquid scintillation counting after extraction into toluene.

An i.v. dose ranging study showed that the pharmacokinetics of propranolol were linear between 1-2 $mgkg^{-1}$ and described by a two-compartment open model with a systemic clearance and terminal half-life of 0.076 ± 0.005 $lmin^{-1}kg^{-1}$ and 47.1 ± 5.41 min (mean ± s.e.mean, n=10) respectively. Propranolol peak-blood concentrations were reached within 6 min. after the oral administration of each formulation. The post-peak half-life of propranolol in the Tween 80 and aqueous systems was significantly longer (t-test, $p<0.05$) than the half-life following i.v. administration, however no difference was found for the emulsion system. The fraction available of propranolol in the Tween 80 formulation was significantly higher compared to the aqueous and emulsion systems, but no difference was observed between the aqueous and Tween 80 formulations (Table). A significant difference was found between the post-peak half-life after oral delivery of the emulsion system compared to both the aqueous and Tween 80 formulation but no difference was found between the aqueous and Tween 80 systems (Table).

Table 1 Bioavailability of Propranolol after Oral Administration to Rats at a Dose of 10$mgkg^{-1}$ (mean ± s.e.mean).

Parameter	Formulation		
	Aqueous (n=5)	Tween 80 (n=6)	Emulsion (n=4)
F (%)	15.9± 1.79	24.1± 3.2	12.9±3.9
$t_{0.5}$ (min)	88.9± 13.6	83.3± 7.6	54.4±1.8

$t_{0.5}$= post-peak half-life, F= fraction available. Results compared using a Student-Newmans-Keuls test at a 5% significance level

It may be concluded that the higher F of the Tween 80 compared to the aqueous control was probably due to the presence of surfactant. The significantly shorter post-peak half-life and reduced F for the emulsion formulation compared to the Tween 80 system may be due to the oil affecting parameters such as gastric empting, the absorption window of propranolol, the first-pass effect or a combination of these phenomena.

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EFFECT OF PLASMA PROTEIN BINDING ON UPTAKE OF SOME STEROIDS BY ISOLATED ADIPOCYTES.

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The influence of plasma protein binding (PPB) of canrenone, SC-26304 (a spironolactone analogue), androstenedione (A), oestrone (E1), oestradiol (E2), progesterone (P), cortisol (C) and testosterone (T) on their uptake by isolated adipocytes was investigated. The possible effects (lipogenesis or lipolysis) of canrenone, SC-26304 and other natural steroids was also investigated using the adipocyte preparation.

Fat cells from epididymal adipose tissue of 2 male rats were prepared by a modification of the method of Rodbell (1964) and made up to a final suspension volume of 15 ml using Krebs Ringer buffer. To aliquots (0.6 ml) of buffer or 50% plasma containing radiolabelled steroid (20,000 - 30,000 dpm/ml) was added fat cell suspension (0.6 ml). The cells were incubated for 30 min, the fat cell layer aspirated off and aliquots (0.2 ml) of the plasma or buffer solution counted for radioactivity. The results are shown in Table 1. Glycerol released from fat cells (as an index of lipolysis) was measured by a modification of the method of Eggstein et al (1974) and lipogenesis was measured by the method of Rodbell (1964).

Table 1 %Uptake of various steroids by rat adipocytes from Krebs Ringer buffer or 50%-human plasma.

Steroid	% Uptake from buffer	% Uptake from plasma
Androstenedione	23.58	20.81
Canrenone	26.47	19.04
Cortisol	14.50	14.21
Oestrone	43.25	29.05
Oestradiol	25.89	16.10
Progesterone	45.03	20.89
SC-26304	31.50	11.94
Testosterone	36.29	31.87

Canrenone, SC-26304, P, E1 and E2 in the range 3 - 50 μg/ml inhibited the action of insulin on glucose uptake (lipogenesis) by isolated adipocytes. SC-26304 and canrenone (0 - 50 μg/ml appeared not to stimulate lipolysis in adipocytes.

The results showed that the less polar the steroid, the greater was their uptake by adipocytes. Therefore, tissue uptake is influenced not only by their lipophilicity but by their relative binding to sites in both the tissue itself and the proteins which would normally be present in the circulating fluids perfusing it.

Rodbell, M. (1964) J. Biol. Chem. 239, 375 - 380.
Eggstein, M., Kuhlmann, E., (1974) Methods in Enzymatic Analysis 4, 1825 - 1831.

THE METABOLISM OF SORBINIL IN THE RAT.

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Sorbinil ((+)6-fluoro-spiro(chroman-4,4'-imidazolidine)-2', 5' dione) is an aldose reductase inhibitor possessing useful activity against diabetic neuropathy and cataracts (Canal and Comi, 1985). We have studied its metabolism in the rat with particular reference to urinary metabolites and induction of sorbinil 2-hydroxylation by chronic administration.

[8-^{3}H]Sorbinil (150mg/kg; 12μCi) in polyethylene glycol 200 (0.5 ml) was administered ip to two groups of four male Wistar rats (200g body wt). Urine was collected every 24h for 72h. From 48h inclusive, one group received unlabelled sorbinil (150mg/kg in PEG) ip every 24h for five days whilst the other received vehicle. A second dose of [8-^{3}H]sorbinil (150mg/kg) was given ip seven days after the first. Urine was collected for 48h, the rats were killed and their major organs removed for determination of residual radioactivity. The urinary and tissue radioactivity was measured by liquid scintillation counting and the 0-24h urinary metabolites assayed by radiometric hplc (5-μm C_8 column; urine containing 36-90 x 10^3dpm eluted with a gradient of acetonitrile in 10mM KH_2PO_4, pH 6.0; ^{3}H recovery 99 ± 4%, $\bar{x}$ ± SD, n = 16). Unchanged sorbinil and its major metabolite, 2-hydroxysorbinil (Dr. R. Ronfeld, personal communication), were identified by co-chromatography with authentic standards initially and then by mass spectrometry ($M^{+\cdot}$ at m/z 236 and m/z 252, respectively).

The sorbinil-treated rats excreted 63.9 ± 1.2% ($\bar{x}$ ± SD) and 69.8 ± 7.1% of the 1st and 2nd doses of [8-^{3}H]sorbinil, respectively, over 24h. The corresponding data for the PEG-treated rats were 67.2 ± 3.7% and 69.9 ± 3.2%. Only 7 - 10% of the dose was excreted in urine during the subsequent 48h. Forty eight hours after administration of the 2nd dose, the lungs, heart, kidneys, spleen and brain each contained less than 0.1% of the dose whilst the liver contained 0.19 - 0.37%. Chronic dosing with sorbinil increased the urinary excretion of 2-hydroxysorbinil and decreased that of unchanged drug (Table).

Table. Urinary excretion (0-24h) of sorbinil and 2-hydroxysorbinil

Rats	Sorbinil	2-Hydroxysorbinil
Before Sorbinil	22.1 ± 1.1	17.0 ± 0.7
After Sorbinil	18.2 ± 2.2**	24.7 ± 3.4*
Before PEG	24.5 ± 3.8	15.0 ± 1.0
After PEG	23.1 ± 3.5	16.3 ± 2.0

Data are mean % ± SD of dose. * $p < 0.05$, ** $p < 0.01$, by Student's paired t-Test. CV of sorbinil and 2-hydroxysorbinil in one collection were 0.9% and 2.4% (n=6), respectively.

In conclusion, sorbinil underwent considerable 2-hydroxylation in vivo and induced its own hydroxylation.

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Canal, N. and Comi, G. (1985) TIPS, August, 328.

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PHARMACOLOGICAL EFFECTS OF HAEM BIOSYNTHETIC INTERMEDIATES IN ISOLATED INTESTINAL PREPARATIONS.

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Delta-aminolaevulinic acid (ALA) has been found to inhibit contractile activity in isolated rabbit jejunal preparations (Cutler et al, 1985), an effect which might be relevant to the gastrointestinal features of acute hepatic porphyrias and lead poisoning in which blood concentrations of ALA rise many fold over normal values (Laiwah et al, 1983). The studies reported here examine the possibility that other haem biosynthetic intermediates may act similarly. Indications of such pharmacological activity have been reported in the earlier literature (Vanotti et al, 1954).

In these experiments, isolated intestinal preparations were bathed in oxygenated Ringer-Locke solution at 37°C and contractions of the preparation were recorded by isotonic transducer and displayed on a calibrated Washington 400 MD2R oscillograph. Effects of ALA (0.6 - 6.0 mM), porphobilinogen (0.4 - 1.1 mM), coproporphyrin I (0.6 - 8.9 mM), protoporphyrin IX (0.2 - 2.3 mM), haemin (0.3 - 3.0 mM) and the precursor amino acids, glycine and succinate (3.0 - 12.0 mM) were examined in isolated rabbit jejunal preparations. As a negative control, effects of similar concentrations of a non-precursor amino acid, leucine, were investigated. Effects of ALA (0.6 - 6.0 mM) were also examined in isolated human taenia coli preparation.

Contractile activity in the rabbit jejunum was inhibited by all compounds tested except leucine. Concentration-dependent decreases in amplitude of contractions followed administration of porphobilinogen, protoporphyrin, haemin and glycine, while tone of the preparation was decreased by ALA, protoporphyrin, coproporphyrin, haemin and succinate. The minimum concentrations giving these effects ranged from 1.5 mM for ALA to 0.4 mM for porpobilinogen, 4.5 mM for coproporphyrin, 0.6 mM for protoporphyrin, 0.8 mM for haemin and 6.0 mM for glycine and for succinate.

Inhibitory effects of ALA were followed by an increase in the amplitude of contractions. This has been reported previously (Cutler et al, 1982) having been found to be inhibited by pretreatment of preparations with indomethacin to block prostaglandin synthesis. In human taenia coli a similar type of effect was observed in the present experiments. There was an initial decrease of tone followed by increase of tone above the levels prior to ALA administration. Sensitivity of human taenia coli to ALA resembled that of rabbit jejunum. The relevance of these findings to acute porphyria warrants further study.

Cutler, M.G. et al (1982) Br. J. Pharmac. 76, 217p
Cutler, M.G. et al (1985) Neuropharmacology 24, 1005-1009
Laiwah, A.C.Y. et al (1983) J. R. Soc. Med. 76, 386-392
Vanotti, A (1954) Porphyrins; their Biological and Chemical Importance, pp146-149, translated by C. Rimington, Hilger and Watts Ltd., London

USEFULNESS OF PHENYLEPHRINE AND SODIUM NITROPRUSSIDE IN BAROREFLEX ASSESSMENT.

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In both human and animal experiments, baroreflex function is usually assessed in terms of the reflex bradycardia to a short acting pressor agent such as phenylephrine (see Gribbin et al., 1971) or in terms of the reflex tachycardia to a short acting depressor agent such as sodium nitroprusside (Docherty et al., 1986). In this study, we have examined whether these two agents give a useful indication of baroreflex function in rats by examining their direct cardiac actions.

Male Wistar rats were anaesthetised with pentobarbitone sodium, or pithed under ether anaesthesia. The carotid artery and jugular vein were cannulated for blood pressure recording and drug injection, respectively.

In pentobarbitone anaesthetised Wistar rats, the baroreflex bradycardia in response to phenylephrine (3-30 μg kg-1) was converted to a tachycardia by atropine (0.6 mg kg-1) or by propranolol (1 mg kg-1). In pithed rats, phenylephrine produced a marked tachycardia over the dose range 1-100 ug kg-1, and this tachycardia was mediated predominantly by beta-adrenoceptors since propranolol (1 mg kg-1) markedly antagonised the response. A component of the tachycardia was mediated by alpha-1 adrenoceptors since the tachycardia to phenylephrine in the presence of propranolol was antagonised by prazosin (1 mg kg-1).

In pentobarbitone anaesthetised rats, the baroreflex tachycardia in response to sodium nitroprusside (1-30 μg kg-1) was markedly attenuated by propranolol (1 mg kg-1) but not by atropine (0.6 mg kg-1). In pithed rats, nitroprusside (1-30 μg kg-1) had no effect on heart rate, but 100 ug kg-1 produced a small tachycardia (7.2 $\pm$ 1.6 min-1) and 1 mg kg-1 produced a marked tachycardia (58.2 $\pm$ 8.2 min-1, n=6).

In conclusion, phenylephrine is an unsuitable agent for assessment of baroreflex function due to its direct cardiac stimulant actions, but sodium nitroprusside in low doses has no direct cardiac actions making it a useful agent in assessing baroreflex function.

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Docherty, J.R. et al. (1986). J. Cardiovasc. Pharmacol. 8, 376-380.
Gribbin, B. et al. (1971). Circ. Res. 29, 424-431.

NEOMYCIN STIMULATES SECRETION AND AGGREGATION IN SAPONIN-PERMEABILISED HUMAN PLATELETS.

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The aminoglycoside antibiotic, neomycin, is known to bind avidly to polyphosphoinositides, and has therefore been widely used as a relatively selective inhibitor of inositol phospholipid metabolism in a number of intact and semi-permeabilised tissues (Gomperts and Cockcroft, 1985; Carney et al, 1985). The present study investigates whether neomycin selectively inhibits inositol phopholipid metabolism in washed human platelets, and can therefore be used to further define the role of this membrane-transducing pathway in platelet activation.

On intact platelets, relatively high concentrations of neomycin (2 - 10 mM), and also a second aminoglycoside, streptomycin (2 - 10 mM), inhibited platelet activation by both thrombin and by the Ca2+ ionophore, A23187, in the presence of indomethacin. Under these conditions platelet activation by thrombin, but not by A23187, is associated with inositol phospholipid hydrolysis (Rittenhouse, 1984). Thus, the inhibitory action of neomycin appeared non-specific, and not related to a selective action on inositol phospholipid metabolism. This result is perhaps not surprising since neomycin does not readily cross cell membranes, and the polyphosphoinositides predominate on the inner leaflet of the membrane.

The action of neomycin was therefore investigated on platelets rendered semi-permeable with saponin in order to allow neomycin access to the cytoplasmic side of the plasma membrane. On permeabilised platelets, however, neomycin (0.06 - 2 mM), and also streptomycin (0.06 - 2 mM), stimulated secretion and aggregation responses, and also the formation of phosphatidic acid. Phosphatidic acid is a metabolite of 1,2-deacylglycerol, and is widely used to reflect the activation of phospholipase C (Lapetina & Cuatrecases, 1979). These responses were fully inhibited by the cyclooxygenase inhibitor indomethacin or by EGTA. It is suggested that on semi-permeabilised platelets the two aminoglycosides stimulate Ca2+ release from intracellular organelles and that this leads to the activation of phospholipase A2. The endoperoxides and thromboxanes that are subsequently formed then induce platelet activation through cell surface receptors leading to the activation of phospolipase C. This action would appear to mask any potential inhibitory effect on inositol phospholipid metabolism by neomycin.

In conclusion, therefore, it would appear that neomycin cannot be used as a selective inhibitor of inositol phospholipid metabolism in either intact or semi-permeabilised human platelets.

We thank Burroughs Wellcome, North Carolina, for financial support for these studies.

Carney, D. H. et al (1985) Cell, 42, 479-488.
Cockcroft, S. & Gomperts, B. D. (1985) Nature, 314, 534-536.
Lapetina, E. G. & Cuatrecasas, P. (1979) Biochem. Biophys. Acta. 573, 394-402.
Rittenhouse, S. E. (1984) Biochem. J. 222, 103-110.

SYNERGISTIC ACTIVATION OF HUMAN PLATELETS IN WHOLE BLOOD.

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Blood platelets contain receptors for, and can be activated by, a number of agents which are normally present in the circulation or which can be synthesised or released by platelets themselves. When added individually to platelets in vitro the concentrations of agonists such as adrenaline, ADP, vasopressin (VP) or platelet-activating factor (PAF) required for stimulation of platelet aggregation markedly exceed those concentrations normally present in blood. Exposure of platelets, in vitro, to low concentrations of two or more agonists can result in the synergistic stimulation of aggregation (i.e. produce a response which is significantly greater than the sum of the individual responses). Such synergistic effects have been observed when platelet aggregation is monitored turbidometrically (Grant and Scrutton, 1980; Culliver & Ardlie, 1981; Vargaftig et al., 1983) but not, it has been suggested, when platelet aggregation is monitored as disappearance of single platelets (Kerry and Scrutton, 1985).

We have examined the effects of low concentrations of adrenaline on loss of single platelets (i.e. platelet aggregation) in human whole blood induced by ADP, PAF and VP. Disappearance of single platelets was measured using an Ultra-Flo 100 whole blood platelet counter (Lumley and Humphrey, 1981). Using this method, ADP (10^{-7} - 10^{-5}M), PAF (10^{-9} - 10^{-7}M) and VP (3×10^{-9} - 3×10^{-7}M) caused concentration-dependent platelet aggregation which reached a peak within one minute of agonist addition and produced a maximum 95% loss of single platelets. In contrast, adrenaline-induced aggregation (3×10^{-8} - 10^{-5}M) peaked at around 5 - 10 minutes after agonist addition and produced a maximum 60 - 80% loss of single platelets. Addition of submaximal concentrations of adrenaline (5 - 15% loss of single platelets) simultaneously with or up to 5 minutes before equi-effective concentrations of ADP, PAF or VP resulted in a synergistic stimulation of aggregation with up to 80% loss of single platelets. The magnitude of the synergistic response was independent of the adrenaline preincubation time (0 - 300s). Addition of subthreshold or suprathreshold concentrations of adrenaline 10 seconds before ADP, PAF or VP resulted in leftward shifts in the agonist concentration-response curves which were dependent upon the concentration of adrenaline used. Maximum leftward shifts (calculated as the ratio of EC_{50} values) obtained were: ADP - 2.8 fold; VP - 22 fold and PAF - 10 fold. In the presence of aspirin (2 mM) to prevent the biosynthesis of thromboxane A_2, the maximum shifts were: ADP - 7.5 fold; VP - 8.3 fold and PAF - 3.4 fold. In some individuals VP produced only a maximum platelet loss of less than 50%. In these cases, adrenaline also increased the maximum VP-induced loss of single platelets by a further 10 - 30%. These effects of adrenaline differed markedly from the merely additive responses seen when a low concentration of an agonist e.g. PAF, was added 10 seconds before increasing concentrations of the same agonist.

In conclusion, synergistic stimulation of platelet aggregation occurs in vitro irrespective of whether aggregation is monitored turbidometrically or as loss of single platelets. This phenomenon may also occur in vivo and may be of major importance in haemostasis and in the pathogenesis of occlusive vascular disease.

Culliver, H.A. and Ardlie, N.G. (1981). Thromb. Haemostas., 46, 0645.
Grant, J.A. and Scrutton, M.C. (1980). Br. J. Haematol., 44, 109-125.
Kerry, R. and Scrutton, M.C. (1985). In 'The Platelets; Physiology and Pharmacology' (G.L. Longenecker, ed.) pp113-157. Academic Press, Orlando.
Lumley, P. and Humphrey, P.P.A. (1981). J. Pharmacol. Methods, 6, 153-166.
Vargaftig et al. (1983). Adv. PG., Thromboxane Leukotriene Res., 11, 429-433.

INHIBITION OF HISTAMINE-N-METHYLTRANSFERASE BY RANITIDINE ANALOGUES

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Histamine-N-methyltransferase (HMT) is principally responsible for the metabolism of histamine in mammalian tissues. It is inhibited by H_1-receptor antagonists (Taylor & Snyder 1972) and H_2-receptor antagonists (Barth et al 1973). The present study investigated the kinetics of the inhibition of HMT produced by ranitidine and four analogues.

HMT from rat kidney was purified 200-fold by chromatofocussing. Enzyme activity was determined by measuring the formation of 1-[methyl-^{3}H]methyl-histamine by the method of Shaff & Beaven (1979). Ranitidine competitively inhibited the enzyme with respect to histamine at non-saturating and saturating concentrations of S-adenosyl-methionine (SAM). With respect to SAM, however, it showed mixed inhibition at non-saturating concentrations of histamine whilst at saturating concentrations of histamine, enzyme activation was observed. Similar findings were found for the H_1-receptor antagonist promethazine. Since the two inhibitors and histamine bind at the same site, this apparent activation was most probably the consequence of relief of substrate inhibition by histamine at saturating concentrations.

Other analogues of ranitidine in which the dimethylamino group had been replaced by a methylamino group, the furanylmethylthioethyl group by a phenoxypropyl, and the S atom by a CH_2 group, all competitively inhibited the enzyme with respect to histamine at saturating concentrations of SAM. Secondary plots of Km app/V app against the concentration of inhibitor for ranitidine and the analogues were linear with Ki values ranging from 50 µM for ranitidine to 100 µM for desmethyl ranitidine.

Another analogue in which the $-\overset{\overset{H\,CNO_2}{\|}}{C}-NHMe$ group of ranitidine was replaced by $-SO_2Me$ showed mixed inhibition with respect to histamine with an intercept, in a double reciprocal plot, above the ordinate. The secondary plot was linear with a Ki of 170 µM.

These studies help to identify the structurally important features of H_2-receptor antagonists for competitive binding to HMT.

Barth,H., Niemeyer, I. & Lorenz,W. (1973) in 'International Symposium on histamine H_2-receptor antagonists': Wood & Simkins (eds), pp 115-126. Deltakos, London.
Shaff, R.E. & Beaven, M.A. (1979) Anal. Biochem. 94, 425-430.
Taylor, K.M. & Snyder, S.H. (1972) Mol. Pharmac. 8, 300-310.

REACTIVE BLUE-2 SELECTIVELY ANTAGONISES THE RELAXANT RESPONSES TO ATP AND ITS ANALOGUES WHICH ARE MEDIATED BY THE P2y PURINOCEPTOR.

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It has recently been proposed that the p_2 purinoceptor should be divided into p_{2x} and p_{2y} subclasses largely on the basis of agonist potency orders (Burnstock & Kennedy, 1985). In addition, it has been found that the p_{2x} purinoceptor can be selectively desensitised by α,β-methylene ATP. In 1979 Kerr and Krantis found that an anthraquinone sulphonic acid derivative, reactive blue-2 (RB2) could antagonise inhibitory responses to ATP in guinea-pig colon. In the experiments reported here, the antagonistic properties of RB2 at p_2 purinoceptors in a number of tissues have been examined with the aim of determining whether RB2 may be of value in clarifying the subdivision of these receptors. The preparations used were the rabbit mesenteric artery and rat coronary circulation in which ATP causes contraction via the p_{2x} purinoceptor and relaxation via the p_{2y} purinoceptor, the guinea-pig taenia coli which exhibits relaxations to ATP mediated by the p_{2y} purinoceptor, and the rat portal vein and rabbit ear artery which both show contraction to ATP via the p_{2x} purinoceptor only.

The perfusion pressure of the rat coronary circulation was measured at constant flow. Mechanical responses of longitudinal muscle of isolated rat portal vein, and of circular muscle of rabbit mesenteric and ear artery (5 mm segments) were measured isometrically. Mechanical activity of taenia coli segments was measured isotonically. Dose response curves to the various drugs were obtained in the absence of RB2 and after exposure of the preparation to RB2 for 20 minutes.

In the guinea-pig taenia coli RB2 competitively antagonised the responses to ATP and its analogues but not to noradrenaline. In both rabbit mesenteric artery and rat coronary circulation, RB2 antagonised the p_{2y} purinoceptor-mediated relaxations in a non-competitive manner. The relaxant responses to adenosine and acetylcholine in rabbit mesenteric artery and adenosine in rat heart were significantly antagonised by RB2, but this antagonism was only 15-20% of the antagonism produced at the p_{2y} purinoceptors. RB2 had no effect on p_{2x} purinoceptor-mediated or αadrenoceptor-mediated contractions in rabbit mesenteric artery. In the presence of RB2 there was a significant potentiation of the p_{2x} purinoceptor-mediated contractions to β,γ-methylene ATP and a significant reduction of αadrenoceptor-mediated contractions in rabbit ear artery. In the rat coronary circulation RB2 reduced the rise in perfusion pressure due to α,β-methylene ATP, but this antagonism was only 23% of the antagonism of the p_{2y} purinoceptor-mediated vasodilations. In rat portal vein it was found that RB2 caused a small but significant reduction of p_{2x} purinoceptor but not αadrenoceptor-mediated contractions.

In conclusion, RB2 has been shown to have selective antagonist action at the p_{2y} purinoceptor in several preparations, although over a narrow dose range (2-100uM), at the top of which non-specific effects are apparent. Nevertheless, RB2 may provide a starting point in the development of specific antagonists for the subdivision of the p_2 purinoceptor.

Burnstock, G. & Kennedy, C. (1985) Gen. Pharmac. 16, 433-440.
Kerr, D.I.B. & Krantis, A. (1979) Proc. Aust. Physiol. Soc. 10, 156.

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CHANGES IN [3H]-NITRENDIPINE BINDING IN GERBIL CORTEX FOLLOWING ISCHAEMIA

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Nicardipine, a 1-4 dihydropyridine calcium antagonist, has protective effects in cerebral ischaemia in the mongolian gerbil, in that neurotransmitter levels are not depleted (Alps et al, 1986). In the heart, Nayler et al (1985) showed that 3-H nitrendipine binding was changed by ischaemia. We have therefore investigated whether 3-H nitrendipine binding to membranes from cortex of mongolian gerbils changes following ischaemia, using two protocols : a 3 h unilateral carotid occlusion or a 10 min bilateral occlusion followed by reperfusion.

All gerbils (50-60 g) were anaesthetised with 6 mg pentobarbitone i.p. 10 min prior to surgery. For unilaterally occuluded animals the right common carotid artery was exposed in the paratrachael region and ligated; sham animals were not ligated. After 3 hours the animals were decapitated and classified as "stroke prone" or "stroke resistant" as previously described (Alps et al, 1984). In animals subject to bilateral occlusion, left and right common carotid arteries were ligated for 10 min followed by termination at 0 min, 1 hr and 2 hr, after re-establishment of blood flow. Binding assays were performed using aliquots of tissue homogenate (80-160 µg protein), incubated with 3-H nitrendipine (0.02 - 4.0 nM) in a total volume of 2.0 ml, 50 mM Tris-Hcl (pH 7.4 at 25°C) in the dark for 120 min, and bound ligand was separated from free by vacuum filtration. Binding parameters for 3-H nitrendipine in gerbil frontal cortex are shown below.

	Left Frontal Cortex		Right Frontal Cortex	
3 hr unilateral ligation	Kd(nM)	Bmax ($F.mol\ mg^{-1}$)	Kd	Bmax
Sham control	0.149	133	0.22	172
"Stroke Prone"	0.056**	49**	0.156	82*
"Stroke Resistant"	0.187	119	0.203	141

	Whole Frontal Cortex		
10 Min Bilateral Ligation	Kd	Bmax	
Sham control	0.21	233	n = 4-5
0 min reperfusion	0.11*	172*	** P < 0.01
1 hr reperfusion	0.16	198	* P < 0.05
2 hr reperfusion	0.34	209	compared to sham control animals

Following a 10 min bilateral occlusion the number of dihydropyridine binding sites was reduced, although affinity increased. These effects were reversed by reperfusion. However, the effects may not be directly dependent upon ischaemia per se because in the unilaterally occluded animals changes in affinity and channel number were greater in the contralateral hemisphere than in the ischaemic hemisphere. In conclusion, cerebral ischaemia may induce changes in 3-H nitrendipine binding to cerebral membranes, but the changes are not simply related to the ischaemic insult.

Alps, B.J. et al (1984), Br. J. Pharmac., 83, 499p.
Alps, B.J. et al (1986), Br. J. Pharmac., 88, 249P
Nayler, W. et al (1985), Eur. J. Pharmacol., 115, 81-89

THE ACUTE EFFECTS OF LOFEPRAMINE AND DESMETHYLIMIPRAMINE ON TRYPTOPHAN METABOLISM AND DISPOSTION IN THE RAT

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Because the tricyclic antidepressant lofepramine is metabolized mainly in the liver to the clinically active desmethylimipramine (DMI), there has been some speculation as to whether lofepramine acts as an antidepressant in its own right, or via DMI. We have previously shown (Badawy & Evans, 1982) that acute administration to rats of a large number of antidepressants (including DMI) elevates brain tryptophan concentration (thereby enhancing cerebral 5-HT synthesis) by inhibiting the activity of hepatic tryptophan pyrrolase (EC 1.13.11.11). It was therefore considered of interest to find out if acute lofepramine administration exerts similar effects and whether any likely such effects could be attributed to the drug itself, rather than its metabolite DMI.

The hydrochlorides of lofepramine and DMI (both gifts from E. Merck) were dissolved in a dimethylformamide-saline mixture (1:3, v/v) and were given i.p. (2ml/kg) to locally bred male Wistar rats (150-170g); control animals received a similar volume of the above vehicle. All chemical, enzymic and other determinations were performed by standard procedures (for references, see Badawy & Evans, 1982).

Rat liver tryptophan pyrrolase activity was inhibited significantly at 2h (by 30% and 43% for total and apoenzyme activities respectively) by a dose of lofepramine as little as 0.5mg/kg and maximally (by 51% and 87% respectively) by a 2.5mg/kg dose. By contrast, the pyrrolase inhibition by DMI was dose-dependent in the dose range of 0.5-10mg/kg (by 21-55% and 37-87% respectively). Lofepramine (and also DMI) inhibited pyrrolase activity directly, after addition *in vitro*, with the inhibition being significant with concentrations as little as 10μM (by 24-29% for lofepramine and 24-42% for DMI). Lofepramine administration also inhibited pyrrolase activity that had previously been enhanced by hormonal induction by cortisol or cofactor activation by haematin.

The above inhibition of liver tryptophan pyrrolase activity by administration of lofepramine or DMI increased the availability of circulating tryptophan to the brain, thereby enhancing cerebral 5-HT synthesis. Thus at 3.5h after lofepramine, brain tryptophan concentration was increased by 14% and 27% by the 0.5 and 2.5 mg/kg doses respectively, with larger doses exerting no further increases. By contrast, the brain tryptophan increases induced by DMI were 17-36% in the dose range of 0.5-10mg/kg. Broadly similar changes reflecting these contrasting differences between lofepramine and DMI were also observed with other parameters of tryptophan metabolism and disposition (i.e. free serum, total serum and liver tryptophan and brain 5-HT and 5-HIAA concentrations). Availability to the brain of an exogenously administered tryptophan load was also enhanced by both drugs.

These results therefore demonstrate that, in common with 19 other antidepressants, acute lofepramine administration enhances rat brain 5-HT synthesis by increasing tryptophan availability to the brain secondarily to inhibition of liver tryptophan pyrrolase activity. The results also suggest that lofepramine itself (and not via its DMI metabolite participation) is capable of exerting all these effects on tryptophan disposition and cerebral tryptophan metabolism by its direct inhibition of liver tryptophan pyrrolase activity.

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Badawy, A. A.-B. & Evans, M. (1982) *Br. J. Pharmac.* 77, 59-67

APOMORPHINE-INDUCED VOMITING IN THE FERRET; ANOMALIES OF RESPONSE TO DOSE AND ROUTE OF ADMINISTRATION.

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Apomorphine causes vomiting in cats, dogs and primates by stimulating dopamine D_2 receptors in the chemoreceptor trigger zone of the area postrema (A.P.). Recently the ferret has been investigated to see how its responses compare with more established models.

The effects of intravenously (i.v.) and subcutaneously (s.c.)administered apomorphine (dose range 10-500 µg/kg) were determined in normal ferrets. Injections were given into the dorsum of the neck (s.c.) and, via a valve connected to a cannula in the left external jugular vein (i.v.).

The emetic response to these injections is shown below. A "bell-shaped" dose-response curve is apparent for s.c. administration. At very high doses of apomorphine (5000 µg/kg) no vomiting or retching was seen. There was a clear difference in response when the s.c. route was used compared with the i.v.. It was expected that the emetic "threshold" using the i.v. route would be more reliably exceeded and with shorter latencies as the dose increased than for the s.c. route. This did not occur. 100 % vomiting response was never achieved via the i.v. route, and there was no response at all below 50 µg/kg. The mean latency at 100 µg/kg i.v. was shorter than that for the s.c. route and despite the discrepancy in % response the actual emetic potential of 100 µg/kg was virtually the same using either route. At all other doses however, the i.v. route seemed less effective in every way.

Apomorphine dose µg/kg		n	Responders (Vomiters/n)	Latency to first vomit and range (mins)	Number of vomits (means ± s.d.)	Number of retches (means ± s.d.)
10	s.c.	4	0/4	-	0	0
25	s.c.	9	2/9	3.8 (1.7-6.0)	0.6 (±1.4)	4.6 (±11.0)
50	s.c.	26	10/26	4.2 (1.5-9.1)	2.8 (±1.8)	18.0 (± 7.0)
100	s.c.	10	10/10	5.2 (3.2-7.9)	2.9 (±1.6)	25.5 (±13.9)
500	s.c.	4	2/4	12.1 (1.0-23.2)	1.5 (±1.9)	19.0 (±15.3)
100	i.v.	12	7/12	2.8 (0.9-8.0)	3.1 (±2.6)	19.3 (±15.8)
500	i.v.	4	1/4	[13.1]	0.3 (±0.5)	9.3 (±18.5)

We suspect that these differences between route of administration may depend upon functions of the rate of presentation of apomorphine to the A.P. and its relative agonist/antagonist activities at given concentrations. It is of interest that similar differences have been found for i.p. and s.c. doses in respect of apomorphine stereotypy in the rat (Melzacka et al, 1979). These differences have to be taken into account when using apomorphine as an emetic stimulus in the ferret during investigations into the neuropharmacology of vomiting.

Melzacka, M. (1979) Pol. J. Pharmacol. Pharm. 31, 309-317

INTRASTRIATAL SKF 38393 REVEALS SPATIAL HETEROGENEITY OF DOPAMINE D_1 RECEPTOR-MEDIATED BEHAVIOURS.

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In unilaterally 6-hydroxydopamine lesioned rats, systemic injection of the D_1 agonist SKF 38393 produces characteristic contraversive circling and orofacial activities (Setler et al, 1978). To investigate the contribution of the super-sensitive striatum to the production of these behaviours, we injected SKF 38393 stereotaxically into different striatal sites. For comparison, parallel experiments were performed with lisuride (D_2 agonist) and apomorphine (D_1/D_2 agonist).

Intrastriatal SKF 38393 (2.5µg) duplicated the systemic effects of the drug in a topographic manner. Robust circling arose from the centromedial striatum, overlapping with and independently of posture. The ventrolateral striatum was a hot-spot for grooming, while forepaw nibbling evolved from the centroventral region and forelimb dyskinesia was site-independent. The origins of sniffing and head movements showed a medial preference. Along the rostro-caudal axis circling was strongest from the head of the striatum, while posture became pronounced posteriorly. Other behaviours were uniformly organised within this plane. All of these behaviours were blocked by SCH 23390 (D_1 antagonist), but not by metoclopramide (D_2 antagonist).

Apart from circling, which showed a bimodal distribution rostro-caudally, behaviours elicited by intrastriatal apomorphine (5 µg) and lisuride (5 µg) were more homogeneously distributed within the denervated striatum. Lisuride was antagonised by metoclopramide, while apomorphine was inhibited by D_1 and D_2 antagonists alike.

The results reiterate the importance of dopamine D_1 receptors in motor control and further show that D_1 and D_2 -mediated behaviours can be distinguished 1) qualitatively, 2) topographically and 3) by their antagonist sensitivity.

Setler, P.E. et al (1978) Eur. J. Pharmac. 50, 419-430

THE DOPAMINE D_1 RECEPTOR AGONIST SKF 38393 POTENTIATES D_2 MOTOR RESPONDING IN RESERPINISED MICE.

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Reserpine depletes brain amines indiscriminately, but only the administration of dopaminomimetics to reserpinised animals effectively overcomes the associated hypokinesia (Arnt, 1985). This study looks at the way in which selective agonists of the dopamine D_1 and D_2 subclasses of receptor, injected alone and in combination, restore motor activity in mice at different times after reserpinisation.

Three hours after receiving reserpine, 5 mg/kg i.p., mice became almost totally akinetic and ceased to display species-typical behaviours such as sniffing, rearing and grooming. Injecting the D_2 agonists RU 24213 or lisuride, or the mixed D_1/D_2 agonist apomorphine, evoked dose-dependent, slow and ponderous forward walking, together with head-down sniffing. The D_1 stimulant SKF 38393, 1.5-15 mg/kg, had no direct effect of its own, but greatly amplified the D_2 response, giving more fluent locomotion, as well as rearing and grooming. This facilitatory effect of SKF 38393 was completely attenuated by a small dose of SCH 23390 (D_1 antagonist), whereas D_2 -mediated responses were sensitive both to SCH 23390 and metoclopramide (D_2 antagonist).

Mice reserpinised for 24 hrs became more sensitive to the motor stimulant actions of D_1 and D_2 agonists. SKF 38393 now reinstated movement directly, while the effects of D_2 stimulation were similar to those at 3 hrs. D_1 stimulation resulted in a greater fluidity and breadth of behaviour than did D_2 stimulation. Combined treatments with D_1 and D_2 agonists were additive, often leading to extinction of certain behaviours, rather than Synergism as occurred earlier. At 24 hrs after reserpine the agonists were blocked selectively by their respective antagonists.

Reserpine caused precipitous falls in the the concentrations of dopamine, noradrenaline and 5-hydroxytryptamine in the striatum, olfactory tubercle and cerebral cortex, with correspondingly elevated metabolite levels, more especially at 24 than 3 hrs.

These results are consistent with the notion that dopamine D_1 and D_2 receptors are normally functionally interdependent, but proliferate and become functionally separate following severe and continued dopamine depletion. If these changes in any way reflect those occurring in Parkinsonism, it is envisaged that the therapeutic management of certain stages of the disease by D_2 agonist drugs might be improved by adjunctive treatment with a D_1 agonist.

Arnt, J. (1985) Eur. J. Pharmac. 113, 79-88.

DOES BUSPIRONE ELICIT FEEDING BY A SIMILAR MECHANISM TO THAT OF 8-OH-DPAT?

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8-OH DPAT, a selective $5HT_{1A}$ agonist, elicits feeding in non-deprived rats (Dourish et al, 1985). The novel anxiolytic buspirone, has been shown to bind with high affinity to the $5HT_{1A}$ receptor site (Peroutka, 1985). We have compared the effects of buspirone and 8-OH DPAT on food consumption in both normal rats and after pretreatment with pCPA or 5HTP.

Male Lister Hooded rats (210-270g) were caged in pairs for 5 days before acute drug effects on feeding were examined. Food (41B pellets) and water were available ad lib. (12:12 light: dark cycle; lights on 08-00h). On day 5, buspirone (0.1-30.0 mg/kg s.c.), 8-OH DPAT (0.03-5.0 mg/kg s.c.) or vehicle (isotonic saline) were administered at 12-00h. The food hoppers were weighed at 12-00h and 15-00h. In a separate experiment, pCPA (200 mg/kg p.o.) or vehicle were administered at 11-30h on days 1 and 2. Food consumption was monitored throughout and, on day 5, buspirone (1 mg/kg s.c.), 8-OH DPAT (0.1 mg/kg s.c.) or vehicle were administered at 12-00h to vehicle - or pCPA-pretreated rats. Food consumption was measured over the period 12-00h - 15-00h. In a third experiment, animals were pretreated with carbidopa (25 mg/kg i.p.) at 10-30h and 5-HTP (10 or 50 mg/kg s.c.) at 11-30h, before receiving buspirone (1 mg/kg s.c.), 8-OH DPAT (0.1 mg/kg s.c.) or vehicle at 12-00h. Food consumption over the period 12-00h - 15-00h was again measured. The rats in each pair received the same treatment and at least 8 pairs were used per treatment. Data were analysed using Student's unpaired t-test.

8-OH DPAT and buspirone both significantly increased feeding, although the dose-response curves were bell-shaped. Maximal increases were recorded at 0.1 mg/kg s.c. for 8-OH DPAT and 1.0 mg/kg s.c. for buspirone (mean ± s.e.m. g food consumed/3h:- vehicle = 1.3 ± 0.7; 8-OH DPAT = 8.0 ± 0.7 ($p < 0.001$): buspirone = 5.0 ± 0.4 ($p < 0.001$)). PCPA pretreatment per se was found to increase food consumption significantly ($p < .01$) (vehicle = 0.4 ± 0.2; pCPA = 3.0 ± 0.8) on day 5. Although 8-OH DPAT and buspirone significantly ($p < 0.01$) increased feeding relative to vehicle control (8-OH DPAT = 4.3 ± 0.4; buspirone = 3.0 ± 0.5), neither drug elicited any further significant increase relative to the pCPA-pretreated animals (pCPA=3.0 ± 0.8; 8-OH DPAT = 4.3 ± 1.0; buspirone = 3.3 ± 0.9). PCPA appeared to change the diurnal cycle of feeding such that food consumption was decreased during the dark period and increased during the light period on days 3 and 4. Administration of carbidopa/5-HTP (50 mg/kg) did not affect feeding but antagonised the increases in food consumption induced by 8-OH DPAT or buspirone. The lower dose of 5-HTP had no effect.

8-OH DPAT-induced feeding has been suggested to be mediated via activation of 5HT autoreceptors, leading to decreased 5HT release (Curzon et al, 1986). Our results with 5HTP, at a dose which increased 5HT levels, and with pCPA, which depleted central 5HT, are consistent with this hypothesis. Buspirone produced effects on feeding similar to those induced by 8-OH DPAT; although unlike 8-OH DPAT buspirone did not induce stereotypy ('5HT syndrome') at higher doses. This is consistent with the existence of pre-and post synaptic $5HT_{1A}$ receptors, implying that buspirone may be more selective for the presynaptic site.

Curzon, G. et al (1986) Br. J. Pharmac 87 21P
Dourish, C.T. et al (1985) Psychopharmacology 86 197-204
Peroutka, S.J. (1985) Biol Psychiatry 20 971-979

RELATIVE POTENCY OF GAMMA-AMINOBUTYRIC ACID (GABA) AND GABA ANALOGUES ON CELL ACTIVITY IN RAT CEREBELLAR SLICES

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Many areas of the mammalian brain have been used for in vitro recording, including the hippocampus, olfactory cortex, lateral geniculate nucleus and spinal cord (Kerkut & Wheal 1981; Dingledine 1984). Electrophysiological properties of rat cerebellar slice neurones have been studied by Llinas and Sugimori (1980). The cerebellum has two inputs, the climbing and mossy fibres and its output is through Purkinje cell axons to the cerebellar nuclei. In the present study activity was recorded extracellularly from neurones in rat cerebellar slices, thickness 500 µm, using glass microelectrodes filled with 4M sodium chloride. Slices were continually perfused with oxygenated Krebs at a temperature of 25°C and a flow rate of 15ml $min.^{-1}$. Activity was recorded from neurones in the Purkinje cell layer of the slice and the firing rate of these cells was in the range 5 - 80 Hz. The effect of ion substitution and GABA analogues was examined by perfusing the slice with either substituted saline or the compound under test. Using this method the concentration of compound could be accurately determined. All experiments were performed at least 5 times and the mean values recorded with S.E.M.

The cell firing rate was sensitive to the addition of magnesium or manganese ions to the Krebs, 10 mM Mg or 4 mM Mn completely blocking activity. The concentration of Mg or Mn to block activity by 50%, EC-50 value, was 3.47 $\pm$ 0.34 mM and 0.15 $\pm$ 0.02 mM respectively. GABA also completely inhibited cell firing, with an EC-50 of 2.9 $\pm$ 0.48 x 10^{-4}M. Analysis of EC-50 values with depth of cell showed a positive correlation, (R 0.86), ie. the deeper cells gave higher EC-50 values compared to surface cells. Muscimol, a GABA analogue not taken up by neurones or glia, also inhibited cell activity with an EC-50 of 2.00 $\pm$ 0.1 x 10^{-7}M. There was no correlation between muscimol EC-50 values and depth. The presence of the GABA neuronal uptake inhibitor nipecotic acid, 1mM, potentiated the effects of GABA, decreasing the EC-50 value in 5 experiments from 3.75 $\pm$ 1.0^{-4}M to 2.30 $\pm$ 1.3 x 10^{-5}M. Taurine and beta-alanine also inhibited cell firing with EC-50 values of 6.88 $\pm$ 0.67 and 6.6 $\pm$ 1.2 x 10^{-4}M respectively. Delta-aminolevulinic acid, a compound thought to have some specificity for presynaptic GABA receptors (Brennan & Cantrill 1979) also inhibited cell activity, EC-50 value of 1.03 $\pm$ 0.13 x 10^{-3}M. Baclofen had no inhibitory effect on cell activity.

Since cell activity could be completely blocked by magnesium it may be synaptic in origin. The potent effect of muscimol is at least partly due to its lack of inactivation by a re-uptake mechanism. Taurine, beta-alanine and delta-aminolevulinic acid are all inhibitory, as is GABA, on this preparation. Studies are in progress to determine the identity of the cells used and the site of action of the compounds.

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Brennan, M.J.W. & Cantrill, R.C. (1979). J. Neurochem. 33, 721-725.
Dingledine, R. (1984). Brain Slices; Plenum Press, New York.
Kerkut, G.A. & Wheal, H.V. (1981). Electrophysiology of Isolated Mammalian CNS Preparations; Academic Press, London.
Llinas, R. & Sugimori, M. (1980). J. Physiol. 305, 171-195.

EXCITATORY AMINO ACID RECEPTOR ACTIVATION AND ENHANCED Ca^{2+} INFLUX INTO RAT HIPPOCAMPAL SLICES.

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Ca^{2+} uptake into hippocampal slices has been stimulated by glutamate, aspartate and by other synthetic excitatory amino acid (EAA) agonists (NMDA, kainate). These effects are inhibited by the appropriate EAA antagonists, or by high Mg^{2+} (responses to NMDA).

Rat hippocampal slices were incubated in Tris-buffered Kreb's medium containing 1.2mM $CaCl_2$ for 30 min before the addition of $^{45}CaCl_2$ (Amersham International, 0.14 Ci/mol). Antagonists were added 5min prior to the addition of $^{45}CaCl_2$ and agonists were added 1min after $^{45}CaCl_2$. The incubation medium normally contained 1.0mM $MgSO_4$; in other experiments 0, 0.1 or 10mM $MgSO_4$ was added. Other details were as described by Bradford et al (1986). L-Glutamate, L-aspartate, NMDA and kainate depolarized the hippocampal slices (as judged by the fall in K^+ content) and evoked a concentration-dependent stimulation of Ca^{2+} influx. Approximate ED_{50} values and maximum evoked increases in Ca^{2+} influx for the agonists were: (n=6), glutamate (100μM; 158 ± 10% of control), aspartate (1mM; 161 ± 12), NMDA (50μM; 153 ± 7), kainate (50μM; 163 ± 3). Neither the selective NMDA antagonist AP7 nor the more effective kainate/quisqualate antagonist GAMS influenced basal Ca^{2+} uptake or slice K^+ content at concentrations up to 1mM. However, AP7 (500μM) completely inhibited Ca^{2+} influx evoked by NMDA (50μM) and partially inhibited the effects of glutamate (100μM) or aspartate (1mM). It did not affect the response to kainate (50μM). In contrast, GAMS (1mM) completely inhibited the latter response and also blocked the action of glutamate plus aspartate when added together with AP7. The fall in K^+ content of the slices induced by the agonists was also reduced by the antagonists and in parallel to the changes in Ca^{2+} influx. The homologue phosphono compounds AP4, AP5 and AP7 all substantially inhibit Ca^{2+} influx and transmitter amino acid release evoked by depolarizing agents (high K^+ and veratrine), with a potency order AP7 $\gg$ AP5 $\gg$ AP4, indicating that a large part of these responses is due to the action of released EAAs (Crowder et al, 1986).

The gating of 'NMDA-receptors' by Mg^{2+} ions (Nowak et al, 1984) was also examined. In 0.1mM Mg^{2+}, basal Ca^{2+} influx was elevated and the responses due to added NMDA (but not kainate) were significantly enhanced (by 21%; $P < 0.005$). Conversely, in 10mM Mg^{2+}, NMDA responses alone were significantly reduced (by 78%; $P < 0.05$).

These results show that depolarization evoked by EAAs via receptor interaction leads to enhanced Ca^{2+} uptake. This Ca^{2+} influx could be via both voltage-dependent or independent Ca^{2+}-channels and these need not necessarily be physically coupled to the EAA receptors. This may be an important element in the purported actions of EAAs in various animal models of neurological disorders including epilepsy and experimental ischaemia where EAA antagonists have shown potent anticonvulsant properties or prevented ischaemic brain damage (Bradford & Dodd, 1976; Coutinho-Netto et al, 1981; Schwarcz & Meldrum, 1985).

Bradford, H.F. & Dodd, P.R. (1976) In: 'Biochemistry & Neurological Disease': Davison, A.N. (ed), pp 114-167. Blackwells, Oxford.
Bradford, H.F. et al (1986). Br.J.Pharmac. 88, 87 - 93.
Coutinho-Netto, J. et al (1981) Epilepsia 22, 289 - 296.
Crowder, J.M. et al (1986) Biochem.Soc.Trans. (in press).
Nowak, L. et al (1984) Nature 307, 462 - 465.
Schwarcz, R. & Meldrum, B.S. (1985). Lancet (II), 140 - 143.

ANTICONVULSANT PROFILE AND WHOLE BRAIN CONCENTRATIONS OF NITRENDIPINE AND NIMODIPINE

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We reported previously that dihydropyridine calcium channel antagonists were very effective against ethanol withdrawal convulsions (Dolin et al, 1986). We now report their activity against convulsions produced by a variety of mechanisms. Male Sprague-Dawley rats (200-250g) were used. Pentylenetetrazol (PTZ) and strychnine were given by intravenous infusion (Nutt et al, 1981) until the first clear signs of clonic and tonic convulsions, respectively, were seen. High pressure (HP) convulsions were studied in a pressure chamber (partial pressure of oxygen 0.4 atm), helium gas being added at 3 atm min^{-1} until clonic convulsions were seen ("pretreatment time" in Table was from injection to control convulsions). N-methyl-d-aspartate (NMDA) was given i.p., followed by observation of the animals for 60 min. In all experiments rectal temperatures were maintained at 37 ± 0.5°C and the observer did not know the prior drug treatment. Nitrendipine and nimodipine were suspended in Tween 80 (1%), under red safe-light, and given i.p. at 100 mg kg^{-1}. Controls, tested concurrently, received vehicle injections. Whole brain concentrations of the dihydropyridines were measured using gas chromatography (RSL-150 Capillary Column; toluene extraction).

Convulsant	Treatment	Pretreatment time	Thresholds, mean ± s.e.m. Controls (n)	Treated (n)	% change	Mann-Whitney 'U' test
PTZ	nitrendipine	30 min	23 ± 1 (8)	38 ± 2* (8)	+65	$P < 0.01$
(mg kg^{-1})	nitrendipine	180 min	25 ± 2 (8)	37 ± 2* (7)	+48	$P < 0.01$
	nimodipine	90 min	25 ± 2 (7)	34 ± 2* (7)	+36	$P < 0.01$
Strychnine	nitrendipine	30 min	0.75 ± 0.04 (8)	0.75 ± 0.05 (7)	0	$P > 0.1$
(mg kg^{-1})	nitrendipine	2h	0.78 ± 0.03 (6)	0.74 ± 0.02 (5)	-5	$P > 0.1$
HP (atm)	nitrendipine	2h	89 ± 5 (6)	113 ± 2* (6)	+27	$P < 0.01$

In contrast, the convulsive effects of NMDA were slightly increased; after 150 mg kg^{-1} NMDA 1/14 control animals showed continuous clonic convulsions compared with 6/11 animals treated 2h previously with 100 mg kg^{-1} nitrendipine, i.p. ($P < 0.05$, Fisher's exact test). After 200 mg kg^{-1} NMDA 6/10 animals treated with this dose of nitrendipine died, compared with 0/10 controls ($P < 0.01$).

Brain concentrations were nitrendipine: 1.7 ± 0.25 $\mu g\, g^{-1}$ (7) (wet weight), 2h after 100 mg kg^{-1}, i.p., and nimodipine: 562 ± 137ng g^{-1} (8), 2h after 100mg kg^{-1} i.p.

The dihydropyridines, therefore, showed anticonvulsant actions against PTZ and the high pressure syndrome, but not against strychnine or NMDA. The mechanism of the anticonvulsant effect is, as yet, uncertain but the action is clearly selective. The extent of the protection against PTZ and high pressure appeared small compared with the effects we found previously on ethanol withdrawal convulsions (Dolin et al, 1986). The results do not support the suggestion of Bowser-Riley (1984) that the effects of drugs on high pressure convulsions are similar to the effects on strychnine convulsions. The doses of the dihydropyridines needed to produce these anticonvulsant actions were higher than those required for their cardiovascular effects, but the central concentrations were in the low micromolar range.

Bowser-Riley, F. (1984) Phil. Trans. R. Soc. Lond., 304, 31-41.
Dolin, S.J. & Little, H.J. (1986) Br. J. Pharmacol., 87, 40P.
Nutt, D.J., Cowen, P.J. & Green, A.R. (1980) Neuropharmacology, 19, 1017-1023.

INCOMPLETE OVERLAP OF INTEROCEPTIVE STIMULI INDUCED BY CL 218872 AND CHLORDIAZEPOXIDE.

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CL218872 is a ligand for benzodiazepine receptors but with less muscle relaxant properties (Lippa et al., 1979).

Male Lister rats were trained to discriminate low doses of either CL218872 (5mg/kg p.o. 1h pre-test, 6 rats) or chlordiazepoxide (CDZP, 5mg/kg p.o. 1h pre-test, 8 rats) from vehicle (demineralised water 2ml/kg) in a two lever Camden operant chamber, using a FR 20 schedule, working for 45mg food pellets. Greater than 85% confidence for every drug or saline lever selection was obtained on a quasirandom dosing schedule before other drugs were tested. Selection confidence was calculated on the scale - first 20 presses on correct lever = 100%, first 20 presses on the incorrect lever = 0%.

Both cues were antagonised by CGS8216 20mg/kg p.o. (99% CL218872, 95% CDZP) although the CL218872 cue was more sensitive to 10mg/kg p.o. (100% antagonism, 34% for CDZP). The CDZP cue generalised to CDZP, CL218872, diazepam, nitrazepam and CGS9896. The CL218872 cue generalised to CL218872 but only partially generalised to all the other drugs, even at higher doses in the case of CDZP:-

Mean % Confidence (No. Rats Tested Chosing Drug Lever)

	Dose mg/kg p.o.	CL218872 Cue	CDZP Cue
CL218872	2	34 (2/6)	
	5	82 (11/16)	36 (3/8)
	10		73 (6/8)
	20		83 (4/4)
CDZP	2		61 (10/16)
	5	42 (3/8)	100 (3/3)
	10	36 (2/7)	
	20	49 (3/6)	
Nitrazepam	2	35 (2/6)	96 (6/6)
Diazepam	5	41 (2/6)	98 (6/6)
CGS9896	10		12 (1/10)
	20	3 (0/5)	70 (7/10)
	100	25 (1/4)	70 (4/6)

These data suggest that both the CL218872 and CDZP cues are mediated via benzodiazepine receptors but that they are not identical interoceptive stimuli.

Lippa, A. S. et al., (1979) Pharmacol. Biochem. Behav. 10, 831-843.

ANXIOLYTIC EFFECTS OF CHLORDIAZEPOXIDE IN SOCIALLY ISOLATED RATS.

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Anxiety in laboratory rodents has been associated with both a reduction in exploration in an elevated X-maze (Parker and Morinan, 1986) and an increase in brain 5-hydroxytryptamine (5-HT) turnover (Iversen, 1984). Using the socially isolated rat as a model of anxiety (Parker and Morinan, 1986), the effect of chlordiazepoxide (CDP) on both exploration and 5-HT turnover was measured to further assess the suitability of this model. The two areas of the brain chosen for investigation were the hippocampus and the amygdala since they have been reported to be involved in the genesis of anxiety (Iversen, 1984).

Male Sprague-Dawley rats were isolated for 21 days immediately after weaning. On day 22 exploratory behaviour in an elevated X-maze (Parker and Morinan, 1986) was tested 1 hour after an intraperitoneal injection of either saline (ISOL-CON) or 2.5mg kg^{-1} CDP (ISOL-CDP). Immediately after the behavioural test, hippocampal and amygdaloid concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured fluorimetrically. 5-HT turnover was calculated from the ratio of 5-HIAA:5-HT concentrations.

The CDP treated rats showed a significant increase in exploration in all the parameters tested (Table 1), and in the amygdala there was a decrease in 5-HT turnover (Table 2).

Table 1 Effect of chlordiazepoxide on the exploratory behaviour of isolated rats

	Arm Entries [E]	Rears [R]	Activity [A]	Product Score [ExAxRx10^{-4}]
ISOL-CON	6.9 ± 0.5	10.2 ± 1.0	672 ± 49	5.08 ± 0.88
ISOL-CDP	12.0 ± 1.1*	16.3 ± 1.4*	823 ± 54*	21.32 ± 4.60*

Each value is the mean ± S.E.M. for 24 rats (obtained from 3 separate experiments)
*P < 0.05 v CON

Table 2 Effect of chlordiazepoxide on brain 5-HT turnover

	Hippocampus	Amygdala
ISOL-CON	1.07 ± 0.07	1.06 ± 0.04
ISOL-CDP	1.13 ± 0.08	0.95 ± 0.03*

Each value is the mean ± S.E.M. for 32 rats (obtained from 4 separate experiments)
*P <0.05 v CON

Therefore the reduction in exploration and the increase in 5-HT turnover seen in isolated rats, which may be related to anxiety (Parker & Morinan, 1986), can be reversed by acute treatment with CDP. These findings lend further support to the validity of the use of the socially isolated rat as a model of anxiety.

Parker, V and Morinan, A (1986) Neuropharmacology in press
Iversen, S (1984) Neuropharmacology 23: 1553-1560

ELECTROPHYSIOLOGICAL AND RADIOLIGAND BINDING STUDIES OF N-METHYL-D-ASPARTATE RECEPTORS IN RAT CEREBRAL CORTEX.

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The N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors is a focus of current interest due to the evidence that it is involved in certain types of learning and memory (Morris et al, 1986) and neurodegenerative states (Schwarcz and Meldrum, 1985). However, only a limited amount of information is available about the affinity of agonists and antagonists at this receptor type. We have used both electrophysiological techniques with in vitro rat cortical slices and radioligand binding experiments using rat cortical membranes to obtain quantitative data on cortical NMDA receptors and to evaluate the proposal that NMDA-sensitive L-[^{3}H]glutamate binding represents the recognition site for the NMDA receptor.

Electrophysiological recordings were made from rat cortical slices (Harrison and Simmonds, 1985). Radioligand binding experiments were carried out using crude postsynaptic density (PSD) preparations from rat cortex with L-[^{3}H]glutamate as the radioligand (Fagg and Matus, 1984). Approximately 80% of the specific L-[^{3}H]-glutamate binding (50nM) to crude PSDs from rat cortex was displaced by NMDA receptor ligands. The agonists, NMDA, trans-2,3-piperidine dicarboxylate (PDA), NMLA and quinolinate inhibited L-[^{3}H]glutamate binding with Ki values of 2.0, 4.5, 10 and 91 μM, respectively. In electrophysiological experiments, compared to NMDA, the relative potencies were: trans-2,3-PDA = 2.5, NMLA = 10, quinolinate = 60, in good agreement with the binding data. The affinity of antagonists was compared directly using pA_2 values from Schild analysis of the electrophysiological experiments and pK_i values for inhibition of L-[^{3}H]-glutamate binding (Table 1). D-2-amino-5-phosphonovalerate (D-AP5), DL-2-amino-7-phosphonoheptanoate (DL-AP7) and β-D-aspartylaminomethylphosphonate (β-D-AspAmp) were competitive antagonists of NMDA-induced depolarisations, and were selective displacers of NMDA-sensitive L-[^{3}H]glutamate binding. The quisqualate/kainate receptor antagonist γ-D-glutamylaminomethylsulphonate (γ-D-GAMS) was weak in both assays.

Table 1

Antagonist	Inhibition of L-[^{3}H]glutamate binding		Antagonism of NMDA-induced depolarisations	
	pK_i (±SEM)	Maximum inhibition(%) (±SEM)	pA_2	Slope of Schild plot (±95% confidence limits)
D-AP5	5.74±0.8	84.0±6.2	5.59	0.95±0.15
DL-AP7	5.09±0.04	72.9±5.7	5.13	1.04±0.13*
β-D-AspAmp	5.09±0.13	83.0±2.1	5.00	0.98±0.16
γ-D-GAMS	>3.0		Dose ratio <2 at 100μM	

* slope for 10-100μM; 100-300μM slope = 0.51± 0.23

These data provide accurate values for the affinity of the above compounds as NMDA-receptor ligands. The close correspondence between the affinity of antagonists as measured in electrophysiological and binding experiments strongly indicates that NMDA-sensitive L-[^{3}H]glutamate binding sites are the recognition sites for NMDA-receptors.

Fagg, G.E. and Matus, A.(1984). Proc. Natl. Acad.Sci.(USA) (1984) 81,6876-6880.
Harrison, N.L. and Simmonds, M.A. (1985). Br. J. Pharmacol. 84, 381-391.
Morris, R.G.M., Anderson, E., Lynch, G.S. and Baudry, M. (1986) Nature 319,744-776.
Schwarcz, R. and Meldrum, B.S. (1985) Lancet ii, 140-143.

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PROTECTION OF N-METHYL-D-ASPARTATE-INDUCED NEURONAL DEGENERATION BY SYSTEMIC ADMINISTRATION OF MK-801

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MK-801, an orally-active anticonvulsant (Clineshmidt et al, 1982), has recently been shown to be a potent and selective antagonist of the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors, acting in a non-competitive manner (Kemp et al, 1986). Recent evidence indicates that NMDA receptors play a major role in the neuronal degeneration induced by cerebral ischaemia (Simon et al, 1984) and hypoglycaemia (Weiloch, 1985), and it has been suggested that NMDA antagonists may be of value in the treatment of neurodegenerative disorders. However, the competitive antagonists available are of limited use due to their poor penetration of the blood-brain barrier. In this study we have evaluated the ability of systemically-administered MK-801 to protect against neuronal degeneration caused by intracerebral injections of NMDA.

Stereotaxic injections of NMDA or kainic acid (in 1µl, pH7.4) were made into the right striatum or dorsal hippocampus of rats under equithesin anaesthesia. MK-801 (dissolved in 0.9% saline) was administered i.p. and saline-injected animals served as controls. For histological analyses, animals were perfused with fixative after 7 days and microtome sections stained with cresyl violet. For neurochemical measurements, animals were killed after 7 days, the striatum or hippocampus dissected and assayed for choline acetyltransferase (CAT) or glutamate decarboxylase (GAD) activity, respectively.

Unilateral injection of NMDA into the striatum (60 nmol) or dorsal hippocampus (20 nmol) in the control group (n=4) resulted in an area of neuronal degeneration extending for several mm around the site of injection. Treatment with 10mg/kg MK-801 1 hour prior to NMDA injection (n=4) caused almost complete protection, with only a few necrotic neurons in the immediate vicinity of the injection site. A quantitative assessment of the neuroprotective affects of MK-801 was made by measuring the activity of marker enzymes for intrinsic neuronal populations. Unilateral injection of 120nmol NMDA into the striatum caused a 33.2$\pm$8.4% reduction in striatal CAT activity, and injection of 40nmol NMDA into the dorsal hippocampus produced a 26.9$\pm$6.4% reduction in hippocampal GAD activity, compared with the contralateral, uninjected side (n=5). MK-801 given i.p. at 1,3 and 10 mg/kg (n=5 at each dose) 1 hour before NMDA injection caused a dose-dependent protection of these effects in both striatum and hippocampus which reached statistical significance at the 10mg/kg dose ($p<0.01$). The specificity of MK-801 for NMDA-induced neurodegeneration was indicated by the finding that kainate-induced CAT decrements in the striatum were not protected by MK-801 up to 10mg/kg.

These results are the first demonstration of protection against NMDA-induced neuronal degeneration by a systemically administered agent. The effectiveness of MK-801 when given by a peripheral route suggests a possible use in the treatment of human neurodegenerative disorders.

Clineschmidt, B.V., Martin, G.E. and Bunting, P.R. (1982) Drug Dev. Res. 2,123-134.
Kemp, J.A., Priestley, T. and Woodruff, G.N. (1986) Br. J. Pharmacol. Proceedings, Amsterdam Meeting.
Simon, R.P., Swan, J.H., Griffiths, T. and Meldrum, B.S. (1984) Science 226, 850-852.
Weiloch, T. (1985) Science 230, 681-683.

INCREASED LOCAL CEREBRAL GLUCOSE UTILIZATION IN LIMBIC REGIONS INDUCED BY FG 7142

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The β-carboline benzodiazepine receptor ligand FG 7142, (N-methyl-β-carboline -3-carboxamide) is anxiogenic both in animals and in man and is proconvulsant in rodents (Pellow & File 1984), putatively via an action at the benzodiazepine/GABA receptor complex. However, the neuroanatomical circuits through which these actions become expressed in vivo have not been defined. We have used the quantitative ^{14}C-2-deoxyglucose technique (Sokoloff et al., 1977) to examine the alterations in function related glucose use after acute administration of FG 7142 in conscious male hooded rats.

Measurements were initiated 10 min after FG 7142 (10 mg/kg i.v.) or vehicle (1% Tween 20 in saline), and the rate of glucose utilization was determined in over 40 brain regions. FG 7142 effected increases in glucose use in selected areas of the limbic system (Table 1). Significant increases in glucose use were observed in the anterior thalamus (increased by 32%), the molecular layer of the hippocampus (20%) and the dentate gyrus (14%). Increases in glucose use in the basolateral amygdala (23%) and the dorsal tegmental nucleus (15%) approached statistical significance. In other limbic regions, such as the septal nucleus and the nucleus accumbens glucose use was unchanged. There was no consistent change in glucose use in the remaining regions examined.

Table 1 Effect on FG 7142 on glucose utilization in limbic structures

Region	Vehicle (n = 4)	FG 7142 (n = 5)
Anterior thalamus	100 ± 5	133 ± 7*
Hippocampus (molecular layer)	73 ± 3	87 ± 5*
Dentate gyrus	61 ± 2	70 ± 3*
Septal nucleus	60 ± 4	58 ± 4
Amygdala (basolateral)	70 ± 6	86 ± 5
Nucleus accumbens	75 ± 4	76 ± 8
Lateral habenular nucleus	102 ± 5	115 ± 5
Dorsal tegmental nucleus	89 ± 3	103 ± 5
Median raphe nucleus	83 ± 4	91 ± 4
Dorsal raphe nucleus	77 ± 6	88 ± 4

Data are presented as mean glucose use (μ mol/100g/min) ± S.E.M.
* $P < 0.05$, Student's t-Test.

These data show that pharmacological manipulation with this anxiogenic β-carboline can produce selective changes in specific components of the limbic circuitry that are readily identifiable by 2-deoxyglucose autoradiography.

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Pellow, S & File S.E. (1984) Psychopharmacology 83, 304-315.
Sokoloff, L. et al. (1977) J. Neurochem. 28, 897-916.

DIFFERENTIAL EFFECTS OF SPECIFIC α-ADRENOCEPTOR ANTAGONISTS ON CATECHOLAMINE MEDIATED BEHAVIOUR.

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Apomorphine and amphetamine induce a wide range of behaviours in rats. Locomotor activity is present at low dosage whereas localised stereotyped activities occur as dose increases. The importance of nigrostriatal and mesolimbic dopamine (DA) systems in the mediation of these responses is well established. However, due previously to the absence of specific pharmacological agents, the influence of noradrenaline (NA) remains in doubt. Recently, the specific NA neurotoxin DSP4 has been shown to differentiate between amphetamine-induced locomotion and stereotypy (Ogren, Archer and Johansson, 1983). The present study investigates the effects of specific and selective α1- and α2-antagonists (prazosin and idazoxan) on apomorphine and amphetamine induced behaviour.

Locomotor/exploratory activity of single rats (locomotion, rearing and hole dipping) was measured at 10 min intervals after injection, using infra-red sensors in an automated arena (60 x 60 cm). Stereotyped activity (sniffing, licking, gnawing etc) was measured in circular perspex cages (diameter 38 cm) with a wire grid floor. During observation periods (1 min every 10 min) behaviours were individually assessed by an observer (double blind) using scales of 0-4 (Dickinson and Curzon, 1983). All rats were familiarised (60 - 120 min) to their environment before testing.

d-Amphetamine sulphate (2mg/kg, i.p.) induced intense locomotion, rearing and hole-dipping commencing 10 min after injection and lasting 90-150 min. Pretreatment with the α1-antagonist prazosin HCl (1mg/kg, s.c., 60 min), a dose without effect alone on normal activity, potently inhibited the hyperactivity at every time point. A similar inhibition has been reported by Pifl and Hornykiewicz (1985) In contrast, the specific α2-antagonist idazoxan HCl (20 mg/kg, p.o.) did not alter amphetamine induced locomotor/exploratory activity.

Idazoxan (20 mg/kg, p.o.) alone induced small but significant increases of activity up to 30 min post-injection. This contrasts with the findings of Chopin et al (1986) who reported a decrease of locomotion and exploration when using the non-specific α2-antagonist yohimbine (which also acts at α1-, DA, 5-HT and benzodiazepine receptors).

Apomorphine HCl (0.5 mg/kg, s.c.)induced biphasic locomotor/exploratory activity. An initial intense period (0-70 min) was followed by a secondary phase of lower magnitude (90 -130 min). Neither α-antagonist altered the initial activity period although prazosin inhibited whereas idazoxan significantly extended the secondary phase.

At higher dosage, amphetamine (6 mg/kg, i.p.) and apomorphine (2mg/kg, s.c.) induced localised stereotyped behaviours. Amphetamine-induced sniffing was significantly decreased by prazosin. Idazoxan decreased amphetamine induced oral behaviour. Apomorphine-induced sniffing and forward movements were also decreased by prazosin whereas gnawing was significantly increased. In contrast, idazoxan increased apomorphine-induced forward movement and sniffing.

These data indicate that the specific α-adrenoreceptor antagonists prazosin (α1) and idazoxan (α2) affect the expression of both locomotor and stereotyped behaviour induced by apomorphine and amphetamine in the rat. A differential regulatory role for NA is suggested in which an increase of NA-functional activity may facilitate locomotion and inhibit stereotyped behaviour whereas a decrease of NA-function may inhibit locomotion and enhance stereotyped behaviour.

Chopin, P., Pellow, S., File, S.E. Neuropharmacol. 25, (1986) 53-57.
Dickinson, S.L., Curzon, G. Neuropharmacol. 22 (1983) 805-812.
Ogren, S.O., Archer, T., Johansson, C. Neurosci. Lett. 43 (1983) 327-331.
Pifl Ch.F., Hornykiewicz, O. Naunyn-Schmiedeberg's Arch. Pharmacol. Suppl. 330 (1985) R71.

EVIDENCE FOR A HUMORAL FACTOR AFFECTING FOOD INTAKE IN RATS

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Experimental evidence suggests that humoral factors are involved in the regulation of food intake and are probably peptides of gastrointestinal origin (Coleman 1973, Parameswaran et al. 1977). Several known gastrointestinal hormone-like peptides have been proposed as food intake regulating factors (Woods et al. 1981). However, their role in the control of food intake remains unclear and questionable (Deutsch et al. 1981).

This study investigates the existence of factors involved in the regulation of food intake by fractionating plasma from fed rats and administering these fractions to fasted rats to investigate their effect on food intake.

Ultrafiltration methods were used to fractionate plasma taken from Wistar rats after feeding. Fractions contained material whose molecular weights ranged from 0 to 10 and from 10 to 30 kD. These fractions were freeze-dried and desalted.

An in vivo food intake monitoring system was developed using Wistar rats trained to bar-press to receive food pellets. A feeding schedule restricted to 3 hours per day was used. The system was standardised using known peptides and fenfluramine. Plasma fractions, redissolved in NaCl (0.9%), were administered intraperitoneally 15 min prior to food presentation.

Both fractions obtained from rat plasma (500ml) were tested. Each test animal (302 ± 11 g) received either 7% (n=3) or 20% (n=3) of the 0 to 10 kD fraction or 30% (n=3) of the 10 to 30 kD fraction. The low dose of the 0 to 10 kD fraction caused a 7% reduction, the high dose a 34% reduction in food intake during the feeding period. Throughout the experiment all animals otherwise exhibited normal behaviour. No reduction in food intake was observed in those animals treated with the 10 to 30 kD fraction.

The results suggest the existence of a humoral factor affecting food intake which is released after feeding and has a molecular weight under 10 kD.

Coleman, D.L., (1973) Diabetologia 9, 294-298.
Deutsch, S.A., Hardy, W.T., (1981) Nature 266, 196.
Parameswaran, S.V. et al., (1977) J. Comp. Physiol. Psychol. 84, 488-495.
Woods, S.C. et al., (1981) Diabetologia 20, 305-313.

CONTRASTING EFFECTS OF (+) AND (-)-N-ALLYL-NORMETAZOCINE DURING DRUG DISCRIMINATION TRIALS

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Whilst there exists a range of biochemical and behavioural evidence that phencyclidine (PCP) and N-allylnormetazocine (NANM) act at a common receptor, other reports suggest that some of the effects of NANM are mediated at a site with which PCP does not interact (Tam and Cook, 1984; Downes et al., 1986). The present studies explore this possibility using an experimental approach based on a standard two lever drug discrimination procedure as described previously (Blackman et al., 1985). Rats were trained to discriminate either (+)NANM or (-)NANM (both 3 mg/kg, s.c.) from saline (1 ml/kg, s.c.). The results of generalisation trials indicated that (+)NANM generalised equally effectively to (+)NANM and (-)NANM, (-)NANM generalised to (-)NANM but not to (+)NANM, (±)NANM generalised to both (+)NANM and (-)NANM, whilst PCP generalised to neither. Although both (+)NANM and (-)NANM generalised in rats trained to discriminate (-)NANM from saline, (-)NANM was five times more potent than (+)NANM in this respect. Buprenorphine and cyclazocine generalised to (-)NANM. Ethylketocyclazocine, nalorphine and morphine failed to generalise to either (+)NANM or (-)NANM. The following data assist in the interpretation of these results: (a) (+)NANM and (-)NANM have almost equal affinities for [3H] PCP binding sites (Zukin et al, 1984). (b) PCP has five times higher affinity for [3H] PCP binding sites than [3H] (+)NANM sites (Downes et al., 1986). (c) (+)NANM has 37.5 times higher affinity than (-) NANM and 30 times higher affinity than PCP for [3H] (+)NANM binding sites (Tam and Cook, 1984; Downes et al., 1986). (d) (+)NANM and (-)NANM were equi-effective in generalising to the effects of PCP in rats but were both 1/5th as potent as PCP in this respect (Shannon, 1982). (e) (-)NANM has 580 times higher affinity for mu opiate binding sites than (+)NANM (Zukin et al., 1984). (f) (-) but not (+)NANM possesses opioid partial agonist effects in vivo (Aceto and May, 1983). (g) (+)NANM has at least ten times higher affinity than (-)NANM for a CNS binding site which is neither an opiate nor a dopamine-2 site (Su, 1981; Tam and Cook, 1984). This site is characterised by its very high affinity for haloperidol and (-) butaclamol. (h) (+)NANM has eight times higher affinity for this non-opiate, non-dopamine-2 binding site than for PCP binding sites (Gundlach et al., 1985). Thus it seems possible that (i)(+)NANM/saline trained rats can use an interoceptive cue provided either by a PCP site or of a non-opiate, non-dopamine-2 brain binding site and (ii) (-)NANM/saline trained rats discriminate not only through opiate receptors (to which buprenorphine generalises) but also through either a PCP or a non-opiate, non-dopamine-2 site (to which (+)NANM and cyclazocine generalise).

Aceto, M.A. and May, E.L. (1983). Eur. J. Pharmac., 91, 267-272.
Blackman, D.E., McCarthy, P.S. and Naylor, V.J.H. (1985). Br. J. Pharmac., 85, 295P.
Downes, C.P., Lewis, P.E. and Stone, M.A.(1986). Br. J. Pharmac., 88, 231-237.
Gundlach, A.L., Largent, B.L. and Snyder, S.H. (1985). Eur. J. Pharmac., 113,465-466.
Shannon, H.E. (1982). Eur. J. Pharmac., 84, 225-228.
Su, T-S (1981). Eur. J. Pharmac., 75, 81-82.
Tam, S.W. and Cook, L. (1984). Proc. Natl. Acad. Sci., 81, 5618-5621.
Zukin, S.R., Brady, K.T., Slifer, B.L. and Balster, R.L. (1984). Brain. Res., 294, 174-177.

NEUROTRANSMITTER RELEASE INTERACTIONS IN THE RAT STRIATUM.

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In addition to intrinsic GABA and cholinergic interneurons, striatum receives neuronal inputs from cortex (glutamatergic) and substantia nigra (dopaminergic). GABA fibres also project from striatum to nigra. We report on the interactions existing between these neuronal systems as assessed using basal and calcium-dependent potassium evoked release of labelled neurotransmitters from rat striatal slices.

Striatal slices (0.3 x 0.3mm) from male Wistar rats were incubated with ^{3}H-dopamine (50 nM), ^{3}H-choline (40 nM) or ^{3}H-GABA (30 nM) and perfused with Krebs medium modified as appropriate to prevent the breakdown of dopamine (plus pargyline 40 µM) or GABA (plus aminooxyacetic acid 0.5 µM), or reuptake of choline (plus hemicholinium-3 10 µM). After 30 min washout, slices were exposed to drugs or neurotransmitter substances usually for 8 min, immediately preceeding and also during 4 min exposure to 15 mM potassium chloride.

The basal release of ^{3}H-acetylcholine from rat striatal slices was increased by exposure to GABA, glutamate or kainate but not dopamine or apomorphine. In contrast, dopamine or apomorphine reduced potassium evoked ^{3}H-acetylcholine release while GABA, glutamate and kainate were without effect (Table 1). Only GABA altered (increased) basal release of ^{3}H-dopamine; all other compounds tested were ineffective. The potassium evoked release of ^{3}H-dopamine was increased by GABA and decreased by both kainic acid and glutamate with no effect of acetylcholine. Basal release of ^{3}H-GABA was increased by glutamate and kainate but not by dopamine, apomorphine or acetylcholine. Potassium evoked ^{3}H-GABA release was decreased by apomorphine, but not dopamine or acetylcholine, and increased by glutamate and kainate.

Table 1 Potassium evoked release of tritiated neurotransmitters

Treatment		Exposure	% control evoked release ^{3}H-acetylcholine	^{3}H-dopamine	^{3}H-GABA
Dopamine	100 µM	8	60 ± 12*	NT	0
Apomorphine	100 µM	8	42 ± 4*	NT	57 ± 6*
Acetylcholine	1 µM	8	NT	0	0
GABA	10 µM - 1 mM	20	0	150 ± 18*	NT
Glutamate	10 mM	8	0	60 ± 10*	156 ± 15*
Kainate	10 mM	8	0	54 ± 11*	130 ± 7*

* $P < 0.05$; NT = not tested

Overall, only GABA, glutamate or kainate altered basal neurotransmitter release. Similarly, the potassium evoked release of ^{3}H-dopamine and ^{3}H-GABA appeared to be modulated by these substances and not by acetylcholine or dopamine respectively. In contrast, dopamine and apomorphine but not GABA, glutamate and kainate altered potassium evoked ^{3}H-acetylcholine release. The present results demonstrate the possibility of studying in one experimental system the complex neuronal interactions in striatum reported by other workers (see Chesselet, 1984).

Chesselet, M-F. (1984) Neuroscience 12, 347-375.

MKB is an MRC Student.

CHRONIC TRIFLUOPERAZINE TREATMENT DOES NOT INDUCE LIPID PEROXIDATION IN RAT CORTEX.

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Recently, haloperidol was shown in vitro to increase lipid peroxidation when incubated with rat cortical synaptosomes (Sawas et al, 1985). We now report on parameters associated with lipid peroxidation in the cortex of rats receiving chronic trifluoperazine treatment.

Male Wistar rats (initial weight 200-250g; Bantin & Kingman Ltd.) received either trifluoperazine dihydrochloride (4.4-4.9 mg/kg/day) dissolved daily in distilled water or distilled water alone for 15 months. In cortical tissue from these animals indices of lipid peroxidation were measured. Formation of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid following incubation of cortical homogenate at 37°C for 90 minutes in air alone or following the addition of 0.01mM $FeSO_4$ plus 0.25mM ascorbic acid or 200mM H_2O_2 plus 2mM $FeSO_4$ to stimulate lipid peroxidation. Basal levels of MDA formation were measured immediately after the homogenization of the cortex. MDA formed was reacted with thiobarbituric acid (TBA) under acid conditions and the product was measured by both spectrophotometric and fluorometric techniques. In the same samples the levels of polyunsaturated fatty acids (PUFA) were measured by a spectrophotometric technique following chloroform:methanol (2:1 v/v) extraction.

The PUFA content of cortical homogenates from control animals and from rats receiving trifluoperazine treatment for 15 months were identical (Table 1). There was no difference in basal levels of MDA in cortical homogenates from trifluoperazine treated rats compared to control rats. Incubation of cortical homogenates in air at 37°C for 90 minutes caused equivalent increases in MDA formation in tissue from both control and drug treated animals. Similarly, incubation of samples with either $FeSO_4$ plus ascorbic acid or H_2O_2 plus $FeSO_4$ also increased MDA formation but again there was no difference in the degree of stimulation between cortical tissue from trifluoperazine or control rats.

Table 1 Levels of MDA formation and PUFA's in rat cortex

Treatment	PUFA's (nmol linolenic acid/mg protein)	Lipid peroxidation (nmol MDA/mg protein above basal levels) Technique	Air	0.01mM $FeSO_4$ + 0.25mM Ascorbic acid	200mM H_2O_2 + 2mM $FeSO_4$
Control	477±30	Fluorometric	3.8±0.2	28.8±1.3	18.9±1.1
		Spectrophotometric	2.0±0.1	27.6±1.1	15.27±0.6
Trifluoperazine	456±25	Fluorometric	3.6±0.2	25.7±0.9	17.4±1.1
		Spectrophotometric	2.1±0.1	26.5±1.4	15.0±0.6

Values expressed as mean ± SEM; n = 12.

In contrast to the in vitro effect of haloperidol, chronic treatment with trifluoperazine does not alter basal levels and the stimulated production of malondialdehyde.

Sawas, A.H. et al. (1985) Arch. Int. Pharmacodyn. 276, 301-312.

DETECTION OF [^{3}H]-SCH 23390 BINDING SITES IN TISSUE SLICES FROM RAT BRAIN STEM.

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The rat brain stem contains previously unidentified populations of D-2 dopamine receptors identified by the specific binding of [^{3}H]spiperone (Chivers et al, 1984) and [^{125}I]iodosulpride (Martres et al, 1985). These regions may also contain dopamine D-1 binding sites and we now report preliminary studies aimed at detecting specific binding sites for [^{3}H]SCH 23390 using slices of rat brain.

Sections (20 µM) of perfused male Wistar rat (150 ± 10g) brain were cut at -20°C between +8.62 to +7.47mm (Konig & Klippel, 1963) to include striatal tissue. The remaining portion of the brain was sectioned horizontally dorsal to the substantia nigra and slices were taken from the upper portion between +1.76 to -0.48mm to include the superior and inferior colliculi, the mesencephalic reticular formation and periaqueductal gray. Thawed slices were incubated for up to 2h with [^{3}H]SCH 23390 (0.1 - 1.0nM; 67.0 Ci/mmol) in the presence and absence of cis-flupenthixol (10^{-8} - 10^{-5}M). Following thorough rinsing, radioactivity per pair of slices was estimated by liquid scintillation spectrometry. In slices containing striatal tissue approx. 85% or more of total [^{3}H]SCH 23390 binding was prevented in the presence of 10^{-5}M cis-flupenthixol. Specific binding was saturable and reached a maximum of approx. 36 fmoles/2 slices at higher [^{3}H]SCH 23390 concentrations (Figure 1). Incorporation of cis-flupenthixol (10^{-8} - 10^{-5}M) caused a dose-dependent inhibition of [^{3}H]SCH 23390 (0.7 nM) binding. In slices of the brain stem approx. 40% of total [^{3}H]SCH 23390 binding was prevented in the presence of 10^{-5}M cis-flupenthixol. Specific binding was saturable and reached a maximum of approx. 2.3 fmoles/2 slices at higher [^{3}H]SCH 23390 concentrations (Figure 1). Incorporation of cis-flupenthixol (10^{-8} - 10^{-5}M) caused a dose-dependent inhibition of [^{3}H]SCH 23390 (0.7 nM) binding.

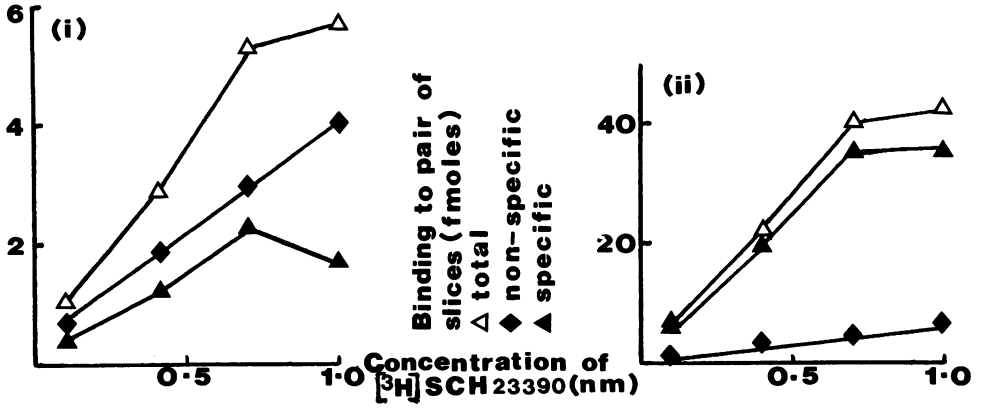

Figure 1 [^{3}H]SCH 23390 binding of slices of (i) brain stem and (ii) striatum

In rat brain stem slices [^{3}H]SCH 23390 binding, as defined by 10^{-5}M cis-flupenthixol appears to identify a population or populations of specific binding sites.

Chivers et al. (1985) J. Pharm. Pharmacol. 36, 484-488.
Martres et al. (1985) Science 288, 752-754.

IN VIVO MICRODIALYSIS STUDIES ON DOPAMINE RELEASE FROM THE STRIATUM EVOKED BY NIGROSTRIATAL STIMULATION

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A considerable body of evidence supports the candidacy of dopamine as the neurotransmitter utilised by a majority of neurones in the nigrostriatal pathway (Graybiel and Ragsdale, 1983). For example, researchers using both push-pull cannulae and voltametric methods have demonstrated that stimulation of this pathway in vivo increases dopamine release from the striatum (Glowinski et al., 1978; Ewing et al, 1983). In the present study we have further examined the in vivo release of endogenous dopamine in response to nigrostriatal stimulation using brain microdialysis.

Wistar rats (260-300 g; either sex) were anaesthetised and tracheotomised. Anaesthesia was then maintained using halothane (< 1%). Burr holes were drilled in the skull directly above the striatum (AP + 0.5; ML ± 2.5) and the medial forebrain bundle (AP - 3.8, ML ± 1.0). A cannula type dialysis probe was positioned into the striatum, and a steel concentric electrode placed in the medial forebrain bundle. The nigrostriatal pathway was stimulated at 0.5 mA intensity at 100 H_Z for 0.5 msec at 1 second intervals for 10 mins. The dialysis probe was perfused continuously (2.5 µl/min) with oxygenated Krebs bicarbonate buffer and dialysate samples (25 µl) were collected at 10 min. intervals. Dopamine (DA), 3,4 dihydroxyphenylacetate (DOPAC) and homovanillic acid (HVA) were measured by high performance liquid chromatography and electrochemical detection.

The basal dialysate concentration of each compound was: DA 6.5 ± 0.7 nM; DOPAC, 0.5 ± 0.1 µM; HVA 0.2 ± 0.1 µM). Electrical stimulation did not affect the release of these compounds. Inclusion of the dopamine uptake inhibitor, nomifensine (1 µM) in the perfusion medium did not affect either basal or stimulated levels of DA, DOPAC and HVA. These parameters were also unaffected when 4-aminopyridine (1 mM; a dose which facilitates Ca^{2+} entry into nerve terminals) was added to the perfusion medium. In a final series of experiments, ouabain (an inhibitor of Na^+/K^+ ATPase) was perfused at a dose which did not influence basal DA or metabolite levels. In the presence of 10 µM ouabain electrical stimulation induced a rapid and reliable increase (180 % of control) in DA release, without affecting metabolite levels.

These data demonstrate that the microdialysis technique can be used to evaluate DA efflux in response to afferent stimulation under appropriate conditions.

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Ewing, A.G., Bigelow, J.W. and Wightman, R.M. (1983). Science 221: 169-170.
Glowinski, J., Nieoullon, A. and Cheramy, A. (1978). In: Dopamine (Roberts, P.J., Woodruff, G.N. and Iverson, L.L.; eds.) pp. 75-88.
Graybiel and Ragsdale (1983). In: Chemical Neuroanatomy (Emson P.C. ed.) pp. 427-504.

ON THE MECHANISMS UNDERLYING AMPHETAMINE INDUCED DOPAMINE RELEASE: IN VIVO MICRODIALYSIS STUDIES.

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The psychostimulant, amphetamine (AMPH) is believed to interact with dopaminergic neurones in the brain. AMPH facilitates dopaminergic responses via a number of mechanisms, including a stimulation of dopamine (DA) efflux (see Zetterstrom et al., 1983; Parker and Cubeddu, 1986). Although the precise mechanism by which AMPH influences DA release is unclear, the finding that this response is inhibited by the dopamine uptake inhibitor, nomifensine, is clearly of great importance (Arbuthnott et al., 1985; Parker and Cubeddu, 1986). In the present study we examined the effects of other agents which influence DA uptake and release on basal and AMPH stimulated DA efflux using the in vivo brain microdialysis method.

Wistar rats (250-280g; either sex) were anaesthetised, tracheotomised and placed in the stereotaxic frame. Anaesthesia was then maintained using halothane (< 1.5%). Cannula type dialysis probes were positioned into both striata (AP + 0.5; ML ± 2.5) and perfused with oxygenated Krebs bicarbonate buffer at 1.25 µl/min. Dialysis samples were analysed for DA and related metabolites (3,4 dihydroxyphenylacetate; DOPAC and homovanillic acid; HVA) by high performance liquid chromatography and electrochemical detection. Ouabain (10-1000 µM) or veratridine (10-500 µM) were included in the perfusion buffer as indicated. AMPH 4mg/kg) was administered by a single intraperitoneal injection.

The basal dialysis concentrations of DA and related compounds were: DA, 18.3 ± 0.5 nM; DOPAC, 1.3 ± 0.2 µM; HVA, 0.6 ± 0.2 µM). AMPH induced rapid increase (450 %) in DA, and a parallel drop in DOPAC and HVA. Although ouabain (10 µM) did not influence basal levels of any compound, a striking facilitation of the AMPH response was noted. Moreover, increasing concentrations of ouabain induced a massive increase in dialysis DA concentration (20-40 fold), which was not affected when nomifensine (1µM) was coperfused. Veratridine also provoked a dose related increase in the extracellular concentration of DA, and a decrease in DOPAC and HVA.

These data demonstrate that AMPH, ouabain and veratridine all increase DA efflux from the striatum. However, it is unlikely that these actions are mediated by a common mechanism.

S.P.B. is supported by an M.R.C. Training Fellowship and I.S.F by an M.R.C. Scholarship.

Arbuthnott, G.W., Ungerstedt, U. and Walker, R. (1985). Brit.J.Pharm. 84, 10P

Parker, E.M. and Cubeddu, L.X. (1986). J.Pharmacol.Exp.Therap. 237: 179-192

Zetterstrom, T., Sharp, T., Marsden, C.A. and Ungerstedt, U. (1983). J.Neurochem. 41: 1769-1773

880P

CHANGES IN AMINO ACID LEVELS IN HIPPOCAMPAL DIALYSATES ASSOCIATED WITH EARLY TRIMETHYLTIN NEUROTOXICITY.

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Trimethyltin (TMT) is a neurotoxin which is a byproduct in the manufacture of dimethyltin. TMT produces hyperactivity and seizures in several animal species and selective lesions in the hippocampus, pyriform cortex and amygdaloid nucleus. The following experiments were designed to try to correlate neurochemical effects of TMT in the hippocampus with the time course of histopathology in that region. The brain dialysis technique was adapted for these studies to monitor changes in levels of GABA, glutamine, glutamate and aspartate in the extracellular compartment of the hippocampus in non-anaesthetised, freely-moving rats before and up to 51 hours after an oral dose of TMT chloride (10mg/kg) or vehicle (arachis oil, 1ml/kg). A looped dialysis probe, implanted stereotaxically into the dorsal hippocampus, was perfused with sterile artificial CSF at 2µl/min. GABA levels were determined in 15µl dialysate by radioreceptor microassay. Amino acids (20µl samples) were analysed by automated ion-exchange chromatography. A separate group of animals dosed in the same way but not dialysed,were prepared for histopathological analysis at 24 and 48 hours after treatment.

TABLE: Percent changes (±sem) relative to predose levels in hippocampal dialysates after TMT chloride (10mg/kg) or vehicle.

		1-4hr	22-28hr	46-51hr
Glutamine	control	84±11	128±12	134±23
	treated	110±12	233±36*	395± 8*
Glutamate	control	102±14	127±19	118±20
	treated	108±21	160±18	205±29*
GABA	control	68±15	57±23	64±23
	treated	95±13	101±18	144±20*
Aspartate	control	114±14	92±12	94± 3
	treated	95±16	85±13	95± 6

*P<0.05,Students t test, control vs treated,(n = 3-5)

A significant elevation in glutamine levels at 22 hrs was the earliest change following TMT treatment. By 46 hrs glutamate and GABA, but not aspartate, were also significantly increased. During the 22-28 hr dialysis period, the behaviour of control and treated rats was the same. However by 46 hr most TMT-treated rats were more active than controls, exhibiting increased noticeability, aggression and irritability. Corresponding histopathological changes were as follows. 24 Hr after dosing there were only a small number of altered pyramidal neurones in the CA3b region of the hippocampus. This consisted of variable numbers of small cytoplasmic vacuoles. By 48 hr, neurones throughout CA1-CA4 regions and fascia dentata were altered. This consisted of vacuolated cytoplasm packed with small granules and some nuclear changes. The results suggest that the early glutamine changes may be associated with neuronal damage induced by TMT.