DIFFERENT ROLES FOR PLATELET-DERIVED THROMBOXANE A_2 (TXA_2) IN PLATELET ACTIVATION AND CORONARY ARTERIAL VASOCONSTRICTION

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In addition to being a powerful platelet stimulating agent, TXA_2 has also been suggested to be a potent, platelet-derived vasoconstrictor (Ellis et al, 1976). The present study was designed (1) to dissociate platelet and vascular actions of endogenous TXA_2 by means of a synthetase inhibitor (dazoxiben) and a platelet PG endoperoxide/TXA_2 receptor blocking agent (BM 13.177) (Patscheke et al, 1984); (2) to separate vasocontractile activities exerted by products of the platelet release reaction (serotonin) (5-HT) from those exerted by cyclooxygenase metabolites of arachidonic acid (AA).

Washed human platelet suspensions (WPS) (3.2×10^8 pl./ml) were incubated at 37^0C with buffer and stimulated with AA (30 µM). Transfer of the incubate into an organ bath, containing a bovine coronary artery strip (BCA) in Krebs-Henseleit buffer and a mixture of blocking agents, including indomethacin, resulted in a biphasic vessel contraction: A first, rapid phase caused by TXA_2 and a second, sustained contraction caused by 5-HT release from the WPS (Schrör et al, 1984).

Treatment of the WPS with BM 13.177 (0.03 - 30 µM) prior to stimulation by AA resulted in a dose-dependent inhibition of 5-HT release (IC_{50}: 0.1 µM), as seen from the inhibition of the 5-HT mediated component of coronary vasoconstriction. BM 13.177 did not antagonize BCA contractions evoked by exogenous 5-HT (1 µM). Addition of BM 13.177 to the BCA prior to AA-stimulated WPS resulted in a dose-dependent (3-300 µM) inhibition of TXA_2 mediated vasoconstriction (IC_{50} about 20 µM). This IC_{50} of BM 13.177 in inhibiting BCA contractions mediated by the natural TXA_2 was considerably higher than that against $PGF_{2\alpha}$ (IC_{50}: 6 µM) or the synthetic TX mimetic U-46,619 (IC_{50}: 0.5 µM). There were no major changes in the PGE_2 and 6-oxo-$PGF_{1\alpha}$ content of the bath fluid (RIA). TX formation (RIA for TXB_2) amounted to 163 + 9 nM under control conditions (AA-stimulated WPS, n = 11), was elevated to 211 $\mp$ 10 nM after pretreatment with 0.3 µM BM 13.177 ($P < 0.05$, n=12) and was reduced to 101 $\pm$ 6 nM after pretreatment with 30 µM BM 13.177 ($P < 0.01$, n = 7). In contrast, treatment of the WPS with dazoxiben (0.1 - 10 µM) prior to AA stimulation resulted in a dose-dependent inhibition of TX formation (IC_{50}: 0.2 µM) with a corresponding increase in PGE_2 and very small elevations in 6-oxo-$PGF_{1\alpha}$, similar to changes previously found with clotting whole blood (Pitzke et al, 1983). This TX inhibition by dazoxiben was paralleled by an inhibition of TXA_2-mediated BCA contraction, whereas the 5-HT mediated component was unchanged. This indicates an undisturbed release reaction even at nearly complete (> 98%) inhibition of the TX synthetase. This may be caused by PG-endoperoxide accumulation, replacing TXA_2 at its binding sites on the platelet surface.

In conclusion: (1) Both TXA_2 and 5-HT are significant platelet-derived coronary vasoconstrictors. However, a primary target of TXA_2 seems to be the platelet rather than the vessel wall. (2) BM 13.177 is a potent antagonist of the platelet PG-endoperoxide/TXA_2 receptor but much less effective against "pure" TXA_2-mediated contractions of the vasculature. This dissociation cannot be seen with the synthetic analogue U-46,619. (3) BM 13.177 is a highly effective blocker of the platelet release reaction in this model whereas dazoxiben is not.

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CHANGES IN CATECHOLAMINE INHIBITORY EFFECTS IN THE RAT ISOLATED UTERUS INDUCED BY OVARIECTOMY AND CYCLO-OXYGENASE INHIBITION

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Catecholamine induced relaxation of rat uterus is mediated mainly through β-adrenoceptors (Boyle & Digges, 1982), but β-adrenoceptor agonists produce different degrees of inhibition throughout the oestrous cycle in both controls and in uteri in which α-adrenoceptors and catecholamine-removal processes are blocked (Boyle & Ohia, 1984). The mechanisms underlying these differences are unclear. Two possible explanations lie in the presence of the ovarian hormones and the generation of prostaglandins within the tissue (Gimeno & Gimeno, 1984). The present study was designed to examine the roles of the hormones and prostaglandins, by investigating adrenoceptor agonist activity in ovariectomized rats and in rats after treatment with the cyclo-oxygenase inhibitor flubiprofen (FBF) to inhibit prostaglandin synthesis.

Virgin female untreated (180-250g) and ovariectomized (200-260g) Wistar rats were used. The four phases of the oestrous cycle were identified from vaginal smears. 2-3cm lengths of each uterine horn were set up in paired 10ml organ baths containing Tyrodes solution at 37°C and gassed with 95% O_2/5% CO_2. Since the rat uterus has no intrinsic tone, inhibitory responses to adrenoceptor agonists and to the smooth muscle relaxant papaverine (PAP) were measured as the percentage inhibition of sub-maximal (60-70%) acetylcholine (ACh)-induced contractions, measured isometrically. The agonists were added to the organ baths 30s before addition of ACh. FBF (10^{-6}M), added to the Tyrodes solution, was in contact with the tissue for at least 30 min before addition of ACh. FBF had no effect on the response to ACh in either control or ovariectomized rats.

Noradrenaline (NA), adrenaline (ADR) and salbutamol (SAL), each produced a dose dependent inhibition of ACh in both control (NA 10^{-9}-10^{-5}M; ADR 10^{-10}-10^{-7}M; SAL $5x10^{-9}$-$5x10^{-6}$M) and in ovariectomized rats (NA $3x10^{-10}$-10^{-6}M; ADR 10^{-11}-$3x10^{-8}$M; SAL $5x10^{-11}$-$5x10^{-8}$M) but in the latter, the effects of the amines were enhanced significantly (e.g. in metoestrus phase, % maximal inhibition of ACh for NA is 44.6±5.4, n=6 whilst in ovariectomized rats, it is 100±0.0, n=6. $P < 0.001$) and each amine consistently abolished the ACh-induced contraction.

FBF treatment also enhanced significantly the effects of the three amines in untreated rats and each amine again abolished ACh contractions. FBF shifted the dose-response curve of each amine to the left in both untreated and ovariectomized rats.

In contrast to the adrenoceptor agonists PAP ($3x10^{-7}$-10^{-4}M) inhibited ACh induced contractions completely in all stages of oestrous and was unaffected by FBF.

These results show that removal of ovarian hormones and inhibition of prostaglandin synthesis enhance β-adrenoceptor agonist inhibitory effects and suggest that the presence of ovarian hormones and prostaglandin generation underlie the differences observed in the inhibitory effects of NA, ADR and SAL. The site and mechanism of the interaction is not known, but the effectiveness of PAP in inhibiting ACh contractions is consistent with an intracellular mechanism which is also linked with adrenoceptor activation.

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PH CL 28A: HIGHLY POTENT INHIBITOR OF PROSTAGLANDIN 15-HYDROXY-DEHYDROGENASE LACKING EFFECTS ON PROSTAGLANDIN SYNTHETASE

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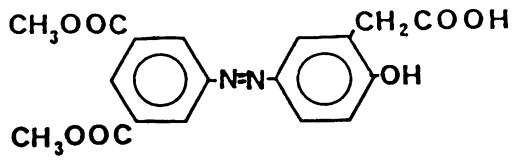
We have shown that structurally related analogues of sulphasalazine inhibit the inactivation of prostaglandins in cytosolic systems and by purified prostaglandin 15-hydroxydehydrogenase, PGDH (Berry et al, 1983). Optimal activity was shown by azo analogues lacking the pyridine ring of sulphasalazine, but containing an additional methylene at the carboxyl group (see illustration). The fact that biological activity is strongly dependent on the nature of the substituent in the B ring prompted attempts to develop more active compounds containing different substituents. This communication describes the activity of Ph CL 28A (3,5-dicarbomethoxy-3'-carboxymethyl-4'-hydroxy-azobenzene) (see illustration for structure), the most active inhibitor tested to date.



The action of CL 28A was studied using purified human placental PGDH, 200 mU/ml, a gift from Dr J Jeffery, Aberdeen University, which was incubated with radiolabelled $PGF_{2\alpha}$ as described in Berry et al, 1983. Using freshly prepared solutions, CL 28A inhibited PGDH with an IC_{50} of 28 ± 6 nM, compared to sulphasalazine 21 ± 1 μM. Solutions kept > 1 day showed much less inhibitory activity (eg, IC_{50} 2.5 μM) due to hydrolysis to the equivalent 3,5- dicarboxylic acid (shown by hplc); the pure dicarboxylic acid had an IC_{50} of 1.4 μM. PGDH inhibition by CL 28A was non-competitive with regard to both substrate and the NAD^+ cofactor; this was confirmed by Dixon plot analysis which yielded a K_i value of 31.6 ± 4.6 nM (cf. 41.1 ± 2.8 μM for sulphasalazine). Furthermore, as shown for other azo analogues, the extent of inhibition was strongly dependent on pH (inhibition 58.7 ± 3.5% for 50 μM CL 28A at pH 7.3 in rat colon supernatant, reduced to 8.7 ± 0.5% at pH 9.8), even though there was no difference in enzyme catalytic activity over this pH range. PGDH inhibition by CL 28A was also reversible by dilution, as shown for other analogues. In experiments using isolated perfused rat lung, CL 28A inhibited the inactivation of 100 ng bolus doses of $PGF_{2\alpha}$ with an IC_{50} of ca. 50 nM (i.e. some 1000 times more active than sulphasalazine).

In experiments using freshly prepared sheep seminal vesicle microsomes incubated with radiolabelled arachidonic acid, we found that like other similar azo analogues, concentrations of CL 28A from 1 to 1000 μM did not inhibit prostaglandin synthesis.

We conclude that CL 28A is a highly active non-competitive inhibitor of PGDH activity lacking anti-synthetase actions and that it may be useful in probing the function of PGDH in both intact organs and in vitro.

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MODULATION OF LEUKOTRIENE B_4 BIOSYNTHESIS IN HUMAN LEUKOCYTES

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Arachidonate metabolism via 5-lipoxygenase yields 5-hydroperoxy eicosatetraenoic acid which is then converted to LTB_4 via an unstable epoxide intermediate, leukotriene A_4 (LTA_4). Although LTB_4 release from intact human leukocytes is well documented, cell-free systems for LTB_4 biosynthesis have been limited to leukocytes from rodents (Maycock et al, 1982). We have previously reported a cell-free preparation of 5-lipoxygenase from human leukocytes and we now show the presence in human leukocyte cytosol of the additional enzymes necessary for LTB_4 biosynthesis. Peripheral human leukocytes were resuspended in 0.05M phosphate buffer containing 0.1% gelatin and 1mM glutathione then disrupted by Polytron homogenisation and sonication. The homogenate was centrifuged at 105,000 (60 min, 4°C) to yield the cytosol, which was used throughout as the enzyme source. Cytosol was incubated with arachidonate or LTA_4 and reactions were terminated using ethyl acetate. Lipids were fractionated on cyanopropyl mini-extraction columns and the diHETE fraction was separated on reverse phase HPLC.

Incubation of human leukocyte cytosol with arachidonate led to formation of LTB_4 as well as its major biological isomers: 6-trans isomers of LTB_4, which are formed by non-enzymic hydrolysis of LTA_4, and 5S12SdiHETE, a product of the concerted action of 5- and 12-lipoxygenases. Formation of LTB_4 and other diHETEs was maximal within 10 minutes at 37°C and no evidence for metabolism of diHETEs was obtained in this cell-free system. Direct evidence for the presence of "LTA_4 hydrolase" was obtained in studies using LTA_4. Incubation of LTA_4 with human leukocyte cytosol led to formation of LTB_4 and its 6-trans isomers whereas control incubations of LTA_4 in physiological buffer or boiled cytosol yielded only the 6-trans isomers. Formation of LTB_4 from LTA_4 was maximal within 10 minutes and no evidence was obtained for production of 5S12diHETE. At low concentrations of LTA_4 ($\leq$ 5μM) the major diHETE formed was LTB_4 but at higher LTA_4 concentrations the 6-trans isomers predominated, which is consistent with saturation of LTA_4 hydrolase at high substrate levels. Biosynthesis of LTB_4 from arachidonate and from LTA_4 exhibited the following differences: (1) LTB_4 biosynthesis from arachidonate had an absolute requirement for calcium ions, in accord with the calcium-dependence of human 5-lipoxygenase (McMillan et al, 1984), whereas LTB_4 biosynthesis from LTA_4 occurred in the absence of calcium. (2) Reduction of the incubation temperature to 21-25°C virtually abolished synthesis of LTB_4 from arachidonate but not from LTA_4. (3) A range of previously reported lipoxygenase inhibitors (BW755C, NDGA, Nafazatrom, Quercetin, Baicalein) potently inhibited production of LTB_4 from arachidonate (IC_{50} values of 10^{-8}M to 10^{-6}M) but at 10^{-5}M caused little inhibition of LTB_4 biosynthesis from LTA_4. Using this cell-free system, in conjunction with LTA_4 and arachidonate, will allow the precise site(s) of action of novel inhibition of LTB_4 biosynthesis to be determined.

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MEASUREMENT OF ARACHIDONATE METABOLISM USING CYANOPROPYL MINI-COLUMNS: EFFECTS OF CYCLO-OXYGENASE AND LIPOXYGENASE INHIBITORS

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Metabolism of arachidonic acid (AA) in rat basophilic leukaemia cells (RBL-1) occurs via both cyclo-oxygenase and 5-lipoxygenase pathways and enzymes from these cells are widely used for investigating potential inhibitors of AA metabolism. In these studies thin layer chromatography is usually employed to analyse AA metabolites. Since this technique is tedious and time-consuming, we have developed a mini-column fractionation procedure which provides a simple, rapid and reproducible technique for evaluating inhibitors of 5-lipoxygenase and cyclo-oxygenase.

The high speed supernatant (cytosol) from RBL-1 cells contained the majority of 5-lipoxygenase activity whilst cyclo-oxygenase activity was predominantly in the microsomes. Enzyme assays, using radiolabelled AA as substrate, were carried out at 37°C after pre-incubation of cytosol or microsomes with drugs at 4°C. Following extraction eicosanoids were reconstituted in hexane:ether (95:5) and applied to cyanopropyl mini extraction columns (Bond-Elut). AA was eluted using hexane:ether (75:25) and its metabolites were eluted using diethyl ether:methanol (50:50). The fractions were collected directly into scintillation vials and AA metabolism was quantified by liquid scintillation counting.

Metabolism of AA by the cytosolic 5-lipoxygenase was calcium-dependent and yielded primarily 5-HETE and diHETEs including LTB_4. The major cyclo-oxygenase product formed in RBL-1 microsomes in the presence of glutathione (1mM) was PGD_2 with smaller amounts of PGE_2 and $PGF_{2\alpha}$. The effects of a range of drugs that interfere with AA metabolism were investigated using these systems.

Quercetin, baicalein, nafazatrom and nordihydro guaiaretic acid potently inhibited 5-lipoxygenase activity (IC_{50} values 4.1×10^{-7}M, 1.8×10^{-6}M, 2.1×10^{-6}M and 5.5×10^{-7}M respectively) but caused less than 50% inhibition of cyclo-oxygenase activity at 10^{-4}M. In contrast, flurbiprofen, indomethacin and naproxen selectively inhibited cyclo-oxygenase (IC_{50} values 5.7×10^{-8}M, 9.0×10^{-7}M and 10^{-6}M respectively). Benoxaprofen profiled as a weak cyclo-oxygenase inhibitor ($IC_{50} = 4.1 \times 10^{-5}$M) but caused less than 50% inhibition of 5-lipoxygenase at 10^{-4}M. The prototype dual inhibitor BW 755C inhibited both 5-lipoxygenase ($IC_{50} = 3.1 \times 10^{-6}$M) and cyclo-oxygenase ($9.7 \times 10^{-6}$M).

We have obtained similar data for each compound with the RBL-1 enzymes using a t.l.c. procedure and with the exception of benoxaprofen, the results are in good agreement with literature values. We conclude that mini-column fractionation offers a simple and reliable alternative to t.l.c. for analyses of the effects of inhibitors of AA metabolism.

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MECHANISM OF CYCLOSPORIN A-INDUCED INHIBITION OF PROSTANOID SYNTHESIS BY MACROPHAGES

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Most studies have indicated the immunosuppressive agent, cyclosporin A (CSA), seems to act only on T-lymphocytes, leaving B cells and macrophages (MØ) relatively unimpaired (Borel, 1981). However it has been shown (Drath & Kahan, 1983) that in rats CSA caused marked inhibition of superoxide release from pulmonary alveolar MØ and reduced C5a- and FMLP-induced chemotaxis of polymorphonuclear leucocytes.

Recently we showed that CSA not only inhibited the rejection of skin grafts in rat but also reduced the formation of prostaglandin (PGs) in the grafts (Fan & Lewis, 1984). However it seems unlikely that this inhibition by CSA is involved in its immunosuppressive activity since the cyclo-oxygenase inhibitor, indomethacin, enhances graft rejection, while suppressing PG formation. We have therefore pursued our earlier work in which it was shown that CSA inhibits PG formation in rat peritoneal MØ (Fan & Lewis, 1984).

The procedure of preparing rat peritoneal MØ was described earlier (Fan & Lewis, 1984). In vitro studies of PG production over a 24h period by the cells when activated by serum-opsonised zymosan (50 µg/ml) revealed that CSA caused a dose-related inhibition of PGI_2 level (measured as 6-oxo-$PGF_{1\alpha}$ by radioimmunoassay). A detailed comparison of the action of CSA with dexamethasone (DEX), indomethacin (IND) as well as with the lipoxygenase and cyclo-oxygenase inhibitor, BW755C, is summarised in the table below, which gives the % inhibition of PGI_2 formation:

Drug concentration (µg/ml)	% inhibition			
	C S A (n=6)	D E X (n=5)	BW755C (n=5)	I N D (n=6)
0.01	8 ± 2	57 ± 8	22 ± 3	31 ± 6
0.1	25 ± 4	66 ± 7	46 ± 9	82 ± 10
1.0	38 ± 4	62 ± 3	70 ± 11	87 ± 6
10.0	61 ± 3	75 ± 2	91 ± 2	94 ± 3

When arachidonic acid was added together with the inhibitors, there was no change in the level of PGI_2 produced by the IND-treated cells whilst the PGI_2 levels of DEX- and CSA-treated cells were elevated to the control level (17.5 ± 1.2 ng per 1.6 10^6 cells). Therefore CSA, like DEX does not inhibit cyclo-oxygenase activity.

Finally CSA has been examined on phospholipase (PLA_2) activity by direct measurement of the hydrolysis of a labelled synthetic substrate by pancreatic PLA_2 in a cell-free system. In contrast to DEX which caused no inhibition as expected, CSA caused 19-80% inhibition of the enzyme activity in concentrations of 1-30 µg/ml. The direct inhibition of PLA_2 might well be a manifestation of the fundamental activity of CSA on immunocompetent cells.

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RELATIVE EFFICACIES OF HISTAMINE H_2-RECEPTOR AGONISTS IN THE GUINEA PIG RIGHT ATRIUM

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The original work of Stephenson (1956) and Nickerson (1956) with irreversible antagonists established the concept of spare receptors and this approach has led to the determination of receptor constants for a number of agonists of different receptor types. Similar calculations for histamine H_2-agonists have not been reported to date due to the lack of a specific H_2-compound which would satisfy all the criteria for being an irreversible antagonist. We now report data to indicate that 3-(2-diethylaminoethylamino)-5-[2-[(2-guanidino-thiazol-4-yl)methylthio]-ethylamino]-4-methyl-1,2,4,6-thiatriazine-1,1-dioxide (E1309) possesses the properties of an irreversible antagonist and also results of its use in the guinea pig right atrium to establish receptor constants for 4 histamine H_2-agonists.

Compound E1309 showed a high affinity for guinea pig cerebral cortex binding sites using a specific ^{3}H-tiotidine binding assay (Gajtkowski et al., 1983), and in the guinea pig right atrium non-surmountable inhibition was observed which gave a calculated Schild slope significantly different from unity. Further studies in the guinea pig right atrium showed E1309 to elicit greater inhibition with longer equilibration times, and to maintain the level of inhibition following continued wash-out (3 washes per 18 min over 90 min) when present at a sub-maximal dose. However, when this latter experiment was repeated in the presence of ranitidine, protection of the H_2-receptor was observed. These data indicate that E1309 in the guinea pig right atrium behaves as an irreversible H_2-antagonist.

Dose response curves were prepared for the four H_2-agonists, histamine, 4-methyl-histamine, dimaprit and impromidine in the absence and presence of E1309. Using

Table 1 Histamine H_2-receptor constants in guinea pig right atrium

Agonist	EC_{50} (μM ± SEM)	K_d (μM ± SEM)	Receptor reserve(%) at max. stimulation	Relative Efficacy
Histamine	0.67 ± 0.33 (10)	15.2 ± 5.1 (8)	16	1
4-Methylhistamine	1.76 ± 0.21 (9)	171 ± 86 (7)	68	3.46
Dimaprit	1.12 ± 0.25 (10)	77.1 ± 26 (5)	56	2.49
Impromidine	0.00116 ± 0.0002(8)	0.0184 ± 0.0058 (8)	19	1.82

the equations derived by Furchgott and Bursztyn (1967), the values shown in Table 1 were calculated. Whereas the order of potency was impromidine > histamine > dimaprit > 4-methylhistamine, the relative efficacies were substantially different. These results indicate that in the guinea pig right atrium the natural agonist histamine has a low receptor reserve, and is less efficacious than other H_2-agonists.

We are indebted to P. Trounson and Victoria Barratt for their technical assistance.

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HISTAMINE STIMULATION OF ADENYLATE CYCLASE IN GUINEA PIG LUNG PARENCHYMA HOMOGENATES

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From work on guinea pig gastric mucosa (Gajtkowski et al, 1983) and guinea pig dorsal hippocampus (Hegstrand et al, 1976) there is evidence that the effect of histamine on H_2 receptors is mediated by raised intra-cellular levels of cAMP resulting from adenylate cyclase activation. Pharmacological studies have shown the presence of H_2 receptors mediating smooth muscle relaxation in the lung parenchymal strip (Chand 1979). However, the link between the H_2 receptor and adenylate cyclase has not previously been examined in this tissue. In this study we describe the stimulation by histamine of adenylate cyclase in the guinea pig lung parenchymal strip and the inhibition of this stimulation by several H_2 antagonists.

Guinea pig lung parenchymal strips were prepared as described previously (Drazen and Schneider, 1978). The strips were then homogenised in 100mM Tris buffer, pH 7.8, using an Ultra-Terrax homogeniser and the homogenate was centrifuged at 700g for 10 minutes. The resulting pellet was resuspended in buffer at a protein concentration determined by a modified Lowry method. Aliquots of homogenate were incubated with varying concentration of histamine essentially as described previously (Gajtkowski et al 1983), and the cAMP content of all samples was determined by the method of Brown et al (1971) using a competitive protein binding assay.

Histamine (1nM-100μM) caused a dose-dependent increase in adenylate cyclase activity from a basal level of 17.0 $\pm$ 2.3 pmoles cAMP/mg protein/minute (n=4), up to a maximal stimulation of 53.0 $\pm$ 6.9 pmoles cAMP/mg protein/minute (n=4) which was achieved with (1000μM) histamine. When histamine (1000μM) was incubated with increasing concentrations of various H_2 antagonists there was a dose-dependent reduction in adenylate cyclase stimulation back to the basal level. Assigning to cimetidine a value of unity the relative potencies of the H_2 antagonists were calculated to be: tiotidine, 140; YM11170,25; ranitidine,1; cimetidine, 1; metiamide,1; burimamide,0.2.

The order of relative potencies is similar to that obtained from inhibition of ^3H- tiotidine binding in guinea pig lung parenchyma homogenates where the relative potencies were: tiotidine,130; YM11170,20; ranitidine,1; cimetidine,1; metiamide,1; burimamide,0.2; and also similar to that obtained for inhibition by these antagonists of histamine stimulation of adenylate cyclase in gastric mucosa (Gajtkowski et al 1983) where the relative potencies were as follows: YM11170-40; tiotidine,14; ranitidine,1; cimetidine,1; metiamide,1; burimamide,0.2. We conclude from these results that in guinea pig lung parenchyma homogenates histamine stimulates adenylate cyclase, with a subsequent rise in cAMP levels, probably by an interaction with H_2 receptors.

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5-HYDROXYTRYPTAMINE-INDUCED HYPERPOLARISATIONS OF THE RAT ISOLATED SUPERIOR CERVICAL GANGLION AND VAGUS NERVE

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5-Hydroxytryptamine (5HT) has been reported to both depolarise and hyperpolarise rabbit peripheral neurones (Dun and Karczmar, 1981; Higashi and Nishi, 1982). In the rat isolated superior cervical ganglion (SCG) and vagus nerve (VN), only 5HT-induced depolarisations have been examined previously (Ireland et al, 1982; Ireland et al, 1983). Therefore, an investigation has been made of the 5HT-induced hyperpolarisations that are also observed on these rat tissues.

Agonist-induced changes in resting membrane potential were recorded extracellularly from freshly-isolated, desheathed SCG or VN preparations (Ireland et al., 1982). Experiments were performed using a low-calcium Krebs-Henseleit medium, since 5HT-induced hyperpolarisations were found to be potentiated when the concentration of calcium chloride was reduced to 1.5×10^{-4}M.

On the SCG, brief (2-3 minute) applications of 5HT (1×10^{-8}-3×10^{-7}M) induced concentration-related hyperpolarisations of 100-400uV peak amplitude. These hyperpolarisations were not blocked by methysergide, 1×10^{-6}M. The responses were also unaffected by phentolamine, 1×10^{-6}M, or phenoxybenzamine, 1×10^{-6}M, suggesting that they were unlikely to have been mediated via adrenoceptors (Brown and Dunn, 1983). Higher concentrations of 5HT (1×10^{-6}-3×10^{-4}M) generally induced triphasic responses consisting of an initial hyperpolarisation (IH), followed by a depolarisation and subsequent after-hyperpolarisation (AH).

On the VN, 5HT (1×10^{-8}-1×10^{-4}M) did not induce IH. However, depolarisations induced by 5HT at concentrations greater than 3×10^{-6}M were generally followed by an AH.

Metoclopramide (1×10^{-6}-1×10^{-4}M) behaves as a competitive antagonist of 5HT-induced depolarisations of the rat SCG and VN (Ireland et al., 1983). In the present study on the SCG, metoclopramide, 3×10^{-5}M, had no effect on the hyperpolarisations induced by 5HT, 1×10^{-8}-3×10^{-7}M, and indeed IH occuring at higher concentrations of the agonist appeared to be increased both in amplitude and duration in the presence of metoclopramide. On the VN, however, this concentration of the antagonist abolished both the depolarisations and any associated AH induced by 5HT, 1×10^{-7}-3×10^{-5}M.

Phenylbiguanide (PBG) mimics the depolarising action of 5HT on both the rat SCG and VN (Fortune et al., 1983). However, on the SCG unlike 5HT, PBG, 1×10^{-6}-3×10^{-5}M, failed to induce IH, even in the presence of metoclopramide, 3×10^{-5}M.

These results suggest that 5HT may hyperpolarise peripheral neurones via two distinct mechanisms. On the SCG, 5HT induced hyperpolarisations that were both independent of depolarisation and unaffected by metoclopramide. In contrast, on the VN, 5HT-induced hyperpolarisations were only observed following depolarisations, and were blocked by metoclopramide.

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50P

EVIDENCE THAT INHIBITION OF BRAIN PROTEIN SYSNTHESIS INHIBITS STRIATAL DOPAMINE RELEASE

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Inhibition of brain protein synthesis inhibits the behavioural hyperactivity syndrome (rat and mouse) and the rotational behaviour in rats with 6-hydroxydopamine- (6-OHDA) induced unilateral nigrostriatal lesions produced by tranylcypromine plus L-DOPA or amphetamine but not the hyperactivity (rats and mice) produced by apomorphine or the rotation (rats) produced by apomorphine, bromocriptine or lergotrile. These findings concur with and add to those reported by Green et al (1976) and suggest that inhibition of brain protein synthesis inhibits dopamine (DA) release.

Rat striatal DA concentrations were unaltered 3h after cycloheximide (Cx: 10 mg/kg i.p.), while homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) concentrations were reduced by 25% and 20% respectively ($p < 0.001$). The rise in rat striatal DOPA and DA concentrations 1h after L-DOPA (50 mg/kg i.p.) was unaffected by Cx (10 mg/kg i.p.) 3h previously, whereas the accumulation of HVA and DOPAC was diminished by 58% and 47% respectively ($p < 0.001$). Cx (10 mg/kg i.p.) followed 3h later by either tranylcypromine (10 mg/kg i.p.) or L-DOPA (50 mg/kg i.p.) inhibited the rise in rat striatal 3-methoxytyramine (3-MT) by 57%. Cx also inhibited the rise in striatal 3-MT produced by pargyline (75 mg/kg) and amphetamine (10 mg/kg) coincidentally inhibiting hyperactivity (motility counts) by 37%.

In rats with a 6-OHDA-induced nigrostriatal lesion, carbidopa (100 mg/kg i.p.) and L-DOPA (50 mg/kg i.p.) produced 60min later rises in striatal HVA and DOPAC on the lesioned side of 62% and 58% respectively as compared with the unlesioned side. Cycloheximide prior to carbidopa plus L-DOPA reduced HVA and DOPAC levels on the unlesioned side only.

Cycloheximide (10 mg/kg i.p.) virtually abolished the decline in striatal dopamine concentrations produced by α-methyl-p-tyrosine (200 mg/kg i.p.) over a 2h period. Inhibitors of protein synthesis did not affect the accumulation of striatal DOPA produced by NSD 1015 (100 mg/kg i.p.).

Cycloheximide (1 mM), anisomycin (1 mM) or puromycin (1 mM) superfused over striatal slices preloaded with [^{3}H]-DA inhibited [^{3}H]-DA efflux produced by K^+, 40 mM, by 33%, 66% and 58% respectively.

The combined behavioural and biochemical data indicate that inhibition of brain protein synthesis inhibits neuronal dopamine release.

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NALOXONE ANTAGONISM OF THE MORPHINE WITHDRAWAL SYNDROME

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In view of data which suggest that some environmental stimuli may activate endogenous opioid-mediated hyperalgesic mechanisms (eg Rodgers & Hendrie, 1983), it has been postulated that this response may be mediated by an unidentified "contra-opioid" agonist (ie a substrate which although acting at opiate receptors has functional consequences opposite to those normally ascribed to opiates). Thus, during opiate withdrawal, where opioid influences are greatly reduced (Kosterlitz & Hughes, 1975) the influence of the contra-opioid may become salient. Further, since this ligand appears to act via opiate receptors, it would be predicted that opiate antagonists should markedly attenuate all contra-opioid influences and hence significantly reduce the severity of the morphine withdrawal syndrome. The present study was undertaken to investigate this hypothesis. 20 individually housed male Sprague-Dawley rats (350-450g) were rendered morphine dependent by ad libitum exposure to solutions of morhpine sulphate (0.5mg/ml sweetened milk) for 9 days. A further 20 animals were exposed to milk alone for the same period. 24 hrs following withdrawal of solutions animals were randomly assigned to receive either 1.5mg/kg naloxone hydrochloride or saline vehicle (0.9%) i.p. in a volume of 1ml/kg. In all there were 4 experimental conditions; milk/saline; milk/naloxone; morphine/saline and morphine/naloxone (n=10/gp). 10 mins post-injection, animals were introduced into an observation arena and the ensuing behaviour recorded on videotape for the next 15 mins. Immediately following this period, hot-plate latencies (HPL) were assessed and rectal temperatures (^{0}C) recorded. The experimenter remained blind to drug condition throughout, with codes only broken after complete analysis.

Analysis of data indicated successful induction of dependence as revealed by (1) increased grooming, body shaking, teeth chattering and sternutation in the morhpine/saline condition as compared to milk/saline control ($p < 0.01$) and (2) withdrawal-induced hypothermia ($p < 0.01$) and hyperalgesia ($p < 0.05$) Naloxone, in the absence of major effects in non-dependent animals, markedly attenuated all abstinence induced behavioural exacerbations and withdrawal hyperalgesia. However, as the antagonist greatly increased sleeping time ($p < 0.01$) it was possible that the apparent antagonism of the morphine withdrawal syndrome was produced by a sedative action of naloxone under these conditions. Consequently, behavioural data were re-analysed (excluding the periods of somnolence) to further investigate this possibility. Standardised behavioural data (rate/min active) expressed as medians (lower-upper quartiles) are presented below.

	Milk/Saline	Milk/Naloxone	Morhpine/Saline	Morphine/Naloxone
Ambulation	5.7(4.6-6.4)	4.8(3.2-5.2)	5.8(4.2-7.5)	4.7(3.5-7.3)
Rearing	3.1(2.3-3.6)	2.4(2.0-2.8)	2.6(1.8-3.9)	1.6(1.6-2.9)
Investigate	2.8(2.4-3.5)	2.1(1.8-2.5)	2.7(1.7-3.6)	3.1(2.1-4.6)
Grooming	1.1(0.8-1.2)	0.7(0.4-1.4)	2.7(2.4-3.9)*	0.6(0-1.4)
Body Shake	0.1(0-0.1)	0.1(0-0.3)	0.7(0.3-1.0)*	0.3(0.1-0.6)*
Teeth Chatter	0 (0-0)	0 (0-0.1)	0.5(0.2-1.3)*	0 (0-0.3)
Sternutation	0.2(0-0.3)	0.1(0-0.4)	0.8(0.1-2.2)*	0 (0-0)
^{0}C ($\bar{x}$ ± S.E.M.)	38.3(± 0.1)	37.6(± 0.2)	36.9(± 0.6)*	37.2(± 0.1)*
HPL($\bar{x}$ ± S.E.M.)	10.2(± 1.1)	8.9 (± 1.6)	6.2(± 0.4)*	9.2(± 1.3)

*$p < 0.05$

Present data indicate that abstinence-induced behavioural exacerbations may be attenuated by naloxone without inducing apparent sedation. These findings are consistent with the existence of an endogenous contra-opioid agonist, the antagonism of which markedly reduces the morphine withdrawal syndromes severity.

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52P

UNEXPECTED PROPERTIES OF [^{3}H]BREMAZOCINE BINDING TO RAT LUMBOSACRAL SPINAL CORD MEMBRANES

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The spinal cord is an important site for opioid action. However, controversy exists as to the nature of binding sites in the cord. We have previously described mu and delta binding in rat lumbosacral spinal cord (Traynor and Rance, 1984) and now report on kappa binding which appears to have different properties from kappa binding previously described in rat brain (Gillan and Kosterlitz, 1982).

Membranes (17,000g) were prepared from rat lumbosacral cord tissue. Binding assays were done in Tris-HCl pH 7.4 for 60min at 37°C and terminated by filtration. Non-specific binding was defined using naloxone (10μM) or bremazocine (1μM).

(^{3}H)Bremazocine bound to spinal cord membranes affording a curvilinear Scatchard plot (Bmax 94 fmols mg^{-1} protein). In the presence of GLYOL (15nM/K_D) and ICI 174864 (1.0μM/K_D) to suppress binding to mu and delta sites bremazocine binding was reduced (Bmax 45 fmols mg^{-1} protein; K_D 0.47nM). The remaining "kappa fraction" was readily displaced by kappa opiates and naloxone with Hill coefficients of 1. However, a number of compounds, including the endogenous kappa ligand dynorphin$_{1-17}$ and the kappa selective U50488H displaced the binding with Hill slopes of less than unity (Table 1).

Table 1 Displacement of (^{3}H)bremazocine from the "kappa" opioid binding site*

Competing Ligand	Ki (nM)	Hill Slope	Competing Ligand	Ki (nM)	Hill Slope
Bremazocine	1.7	1.0	DADLE	606	0.32
EKC	5.2	0.92	GLYOL	160	0.62
Tifluadom	12	0.9	U50488H	280	0.45
Naloxone	21	0.9	Dynorphin$_{1-17}$	31	0.44

*Abbreviations: EKC (ethylketocyclazocine): DADLE (D-Ala^2,D-Leu^5-enkephalin); GLYOL (D-Ala^2,MePhe^4,Gly-ol^5-enkephalin); U50488H (Trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide).

The most easily reached conclusion is that [^{3}H]bremazocine is binding in more than one fashion to cord membranes, after blockade of mu and delta sites, and that U50488H and the opioid peptides tested are able to separate these components. The fact that binding to the kappa fraction was totally suppressed by naloxone appears to rule out the involvement of a sigma component in the observed effects. Others have postulated the existence of kappa-subtypes in spinal cord (Attali et al., 1982). Further work will be necessary to determine whether the binding data reported here should be interpreted as an indication of binding site heterogeneity.

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DSP-4 LESIONING ABOLISHES THE ENHANCED MONOAMINE-MEDIATED BEHAVIOUR FOLLOWING REPEATED ELECTROCONVULSIVE SHOCK

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Repeated administration of electroconvulsive shock (ECS) to rats enhances the behavioural responses to 5-hydroxytryptamine (5-HT) and dopamine (see Green, 1984) and Green and Deakin (1980) have suggested that these effects are dependent on intact central noradrenergic function.

DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) is a novel noradrenergic neurotoxin which on peripheral administration produces central denervation similar to destruction of the locus coeruleus (Jonsson et al, 1981). We have now studied the effects of DSP-4 lesioning on the above behavioural parameters and also on the effects of repeated ECS.

Young male Sprague-Dawley rats (2 weeks) were obtained with lactating females. DSP-4 (50 mg/kg) or saline were injected on two occasions 7 days apart. All rats were pretreated with zimeldine (5 mg/kg) to protect 5-HT neurons. DSP-4 reduced noradrenaline concentrations by approximately 80% in cortex and 60% in mid/hind brain but did not alter 5-HT or dopamine levels. ECS (110V, 1s) was given via earclip electrodes once daily for up to 10 days, commencing 2 days after the second DSP-4 injection. Activity was measured on pairs of rats using LKB Animex meters (sensitivity and tuning 30 μA). DSP-4 lesioning did not alter the behavioural responses to apomorphine (0.2 mg/kg s.c.) or the 5-HT agonist, quipazine (7.5 mg/kg i.p.), but abolished the enhancement of these seen 24h after ECS x 10.

	Apomorphine		Quipazine	
	Pre-ECS	ECS x 10	Pre-ECS	ECS x 10
Sham lesion	1466 ± 79	2216 ± 229*	2663 ± 148	3966 ± 308*
DSP-4	1091 ± 120	431 ± 85	1823 ± 180	2036 ± 249

Results are mean total movements ± S.E. *Significantly different from pre-ECS $P < 0.01$. Number of groups $\geqslant 5$.

These results complement those of Green and Deakin (1980) who obtained identical results following lesioning of the locus coeruleus and both ascending and descending noradrenergic fibres using 6-hydroxydopamine.

In conclusion, therefore, these results confirm that central noradrenergic function is intimately involved in the mechanism for enhancement of 5-HT- and dopamine-mediated behaviours.

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54P

A SIMPLIFIED HPLC METHOD FOR 3-METHOXYTYRAMINE: CORRELATION WITH BEHAVIOURAL ACTIVITY IN RATS

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Under normal conditions in the brain 3-methoxytyramine (3-MT) is a minor, intermediate metabolite of dopamine, produced by the enzyme catechol-O-methyl transferase (COMT) (Westerink & Korf, 1976). However, inhibition of monoamine oxidase (MAO) radically alters this situation and 3-MT then becomes the sole product of dopamine metabolism.

We have now developed a simplified method for analysing 3-MT using HPLC and electrochemical detection. We have used this method to investigate whether in rats there was any relationship between behavioural activity and the accumulation of 3-MT in the n. caudatus or n. accumbens following injection of an MAO inhibitor and L-DOPA. Individual male Sprague-Dawley rats (125-200 g) were placed on LKB Animex meters (sensitivity and tuning 30 μA). They were injected with tranylcypromine (10 mg/kg), followed 30min later by L-DOPA (10-50 mg/kg). Activity was measured for the subsequent 60min period and rats were then killed and brains dissected. Tissues were homogenised in 10 vols (w/v) of 0.1 M $HClO_4$ containing 400 μM $Na_2S_2O_5$ (antioxidant) and 2.5 μM 3-methoxy-4-hydroxybenzylamine (internal standard). Following centrifugation a suitable aliquot of the supernatant was injected directly onto the chromatograph. The liquid chromatograph consisted of an Altex 100A pump, a 25 cm x 4.6 mm 5 μm Spherisorb I ODS analytical (fitted with a pre-column) and a Bioanalytical Systems model LC-4B amperometric detector with TL-5 glassy carbon electrode. The mobile phase (2 ml/min) consisted of 0.07 M HNO_3 (88%, v/v) methanol (12%), octane-1-sulphonic acid (5 mM) adjusted to pH 2.3. The detector potential was +0.85V.

Using this method 3-MT was not detectable (< 0.1 μg/g wet weight) either in the n. caudatus or n. accumbens of untreated rats. In contrast, 3-MT was measurable in brain tissues of tranylcypromine pretreated rats and, furthermore, this dopamine metabolite increased in a dose-dependent manner in both brain regions following injection of L-DOPA (10-50 mg/kg). The activity of these rats was also dose-dependently increased by L-DOPA, and a highly significant correlation was found between this parameter and the 3-MT accumulation in the 60min period following L-DOPA administration in both the n. caudatus ($R = 0.76$, $P < 0.005$) and n. accumbens ($R = 0.84$, $P < 0.001$).

In conclusion, therefore, these results suggest that under the above conditions 3-MT may provide a useful marker of central dopaminergic function.

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TURNING INDUCED BY INTRASTRIATAL INJECTION OF GABAERGIC DRUGS: EVIDENCE FOR A GABA-MEDIATED INHIBITION OF DOPAMINERGIC FUNCTION

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There is now ample evidence to suggest that γ-aminobutyric acid (GABA) can both inhibit and facilitate dopaminergic (DA) functions in the basal ganglia (Dray, 1979). The turning-inducing effect of GABAergic drugs was extensively studied after intranigral injections, whereas only few studies involved direct injections in the striatum (Pycock, 1980). We report here the effect of intrastriatal injections of GABA agonists and antagonists, in conscious mice.

Female Swiss CD_1 mice (25-30 g, Charles River, France) were used. Drugs were solubilized in phosphate buffer, with a final pH of 6, and were injected into the right striatum of conscious mice, directly through the skin and skull, via a Hamilton microsyringe, under a volume of 1 µl. The injection point was slightly internal and caudal to the orbitus, with a needle length of 3.5 mm. The animals were then placed in small individual square cages.

The number of complete turns was counted between 0 and 2, 2 and 4, 8 and 10 min after injection.

The vehicle-injected mice exhibited a weak, short lasting, ipsilateral turning (2-4 turns/6 min). Muscimol (M) induced a dose-dependent ipsilateral turning (mean ± SEM nb. of turns/6 min - 0.01 µg : 5.2 ± 0.8 ; 0.05 µg : 7.1 ± 1.0 ; 0.1 µg : 8.1 ± 0.8 ; 0.5 µg : 16.6 ± 2.5). This effect was blocked by i.p. bicuculline (15 min before) (M 0.1 µg : 14.5 ± 2.3 ; M + bic. 0.5 mg/kg : 5.9 ± 1.1**) or SR 95103, a new GABA-A receptor antagonist (2-(carboxy-3'-propyl)-3-amino-4-methyl-6-phenyl pyridazinium chloride ; Chambon et al, 1985) (M 0.1 µg : 18.7 ± 1.6 ; M + SR 10 mg/kg : 9.5 ± 1.6** ; M + SR 30 mg/kg : 3.9 ± 1.0**). Strychnine (0.1 and 0.3 mg/kg, i.p. ; 15 min) did not affect M-induced turning, whereas haloperidol (i.p. ; 30 min) potentiated it (M 0.1 µg : 14.5 ± 2.3 ; M + hal. 0.3 mg/kg : 35.3 ± 6.9*). The agonist SL 75102 (Lloyd et al, 1982) also induced a bicuculline-sensitive ipsilateral turning (1 µg : 15.5 ± 1.1 ; 1 µg + bic. 0.5 mg/kg : 2.2 ± 0.6**). Bicuculline methiodide (BMI) induced contralateral rotations (0.005 µg : - 3.3 ± 0.5 ; 0.01 µg : - 11.4 ± 1.3 ; 0.05 µg : - 18.7 ± 3.0) which were blocked by prior injection of muscimol (15 min ; i.p.) (BMI 0.05 µg : - 17.9 ± 1.7 ; BMI + M 0.03 mg/kg : - 11.0 ± 1.6* ; BMI + M 0.1 mg/kg : - 6.0 ± 0.8**), or haloperidol (30 min ; i.p.) (BMI 0.05 µg : - 17.9 ± 1.7 ; BMI + hal. 0.3 mg/kg : - 5.6 ± 1.8**). The new antagonist SR 95103 also induced a muscimol-sensitive contralateral turning (0.5 µg : - 23.8 ± 4.0 ; 0.5 µg + M 0.1 mg/kg, i.p. : - 5.8 ± 1.4**).

In this model, apomorphine (0.01-0.5 µg) and d-amphetamine (20-50 µg) induced contralateral rotations whereas haloperidol (0.001-1 µg) did not induce rotations. These data indicate that, after direct intrastriatal injection, GABA antagonists mimick the effects of dopamine, whereas agonists induce the inverse effect. In addition, neuroleptics antagonize the turning induced by GABA antagonists, and potentiate that of agonists.

This suggests that, in the striatum, GABA inhibits the dopamine-mediated mechanisms which are involved in the control of turning behaviour. Additional studies are presently being done to further investigate the precise site and mechanism of this effect.

* $p < 0.05$; ** $p < 0.01$ vs intrastriatal drug alone (Student's t test).

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BEHAVIOURAL AND BIOCHEMICAL CONSEQUENCE OF MPTP ADMINISTRATION TO THE COMMON MARMOSET

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Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to man and other primate species induces a persistent Parkinsonian syndrome associated with loss of nigral dopamine containing neurones (Davis et al, 1979; Burns et al, 1983; Langston et al, 1984). We now report on the behavioural and biochemical effects of MPTP administration in the common marmoset.

Marmosets (290-440 g) of either sex received MPTP (1-4 mg/kg ip dissolved in a minimum quantity of 70% alcohol and diluted to volume with sterile 0.9% saline) for up to 5 days. Within 2-3 days of starting drug treatment the animals exhibited varying degrees of bradykinesia or akinesia, rigidity, postural abnormalities, loss of vocalisation and some postural tremor. This state was maintained at 10 days following the start of MPTP treatment but by 4-6 weeks some reversal of the motor deficits had occurred. However, animals still exhibited fewer and less co-ordinated movements compared to control animals. The syndrome induced by MPTP was reversed by the administration of L-DOPA (20 mg/kg ip) in conjunction with carbidopa (α-methyldopa-hydrazine; 5 mg/kg ip).

At 10 days following the start of MPTP treatment caudate concentrations of dopamine, HVA and DOPAC were reduced by more than 90% compared to values from control animals (Table 1). The uptake of ^{3}H-dopamine into putamen synaptosomal preparations was also markedly reduced (Table 1) but to a lesser extent than the caudate dopamine loss. The number of specific ^{3}H-spiperone binding site (Bmax) and the dissociation constant (K_D) in caudate were unchanged. At 4-6 weeks following the start of MPTP treatment caudate concentration of dopamine, HVA and DOPAC remained reduced. Similarly the uptake of ^{3}H-dopamine in putamen was decreased but this loss now exceeded that for dopamine concentrations. At this time more variation between individual animals was observed perhaps reflecting differing degrees of behavioural recovery. Again, the Bmax and K_D for specific ^{3}H-spiperone binding in caudate was unchanged following MPTP treatment.

Table 1 Alterations in dopaminergic parameters induced by MPTP treatment

Treatment group	Dopamine	HVA (ug/g)	DOPAC	^{3}H-dopamine uptake (pmoles/mg protein/h)	^{3}H-spiperone Bmax (pmoles/g)
Controls	8.9±0.6	5.6±0.6	5.2±0.5	228±47	17.2±1.1
10 days	0.35±0.20*	0.26±0.03*	0.28±0.08*	77±10*	19.2±1.5
4-6 weeks	0.89±0.32*	1.4±0.4	0.87±0.17*	34±12*	18.9±0.9

* $p < 0.05$ Student t test; n = 6-10

Administration of MPTP to the common marmoset induces a partially reversible Parkinsonian syndrome responsive to L-DOPA. The motor deficits are accompanied by a loss of striatal dopamine function reflecting the selective neurotoxic actions of MPTP.

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EVIDENCE FOR A PREJUNCTIONAL INHIBITORY 5-HT_1 RECEPTOR IN RAT KIDNEY

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The terminal regions of some (but not all) postganglionic sympathetic neurons are endowed with 5-hydroxytryptamine (5-HT) receptors, activation of which results in inhibition of transmitter release. Evidence has been presented that these prejunctional receptors in dog saphenous vein are of the 5-HT_1 subtype (Engel et al, 1983). We now report that the release of [^{3}H] noradrenaline ([^{3}H]NA) from the sympathetic nerves of rat kidney is inhibited by 5-HT and that the receptor involved also conforms to criteria defining the 5-HT_1 recognition site.

Isolated rat kidneys were perfused in vitro with [^{3}H]NA (3.17nM) for 30 min in Kreb's solution of the following composition (mM): NaCl 118.5, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5, and EDTA 0.1. After a 45 min wash-out period, the renal periarterial nerves were stimulated at supramaximal voltage with square wave pulses of 1 msec at 2 Hz for periods of 20 sec. Stimulus-induced overflow of tritium was calculated by subtracting the tritium measured in the venous effluent under resting conditions from that measured during the 20 sec stimulation period. (Previous experiments have shown that over 80 percent of the tritium in the venous effluent is as authentic NA.)

5-HT (perfused through the kidney for 15 min) produced statistically significant ($p<0.05$) concentration related decreases in stimulus-induced tritium overflow, ranging from 10 to 15 percent at 10nM to 50 percent inhibition at 1μM. These reductions were well maintained over the 15 min perfusion period and were fully reversed by perfusion with Kreb's free of 5-HT. Basal release of tritium was not altered by 5-HT (10 and 100nM) but was increased by 20 percent at 1μM. The inhibitory action of 5-HT (100nM) on stimulus-induced overflow was antagonized by co-perfusion with either methiotepin (0.3 to 30nM) or metergoline (30 and 100nM). The approximate IC_{50} values for these antagonists were 3nM and 30nM, respectively. In contrast, cyproheptadine (30nM), a preferential 5-HT_2 antagonist, and phentolamine (1μM) failed to antagonize inhibition by 5-HT on stimulus-induced overflow. Thus, 5-HT_2 and alpha receptors are not involved. As with 5-HT, 5-carboxamide tryptamine (1nM to 100nM) inhibited stimulus-induced tritium overflow in a reversible and concentration dependent fashion. 5-Carboxamide tryptamine proved to be about 6 times more effective in this regard than 5-HT and, like 5-HT, was inhibited by methiotepin but not phentolamine. Finally, the putative 5-HT_{1A} agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, 100nM) failed to inhibit stimulus-induced tritium overflow.

In conclusion, we have demonstrated the presence of an inhibitory prejunctional receptor to 5-HT on the sympathetic nerves to the rat kidney. The fact that the inhibitory action of 5-HT is antagonized by methiotepin and metergoline and is mimicked by 5-carboxamide tryptamine suggests strongly that a 5-HT_1 recognition site is involved. The result with 8-OH-DPAT may further indicate that this site is of the 1B subtype.

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INITIAL CHARACTERIZATION OF [^{3}H] PAROXETINE BINDING IN RAT CORTICAL MEMBRANES

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Paroxetine is a very specific and potent inhibitor of serotonin uptake with a chemical structure different from that of tricyclic antidepressants (Buus-Lassen, 1978). Recently, specific, high affinity ^{3}H-paroxetine binding sites in platelets have been described, which are thought to be associated with the serotonin transport mechanism (Mellerup et al., 1983).

^{3}H-Imipramine, a tricyclic antidepressant drug, labels a site associated with the serotonin transporter in serotonergic neurons in the central nervous system and in platelets (Langer et al., 1980 ; Raisman et al., 1980). The following study was therefore undertaken to investigate ^{3}H-paroxetine binding in membranes of the rat cerebral cortex.

Rat cortical membranes were prepared using the procedure of Sette et al. (1983). A rapid filtration procedure was used to measure ^{3}H-paroxetine bound to rat cortical membranes after an incubation period of 60 min at 22°C. Using the specific serotonin uptake inhibitor, fluoxetine, at a final concentration of 10 µM to define non specific binding, ^{3}H-paroxetine displayed specific, saturable binding of very high affinity to rat cortical membranes. Scatchard analysis of the equilibrium binding isotherm indicated the labelling of a single class of binding sites (Kd=0.15±0.01 nM and Bmax=549±36 fmoles/mg protein). Specific binding represented 83 % of total binding at a ^{3}H-paroxetine concentration of 0.15 nM. The dissociation affinity constant of ^{3}H-paroxetine binding to rat cortical membranes derived from kinetic experiments gave a value of 0.034 ± 0.008 nM.

^{3}H-Paroxetine binding to rat cortical membranes was inhibited by the serotonin uptake inhibitors, indalpine (Ki = 1.73 nM), chlorimipramine (Ki = 2 nM), fluvoxamine (Ki = 9.9 nM), fluoxetine (Ki = 13.63 nM) and imipramine (Ki = 40.9 nM). Also, the serotonin transporter substrates, serotonin and tryptamine inhibited ^{3}H paroxetine binding with Ki values of 698 nM and 880 nM, respectively. Other neurotransmitters tested, including dopamine, GABA, histamine and noradrenaline did not inhibit ^{3}H-paroxetine binding to rat cortical membranes. A good correlation (r=0.96) was obtained between the potency of various drugs to inhibit ^{3}H-paroxetine binding to rat cortical membranes and to inhibit the uptake of ^{3}H-serotonin into synaptosomes.

Also, compared to sham controls, a 90 % decrease in specific binding of ^{3}H-paroxetine to cortical membranes prepared from rats lesioned with the neurotoxin, 5,7-dihydroxytryptamine was obtained ; this confirms that the specific ^{3}H-paroxetine binding sites are located on serotonergic neurones.

Thus, the binding properties of ^{3}H-paroxetine in the rat cortex indicates that this ligand is specifically labelling the transporter complex of serotonergic neurones.

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COMPARATIVE STUDIES ON [^{3}H]-PAROXETINE AND [^{3}H]-IMIPRAMINE BINDING TO HUMAN PLATELETS

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In the brain and platelets of several species, including man, ^{3}H-imipramine labels a regulatory site associated with the high affinity serotonin transporter (Langer et al., 1980 ; Langer and Raisman, 1983). As the pseudo Hill slopes for the inhibition of ^{3}H-imipramine binding in human platelets by tricyclic and non-tricyclic 5-HT uptake inhibitors is close to unity, it was suggested that the mechanism of inhibition by these drugs was purely competitive. On the other hand, transporter substrates such as 5-HT and tryptamine interact with the ^{3}H-imipramine receptor through an allosteric mechanism (Segonzac et al., 1984). Recently, the non-tricyclic 5-HT uptake inhibitor paroxetine became available as a radioligand (Mellerup et al., 1983) and we compared ^{3}H-imipramine and ^{3}H-paroxetine binding to the 5-HT transporter complex in human platelets.

Platelet membranes prepared from outdated frozen platelet-rich-plasma were incubated with ^{3}H-imipramine (Amersham, 21 Ci/mmol) at 0°C as described previously (Segonzac et al., 1984). ^{3}H-Paroxetine (New England Nuclear, 20 Ci/mmol) binding was studied after a 180 min incubation at 0°C or 20°C as described for ^{3}H-imipramine binding using a final incubation volume of 2 ml. When ^{3}H-imipramine binding was studied at 20°C, the final incubation volume was 1 ml.

At 0°C, ^{3}H-imipramine reversibly binds to human platelet membranes with a Kd of 0.6 nM. ^{3}H-Paroxetine binding at 0°C can also be demonstrated, but it is only weakly inhibited by desipramine (100 µM) and the binding of ^{3}H-paroxetine at 0°C would appear to be irreversible. At an incubation temperature of 20°C, high affinity (Kd : 0.02 nM) ^{3}H-paroxetine binding is inhibited potently by tricyclic and non-tricyclic 5-HT uptake inhibitors and uptake substrates. Representative Ki values are (nM) : imipramine : 4.6 ; fluoxetine : 7.1 ; indalpine : 6.8 ; 5-HT : 1920 ; tryptamine : 4600. These values agree closely with those obtained studying ^{3}H-imipramine binding at 0°C (see Segonzac et al., 1984) and ^{3}H-imipramine binding at 20°C (Ki, nM : imipramine, 4.1 ; fluoxetine, 5.6 ; indalpine, 2.7 ; 5-HT, 1870 ; and tryptamine, 9380), indicating that both binding sites are closely related. Kinetic experiments, however, showed that ^{3}H-paroxetine-receptor dissocation at 20°C is significantly faster when studied in the presence of 100 µM desipramine (t1/2 : 68 min) than in the presence of unlabelled paroxetine (100 µM, t1/2 : 108 min). Similarly, the substrate recognition site of the transporter complex allosterically modulates ^{3}H-paroxetine binding since the t1/2 of dissociation was significantly increased in the presence of 500 µM 5-HT (205 min).

The present experiments indicate that at 20°C, ^{3}H-paroxetine binding to human platelets is reversible and of high affinity (see also Mellerup et al., 1983). On the basis of the pharmacological characterization of ^{3}H-paroxetine binding it would appear to be closely related to the well-defined ^{3}H-imipramine receptor in these membranes although kinetic experiments indicated a possible non-identity of these binding sites.

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BENZODIAZEPINES REGULATE COUPLING TO ANION CHANNELS IN ONLY SOME $GABA_A$ RECEPTOR COMPLEXES

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We have recently shown that only some subtypes of 'central-type' benzodiazepine (BZ) binding sites are allosterically influenced by $GABA_A$ receptors (Mitchell and Wilson, 1984a, b). There is further heterogeneity within the population of GABA-linked BZ binding sites, since the potency of this interaction is many fold greater at cerebellar sites than at the GABA-linked sites in hippocampus (Harris and Mitchell, 1984). Here we report the effects of benzodiazepines on the coupling between GABA receptors and a site labelling the anion channel, at the different types of complex in cerebellum and hippocampus.

Tissue from male Wistar rats was homogenised in 100 volumes of cold buffer (5mM Tris HCl, 1mM EDTA, pH 7.5) using a high-frequency homogeniser. Membrane fragments were washed four times by centrifugation (48,000g, 10 min) and rehomogenisation in fresh buffer. Binding of [^{35}S] t-butylbicyclo-phosphorothionate ([^{35}S]TBPS) was investigated using the method of Squires et al (1983), with a ligand concentration of 2nM and non-specific binding defined by either 10μM TBPS or 100μM picrotoxinin.

Picrotoxinin displaced [^{35}S] TBPS binding as a single linear component of almost identical affinity in cerebellum (Ki, 167 ± 4nM, n = 9) and hippocampus (Ki, 178 ± 5nM, n = 5) suggesting a close similarity of the ion channel part of the complexes in each area. GABA caused complete displacement of [^{35}S] TBPS binding in both areas but with some difference in IC_{50} values: cerebellum, 445 ± 28nM (n = 12); hippocampus, 1290 ± 100 (n = 9). Hofstee plots of [^{35}S] TBPS displacement by GABA (or other agonists) revealed a concave curvilinearity in cerebellum, in contrast to hippocampus where only a single component was apparent (Ki, 1503 ± 101nM, n = 6). In cerebellum, the plots could be resolved into two apparent linear components (Ki_1, 10 ± 2nM, 15 ± 2% of sites; Ki_2, 658 ± 22nM, 85 ± 2% of sites, n = 5). The benzodiazepine Ro 11-6896 (10nM), (which had no effect alone in these conditions) increased the potency of GABA in hippocampus by a factor of 1.77 ± 0.10, (IC_{50} shift, $p < 0.001$, n = 8). In cerebellum there was a prominent shift in IC_{75}, (x 1.49 ± 0.07, $p < 0.01$, n = 6) but not in IC_{25}, (x 1.10 ± 0.08, $p > 0.05$) indicating that only the low affinity component (Ki_2) but not the high affinity component (Ki_1) (which will predominate in the IC_{25} measurement) is affected.

These results suggest that in addition to BZ binding sites independent of GABA receptors (in hippocampus), there can exist $GABA_A$ receptor complexes that are independent of BZ regulation (in cerebellum). Those complexes that do have interacting GABA and BZ sites show differences between areas which suggest that there may be subtypes with distinct characteristics.

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ENHANCEMENT OF THE PHOSPHOTIDYLINOSITOL RESPONSE BY RAISED EXTRACELLULAR POTASSIUM IN RAT CEREBRAL CORTEX

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Previous work has shown that 100mM K^+ provokes an increase in phosphotidylinositol (PI) turnover in rat brain cortex (Brossard and Quastel, 1963). Cholinergic muscarinic agonists have been shown to both stimulate PI hydrolysis in cortical slices (Berridge et al, 1982) and inhibit K^+ currents in cortical neurons (Brown, 1983). Here we have investigated the possible interaction of raised extracellular K^+ on the PI hydrolysis elicited by carbachol in isolated rat cerebral cortical miniprisms.

PI hydrolysis was measured according to Berridge et al (1982). Miniprisms (350x350μm) were labelled with myo-[2-^{3}H] inositol and 50μl aliquots were incubated for 1h with 10mM LiCl with or without added agonists. Added ions were substituted for Na^+. Results are expressed as the ratio of cpm in inositol phosphate fraction/cpm in phospholipid fraction x 100.

Table 1 The Effect of Carbachol and external K^+ on PI response in rat cortex

Carbachol M	External K^+mM 6	20	50
0	7.75 ± 1.11	8.57 ± 1.62	12.58 ± 2.30
10^{-5}	7.95 ± 0.99	12.09 ± 1.76	18.44 ± 2.56
10^{-4}	9.74 ± 1.12	17.74 ± 3.53	33.20 ± 5.50

n = 6, $P < .01$ for effect of 50mM K^+ and 10^{-4}M carbachol alone and their combinations (2-way ANOVA).

A marked stimulation of PI hydrolysis was elicited by carbachol in the presence of 20 and 50mM K^+ (Table 1). 10^{-4} Carbachol or 50mM K^+ alone produced a small but statistically significant increase in PI hydrolysis whereas 10^{-5}M carbachol or 20mM K^+ did not (Table 1). However, both 20 and 50mM K^+ considerably enhance carbachol stimulated hydrolysis. Atropine (10^{-5}M) had no effect on the hydrolysis caused by raised K^+ alone but atropine and also tetraethylammonium (TEA) (20mM), which at this concentration blocks K^+ channels and is a muscarinic antagonist, prevented the enhanced PI response elicited by carbachol both in the presence and absence of raised K^+. This enhancement is unlikely to be due solely to depolarisation induced release of endogenous transmitters as veratrine ($7.5x10^{-5}$M) did not cause a similar enhancement in combination with carbachol, although it also had a significant effect on PI hydrolysis on its own and in addition inhibition of presynaptic release by 10mM Mg^{2+} did not block the K^+ mediated enhancement.

Preliminary results indicate that PI hydrolysis induced by agonists such as noradrenaline (10^{-4}M), histamine ($5x10^{-4}$) and 5-hydroxytryptamine ($5x10^{-4}$) may also be enhanced by raised K^+ but the effect is much less marked than that observed with carbachol. Interestingly while Cs^+ and Rb^+ could substitute for K^+, Li^+ and Tris-HCl could not.

In conclusion K^+ markedly potentiates the PI hydrolysis elicited by carbachol and the effect is not dependent on presynaptic release mechanisms.

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(+)-TUBOCURARINE ACTIVATES THE NICOTINIC CHANNELS OF BOVINE CHROMAFFIN CELLS

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(+)-Tubocurarine (Tc) has been shown to act as an agonist on the nicotinic receptors of cultured rat myotubes (Trautmann, 1982). A possible agonist action of Tc on nicotinic receptors of ganglia has not been investigated. Using the patch clamp technique we have investigated the actions of Tc on cultured bovine chromaffin cells which possess nicotinic receptors pharmacologically similar to those found in vertebrate autonomic ganglia (Fenwick et al, 1982) and report that in this preparation Tc activates single channel currents of a similar conductance to those activated by acetylcholine (ACh).

Bovine chromaffin cells were isolated, cultured and used 1-6 days after plating (Fenwick et al, 1982). "Whole-cell" and "outside-out" configurations of the patch clamp technique were used (Hamill et al, 1981) and all experiments were performed at room temperature (20-22°C).

In the "whole-cell" recording mode chromaffin cells had an input resistance of 5-8 GΩ and a mean resting membrane potential of -55 $\pm$ 12.8 mV ($\pm$ S.D., n = 72 cells). Microperfusion of ACh (1-100 μM) onto "outside-out" membrane patches activated single ion channels on 27 membrane patches out of 36 tested (75%). Similarly, microperfusion of Tc (5-100 μM) activated single channels on 16 membrane patches out of 21 tested (76%). The main single channel conductances for ACh, 42.9 $\pm$ 5.7 pS (mean $\pm$ S.D., n = 6) and Tc 43.6 $\pm$ 5.6 pS (mean $\pm$ S.D., n = 4) determined between -50 mV and -140 mV were not significantly different. Similarly the extrapolated reversal potentials for ACh, -8.2 $\pm$ 6.6 mV (mean $\pm$ S.D., n = 6) and Tc, -12.1 $\pm$ 11.0 mV (mean $\pm$ S.D., n = 4) were not significantly different. The similar values for single channel conductance and reversal potential suggest that ACh and Tc are activating the same receptor-channel complexes.

In an attempt to compare the agonist potency of Tc and ACh we have estimated the number of channels activated simultaneously by microperfusion of "agonist" onto the whole cell. The peak number of channels activated by ACh showed a clear dose dependency activating on average 2, 23 and 270 channels for 1, 10 and 100 μM ACh respectively. In contrast the peak number of channels activated by Tc ranged between 1 and 3 for 5-100 μM Tc.

In conclusion Tc can activate the nicotinic receptors of bovine chromaffin cells, although in comparison with ACh it is a relatively weak agonist. Future experiments will investigate the actions of other "antagonists" to determine if agonist activity is a general property of cholinergic antagonists.

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NORMAL α_2-ADRENOCEPTOR COUPLING IN ADRENALINE INSENSITIVE HUMAN PLATELETS

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A subgroup of patients with myeloproliferative disorders (MPD) can be identified whose platelets show insensitivity to the aggregating effects of adrenaline in vitro. These platelets, however, have normal numbers of alpha$_2$-adrenoceptors as identified by means of ^{3}H-yohimbine binding (Swart et al, 1984). We have investigated the coupling of platelet alpha$_2$-adrenoceptors to adenylate cyclase both in lysates by means of radioligand binding studies and in whole cells by measurement of cyclic AMP accumulation. Normal platelets were compared to adrenaline sensitive and insensitive platelets in both types of study.

Binding studies were performed using platelet lysates prepared as previously described (Cheung et al, 1982). The effects of Gpp(NH)p and NaCl on adrenaline inhibition of ^{3}H-yohimbine binding in the presence of $MgCl_2$ were then assessed in competition experiments. These were analysed using computer modelling techniques for meaned curves (n = 5).

Whole platelet preparations were prepared by gel filtration of PRP. Cyclic AMP accumulation was then measured using a protein binding technique (Brown et al, 1971) to assess basal cyclic AMP, PGE_1 (0.3μ M) stimulated cyclic AMP and the maximum % inhibition of stimulated cyclic AMP by adrenaline.

The effects of guanine nucleotides and sodium ions on the affinity of adrenaline for yohimbine binding sites and the slope of the competition curves was identical in all 3 groups (Table 1).

Table 1*

Additions	Controls	Adrenaline Sensitive MPD	Adrenaline Insensitive MPD
None	105.3(0.81)	134.2(0.79)	70.5(0.65)
10^{-4}Gpp(NH)p	718.4(0.93)	749.8(0.87)	598.2(0.87)
100mM NaCl	1234.0(0.74)	1139.0(0.63)	891.0(0.72)
10^{-4}Gpp(NH)p	7684.0(1.04)	5150.0(0.90)	6678.0(1.1)

*Values shown are IC_{50}nM (slope factor)

Basal and PGE_1 stimulated cyclic AMP levels were no different in normals from our two groups of patients. The percentage inhibition of PGE_1 stimulated cyclic AMP levels by adrenaline was significantly lower in the MPD patients (64.0 ± 5.1%) than in normal controls (89.8 ± 1.9%) but it was not significantly different in the patients sensitive (62.5 ± 8.0%) or insensitive (65.0 ± 6.6%) to adrenaline.

The results indicate that the normal numbers of alpha$_2$-adrenoceptors in patients with MPD insensitive to adrenaline are associated with normal receptor coupling to adenylate cyclase as measured in the binding studies. The fact that the effect of adrenaline on cyclic AMP accumulation was no different in the patients sensitive or insensitive to adrenaline provides support for the view that changes in cyclic AMP alone do not explain the mechanism of adrenaline induced platelet aggregation and suggests that there may be an alternative second messenger for the platelet alpha$_2$-adrenoceptor.

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INVOLVEMENT OF DOPAMINE RECEPTORS AND α_2-ADRENOCEPTORS IN THE HYPOTENSIVE EFFECT OF B-HT 958 IN THE RAT

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B-HT 958 (2-amino-6-(p-chlorobenzyl)-4H-5,6,7,8-tetrahydrothiazolo-(5,4-d)-azepine has been described as a partial agonist at α_2-adrenoceptors with a high pre:post-junctional activity ratio (Pichler et al, 1982). This compound has also been reported to lower blood pressure in anaesthetised rats and cats by a mechanism apparently involving α-adrenoceptor stimulation, but with only a small central nervous system component (Kobinger & Pichler, 1984). In this study we have investigated whether the hypotensive effect of B-HT 958 is due to its agonist action at peripheral prejunctional α_2-adrenoceptors.

Groups of 6 male Wistar rats (250-350 g) anaesthetised with Inactin (thiobutobarbitone Na 100 mg/kg ip) received the α_2-adrenoceptor antagonist idazoxan (Doxey et al, 1983), (±)sulpiride (dopamine antagonist) or saline either iv or into a lateral cerebral ventricle (icv volume 10 µl), as a 5 min infusion 5 min before B-HT 958 350 µg/kg iv. Blood samples were withdrawn from the carotid arterial cannula for determination of plasma noradrenaline (NA) by radioenzymatic assay (Brown & Jenner, 1981) 5 min before and immediately after idazoxan, sulpiride or saline and 5, 10, 30, and 60 min after B-HT 958.

In control animals (10 µl saline icv) mean arterial pressure (MAP) fell by 27.2 ± 3.9 mm Hg 5 min after B-HT 958 and heart rate (HR) by 45 ± 4.3 beats/min. Plasma NA fell by 37.9 ± 3.4% from a control value of 0.254 ± 0.02 ng/ml. Idazoxan 20 µg icv did not inhibit the falls in MAP and HR produced by B-HT 958. Icv idazoxan produced a rise in plasma NA to 140 ± 9.6% of the basal value (0.275 ± 0.02 ng/ml) but did not attenuate the B-HT 958 induced fall in plasma NA. In the control group receiving saline 1 ml/kg iv, B-HT 958 caused similar falls in MAP and HR as before with a concomitant fall in plasma NA of 35 ± 5.3%. Idazoxan 300 µg/kg iv produced slight but significant inhibition of B-HT 958 hypotension ($p < 0.05$ analysis of variance) and had no effect on the bradycardia. Idazoxan 1 mg/kg iv produced further inhibition of the hypotension and some inhibition of the fall in HR. Both doses of idazoxan caused large rises in plasma NA to 300 ± 36% and 285 ± 32% of basal values but did not attenuate the B-HT 958 induced fall in plasma NA. Sulpiride 300 µg/kg iv inhibited B-HT 958 hypotension and bradycardia by 60%. Sulpiride itself had no effect on plasma NA levels and significantly inhibited the fall in plasma NA 5 and 10 min after B-HT 958 (-12 ± 8.6% and -16.1 ± 6.9% from 0.235 ± 0.02 ng/ml, $p > 0.05$). Sulpiride 10 µg and 50 µg icv caused slightly more inhibition of B-HT 958 hypotension than iv sulpiride and 50% inhibition of the fall in HR. After icv sulpiride B-HT 958 did not cause a significant fall in plasma NA. A combination of idazoxan 1 mg/kg iv and sulpiride 300 µg/kg iv was most effective in preventing the falls in MAP and HR produced by B-HT 958 and almost completely inhibited the fall in plasma NA.

These results suggest that in anaesthetised rats, stimulation of peripheral prejunctional α_2-adrenoceptors may make some contribution to the hypotensive effect of B-HT 958, but that this is due mainly to stimulation of dopamine receptors located both within the central nervous system and at peripheral sites.

D.H. is an MRC Scholar

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STEREOSELECTIVE ANTAGONISM AT THE 5-HT AUTORECEPTOR BY THE OPTICAL ISOMERS OF METHIOTHEPIN

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Recent studies of the pharmacology of the 5-HT autoreceptor in the rat brain have identified four compounds which consistently display antagonist activity at this site - methiothepin, quipazine, metergoline and (-)-propranolol (Engel et al., 1983 ; Middlemiss, 1984a,b). Of these antagonists propranolol is of particular interest since only the (-) and not the (+) isomer possesses antagonist activity at the 5-HT autoreceptor (Middlemiss, 1984a). This observation suggests that a chiral association is possible between the 5-HT autoreceptor and antagonists. Methiothepin also has an optically active chiral centre at the point of connection of the piperazine and the tricyclic nucleus. The present study was carried out to evaluate the antagonist activity of the optical isomers of methiothepin at the 5-HT autoreceptor in the rat frontal cortex.

The enantiomers of methiothepin were prepared by the classical technique of enantioselective crystallisation with optically pure (+) and (-) binaphthyl phosphoric acid. Pharmacological evaluation of the isomers of methiothepin was carried out using optically pure hydrochloride salts of (+) and (-)-methiothepin as compared with (±)-methiothepin maleate. Antagonist potency of the isomers of methiothepin at the 5-HT autoreceptor was quantified using 5-HT inhibition of depolarization-induced transmitter overflow from rat brain slices as the index of autoreceptor activity. Briefly, the endogenous transmitter stores of 5-HT neurones in slices of the rat frontal cortex were labelled with [^{3}H] 5-HT and superfused with Krebs solution containing elevated K^+ ions (25 mM) and the 5-HT uptake inhibitor paroxetine (3.2 µM). Antagonists were perfused from 24 min before the start of the cumulative addition of 5-HT (30 nM to 1 µM). The inhibition of the K^+ evoked overflow of tritium by 5-HT was expressed as % of control and was calculated as previously described (Middlemiss, 1984a, b).

Addition of (±)-methiothepin (1 µM) caused a significant enhancement of K^+ evoked tritium overflow (+ 26%) and an attenuation of the inhibitory effects of 5-HT with an apparent pA_2 of 6.62. Under identical conditions, (-)-methiothepin (1 µM) was somewhat more effective both in augmenting K^+ evoked overflow (+33%) and in attenuating the inhibitory effects of 5-HT (apparent pA_2 6.81). In contrast (+)-methiothepin (1 µM) caused a small but non-significant enhancement of K^+ evoked overflow of tritium (+ 16%) and was less effective in attenuating the inhibitory effects of 5-HT (apparent pA_2 5.95).

The present results provide further evidence that a chiral interaction is possible between the 5-HT autoreceptor and antagonists. This property may lead to the design of more potent and selective antagonists at the receptor controlling 5-HT release.

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66P

LOW-AFFINITY SUBSTANCE P RECEPTORS MODULATE TRH-RECEPTOR INTERACTIONS: TISSUE SPECIFICITY AND EFFECTS OF OTHER DRUGS

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Numerous transmitters and drugs unrelated in structure to alkaloid and peptide opioids are known to suppress pain in mammals. Recently antinociceptive properties of the tripeptide thyrotropin-releasing hormone (TRH) have been demonstrated in rodents upon infusion into the brain (Rips et al.,1983; Webster et al.,1983) and TRH even surpassed morphine in its efficacy in some analgesic tests. Substance P (SP) ,an undecapeptide,in contrast,mediates pain messages at the spinal cord (SC) substantia gelatinosa (SG) and intrathecal SP causes pain. In view of the co-localization of SP and TRH in medullary efferents to the SC ,brainstem and mid-brain possible interactions between these neuropeptides at the receptor level were investigated using ligand binding techniques.

TRH receptors were labelled in vitro with [^{3}H](3-Me-His^2)TRH ([^{3}H]MeTRH) (1-2 nM; 5h/ 0°C). High-affinity (K_d = 2-5 nM) receptors have been found concentrated in the SG and the ventral gray of the SC,and in amygdala, n.accumbens, septum ,hypothalamus and c.cortex of rodent brain (Sharif et al.,1983a; Sharif and Burt,1985) by assays performed on thin sections and in membranes. SP added to washed SC membranes(+ 10μM bacitracin) caused a dose-related noncompetitive inhibition of specific [^{3}H]MeTRH binding (IC_{50} = 40-50 μM ,n = 6) ,while its C-terminal frgments were less potent : SP > Hepta- > Nona- > Hexa- > Pentapeptide. SP was more potent in ventral gray membranes than dorsal gray of rat (but not rabbit) SC, equipotent in rat limbic and whole spinal areas but substantially less active in pons,olfactory bulb and pituitary . In contrast, neurotensin,somatostatin,Leu- and Met-enkephalins,bombesin, angiotensin II , CCK (4), LHRH and numerous amino acids and biogenic amines were relatively inactive in competing for [^{3}H]MeTRH binding in rat spinal and amygdaloid preparations. SP reduced receptor availability without altering the affinity for TRH .SP was without effect against receptor binding of [^{3}H]glutamate, [^{3}H]NMS and [^{3}H]5HT to rat amygdala membranes even at 50μM final concentration.SP tested in rat and rabbit SC and amygdala at 100μM produced a biphasic dissociation of [^{3}H]MeTRH from TRH receptors with faster kinetics than TRH (10 μM).

These relatively specific actions of SP on TRH recptors in the SC and limbic areas (regions involved in the transduction and perception of pain) suggest that high levels of SP released by noxious stimuli may potentiate the percieved pain by inhibiting the interaction of TRH with its population of "antinociceptive receptors". The differential brain regional sensitivity to SP may be related to TRH/SP coexistence (Sharif et al.,1983b) and/or other anatomical relationships of such peptidergic innervations. Although the nature and class(es) of SP receptors mediating these effects need to be identified, the rank order of potency of SP analogs does suggest the involvement of novel low-affinity sites of the SP-P-type. Future studies with tachykinins,preferably tested in functional assays,may provide further evidence to support the physiological relevance of the present observations.

I thank Dr.David Burt for his continued support during these studies.

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IS IDAZOXAN A PARTIAL AGONIST AT PRESYNAPTIC INHIBITORY α_1-RECEPTORS IN SHR TAIL ARTERIES?

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Idazoxan (Doxey et al., 1983), an α_2-receptor antagonist can inhibit NA release in vitro by stimulating α_2-receptors under conditions o low frequency stimulation (Limberger and Starke, 1983). Idazoxan is also a v: oconstrictor agent in vivo (Paciorek and Shepperson, 1983), an effect mediated by stimulation of vascular α_1-receptors. We now describe the effects of idazoxan on ^{3}H-transmitter release from SHR-tail arteries.

Male SHR (12-15 weeks) were anaesthetized with pentobarbitone (60 mg/kg i.p.) and the proximal tail artery prepared by the methods of Hicks et al. (1984). Vessels were prelabelled (1 h) with 0.33 µM dl [7-^{3}H(N)-]-NA (specific activity : 13.5 Ci/mmol) followed by perfusion at 4 ml/min in Krebs' bicarbonate at 37°C for 1 h. The Krebs' medium contained propranolol (1 µM), cocaine (4 µM) and indomethacin (2.5 µM). Spontaneous outflow of radioactivity and the radioactivity released during two periods of periarterial field stimulation (1 Hz, 2 min, 40 v, 0.6 ms) was measured. The stimulation induced release of radioactivity at this frequency is entirely Ca^{2+}-dependent and reproducible. Absolute values of overflow in S_1 = 0.72 ± 0.08 % of tissue radioactivity (n = 24).

Table 1 : S_2/S_1 = ratio of second to first period of stimulation. * $p < 0.05$ significantly different from control (Duncan test). ** $p < 0.05$ significantly different from idazoxan alone data. a) yohimbine and prazosin added before S_1.

Drugs present in S_2	[concn µM]	n	Fractional release S_2/S_1
Control		9	1.07 ± 0.08
Idazoxan	(0.1)	10	1.12 ± 1.13
Idazoxan	1.0	16	1.72 ± 0.16*
Yohimbine	(0.1)	4	2.23 ± 0.30*
Phentolamine	(1)	5	3.59 ± 0.50*
Prazosin	(0.3)	4	1.98 ± 0.19*
Clonidine	(1)	11	0.37 ± 0.10*
Idazoxan a) + Yohimbine	(1) (0.1)	4	1.56 ± 0.24
Idazoxan a) + Prazosin	1.0 0.3	4	2.68 ± 0.45**

Yohimbine and phentolamine significantly increased the stimulation evoked release of tritium (Table 1). Idazoxan (0.1 µM) failed to increase tritium release and was only marginally active at 1 µM, however, idazoxan (0.1 µM in S_3) completely antagonized the inhibition by clonidine (1 µM) of tritium release (S_3/S_1 = 1.4 ± 0.18, n = 4). Prazosin (0.3 µM) also significantly increased transmitter release in this model (Table 1). Idazoxan probably failed to elicit marked facilitation of release because of a concomitant inhibition by the drug on tritium release. This "apparent inhibition" of release in the presence of idazoxan was blocked by prazosin but not by yohimbine, unmasking a potent facilitory effect of idazoxan (Table 1). In SHR tail arteries, idazoxan failed to markedly facilitate the release of transmitter, at concentrations which blocked prejunctional α_2-receptors. A facilitory effect of idazoxan was unmasked in the presence of prazosin, which suggests that prejunctional α_1- as well as α_2-receptors can inhibit transmitter release in the SHR tail arteries. These inhibitory α_1-receptors can be stimulated by idazoxan.

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IRREVERSIBLE BLOCK OF κ RECEPTORS IN THE GUINEA PIG ILEUM

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We have shown that the irreversible μ-selective opioid antagonist β-funaltrexamine can be used to determine the μ selectivity of opioid agonists (Sheehan, Hayes and Tyers, 1984). There is no comparable κ-selective antagonist, but we report here on the use of the irreversible non-selective opioid receptor blocker β-chlornaltrexamine (β-CNA, Portoghese et al, 1979), in combination with selective μ receptor protection with [D-ala^2, MePhe^4, Gly(ol)^5]-enkephalin (DAGO), to produce an effective κ receptor antagonism and thus to analyse the κ activity of some opioid agonists.

In the field-stimulated guinea-pig ileum preparation, set up as described previously (Sheehan, Hayes and Tyers, 1984), opioid agonists cause an inhibition of the twitch response. Dose-response curves were constructed before and after treatment with β-CNA for various opioid agonists. β-CNA ($1x10^{-9}$ - $3x10^{-7}$M produced a time- and concentration-dependent block of the response to DAGO and the κ-selective agonist U50488 (Vonvoigtlander, Lahti and Ludens, 1983). No significant recovery of the response was seen up to 4.5 hours after β-CNA treatment despite frequent washing of the tissue.

For study of the ability of DAGO to protect μ receptors, a standard treatment of $1x10^{-7}$M β-CNA (15 min incubation) was used. DAGO was incubated with the tissue 10 min before and during the addition of β-CNA, and was also added again after each of the first four washes. After 45 min of intermittent washout, the twitch height stabilised at about 25% greater than that before β-CNA treatment. The effect of various protecting concentrations of DAGO on the parallel shifts in the dose-response curves to normorphine (μ-selective) and U50488 (κ-selective) are shown in table 1.

Table 1 Dose ratios after β-CNA (10^{-7}M, 15 min) in the presence of various protecting concentrations of DAGO (n=6 or more)

	Dose ratios (±SEM) after β-CNA				
Conc of DAGO (M)	0	$1x10^{-6}$	$3x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$
Normorphine	1260 ±540	35.0 ± 3.2	38.7 ± 18.0	5.9 ± 0.6	6.1 ± 1.7
U50488	1210 ±490	183 ± 67	288 ± 195	151 ± 39	67 ± 28

DAGO selectively reduced the antagonism of normorphine by β-CNA while having a lesser effect on U50488. The concentration of DAGO which apparently gave the greatest selective protection of μ over κ receptors was $1x10^{-5}$M, and this treatment was thus chosen to investigate the selectivity of some opioid agonists. The changes in potency, expressed as dose ratios ± SEM, after β-CNA/DAGO treatment were as follows: DAGO 2.5±1.5 (6); morphine 7.5±2.7(5); pentazocine 9.4±4.6 (9); ethylketocyclazocine 28.5±5.9 (12); Mr 2034 137 ±44 (6); bremazocine 112±23 (6); and tifluadom 142±35 (9). This study suggests that tifluadom and U50488 have the greatest κ:μ selectivity of the agonists tested.

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THE CARDIOVASCULAR RESPONSE TO 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALIN (8-OH-DPAT) IN THE RAT

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8-Hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) has biochemical and behavioural effects consistent with agonist activity at central 5-hydroxytryptamine (5-HT) receptors (Hjorth et al. 1982). Direct evidence for this has come from binding studies in which 8-OH-DPAT shows selectivity for the high affinity 5-HT_{1A} subtype of the 5-HT_1 recognition site (Middlemiss & Fozard, 1983). In this report we describe the cardiovascular effects of 8-OH-DPAT in both normotensive and spontaneously hypertensive (SH) rats and present the results of a preliminary pharmacological analysis of the response.

Normotensive (male Sprague-Dawley ; 330-440g) or SH (Wistar-Kyoto ; 320-370 g) rats were prepared for recording blood pressure (BP) or heart rate (HR) in either the conscious state or under pentobarbitone anaesthesia (Fozard, 1982). The field-stimulated guinea-pig ileum and rat vas deferens were used to detect agonist activity at α_2-adrenoceptors.

In conscious SH rats, i.v. injections of 8-OH-DPAT, 8 and 32 µg/kg, caused dose-related and prolonged (50-80 min) falls in both BP and HR ; with 128 µg/kg the sustained (> 90 min) hypotension (-35 mmHg) and bradycardia (-45 b/min) were preceded by transient (< 5 min) increases in both BP (+12 mmHg) and HR (+30 b/min). Pretreatment of SH rats with p-chlorophenylalanine, 300 mg/kg i.p. 3 days prior to the experiment, reduced selectively the brain 5-HT (-89%) and 5-hydroxyindole acetic acid (-80%) concentrations but did not significantly change resting BP and HR. In these animals, responses to 8-OH-DPAT were similar to those in control animals except that the initial increases in BP and HR were now evident at the lower doses (8 and 32 µg/kg) and were particularly prominent at the 128 µg/kg dose.

A pharmacological analysis of the cardiovascular response to 8-OH-DPAT was carried out in normotensive anaesthetized rats which responded to 8-OH-DPAT, 8-128 µg/kg i.v., in a qualitatively similar fashion to SH rats. Comparisons were made using just submaximal doses of 8-OH-DPAT (32 µg/kg, i.v.) and clonidine (2 µg/kg i.v.), a centrally acting hypotensive agent with negligable affinity for central 5-HT receptors. The classical 5-HT receptor agonist, metergoline, 2.5 mg/kg, i.p. -60 min, and a putative irreversible antagonist at central 5-HT receptors, 8-methoxy-2-(N-2-chloroethyl-N,n-propyl) aminotetralin, 1 mg/kg, i.v. -10 min, abolished the cardiovascular response to 8-OH-DPAT without significantly affecting the response to clonidine. In contrast, the effects of both agonists were blocked dose-dependently and to a similar extent by the α_2-adrenoceptor antagonists, yohimbine, 0.5-2 mg/kg, idazoxan, 0.125-0.5 mg/kg and WY 26392, 0.125-0.5 mg/kg, all given s.c. 60 min prior to the agonists. In *in vitro* experiments (guinea-pig ileum ; rat vas deferens) no *direct* agonist effects at α_2-adrenoceptors could be demonstrated.

Thus, 8-OH-DPAT lowers BP and HR in both conscious SH and normotensive anaesthetized rats. The effects appear to be independent of intact central 5-HT transmitter stores. A direct effect on 5-HT receptors but involving a catecholamine link is suggested by the fact that 5-HT receptor antagonists selectively inhibit the response and that α_2-adrenoceptor antagonists are also inhibitory despite 8-OH-DPAT having no direct agonist effects at α_2-adrenoceptors *per se*.

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THE DISCRIMINATIVE STIMULUS PROPERTIES OF 5-HTP ARE MEDIATED BY THE 5-HT_1 RECEPTOR

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Recent reports using 5-hydroxytryptophan (5-HTP) as a discriminative stimulus (DS) (Friedman et al., 1983) have shown that the effectiveness of 5-HTP as a DS depends on stimulation of a central 5-HT receptor pharmacologically distinct from those involved in the head-twitch response, which have been identified with 5-HT_2 receptors (Green et al., 1983). In an attempt to confirm this the present experiments were designed to show that 5-HTP could act as a DS using shock escape in a T-maze and to investigate the involvement of subtypes of the 5-HT receptor using pirenperone, a selective 5-HT_2 antagonist (Green, et al., 1983) and RU 24969, a selective 5-HT_1 agonist (Gardner and Guy, 1983).

Groups of 4 male Wistar rats (300-350g at the start of the experiment) were maintained on a 14 hr light- 10 hr dark cycle with lights on at 0500. A wooden T-maze with an electrifiable grid floor (0.25mA) was used, with safe areas at the end of each side arm, out of sight of the choice point. After establishing shock escape, discrimination training started. Carbidopa (25mg/kg i.p.) was given 60 min before training; 5-HTP or saline was given i.p. 30 min before training. Training sessions, carried out on weekdays only, consisted of 8 trials one minute apart, with drug and saline given in a double alternation sequence. This continued until rats reached a criterion of 8 out of 10 consecutive correct first trial responses. To allow for position preference, half the rats were trained to turn left, and half right, after saline. The opposite direction choice was required after 5-HTP. Once criterion had been reached experiments were carried out on Tuesdays and Fridays, with training continuing on other days. On experimental days both safe areas were available and only one trial was carried out to avoid reinforcing incorrect responses.

Discrimination training started with a dose of 30mg/kg 5-HTP but no separation from saline was apparent after 15 sessions and the dose was therefore increased to 50mg/kg. At this dose rats reached criterion after 12.4±2.2 (mean±sem, n=8). Pirenperone (60 min before testing) was found to be ineffective as an antagonist of the cue properties of 5-HTP (No inhibition at 400µg/kg i.p. n=8). The ED_{50} for pirenperone against 5-HTP head shakes using the same dosing schedule as for discrimination training was found to be 2.3µg/kg i.p.. RU 24969 generalised to the 5-HTP cue in a dose dependent manner with an ED_{50} of 0.41mg/kg i.p. 5-Methoxy-N,N-dimethyltryptamine, which is not selective for either 5-HT receptor (Green et al., 1984) also generalised to 5-HTP, with an ED_{50} of 1mg/kg i.p.. Quipazine, whose DS properties are mediated by the 5-HT_2 receptor (Friedman et al., 1984) did not generalise with the 5-HTP cue (11% at 2.5mg/kg i.p., n=18).

These results confirm that 5-HTP can act as a DS and provide evidence that this response is mediated by the 5-HT_1 receptor subtype with no involvement of the 5-HT_2 receptor. This technique may thus prove useful for further study of the 5-HT_1 receptor and for the development of more specific agonists and antagonists than are presently available.

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SPONTANEOUS OROFACIAL DYSKINESIA DURING LONG-TERM DEPOT NEUROLEPTIC TREATMENT: INTERACTIONS WITH AGE

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Abnormal,involuntary orofacial movements can arise during long-term neuroleptic treatment, and are commonly referred to as 'tardive' dyskinesia. Elderly patients appear to be more likely to develop the syndrome, both with and without exposure to neuroleptics(Kane & Smith, 1982; Waddington, 1984). To extend our previous studies in young animals (Waddington et al, 1983), we have investigated neuroleptic effects in aged animals.

Male Sprague-Dawley rats of 3 and 22 months were given monthly injections (i.m.) of the depot neuroleptic fluphenazine decanoate (2.5 mg/kg) or haloperidol decanoate (25 mg/kg) or of oil vehicle. They were observed for a 3 month period and mouth movements, most commonly vacuous chewing, were assessed on a 0-4 scale (Waddington et al, 1985 a,b). In young animals an excess of mouth movements emerged towards the end of the second month and continued up to the 3 month point; while mean scores at 3 months were higher in each aged group, this was only significant between the vehicle groups (Table).

3 month treatment	Young	Old
Control	0.79 ± 0.25	2.20 ± 0.44[a]
Haloperidol	2.43 ± 0.28**	2.50 ± 0.51
Fluphenazine	2.13 ± 0.28**	2.29 ± 0.39

Mean ± S.E.mean (n=5-15). **$p<0.01$; [a]$p<0.05$ v/s young control

These two depot neuroleptic treatments produced similar excesses of late-onset orofacial movements in young animals, exaggerating movements already present in controls. In aged animals the excess of mouth movements arose more from age-related processes than from neuroleptic treatment. The present results, along side studies of dopamine receptors (O'Boyle & Waddington, this meeting), indicate the importance of age-related processes unrelated to neuroleptic treatment in the emergence of the syndrome in older subjects.

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THE ROLE OF CHRONIC STRESS IN THE DEVELOPMENT OF DIAZEPAM DEPENDENCE

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There is evidence to suggest that adaptation to an aversive stimulus is associated with regionally selective changes in the concentration of 5-hydroxytryptamine (5-HT) in the hippocampus, and that dependence upon the anxiolytic properties of drugs may be related to effects on the adaptive process (Benwell and Balfour, 1982; Balfour, 1982). The purpose of the present study was to examine the effects of the chronic administration of the established anti-anxiety drug diazepam (Dz) on the changes in plasma corticosterone (PC) and brain 5-HT which occur during adaptation to an aversive stimulus.

Male Sprague Dawley rats were treated intragastrically with Dz (25 mg kg^{-1}) or vehicle (40% v/v propylene glycol in water) either acutely or chronically (40 days) . In the acute studies, 30 minutes after the injection half the rats in each group were placed on elevated platforms (Balfour and Reid, 1979) for 30 minutes (n = 6), the remainder being left in their home cages for a further 30 minutes (n = 6). All chronically treated rats, with the exception of one group of vehicle-treated control rats, were placed on the platforms 30 minutes after each injection. On the last day of the chronic studies Dz withdrawal was studied by giving vehicle to half the rats previously treated with Dz. At the end of each experiment the animals were killed by cervical dislocation and the brains rapidly removed for the determination of 5-HT and 5-hydroxyindole acetic acid (5-HIAA) Reinhard et al, 1980). A blood sample taken from the trunks was used for the estimation of PC levels (Mattingly, 1962).

Dz had no significant effect on the raised PC levels observed in the acutely stressed rats, but appeared to enhance the reduction in the levels in rats exposed to the test procedure for 40 days. This difference approached statistical significance. The PC levels in Dz-withdrawn rats (24.8 $\pm$ 2.0μg/100 ml) were significantly greater ($p < 0.01$) than those found in the stress-adapted rats given Dz (9.2 $\pm$ 1.8 μg/100 ml) or vehicle (15.2 $\pm$ 1.5 μg/100 ml) (n = 7 per group). The PC levels in Dz withdrawn rats were not significantly difference to those observed in acutely stressed vehicle-treated rats (27.6 $\pm$ 3.1μg/100 ml). These effects of Dz appeared to be dose dependent. The effects of Dz and its withdrawal on PC levels in the stressed and stress-adapted rats were not associated with significant changes in 5-HT or 5-HIAA in any of the brain regions studied.

It is concluded that Dz dependence may be associated with its effects on the process of adaptation to an aversive stimulus but that these effects appear unrelated to changes in hippocampal 5-HT.

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STRIATAL [^{3}H]- SPIPERONE BINDING AFTER LONG-TERM DEPOT NEUROLEPTIC TREATMENT: INTERACTIONS WITH AGE

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Chronic treatment with neuroleptics can result in the development of dopamine (DA) receptor supersensitivity (Muller & Seeman, 1978), and the syndrome of tardive dyskinesia is commonly thought to derive from such events in man. Since age is considered to predispose man to tardive dyskinesia (Smith & Baldessarini, 1980) we have examined this hypothesis by assessing D-2 DA receptor status after chronic treatment with neuroleptics (Waddington et al, 1983) in young and aged animals.

Male Sprague-Dawley rats (young, 3 months; aged, 22 months) were given monthly i.m. injections of the depot neuroleptics haloperidol decanoate (25 mg/kg) and fluphenazine decanoate (2.5 mg/kg) or of oil vehicle alone. After 3 months of treatment animals were sacrificed and striatal D-2 receptors assayed using ^{3}H-spiperone (^{3}H-SPIP) (O'Boyle & Waddington, 1984).
Saturation analysis of the binding of ^{3}H-SPIP revealed that striatal D-2 receptor density (B_{max}) was elevated in young animals treated with haloperidol decanoate ($p<0.01$), but not in those treated with fluphenazine decanoate, at this low dose. Ageing was associated with a decrease in the number of D-2 receptors ($p<0.01$) and the degree of elevation induced by chronic haloperidol decanoate treatment was reduced in the aged animals. Receptor affinity (K_d) was not influenced by drug treatment or age (Table).

	YOUNG		OLD	
	K_d	B_{max}	K_d	B_{max} **a
Control	0.07±0.01	13.4±1.4	0.09±0.02	11.5±1.4
Haloperidol	0.08±0.01	20.8±1.7**	0.09±0.02	14.5±1.2
Fluphenazine	0.09±0.01	12.7±0.6	0.09±0.01	10.1±0.8

mean ± S.E.mean, n=5-6. **$p<0.01$. [a]significant overall effect of age

In behavioural studies, these two neuroleptic treatments were indistinguishable as inducers of late-onset orofacial movements in young animals (Molloy & Waddington, this meeting). These results therefore suggest that factors other than D-2 DA receptor blockade and resultant supersensitivity may be involved in the development of such orofacial movements. Also, aged rats appear to have impaired adaptive responses to long-term neuroleptic treatment. This would be consistent with recent data suggesting that the pathophysiology of tardive dyskinesia does not necessarily involve changes in the characteristics of striatal DA receptors (Crow et al, 1982; Waddington et al, 1983; Waddington, 1984).

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EFFECTS OF CHLORDIAZEPOXIDE AND ETHANOLAMINE-O-SULPHATE ON DISCRIMINATION LEARNING AND PERFORMANCE IN RATS

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Interactions between chlordiazepoxide (CDP) and the GABA-transaminase inhibitor ethanolamine-O-sulphate (EOS) were investigated using the acquisition and performance of continuous reinforcement - time out (CR-TO) and variable interval reinforcement - time out (VI-TO) discriminations in 83 male hooded rats (OLAC). The rats were trained in Skinner boxes to press a lever for 45 mg pellet rewards either after each press (CR) or after random intervals averaging 20 s (VI) and required to distinguish between 5 min reward periods, cued by lights, and 5 min time out periods, without lights or reinforcements, the two components alternating in a 20 min test session per rat.

CDP (10.0 mg/kg i.p., 1 h before testing) exerted differential effects on the acquisition of CR-TO and VI-TO discriminations tested for 12 and 16 consecutive days, respectively. In the CR-TO condition control animals reached a 70% level of efficiency (percentage of total responses occurring in the rewarded periods) within 3 days, whilst CDP-treated rats were initially disrupted but reached control level by days 11/12. In the VI-TO condition, where the discrimination was more difficult to learn, CDP-treated rats did not initially perform more poorly than controls, but acquired the discrimination more slowly remaining significantly below control level from days 8 to 16 and showing little improvement over days.

CDP (5.0 and 10.0 mg/kg) did not disrupt performance of a learned CR-TO discrimination, where animals had reached 80% efficiency. Neither did CDP (10.0 mg/kg) impair reversal learning in these trained rats relative to controls. In contrast CDP (2.5, 5.0 and 10.0 mg/kg) dose-relatedly impaired efficiency ($F\ 3, 16 = 10.90$, $p < .0005$) in the VI-TO condition despite an 85% pre-drug efficiency level.

EOS (5.0 mg/ml) in drinking water for 1 week prior to and throughout all experiments, a dose reported to increase rat brain GABA levels by 240% (Fletcher & Fowler, 1980), exerted no significant effects on the acquisition, performance or reversal of the discriminations. In rats given joint treatment with EOS and CDP no interactive or additive effects were apparent - efficiency was equivalent to that found with CDP alone.

The results suggest that CDP affects discrimination only in conditions of new learning and uncertainty, consistent with a benzodiazepine-induced disruption of information processing and stimulus analysis (Gray 1982). For a TO-CR discrimination only a single lever press is required to distinguish a rewarded from a non-rewarded period, whereas efficient performance in a VI-TO schedule requires attention to the cues signalling reward. The findings also suggest that benzodiazepine effects on discrimination may not be mediated by GABA. These results will be discussed in relation to previous evidence (Hodges & Green, 1984) that effects of CDP on punished operant responding are substantially increased by EOS, suggesting that GABA is involved in the anti-conflict activity of benzodiazepines.

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HYPERPHAGIA INDUCED BY κ OPIATE AGONISTS IN NON-DEPRIVED RATS: DISSOCIATION FROM A DIURETIC EFFECT

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Agonists at kappa opiate receptors produce modest increases in the intake of standard laboratory diet in rats (Morley et al. 1983; Sanger & McCarthy 1981), and also enhance water consumption in nondeprived rats (Turkish & Cooper 1984). We have reported that the kappa agonists, ethylketocyclazocine (EKC) and U-50,488H, strongly enhance the consumption of a highly palatable sweet diet, in partially pre-satiated rats (Cooper et al. 1984). The increased food consumption occurred with short latency (within 30 min access to the diet), following small doses of the drugs.

We carried out a study to determine if the hyperphagia induced by the specific kappa agonist, U-50,488H, was reversible by the opiate receptor antagonist, naloxone. Male rats (General strain), bred in our laboratory, were familiarised with the highly palatable diet (150 ml powdered small animal diet, mixed with 50 ml sweetened condensed milk and 200 ml water). Before drug administrations, they were partially pre-satiated by having access to 7.0 g of the diet. U-50,488H and naloxone were injected subcutaneously, either alone or in combination, and then 30 min later, access to the diet was restored, and food intake was recorded for 30 min. U-50,488H, administered alone achieved a peak hyperphagic effect at 0.3 mg/kg. Concurrent administration of naloxone (0.3 mg/kg) shifted the peak of the dose-response curve to 3.0 mg/kg. There was no reduction in the magnitude of the peak effect for U-50,488H, and we conclude that naloxone acted as a competitive antagonist at the receptors mediating the kappa effect on food intake.

Kappa agonists are also potent diuretics (Leander 1984), and act to inhibit vasopressin release. We carried out a second study to determine whether or not EKC-induced hyperphagia was a secondary consequence of water and electrolyte loss. Twenty-four nondeprived male rats (150-250 g) were adapted to eating the highly palatable diet. Following the familiarisation period, they consumed 12.6 $\pm$ 0.6 g (mean $\pm$ S.E.M) in 30 min. They were allocated to two equal groups, one receiving bilateral ureteric ligation under Sagatal anaesthesia, and the other acting as sham-operated controls. Twenty-four hours later, the effects of EKC (0.1 mg/kg^{-1}, subcutaneously injected), or a distilled water control injection, were tested in ligated and sham animals. They were first partially pre-satiated, the injections were administered; 30 min later, access to the diet was restored and food intake was recorded at 30 min intervals over the next 2h. An analysis of variance revealed a significant drug effect, which was apparent within 30 min after the return of the food ($p < 0.01$). Ligated animals injected with EKC consumed 12.3 $\pm$ 1.1 g in the first 30 min compared to ligated animals injected with vehicle (5.5 $\pm$ 0.6 g intake). While EKC-induced drinking in nondeprived rats may be secondary to water loss produced by diuresis, the present results rule out the possibility that the hyperphagia produced by kappa treatment is secondary to water loss.

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β-BLOCKADE WITH PROPRANOLOL CAUSES RESISTANCE TO EXTINCTION IN RATS

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Selective lesion to brain noradrenaline (NA) systems with the neurotoxin 6-hydroxydopamine was reported by Mason & Iversen in 1975 to cause increased responding in extinction in the rat. This dorsal bundle extinction effect (DBEE) has since been extensively replicated and extended to include increased responding in extinction of runway tasks (Mason & Iversen, 1975; Owen et al., 1982), of fixed interval (Mason & Iversen, 1978) and go/no-go lever pressing paradigms (Tremmel et al., 1977) and in various avoidance tasks (Ashford & Jones, 1976; Mason & Fibiger, 1978; 1979; Plaznik & Kostowski, 1980). It is even seen in extinction of classically conditioned responses such as rabbit eyeblink (McCormick & Thompson, 1982). As yet, however, the type of receptor mediating the DBEE is unknown. The present study demonstrates that beta-blockade with propranolol can mimick the DBEE and increase responding during extinction.

Male albino rats (OLAC, Bicester) weighing 200g were housed in a temperature controlled vivarium on a 12:12 light dark cycle and given access to food for one hour per day until their weights has dropped to 90% of free-feeding. Water was available ad libitum. Ten rats received intraperitoneal (IP) injection of saline 30 min prior to behavioural testing while a further ten animals received IP injection of propranolol hydrochloride (20 mg/kg in a volume of 2 ml/kg, ICI Ltd.). After magazine and lever shaping as described elsewhere (Mason & Iversen, 1978) thirteen acquisition sessions of 15 min duration were given in which every lever press produced one food pellet from the automatic dispenser (45mg Noyes). On the next three days the feeder was empty and extinction commenced. Here, no food was ever given and a lever press produced only the click of the empty feeder. Response rates were recorded for successive four minute periods on each of the three 16 min extinction sessions.

Injection with propranolol did not greatly affect acquisition of lever pressing so that on day thirteen both groups were pressing equally (saline mean = 180.4, propranolol mean = 177, Mann-Whitney U = 41, NS). However, when extinction testing commenced the propranolol-injected group showed significantly more responding in the face of non-reward than did the controls (day 1; saline = 132.5, propranolol = 280.7, Mann-Whitney U = 16, $p < 0.02$). This effect became smaller on day two (saline = 65.9, propranolol = 112.9, Mann-Whitney U = 27, $0.05 < p < 0.10$) and by day three both groups had extinguished equally.

In conclusion, the present study suggests that the receptor involved in the DBEE is probably beta in nature and constitutes a further demonstration of the role of brain NA systems in extinction behaviour.

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PROPRANOLOL PRETREATMENT REVERSES THE DEPRESSANT EFFECT OF ARGININE-8 VASOPRESSIN ON THE SPONTANEOUS ACTIVITY OF RATS

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The neuropeptide vasopressin depresses the spontaneous motor activity of rats (Ebenezer, 1984). However, the mechanisms of action are unknown. It is possible that vasopressin may interact with one or more of the known neurotransmitter systems to produce its effects. Indeed, it has been suggested that vasopressin may modulate both central (Kovacs et al. 1980) and peripheral (Hanley et al. 1984) noradrenergic systems, and recently we have demonstrated that the effects of the neuropeptide on the activity of the cerebral cortex of rats (Burns et al. 1984) can be antagonised by pretreatment with the B-adrenoceptor blocking drug propranolol. The present study was designed to investigate the effects of vasopressin on the spontaneous activity of rats pretreated with propranolol.

Spontaneous activity was measured in a 'grid-floor, short circuit' type of activity box (Ebenezer, 1983). Forty eight male Wistar rats (wts:250-330g) were randomly divided into 6 groups: Group 1 received saline 30 min before another injection of saline (control group); Group 2 received propranolol hydrochloride (5mg/Kg) 30 min prior to saline; Group 3 received saline 30 min prior to arginine vasopressin (AVP) (1.0 IU/Kg); Group 4 received propranolol (5mg/Kg) 30 min prior to AVP (1.0 IU/Kg); Group 5 received saline 30 min prior to AVP (4.0 IU/Kg); Group 6 received propranolol (5mg/Kg) 30 min prior to AVP (4.0 IU/Kg). Drugs were administered subcutaneously. Rats were placed singly in the activity box immediately after the 2nd injection and the activity scores were read at 5 min intervals over a 30 min period.

The activity of rats treated with propranolol prior to saline (Group 2) did not differ significantly from the activity of the control rats (Group 1). AVP (1.0 IU/Kg, Group 3; and 4.0 IU/Kg, Group 5) produced significant decreases in the activity of the rats compared with control values. This reduction was most pronounced during the 1st 15 min after injection of AVP (1.0 and 4.0 IU/Kg). The reduction in activity was greater with the high dose of AVP compared with the low dose. These results are consistent with those reported previously (Ebenezer, 1984). However, pretreatment with propranolol totally prevented the reduction in activity that was evident when AVP (1.0 IU/Kg) was administered alone. Propranolol also prevented the depressant effect of AVP (4.0 IU/Kg) during the 1st 10 min after injection. There was, however, a significant ($p < 0.05$) reduction in activity during the 10-15 min measurement interval. This reduction was relatively short lasting and the activity of these rats returned to control values shortly thereafter.

The depressant effect of AVP on spontaneous motor activity is therefore largely prevented by pretreatment with propranolol. This suggests that vasopressin may produce some of its behavioural effects by interacting with a noradrenergic system. The site, or sites, of this interaction (whether central, peripheral or both) is not known.

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