THE EFFECTS ON CARRAGEENIN-INDUCED INFLAMMATION OF COMPOUNDS WHICH INTERFERE WITH ARACHIDONIC ACID METABOLISM

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Prostaglandins and leukotrienes, arachidonic acid derivatives of cyclo-oxygenase and lipoxygenase respectively, have potent inflammatory properties. Prostaglandins mediate vascular and pain responses in inflammation while leukotrienes may contribute to leukocyte activation. Aspirin-like drugs selectively inhibit prostaglandin production whereas anti-inflammatory corticosteroids prevent arachidonic acid release from phospholipids, thereby indirectly inhibiting both cyclo-oxygenase and lipoxygenase. Prostaglandin and leukotriene synthesis is inhibited by ETYA (eicosatetraynoic acid; Hamberg and Samuelsson, 1974), BW755C (3-amino-1-(m-(trifluoromethyl)-phenyl)-2-pyrazoline; Higgs et al., 1979), benoxaprofen (Walker et al. 1980), sulindac (Humes et al. 1981) and diethylcarbamazine (Piper and Temple, 1981). NDGA (nor-dihydro-guaiaretic acid) is a selective lipoxygenase inhibitor (Hamberg, 1976). We have now compared the effects of these compounds on carrageenin-induced inflammation.

Inflammatory exudates were collected 24 h after the subcutaneous implantation of carrageenin-impregnated polyester sponges in male rats (Higgs et al. 1979). Each drug was administered orally at the time of sponge implantation, 5-8 h later and 3 h before sponge removal. Prostaglandin E_2 concentrations in extracts of sponge exudates were determined by bio-assay and total leukocyte numbers were counted by phase contrast microscopy. Dose response curves were submitted to regression analysis and relative potencies were calculated. ED_{50} values for each variable are shown in Table 1.

Table 1 (* indicates a significant difference from 1; p 0.01)

Compound	ED_{50} (mg/kg; 95% confidence limits)		Relative Potency (WBC/PG)
	Leukocyte (WBC) Inhibition	Prostaglandin (PG) Inhibition	
BW755C	14.2 (8.6-21.5)	16.1 (10.2-24.1)	0.9
Dexamethasone	0.31 (0.19-0.46)	0.15 (0.07-0.23)	2.1
Benoxaprofen	8.5 (6.0-11.9)	0.9 (0.5-1.4)	9.7*
Sulindac	11.3 (6.5-65.0)	1.1 (0.6-1.7)	10.3*
Phenylbutazone	46.0 (28.5-72.9)	2.9 (0.9-5.5)	16.1*
NDGA	100	32.7 (23.0-41.6)	-
ETYA	100	100	-
Diethylcarbamazine	200	200	-

These results support the theory that BW755C has a steroid-like profile of anti-inflammatory activity. The inhibition of leukocyte migration by the drugs tested could be due to effects on lipoxygenase. In this model however, sulindac and benoxaprofen are selective cyclo-oxygenase inhibitors and in this sense they are similar to conventional aspirin-like drugs. The effects of NDGA, ETYA and diethycarbamazine on arachidonic acid metabolism in vitro are not reflected in vivo.

Hamberg, M. (1976). Biochim. Biophys. Acta. 431:651-654.
Hamberg, M. and Samuelsson, B. (1974). Proc. Nat. Acad. Sci. 71:3400-3404.
Higgs, G.A. et al. (1979). Biochem. Pharmacol. 28:1959-1961.
Humes et al. (1981). Symposium on Leukotrienes, Florence, Abstract book, p. 11.
Piper and Temple (1981). J. Pharm. Pharmacol. 33:384-386.
Walker et al. (1980). J. Pharm. Pharmacol. 32:866-867.

THROMBOXANE AND THE PULMONARY HYPERTENSIVE RESPONSE TO ENDOTOXIN

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The acute response to intravenously administered E. coli endotoxin in anaesthetized cats consists of an increase in pulmonary artery pressure (PAP) and airways resistance, a reduction in lung compliance and a transient systemic hypotension. It has been demonstrated previously (Coker et al., 1981) that endotoxin elicits a marked early release of thromboxane (Tx) and prostaglandin (PG)$F_{2\alpha}$ from the lungs. The aortic concentrations of these vasoconstrictor substances were shown to correlate with mean PAP. We now report the results of a study designed to establish the relative importance of TxA_2 and $PGF_{2\alpha}$ in mediating this pulmonary vascular response to endotoxin.

Cats of either sex were anaesthetized with sodium pentobarbitone and prepared for haemodynamic measurements (Parratt, 1973). UK-37,248-01, a thromboxane synthetase inhibitor (Tyler et al., 1981) or saline was given 15 min prior to E.coli endotoxin (2 mg/kg i.v.). Blood samples were obtained from the aorta and pulmonary artery 16 and 5 min before, and 2,7 and 30 min after, endotoxin. Plasma was analysed for TxB_2 (the stable breakdown product of TxA_2) and $PGF_{2\alpha}$ by radioimmunoassay.

Endotoxin significantly ($P<0.01$) increased mean PAP from 16±1 mmHg to 34 mmHg and 33±3 mmHg at 2 and 7 min respectively, and increased aortic TxB_2 from 114±25 pg/ml to 1078±232 pg/ml ($P<0.05$) and 630±108 pg/ml ($P<0.01$) at these times. UK-37,248-01 markedly reduced the effect of endotoxin on PAP to (22±2 mmHg and 18±1 mmHg at 2 and 7 min respectively; $P<0.01$) and significantly ($P<0.05$) reduced aortic TxB_2 concentrations to 197±26 pg/ml and 143±25 pg/ml respectively. Release of $PGF_{2\alpha}$ was not reduced by pretreatment with UK-37,248-07.

The results suggest that TxA_2 and not $PGF_{2\alpha}$ is mainly responsible for the increase in pulmonary artery pressure that occurs following administration of endotoxin.

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Coker, S.J. et al. (1981) Br. J. Pharmac. 74, 792P-793P.
Parratt, J.R. (1973) Br. J. Pharmac. 47, 12-25.
Tyler, H.M. et al. (1981) Lancet, i, 629-632.

ROLE OF KININS IN THE METABOLIC EFFECTS OF ENDOTOXIN

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Kinins have been implicated in endotoxin-induced shock (Nies et al, 1968) and the syndrome has been shown to be modified beneficially by aprotinin, a kallikrein inhibitor (Sumida, 1979). One feature of endotoxin shock is profound hypoglycaemia (Hinshaw, 1976) and bradykinin can itself produce hypoglycaemia (Wicklmayr et al, 1980). The aim of the present work was to obtain information about the metabolic effects of bradykinin and to determine the involvement of kinins in endotoxin-induced hypoglycaemia. The experiments were performed in conscious rats using methods and biochemical determinations described previously (Adeleye et al, 1981).

Bradykinin (2 ng $kg^{-1}min^{-1}$ i.v. preceded by a bolus of 20 ng/kg i.v.) produced a marked but transient hyperglycaemic response (control 6.2 ± 0.24 mmol/l; bradykinin 10.9 ± 0.3 mmol/l at 30 min; $P < 0.01$). During continued infusion the plasma glucose fell progressively to 3.9 ± 0.15 mmol/l by the 5th hour and returned promptly to the control value on stopping the infusion. Plasma lactate was also increased (e.g. from 0.27 ± 0.04 to 0.88 ± 0.05 mmol/l at 30 min). These increases in the plasma concentrations of lactate and glucose in response to bradykinin were prevented by sympathectomy (adrenal demedullation followed by 7 day treatment with guanethidine, 25 mg kg^{-1} day^{-1}, i.p.). In sympathectomised rats bradykinin infusion produced prompt hypoglycaemia evident at 30 min although the magnitude of this response was no greater than that observed in intact animals.

Endotoxin (E. Coli lipopolysaccharide, 8 mg/kg i.v.) produced an initial hyperglycaemic response (control-plasma glucose 6.7 ± 0.22 mmol/l; endotoxin treated 8.3 ± 0.15 mmol/l at 1 h; $P < 0.05$) followed by the development of profound hypoglycaemia (2.7 ± 0.18 mmol/l at 5 h). These plasma glucose changes were accompanied by significant and sustained increases in plasma lactate concentration (e.g. control 0.37 ± 0.03 mmol/l; endotoxin-treated 0.66 ± 0.06 mmol/l at 2 h; $P < 0.01$). The effects of endotoxin on the plasma concentrations of glucose and lactate were prevented by aprotinin (142,000 units/kg administered 15 min before endotoxin). Aprotinin itself did not modify the plasma glucose concentration and did not impair the hypoglycaemic response to insulin (1 unit/kg i.v.). However it did reduce the hypoglycaemic response to bradykinin (e.g. plasma glucose at 3 h in rats infused with bradykinin 3.0 ± 0.09 mmol/l; plasma glucose in bradykinin-infused rats pre-treated with aprotinin 4.8 ± 0.08 mmol/l; $P < 0.05$).

The ability of bradykinin infusion to mimic the metabolic effects of endotoxin and the prevention of these metabolic effects by the kallikrein inhibitor aprotinin suggest the possible involvement of kinins in the metabolic events that occur after endotoxin injection. This possibility merits further investigation. On the other hand the attenuation of bradykinin-induced hypoglycaemia by aprotinin complicates the interpretation of these data.

Adeleye, G.A. et al (1981) Circ. Shock 8, 543-550.
Hinshaw, L.B. (1976) Circ. Shock 3, 1-10.
Nies, A.S. et al (1968) Circ. Res., 22, 155-164.
Sumida, S. (1979) Adv. exp. Med. Biol., 120, 395-401.
Wicklmayer, M. et al (1980) Agents and Actions 10, 339-343.

CITALOPRAM ANTAGONIZES THE EFFECTS OF LSD ON THE PRESYNAPTIC SEROTONIN AUTORECEPTORS IN SLICES OF THE RAT HYPOTHALAMUS

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In the slices of rat occipital cortex, inhibition of neuronal uptake of noradrenaline by cocaine or desipramine reduces the presynaptic effects of α_2-adrenoceptor agonists of the imidazoline type such as oxymetazoline and clonidine (Pelayo et al., 1980).
The presence of presynaptic 5HT inhibitory autoreceptors has been shown in cerebral cortex slices (Göthert and Weinheimer, 1979 ; Baumann and Waldmeier, 1981) and in synaptosomes of the rat hypothalamus (Cerrito and Raiteri, 1979). We have recently shown that LSD is a potent agonist at presynaptic 5HT autoreceptors in the rat hypothalamus (Langer and Moret - submitted).
The aim of the present experiments was to examine the interaction between citalopram, a selective 5HT uptake blocker, and the inhibition by LSD of ^{3}H-5HT release elicited by electrical stimulation. The experiments were carried out in slices of rat hypothalamus prelabelled with ^{3}H-5HT. Each slice was stimulated twice at 3 Hz for 2 min (2 msec duration, 20 mA). The overflow elicited by electrical stimulation was entirely calcium dependent. In the controls, the percentage of total tissue radioactivity released was 1.89 $\pm$ 0.25 during the first period of electrical stimulation (S_1) and 1.89 $\pm$ 0.21 in S_2 (n = 7). Expressed as the ratio between two consecutive periods of electrical stimulation the value S_2/S_1 was 1.03 $\pm$ 0.06 (n= 7). Exposure to LSD before S_2 reduced significantly the stimulation-evoked overflow of the tritiated transmitter (S_2/S_1 : 0.62 $\pm$ 0.11, at 0.01 µM, n = 8 ; S_2/S_1 : 0.32 $\pm$ 0.07, at 0.1 µM, n = 7 ; and S_2/S_1 : 0.19 $\pm$ 0.06, at 1 µM, n=7).
The 5HT receptor blocking agent, methiothepin, antagonized competitively the inhibition by LSD of ^{3}H-5HT release elicited by electrical stimulation indicating that LSD stimulates presynaptic 5HT autoreceptors. Exposure to 0.01 ; 0.1 or 1 µM of citalopram before S_2 did not modify ^{3}H-transmitter overflow. Under these experimental conditions, citalopram at 0.1 and 1 µM completely antagonized the inhibition by LSD (0.01 ; 0.1 and 1 µM) of ^{3}H-5HT release elicited by electrical stimulation. When citalopram 0.01 µM was used, the inhibitory effect of LSD was significantly reduced but not completely antagonized.
It is concluded that citalopram, in concentrations in which it does not affect per se the electrically-evoked release of ^{3}H-5HT, antagonizes the inhibition of neurotransmission induced by the 5HT agonist LSD. The presynaptic 5HT autoreceptor differs pharmacologically from both the $5HT_1$ and the $5HT_2$ subtypes of receptors (Moret and Langer - submitted). Yet the interaction between citalopram and LSD at the level of the 5HT autoreceptor does not seem to involve a competitive interaction at the same receptor site. One possible explanation of our results is that there is a specific interaction between inhibition of neuronal uptake of 5HT and the presynaptic 5HT autoreceptor. It is tempting to speculate that neuronal uptake of 5HT and the presynaptic 5HT autoreceptor may be linked in a functional manner.

Baumann, P.A. and Waldmeier, P.C. (1981) Naunyn Schmiedeberg's Arch. Pharmacol. 317, 36-43.
Cerrito, F. and Raiteri, M. (1979) Eur. J. Pharmacol. 57, 427-430.
Göthert, M. and Weinheimer, G. (1979) Naunyn-Schmiedeberg's Arch Pharmacol. 310, 93-96.
Langer, S.Z. and Moret, C. J. Pharmacol. Exp. Ther. (submitted).
Moret, C. and Langer, S.Z. Eur. J. Pharmacol. (submitted)
Pelayo, F. et al. (1980) Eur. J. Pharmacol. 64, 143-155.

THE EFFECTS OF CLOMIPRAMINE ON THE 24 HOUR VARIATION OF 5HT AND TRYPTOPHAN CONCENTRATIONS IN THE RAT BRAIN

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In man the occurrence of depressive episodes has been associated with disturbances of circadian rhythms (Pflug et al, 1976). It was therefore of interest to examine the effects of antidepressant drugs on the well documented 24h variation in brain 5HT metabolism (inter alia Wurtmann & Fernstrom, 1972).
As part of this study we have investigated the effects of clomipramine HCl, administered for 2 or 14 days, on 5HT and tryptophan (TRY) concentrations in rat brain.

Groups of 6 male Wistar rats (University of Bath strain), weighing 150-200g, were housed for 14 days on a 12:12h L:D lighting regime (lights on 0600h). Clomipramine HCl (50, 100 or 200 mg/l) was administered in the drinking water. None of these concentrations affected the fluid intake or the amount of food eaten.
After 2 or 14 days groups of animals were killed at 0100, 0700, 1300 and 1900h and the brains removed and frozen in liquid nitrogen. Concentrations of 5HT and TRY were determined using HPLC and electrochemical detection (Pleece et al, 1982).

In control animals a significant variation in both 5HT ($F(3,17) = 3.32$) and TRY ($F(3,15) = 4.95$) was obtained with time of day (Table 1).
After 2 days, clomipramine at 50 and 100 mg/l had relatively little effect on either 5HT or TRY. Significantly elevated concentrations were obtained at all clock hours with 200 mg/l.

After 14 days all doses significantly reduced 5HT and TRY concentrations at all clock hours, 100 mg/l being the optimum dose (see Table 1).

TABLE 1. Effects of clomipramine HCl after 14 days.

TIME	0100h		0700h		1300h		1900h	
TREATMENT	TRY	5HT	TRY	5HT	TRY	5HT	TRY	5HT
CONTROL	2.01±0.19	5.02±0.62	2.25±0.12	7.41±0.81	2.39±0.09	7.41±0.62	3.12±0.31	7.35±0.43
Clomipramine HCl 50 mg/l	1.57±0.11	4.39±0.30	0.40±0.03	5.22±0.26	0.25±0.01	4.74±0.39	0.44±0.01	4.97±0.43
- " - 100 mg/l	1.22±0.06	3.21±0.19	1.31±0.19	3.78±0.36	1.17±0.13	3.98±0.31	1.28±0.11	3.21±0.12
- " - 200 mg/l	0.37±0.06	4.73±0.12	0.21±0.05	4.68±0.22	0.27±0.07	5.42±0.49	0.21±0.03	4.40±0.12

CONCENTRATIONS OF TRY AND 5HT (x 10) ARE EXPRESSED AS µg/g WEIGHT (MEAN ± S.E.M.: n = 4, 5 or 6)

This reduction in concentration was associated with the disappearance of the 24h variation in both 5HT and TRY. Our results therefore indicate that clomipramine can modulate 24h rhythms in central indoleamines, with a time course similar to that of the drugs antidepressant action.

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Pflug, B. et al (1976) Acta Psychiat.Scand. 54, 254-266.
Pleece, S.A. et al (1982) In press.
Wurtmann, A. & Fernstrom, J.D. (1972) In: "Perspectives in Neuropharmacology", Ed: S.N.Snyder, pp.143-193, Oxford University Press, New York.

MULTIPLE GABA-RECEPTORS ON Aδ AND C PRIMARY AFFERENT NEURONES IN THE ADULT RAT

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Under various experimental conditions, in vivo and in vitro, GABA elicits a rapid increase of Cl^- conductance of dorsal root ganglion (DRG) neurones. This effect, which undergoes desentization and is not mimicked by β-chlorophenyl-GABA (baclofen) has been demonstrated on a variety of sensory cells, including those with slow conducting axones (Desarmenien et al. 1981). In addition, GABA can shorten the Ca^{++} component of intracellularly-evoked action potentials ; this, and a similar effect of (-)baclofen, is probably due to the activation of a second class of GABA receptors recently described (see Bowery et al., 1981 ; Dunlap, 1981). Our aim was to specify this dual action of GABA on mammalian Aδ and C neurones, which can be identified by anti- or orthodromic stimulation. In the mature DRG, these cells commonly display action potentials with a delayed Ca^{++} current. Only small DRG cells (15-35 µm) were impaled in vitro under direct microscopic vision (x 320) by a single micropipette (2M CsCl or Cs_2SO_4). Drugs were applied in the superfusate at equilibrium concentration to study their action on Ca^{++} spikes, and by means of fast microperfusion when effects on Cl^- conductance were monitored (see methods in Desarmenien et al. 1980).

K^+ conductances were blocked by TEA (7.5 mM outside) and by intracellular injection of Cs^+ in identified Aδ and C neurones (conduction velocity of 9.6 ± 1 $m.s^{-1}$, n=29; and 0.7 ± 0.08 $m.s^{-1}$, n=8 ; group Aδ and C cells respectively). Action potentials then display a long lasting plateau (0.05 to 8s) due to an inward Ca^{++} current as: (1) Ba^{++} can substitute for Ca^{++}, (2) the plateau is reduced by 10^{-6}, 10^{-5} M methoxy-verapamil (D600), (3) it is shortened in a Ca^{++}-free medium with or without Cd^{++} (10 µM). Care was taken to study stable Ca^{++} currents (duration and amplitude) by using a low stimulation rate (<0.1 Hz) and by keeping the membrane potential constant, under both current- and voltage-clamp conditions (single electrode clamp circuit). The duration of these Ca^{++} spikes was reversibly decreased during the superfusion of low concentrations of GABA or (±) baclofen (10^{-6} to 10^{-7}M). A similar effect was obtained with muscimol, but not with THIP or isoguvacine, eventhough all three GABA-like compounds are agonists on the GABA/Cl^- receptor. Of particular interest is the fact that, in the concentration range used, the GABA/Cl^- receptors were neither activated nor desensitized (Desarmenien et al., 1980). Moreover the specific inhibition of Ca^{++} spikes was insensitive to bicuculline (10^{-6} M).

In conclusion, these results show that, perhaps in contrast with the study of Ia afferents, the action of GABA on Aδ and C fibers has to be considered with respect to both classes of receptors and along with the assumption that $GABA_B$ receptors exist on peptidergic primary afferents.

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Bowery, N.G. et al (1981) Eur. J. Pharmacol. 71, 53-70
Desarmenien, M. et al. (1980) J. Physiol. (Lond) 307, 163-182
Desarmenien, M. et al. (1981) In : amino acid neurotransmitters. De Feudis, F.V. & Mandel, P. Raven Press. New York pp. 309-319
Dunlap, K. (1981) Br. J. Pharmacol. 74, 579-585.

GABAERGIC AND DOPAMINERGIC INFLUENCES ON GLUCOSE UTILIZATION IN THE EXTRAPYRAMIDAL SYSTEM

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The purpose of the present investigation was to examine the interaction of GABAergic and dopaminergic mechanisms in striatonigral function, utilising the selective lesioning properties of kainic acid (Coyle et al, 1978) and mapping cerebral functional alterations with the 2-deoxyglucose (2-DG) technique (Sokoloff et al, 1977).

Unilateral striatal lesions were induced in 16 male SD rats by the stereotactically placed infusion of kainic acid (2µg in 2µl mock CSF). In sham rats, vehicle alone was infused. After 10 days, local cerebral glucose utilization was measured in the lightly restrained, conscious animals using the quantitative autoradiographic 2-DG technique as described previously (Sokoloff et al, 1977). The experiments were initiated by the injection of (^{14}C) 2-DG (125µCi/kg) delivered either 20 min after the administration of muscimol (1.5mg/kg i.v.) or 10 min after apomorphine (1.0mg/kg i.v.). All brains were analysed histologically to ensure that damage was confined to the striatum.

The lesions of striatal efferents and interneurones with kainic acid resulted in large (50%) increase in glucose use in globus pallidus (GP) and substantia nigra reticulata (SNR) (Table 1), the areas to which striatal efferents project. Moreover, these increases could be markedly attenuated by i.v. administration of muscimol at a dose which had little effect upon glucose use in sham animals. In contrast, i.v. administration of apomorphine had no effect upon glucose use in the GP of lesioned animals, but resulted in moderate increases (20%) in both pars reticulata and pars compacta of substantia nigra (SNC). However, only within SNR was the increase observed in lesioned animals following apomorphine significantly greater than the sham group (Table 1).

Table 1 Glucose utilization (umol/100g/min) following muscimol and apomorphine (i.v.) in areas ipsilateral to striatal lesions.

		Saline	Muscimol (1.5mg/kg)	Apomorphine (1.0mg/kg)
GP	Sham	44 ± 1	38 ± 2	71 ± 5*
	Lesion	80 ± 4	56 ± 1**	76 ± 6
SNR	Sham	45 ± 1	40 ± 2	80 ± 9*
	Lesion	80 ± 5	58 ± 1**	98 ± 6**
SNC	Sham	56 ± 1	50 ± 4	77 ± 8*
	Lesion	62 ± 3	57 ± 1	75 ± 5*

Data are presented as mean ± S.E. mean. *$p < 0.05$ between sham and lesioned groups. *$p < 0.05$ between saline, muscimol and apomorphine treatment.

The effects of lesions of the striatum in increasing glucose use in GP and SNR, and their reversal by muscimol confirm the inhibitory, mainly GABA mediated, striatal influence upon these areas. However, the effects of apomorphine administration also appear to be mediated via striatal efferents, with only SN showing any evidence for a direct dopaminergic effect. Thus the use of the 2-DG technique together with kainic acid lesioning provides a novel approach to mapping functional alterations associated with specific lesions of CNS.

Coyle, J.T. et al (1978) J. Comp. Neurol. 180, 301-324.
Sokoloff, L. et al (1977) J. Neurochem. 28, 897-916.

MOTOR INHIBITION AND FACILITATION BY DOPAMINE AGONISTS FROM THE OCTAHYDROBENZO(g)QUINOLINE SERIES

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Trans-1-aza-6,7-dihydroxy-1-propyl-1,2,3,4,5,5a,10,10a-octahydrobenzo(g)quinoline reduces locomotor responding of mice (Costall et al, 1981). Here we examine a series of N-alkylated octahydrobenzo(g)quinoline derivatives with hydroxyl functions in the -6,7- or -7,8- positions as potential dopamine agonists (DA) to determine a) whether the α- or β-rotameric conformation is relevant to locomotor depression, b) whether locomotor depression and locomotor stimulation can be differentiated and c) to characterise the receptor types involved.

Studies utilised male BKW mice. Spontaneous locomotor activity (SLA) was measured in photocell cages over 20 min , stereotypy was scored (1 - sniffing, 2 - periodic biting, 3 - continuous biting), and climbing measured in wire-lined cages as % time spent climbing (Costall et al, 1978).

Compounds with the α-rotameric conformation, 1-aza-6,7-dihydroxy-1,2,3,4,5,5a,10, 10a-octahydrobenzo(g)quinoline (64-(140)-1000 μg/kg s.c.), the N-methyl derivative (0.064-(0.9)-64 μg/kg s.c.), the N-ethyl derivative (0.05-(0.29)-3.2 μg/kg s.c.) and the N-propyl derivative (0.064-(0.4)-4.0 μg/kg s.c.), all caused dose-related decreases in SLA (range of doses indicated , that causing a 50% decrease in SLA shown in parentheses). The less marked reduction by the non-alkylated benzo(g)-quinoline was accompanied by muscular hypotenia and piloerection; these non-specific actions were not seen using the N-alkylated agents. A 45 min pretreatment with spiroperidol (0.025 mg/kg i.p.) partially, but significantly, antagonised the locomotor depression caused by the three N-alkylated derivatives; yohimbine (1.25 mg/kg i.p.) and prazosin (0.125 mg/kg i.p.) were ineffective. The reduction in SLA by the non-alkylated compound was not affected by any of the antagonists. The doses of N-methyl-, N-ethyl- and N-propyl-benzo(g)quinolines to induce stereotypy (score 3) and climbing (50% of max. response to apomorphine) were, respectively, 1.0 and 0.28 mg/kg s.c., 0.1 and 0.07 mg/kg s.c., and 0.15 and 0.04 mg/kg s.c., all actions being haloperidol-sensitive (0.1 mg/kg i.p.). The non-alkylated -6,7-dihydroxy compound failed to induce stereotypy or climbing at 10.0 mg/kg s.c. Compounds with the β-rotameric conformation, 1-aza-7,8-dihydroxy-1,2,3,4,5,5a,10, 10a-octahydrobenzo(g)quinoline and analogous N-alkylated derivatives, were inactive to reduce SLA at doses up to 4.0 mg/kg s.c. and failed to induce climbing or stereotypy at doses up to 10.0 mg/kg s.c.

Thus, N-alkylated octahydrobenzo(g)quinolines having 6,7-dihydroxy substitution have potent ability to reduce and, at higher doses, enhance motor function, at a neuroleptic sensitive site. Analogous agents having a 7,8-dihydroxy substitution were inactive, confirming the importance of an α-rotameric conformation for the induction of behavioural change at a dopamine (neuroleptic) receptor. The exceptional potency to inhibit SLA would suggest the development of this series for the crucial differentiation of motor inhibitory-motor facilitating action.

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Costall, B. et al (1981). Eur. J. Pharmac., 73, 175-188.
Costall, B. et al (1978). Eur. J. Pharmac., 50, 39-50.

EVIDENCE FOR A PRESYNAPTIC DOPAMINE RECEPTOR IN RAT HIPPOCAMPUS

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Previous studies have shown that the administration of nicotine selectively reduces the concentration and biosynthesis of 5-hydroxytryptamine (5-HT) in rat hippocampus (Balfour & Benwell, 1981; Benwell & Balfour, 1979) and that the administration of dopamine (DA) directly into the hippocampus can substitute for nicotine in rats trained to discriminate nicotine from saline (Rosecrans & Chance, 1978). It is possible, therefore, that the effects of nicotine on hippocampal 5-HT may be mediated through DA release and the results presented here form part of a study designed to investigate this possibility.

In vitro release studies were performed using the procedure described previously (Balfour, 1980). Dilute suspensions of synaptosomes, preloaded with ^{3}H-5-HT, were incubated in 1 ml of Krebs medium, buffered to pH 7.4 with Tris/HCl buffer. Samples (0.1 ml) were removed at 5 min intervals, filtered through cellulose filters of 0.65 μm pore size (Sartorius) and the radioactivity trapped in the tissue on the filters measured by scintillation counting. 5-HT release was calculated from the decrease in radioactivity remaining on the filters as the incubation progressed. The drugs were added at the beginning of the incubation (haloperidol) or in 0.1 ml of medium 15 seconds after the collection of the first sample (DA, apomorphine, noradrenaline). The same volume of medium was added to control incubations.

DA (10^{-5}M) significantly ($P < 0.01$) increased the spontaneous release of ^{3}H-5-HT during a 5 minute incubation period from 14 $\pm$ 2 percent of bound radioactivity to 29 $\pm$ 4 percent ($n = 9$). The increased release elicited by DA, which was dose-dependent, was antagonised by haloperidol. The DA receptor agonist, apomorphine (10^{-5}M) also caused a significant ($P < 0.01$) increase ^{3}H-5-HT release whereas the addition of noradrenaline (10^{-5}M) to the incubation medium had no significant effect on release. DA (10^{-5}M) did not increase the spontaneous release of ^{3}H-5-HT from synaptosomes prepared from cerebral cortex, hypothalamus or pons and medulla.

It is concluded that the data provide evidence for the presence of a DA receptor on the 5-HT nerve terminals of rat hippocampus whose purpose could be to regulate the localised release of 5-HT in this region of the brain. The possible relationship of these results to the changes in 5-HT observed following nicotine administration in vivo is difficult to predict with certainty although it is interesting that other drugs which increase 5-HT release or decrease 5-HT re-uptake and metabolism are also reported to decrease 5-HT biosynthesis (Costa & Meek, 1974; Green & Graham-Smith, 1976).

Balfour, D.J.K. (1980) Eur. J. Pharmac. 68, 11.
Balfour, D.J.K. & Benwell, M.E.M. (1981) Br. J. Pharmac. 72, 490p.
Benwell, M.E.M. & Balfour, D.J.K. (1979) Psychopharmacology 63, 7.
Costa, E. & Meek, J. (1974) Ann. Rev. Pharmac. 14, 491.
Green, A.R. & Graham-Smith, D.G. (1976) Nature 260, 487.
Rosecrans, J.A. & Chance, W.T. (1978) in Drug Discrimination and State Dependent Learning, (eds Ho, B.T. Richards, D.W. & Chute, D.L.) p 119, New York, Academic Press.

INHIBITION OF DOPAMINE OXIDATION BY (+)-AMPHETAMINE IN MOUSE BRAIN IN VIVO

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The stimulant effects of amphetamine are generally attributed to catecholamine release plus blockade of re-uptake. The characteristic stereotyped behaviour appears to be mediated by dopamine (DA). At 5-10 mg/kg, (+)-amphetamine raises the level of brain DA (Harris et al, 1975), and lowers that of its principal metabolite, 3,4-dihydroxyphenylacetic acid (Braestrup, 1977). These biochemical changes could arise from monoamine oxidase (MAO) inhibition, although other mechanisms may be involved (Braestrup, 1977). There is now good evidence, based on the protection exerted against irreversible MAO inhibition by phenelzine, that these doses of (+)-amphetamine cause appreciable, although short-lived,inhibition of 5-HT oxidation by mouse or rat brain in vivo (Green & El Hait, 1980a, 1980b; Miller et al, 1980),and a similar effect might consequently be expected on the oxidation of DA.

Table 1 shows the effect of 15 min pre-treatment of mice with subcutaneous (+)-amphetamine sulphate on the extent of inhibition of brain MAO produced 24 h after phenelzine. MAO was assayed radiochemically on whole brain homogenates. The % MAO inhibition by (+)-amphetamine itself was calculated as described by Green & El Hait (1980b). Oxidation of DA is inhibited almost to the same extent as that of 5-HT, 5 mg/kg of (+)-amphetamine giving nearly 50% inhibition.

Table 1 Effect of pre-treatment with (+)-amphetamine on the residual activity of brain MAO in mice injected with phenelzine (4 mg/kg s.c.).

(+)-Amphetamine (mg/kg)	Residual MAO (% of control) 5-HT	DA	% MAO inhibition by amphetamine 5-HT	DA
0	23 ± 4	26 ± 3	0	0
2	36 ± 4	38 ± 5	30	28
5	48 ± 6	47 ± 8	50	44

In rat brain in vitro, DA is oxidized by both the highly clorgyline-sensitive (A) and less clorgyline sensitive (B) forms of MAO, but in vivo the A-form appears to predominate (Waldmeier et al, 1976). The above results suggest the same is true of mouse brain. (+)-Amphetamine is only a weak inhibitor of the B-form in vitro (Green & El Hait, 1980a), and appears not to inhibit this form in vivo (Miller et al, 1980). Clorgyline (0.1 or 0.3 mg/kg s.c.) caused only slightly less inhibition of DA oxidation by mouse brain (37 and 67%) than of 5-HT oxidation (41 and 80%), again suggesting that the A-type enzyme contributes at least 80% of the total MAO activity towards DA in vivo.

At the doses of (+)-amphetamine (>10 mg/kg) required to produce stereotyped behaviour in mice (Thomas & Handley, 1978), inhibition of brain DA oxidation is likely to be substantial. In so far as MAO inhibition would preserve the DA released from intraneuronal stores from destruction, this action could be an important factor contributing to these behavioural effects.

Braestrup, C. (1977) J. Pharm. Pharmac. 29, 463
Green, A.L. & El Hait, M.A.S. (1980a) J. Pharm. Pharmac. 32, 262
Green, A.L. & El Hait, M.A.S. (1980b) Biochem. Pharmac. 29, 2781
Harris, J.E. et al (1975) Neuropharmac. 14, 457
Miller, H.H. et al (1980) Biochem. Pharmac. 29, 1347
Thomas, K.V. & Handley, S.L. (1978) Arzneimittelforsch. 28, 827
Waldmeier, P.C. et al (1976) Naunyn-Schmiedeberg's Arch. Pharmac. 292, 9

α-ADRENOCEPTOR SELECTIVITY STUDIES WITH RX 781094 USING RADIOLIGAND BINDING TO CEREBRAL MEMBRANES

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RX781094 is a potent and selective α_2-adrenoceptor antagonist (Chapleo,Doxey, Myers and Roach,1981),which has central activity as shown by its ability to block the sedative and EEG effects of clonidine and guanoxabenz (Dettmar,Lynn and Tulloch, 1981). This report describes the selectivity of RX 781094 for α-adrenoceptors on CNS membranes using radioligand binding techniques. (^{3}H)-Prazosin and (^{3}H)-clonidine were used to label α_1 and α_2-adrenoceptors respectively. The inhibitor constants (Ki values) of a number of agents for the displacement of (^{3}H)-clonidine are compared with those for displacement of (^{3}H)-RX781094.

(^{3}H)-RX781094 was prepared by catalytic bromine-tritium exchange (Radiochemical Centre,Amersham) and purified by preparative tlc (>99%).Rat brain membranes (minus cerebellum) were prepared by homogenisation in 20 vols of buffer (50 mM Tris HCl,pH 7.1, used throughout), filtration through cheesecloth and centrifugation (3x) at 50,000 x g for 10 min. The final pellet was resuspended in 10 vols of buffer and stored at -20°C. Cerebral membranes were incubated at 25°C with one of : 1nM (^{3}H)-prazosin (Pfizer, 33 Ci/mmol) for 20 min; 1nM (^{3}H)-clonidine (NEN, 22.2 Ci/mmol) for 30 min; 1nM (^{3}H)-RX781094 (30 Ci/mmol) for 15 min. L-Adrenaline bitartrate,300uM(as base, final concentration) was used to define specific binding. Bound radioactivity was trapped on Whatman GF/B filters and washed with ice-cold buffer containing 120 mM NaCl.

The specific binding of (^{3}H)-RX781094 to rat cerebral membranes was found to be saturable (80% specific binding), reversible and of high affinity. In 50 mM Tris HCl buffer, pH 7.1, the mean apparent dissociation constant (K_D) was 2.8±0.2nM(SEM) and the mean density of binding sites (Bmax) was 230 ± 14 fmole/mg protein. The inhibitor constants for 5 α-adrenoceptor antagonists are shown in Table 1. RX 781094 was about 7 times more potent than yohimbine at displacing (^{3}H)-clonidine from α_2-adrenoceptors and equipotent with yohimbine at displacing (^{3}H)-prazosin from $\alpha_1$adrenoceptors
Thus, the α_2/α_1 selectivity (B/A) of RX781094 (94) was seven times greater than the selectivity of yohimbine (14).

Table 1. Ki values (nM ± SEM for α-adrenoceptor antagonists.

COMPOUND	(^{3}H)-CLONIDINE,A	(^{3}H)-PRAZOSIN,B	(^{3}H)-RX781094	B/A
RX 781094	5.8 ± 3.5	547 ± 187	4.0 ± 0.8	94
Yohimbine	39 ± 19	544 ± 292	19 ± 4	14
Prazosin	566 ,7805	0.21± 0.06	568 ± 14	0.00005
WB 4101	216 ± 67	2.5 ± 0.9	96 ± 32	0.012
Phentolamine	5.1 ± 0.6	4.8 ± 1.6	2.6 ± 0.9	0.94

Chapleo,C.B.et al.Brit.J.Pharmac.Oxford.1981,in press.
Dettmar,P.W.et al.Brit.J.Pharmac,Oxford,1981,in press.

SODIUM SHIFT OF THE INHIBITION OF (^{3}H)-IMIPRAMINE BINDING BY 5HT AND 5HT-UPTAKE BLOCKERS BUT NOT BY TRICYCLIC ANTIDEPRESSANTS

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^{3}H-imipramine binding to rat cortex membranes in the absence of added sodium ions has been shown to have an affinity four times lower than in the presence of the 120 mM sodium used routinely (Briley and Langer, 1981). The maximum binding was unaltered by the removal of sodium.

We report here the effect of sodium ions on the affinity of certain drugs for the inhibition of ^{3}H-imipramine binding in rat cortical membranes. Experiments were carried out in the absence of added sodium or in the presence of 120 mM sodium chloride. ^{3}H-imipramine binding was inhibited by tricyclic antidepressant drugs such as amitriptyline, chloripramine and desipramine with similar or only slightly altered IC_{50} values in the presence and absence of sodium (amitriptyline, +NaCl 35.0 $\pm$ 9.1 nM n = 4 ; -NaCl 36.5 $\pm$ 11.8 nM n = 3 ; chloripramine, +NaCl 23.6 $\pm$ 2.8 nM n = 5 ; -NaCl 171 $\pm$ 71 n = 6 ; desipramine, +NaCl 177 $\pm$ 9.7 n = 4 ; -NaCl 152.6 $\pm$ 20.8 n = 3). On the other hand, a very large difference of between 40 and 100 fold was found when 5HT-uptake blockers such as fluoxetine, citalopram and paroxetine were studied (fluoxetine +NaCl 24.8 $\pm$ 5.7 nM, n = 4 ; -NaCl 1187 $\pm$ 240 nM n = 4 ; citalopram +NaCl 41.0 $\pm$ 3.8 nM, n= 4 -NaCl 3350 $\pm$ 1416 nM, n = 9 ; paroxetine +NaCl 66.3 $\pm$ 16.4 nM n = 4, -NaCl 6075 $\pm$ 1738 nM n = 4).

The affinity of 5HT for the inhibition of ^{3}H-imipramine binding was also greatly decreased in the absence of sodium (+NaCl 3.18 $\pm$ 0.09 µM, n =7, -NaCl 139.7 $\pm$ 64.2 µM, n = 7).

These effects were all due to the removal of sodium. Similar results were obtained whether NaCl was removed or replaced by choline chloride.

The Hill coefficient calculated from the inhibition of ^{3}H-imipramine binding by 5HT and the 5HT-uptake blockers was significantly less than 1 both in the presence and absence of sodium ions suggesting a non-competitive inhibition. On the other hand the tricyclic antidepressants inhibited ^{3}H-imipramine binding with Hill coefficients close to unity.

We conclude that the non-competitive nature of the inhibition of ^{3}H-imipramine binding by 5HT and the 5HT-uptake blockers (Briley et al., 1982) involves a highly sodium-dependent step. On the other hand, the competitive inhibition by tricyclic drugs is relatively sodium insensitive.

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Briley, M. and Langer S.Z. (1981) Eur. J. Pharmacol. 72, 377-380.
Briley, M.S., Langer S.Z. and Sette, M. (1982) Brit. J. Pharmacol. (in press).

NUMBERS OF STRIATAL (^{3}H).NPA BINDING SITES CHANGE TO COMPENSATE FOR CONSEQUENCES OF CHRONIC MESOLIMBIC DOPAMINE INFUSION

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Following acute manipulation of a component part of the motor system, changes in neurotransmitter activity can be recorded in other regions (Costall & Naylor,1979). In the present experiments we chronically infuse dopamine (DA) into a mesolimbic brain area and monitor changes in the binding of a DA agonist to mesolimbic and striatal tissue, attempting to relate such changes to behavioural modifications.

Male Sprague-Dawley rats were selected as 'high' or 'low' locomotor hyperactivity responders to the DA agonist (-)N-n-propylnorapomorphine ((-)NPA). Continued DA (50 µg daily) or vehicle infusion into the nucleus accumbens of selected rats was effected via bilateral intra-accumbens injection units attached to two Alzet osmotic minipumps (implanted subcutaneously in back neck region); injection units were accurately located via guide cannulae previously implanted using standard stereotaxic surgery. Locomotor hyperactivity responding to (-)NPA, measured in photocell cages, was monitored both during the 13 day infusion period and after pump withdrawal. Tissue was taken from the mesolimbic (nucleus accumbens + tuberculum olfactorium) and striatal brain areas of normal, vehicle or DA treated rats and subject to radioligand binding assays using ^{3}H.NPA.

During DA infusion animals selected as high (normally 60-80 counts/5 min from photocell cages to 0.05 mg/kg s.c. (-)NPA) and low (normally 10-25 counts/5 min) activity responders both showed reduced responsiveness to (-)NPA (3-7 counts/5 min). This reduced responsiveness was maintained by the initial 'high-activity' animals after discontinuing infusion (22-27 counts/5 min) whilst, over a period of several weeks after infusion, the animals initially categorised as 'low activity' developed a markedly exaggerated response to (-)NPA (42-53 counts/5 min). Hyperactivity responses were all shown to be neuroleptic sensitive. Radioligand binding assays showed animals categorised as 'high activity' responders to have significantly more mesolimbic binding sites for ^{3}H.NPA than 'low activity' animals (B_{max} 742 compared with 185 fmol/mg protein) although striatal sites were only slightly raised (B_{max} 315 compared with 268 fmol/mg protein). After discontinuing the DA infusion which had resulted in reduced behavioural responding to (-)NPA of 'high activity' animals but enhanced responding of 'low activity' animals, the numbers of mesolimbic sites for ^{3}H.NPA had been respectively reduced and increased (to B_{max} 408 and 394 fmol/mg protein). Increased mesolimbic sites were associated with reduced numbers of striatal sites for ^{3}H.NPA (to B_{max} 143 from 268 fmol/mg protein) although lowered numbers of mesolimbic sites were not associated with any significant change in numbers of striatal sites (B_{max} 310 fmol/mg protein).

Thus, the studies show that changes in DA agonist binding to striatal tissue can occur as a consequence of mesolimbic DA manipulation, that these may persist for many weeks, and have behavioural correlates linked to the normal locomotor responsiveness of rats to the DA agonist.

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Costall, B. & Naylor, R.J. (1979). Br. J. Pharmac., 66, 105P.

ACTIONS OF ETHYLENEDIAMINE ON LIMULUS AND HELIX CENTRAL NEURONES AND ON RAT CEREBELLAR AND SYMPATHETIC GANGLION NEURONES

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Ethylenediamine (EDA) has been shown to mimic the action of GABA on cortical and pallidal neurones of the rat (Forster et al, 1981; Perkins & Stone 1982). EDA is about 20 times less potent than GABA in depolarising rat superior cervical ganglion and this effect and that of EDA on rat pallidal neurones is blocked by bicuculline (Perkins et al 1981).

Intracellular recordings were made from Limulus polyphemus and Helix aspersa central neurones and extracellular recordings from rat cerebellar Purkinje cells and amplified and displayed using conventional electrophysiological techniques. Surface potentials were recorded with respect to the postganglionic trunk from rat superior cervical ganglion using $Ag^+/AgCl$ electrodes. All recordings were displayed continuously on chart recorders. Compounds were applied either iontophoretically or directly into the bath containing the preparations. The following compounds were used: GABA, EDA, piperazine, diaminopropane, diaminobutane, diaminopropionic acid, bicuculline, picrotoxinin and flurazepam.

EDA inhibited 13/18 Limulus neurones, being 81.44 ± 8.67 times less potent than GABA which inhibited 18/18 cells. The EDA response is fast in onset but often longer in duration compared to GABA and persists in high magnesium Ringer. The reversal potential for EDA and GABA are both at -65mV. Both EDA and GABA inhibitions are associated with an increase in chloride conductance and are reversibly blocked with picrotoxinin, 3.5×10^{-7}M. Piperazine inhibited 5/5 Limulus neurones, being 186.0 ± 31.65 times less potent than GABA. Diaminopropane and diaminobutane inhibited 2/7 and 1/5 cells respectively and were more than 100 times less potent than GABA. Helix neurones which were excited by GABA, were also excited by EDA, EDA being 9.25 ± 2.52 (n=6) times less potent than GABA. Helix neurones which were inhibited by GABA, were also inhibited by EDA, EDA being 92.25 ± 11.9 (n=6) times less potent than GABA. Piperazine, diaminopropane and diaminopropionic acid weakly excited both Helix cell types. EDA was equipotent with GABA on rat cerebellar Purkinje cells, producing inhibition on 40/40 cells tested. Potentiation of the EDA inhibition with flurazepam (10-30nA) occurred in only 3/23 cells. The benzodiazepine potentiated GABA on 15/15 of these 23 cells. Bicuculline (30nA) reversibly blocked EDA responses in 10/13 cerebellar cells. On the isolated superior cervical ganglion, perfusion with glutamic acid decarboxylase inhibitors thiosemicarbazide and mercaptopropionic acid did not change the response to GABA or EDA. Desensitisation of the ganglion to GABA also caused a loss of sensitivity to EDA but not to carbachol or 5-hydroxytryptamine. These results present further data to suggest that EDA can interact with neuronal postsynaptic GABA receptors.

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Forster, P., Lloyd, H.G.E., Morgan, P.F., Parker, M., Perkins, M.N. & Stone, T.W. (1981). Br. J. Pharmac. 74, 274P.
Perkins, M.N., Bowery, N.G., Hill, D.R. & Stone, T.W. (1981). Neurosci. Letts. 23, 325-327.
Perkins, M.N. & Stone, T.W. (1982). Br. J. Pharmac. (in press).

EFFECTS OF CLONIDINE-LIKE IMIDAZOLIDINES ON TRANSMISSION IN RAT ISOLATED SUPERIOR CERVICAL GANGLION

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Catecholamines depress ganglionic transmission by inhibiting acetylcholine release (Christ & Nishi, 1971). In rat superior cervical ganglia the receptors involved in this effect have been provisionally characterized as α_2-adrenoceptors (Brown & Caulfield, 1981). I have tested this further using clonidine (2,6-dichlorophenylimino-2-imidazolidine), the 2,3-, 2,4- and 2,5-dichloro isomers St 476, St 363 and St 475, and the 3,4-dihydroxy analogue DPI.

Compound action potentials (CAP; supramaximal preganglionic stimulation at 0.2 Hz) were recorded from desheathed rat SCG using a three-chambered bath (Brown & Marsh, 1978). Ganglia were superfused continuously with oxygenated Krebs solution at 22-25°C. Concentration-response relationships for the effects of the imidazolidines on the height of the CAP were established and compared with those of adrenaline (AD).

Clonidine, DPI and AD (1 nM - 100 µM) caused concentration dependent inhibition of the CAP. The curves plateaud around 1 - 10 µM; EC_{50} values were approximately 0.02, 0.1 and 0.1 µM, respectively. For DPI and AD, the maximum inhibition of the CAP in any one experiment was 30 - 40%; this may represent the effect of maximal presynaptic α-adrenoceptor activation with stimulation at 0.2Hz. Phentolamine (1 µM) antagonised the inhibitory effects of all three compounds to a similar extent. At 100 µM, clonidine, but not DPI or AD, nearly abolished the CAP; it is likely that this effect is non-specific, possibly being related to clonidine's known local anaesthetic activity. The effects of St476, St 363 and St 476 were complex. Like clonidine, at 100 µM each of the drugs almost abolished the CAP. At lower concentrations (10 and 30 µM), inhibition was much less pronounced, being less than that of maximally effective concentrations of DPI or AD; subsequent responses to AD (1 µM) were substantially reduced by all three imidazolidines, suggesting a partial agonist action. At the lowest concentration tested (1 µM), St 363 and St 475 caused small but significant increases in the height of the CAP in a similar manner to phentolamine (1 µM; Brown and Medgett, 1981); these compounds may thus be blocking the effects of endogenous catecholamines on presynaptic α-adrenoceptors.

The order of inhibitory effectiveness of the clonidine isomers was; clonidine >> St 476 > St 363 > St 475; this is the same order as that for hypotensive effects in the anaesthetised rat (Timmermans & van Zwieten, 1977) and for inhibition of stimulation-induced noradrenaline release in guinea pig atria (Medgett & McCulloch, 1980). It may be concluded that the receptors in each system are α_2-adrenoceptors; however, release-inhibiting effects of some imidazolidines may not be solely a result of prejunctional α_2-adrenoceptor activation.

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Brown, D.A. & Caulfield, M.P. (1981). In: Adrenoceptors and Catecholamine Action. Ed. Kunos, G. (Wiley).(In press)
Brown, D.A. & Marsh, S. (1978). Brain Res., 156, 187-191
Brown, D.A. & Medgett, I.C. (1982). Brit. J. Pharmacol., (In press)
Christ, D.D. & Nishi, S. (1971). J. Physiol., 213, 107-117
Medgett, I.C. & McCulloch, M.W. (1980). J. Pharm. Pharmacol., 32, 137-138
Timmermans, P.B.M.W.M. & van Zwieten, P.A. (1977). Arch. Int. Pharmacodyn., 228, 237-250

RELATIONSHIP BETWEEN α-ADRENOCEPTOR SELECTIVITY AND ANTICONVULSANT ACTIVITY AFTER CHRONIC CLONIDINE-LIKE DRUGS IN RATS

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Clonidine-like compounds have been shown to protect against pentylenetetrazol (PTZ) induced convulsions in rats (Summers et al, 1982), and to vary widely in their selectivity for α_2 and α_1 adrenoceptors as judged by radioligand assays (Summers et al, 1980). The degree of anticonvulsant activity and the dose range over which the effect is seen has been shown to correlate with the selectivity of the compounds for α_2 adrenoceptors (Summers et al, 1982). Convulsions were induced in Sprague-Dawley rats (150-200 g) with PTZ (50 mg/kg i.p.) and their characteristics and duration noted and compared to vehicle treated controls. Acute guanfacine, clonidine or lofexidine (0.01-1 μg/kg i.p.) 15 min before PTZ challenge, produced dose-dependent reductions in duration and intensity of convulsions. Similar effects were produced with 100 fold lower dose levels given intracerebroventricularly. Chronic guanfacine, clonidine or lofexidine were given subcutaneously (2.3 ± 0.03 μg/kg/day, n = 71) for 7 days using ALZET osmotic minipumps. Groups of animals underwent PTZ challenge during infusion and at 6, 14, 24 and 72 h after minipump withdrawal. Rats challenged with PTZ during infusion showed a reduced duration of convulsions compared to control whereas in the withdrawn animals there was a marked increase in both duration and intensity of convulsions which was most marked at 14 hrs and had returned to normal by 72 hrs (Table 1).

Table 1 Relationship between time of withdrawal of clonidine-like drug and duration of PTZ induced convulsions in rats

Time after withdrawal h	Guanfacine	Clonidine	Lofexidine
Control	59.2 ± 1.3s(4)	-	-
0	13.0 ± 1.2s(4)*	9.0 ± 3.1s(4)*	19.2 ± 0.9s(4)*
6	95.0 ± 6.0s(4)*	253.3 ± 14.5s(3)*	387.5 ± 8.5s(4)*
14	205.0 ± 6.4s(4)*	372.5 ± 42.0s(4)*	745.0 ± 90.7s(4)*
24	107.5 ± 4.8s(4)*	174.0 ± 5.7s(4)*	282.6 ± 8.5s(4)*
72	62.8 ± 1.1s(4)	61.0 ± 1.7s(4)	57.5 ± 1.3s(4)

*Significantly different ($P < 0.001$) from control. Numbers of animals in parentheses.

The intensity of the withdrawal syndrome was related to the selectivity of the compounds for α_1 and α_2 adrenoceptors which is guanfacine > clonidine > lofexidine (Summers et al, 1980). As the compounds become less selective for α_2 adrenoceptors the intensity of the withdrawal syndrome is increased.

In conclusion, chronic administration of clonidine like drugs has a protective effect against convulsions induced by PTZ whereas withdrawal is associated with an exaggerated response, the severity of which is related to the selectivity of the compounds for α_2 adrenoceptors.

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Summers, R.J., Jarrott, B. & Louis, W.J. (1980) Neuroscience Letts. 20, 347-350.
Summers, R.J., Papanicolaou, J., Vajda, F.J.E. & Louis, W.J. (1982) Clin. exp. Pharmacol. Physiol., in press.

THE SEDATIVE AND ANTICONVULSANT EFFECTS OF MELATONIN ARE NOT BLOCKED BY A SELECTIVE BENZODIAZEPINE ANTAGONIST

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The pineal hormone, melatonin, possesses anticonvulsant and sedative properties (Anton-Tây, 1974; Albertson et al, 1981). Recently it has been reported that both melatonin and its brain metabolite, N-acetyl 5-methoxykynurenamine, will specifically displace membrane-bound {^{3}H}-diazepam in vitro (Marangos et al, 1981). In order to examine whether the anticonvulsive and sedative effects of melatonin are mediated via an interaction with benzodiazepine receptors, we have attempted to block these effects by using the selective and potent benzodiazepine antagonist Ro 15-1788 (Hunkeler et al, 1981; Cowen et al, 1981a).

Melatonin (200 mg kg^{-1} i.p. as a 40 mg ml^{-1} suspension in Tween 80) or Tween (5 ml kg^{-1} i.p.) was injected into male SD-derived rats of 180-200 g. 45 min later either Ro 15-1788 (10 mg kg^{-1} i.p. as a 2 mg ml^{-1} suspension in Tween 40) or Tween alone was administered i.p. After a further 15 min the rats were tested in one of two ways. In one group seizure thresholds were determined by infusion, via a tail vein, of pentylenetetrazole (10 mg ml^{-1}) until the onset of seizure (Nutt et al, 1980). Other rats were placed individually on Automex activity meters where the sedative effects of melatonin were assessed as lack of exploratory activity in a new environment over a 10 min period (see Cowen et al, 1981b).

Table 1 Changes in seizure threshold and exploratory activity following melatonin

	Tween/Tween	Melatonin/Tween	Melatonin/Ro 15-1788
Seizure threshold (mg kg^{-1} PTZ)	28 ± 4 (6)	35 ± 6 (8)*	42 ± 10 (4)†
Activity counts	402 ± 90 (4)	48 ± 18 (4)*	29 ± 9 (5)*

Numbers represent mean ± S.D. with number of animals in brackets. *Significantly different from Tween/Tween $P < 0.05$. †Significantly different from Tween/Tween $P < 0.02$.

As can be seen from Table 1, Ro 15-1788 did not antagonise the anticonvulsant or sedative effects of melatonin. At a dose of 10 mg kg^{-1} Ro 15-1788 has no intrinsic activity but potently antagonises the sedative and anticonvulsant properties of benzodiazepines (Hunkeler et al, 1981; Cowen et al, 1981a). Our results therefore make it unlikely that either the sedative or the anticonvulsant effects of melatonin are mediated via the benzodiazepine receptor. These findings may reflect the rather low affinity of both melatonin and N-acetyl 5-methoxykynurenamine for the benzodiazepine receptor, their K_i values being considerably less than those of the benzodiazepines (Marangos et al, 1981).

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Albertson, T.E. et al (1981) Neuropharm. 20, 61
Anton-Tây, F. (1974) Adv.Biochem.Psychopharm. 11, 315
Cowen, P.J. et al (1981a) Br.J.Pharmac. London Meeting December 1981 P.8
Cowen, P.J. et al (1981b) Nature 290, 54
Hunkeler, W. et al (1981) Nature 290, 514
Marangos, P.J. et al (1981) Life Sci. 29, 259
Nutt, D.J. et al (1980) Neuropharm. 19, 1017

BENZODIAZEPINES INHIBIT THE BINDING OF NITROBENZYLTHIONOSINE, A NUCLEOSIDE TRANSPORT INHIBITOR, TO CNS MEMBRANES

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Inhibition of nucleoside transport, which might be expected to potentiate the effects of adenosine, has been invoked in postulates about the central (Phillis, et al, 1980) and peripheral (Clanachan & Marshall, 1980a) actions of diazepam and other benzodiazepines (BDZs). Diazepam, for example, potentiates the inhibitory neuromodulatory action of adenosine in the CNS (Phillis, 1979) and potentiates adenosine-induced coronary vasodilation in anaesthetized dogs (Clanachan & Marshall, 1980b). In a previous study (Hammond et al, 1981), we estimated the affinities of a series of BDZs for the nucleoside transport system in human erythrocytes through the inhibition of the binding of (G-^{3}H)nitrobenzylthioinosine (NBMPR), a potent, specific nucleoside transport inhibitor. NBMPR binds tightly (K_d about 0.32 nM), but reversibly, to transport inhibitory sites on human erythrocytes, a system in which inhibition of transport is related to site occupancy (Cass et al, 1974). We have now characterized the binding of (G-^{3}H)NBMPR to guinea pig cortical membranes and evaluated the affinities of dipyridamole, a recognized nucleoside transport inhibitor, and several BDZs for this site.

Under optimal conditions (pH 7.4, 22°C, 20 min incubation), site-specific binding of NBMPR to CNS membranes took place at sites of a single class (B_{max} (mean ± s.e. mean, $n \geq 6$) of 300 ± 10 fmoles/mg protein) at which the K_d for NBMPR was 0.25 ± 0.01 nM. Mass law analysis of binding data by the double reciprocal plot method showed that dipyridamole was a potent (K_i = 11 ± 2 nM), competitive inhibitor of the binding of NBMPR. Similarly, the BDZs, Ro5-4864, diazepam, clonazepam and lorazepam, also inhibited NBMPR binding competitively (K_i values of 5.4 ± 0.6μM, 16 ± 2 μM, 34 ± 5 μM and 52 ± 14 μM, respectively), but were considerably less potent in that respect than dipyridamole.

These results demonstrate the existence of a high affinity, saturable binding site for NBMPR in CNS membranes. Human erythrocytes and cultured cells possess similar membrane-located, high affinity sites for NBMPR, the occupation of which is associated with inhibition of nucleoside transport. In addition, the affinities of dipyridamole and the BDZs for the NBMPR site on CNS membranes are similar to those measured for the NBMPR site on erythrocytes (K_i values for dipyridamole, Ro5-4864, diazepam, clonazepam and lorazepam are 2.0 ± 0.4 nM, 2.2 ± 0.6 μM, 6.8 ± 1.1 μM, 24 ± 5 μM and 45 ± 7 μM, respectively. These findings support the hypothesis that some BDZs may inhibit nucleoside transport in the CNS and consequently, potentiate the action of adenosine. However, the low apparent affinity of the BDZs for the NBMPR site and the lack of correlation between the order of affinity of these compounds for the CNS BDZ site and the NBMPR site indicates that these sites are unrelated. Furthermore, the low apparent potencies of the BDZs for the nucleoside transport system suggest that nucleoside transport inhibition is likely only to occur with concentrations of the BDZs which are higher than those associated with their anxiolytic action.

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Cass, C.E. et al (1974) Biochim. Biophys. Acta 345, 1-10
Clanachan, A.S. & Marshall, R.J. (1980a) Br. J. Pharmac. 71, 459-466
Clanachan, A.S. & Marshall, R.J. (1980b) Br. J. Pharmac. 70, 66P
Hammond, J.R. et al (1981) Life Sciences 29, 2207-2214
Phillis, J.W. (1979) Can. J. Physiol. Pharmac. 57, 432-435
Phillis, J.W. et al (1980) Brain Research 195, 494-498

INHIBITION OF (^{3}H)-FLUNITRAZEPAM BINDING IN MOUSE BRAIN IN VIVO BY AGENTS SELECTIVE FOR THE BZ_1 BENZODIAZEPINE RECEPTOR SUBTYPE

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The specific binding sites for benzodiazepines (BZ's) found in mammalian brain are probably synonymous with the receptor sites that mediate the pharmacological actions of these drugs (see Muller, 1981). Squires et al (1979) subdivided BZ receptors into two types (BZ_1 and BZ_2) on the basis of the binding characteristics of the triazolopyridazine, CL 218,872. The distributions of the subtypes showed regional differences: cerebellum contained mainly BZ_1 while hippocampus and cerebral cortex contained both BZ_1 and BZ_2 in the ratios 50:50 and 70:30 respectively. Since then, a second group of compounds, the β-carboline-3-esters, have been shown to have preferential affinity for the BZ_1 receptor subtype (Nielsen & Braestrup, 1980). We have attempted to show whether the selectivity for BZ_1 binding sites in vitro shown by CL 218, 872 and the β-carboline esters (ie 3-methyl, ethyl and propyl) can also be demonstrated in vivo. To achieve this, we have measured the amount of ^{3}H-flunitrazepam bound to cerebral cortex and cerebellum after intravenous injection of the radioligand in conscious mice.

Male mice (Glaxo CR/H strain, 22-26G) were injected intravenously (iv) with 150μCi/kg ^{3}H-flunitrazepam (Amersham) and killed 20 min later. CL 218,872 (0.5-128mg/kg) or diazepam (0.5-128mg/kg) were administered orally 1 hour before the radioligand and the β-carboline esters (0.125-32mg/kg) were injected intravenously 10 min after the radioligand. Cerebella and cerebral cortices were homogenised in 80 vol Tris-HCl buffer. Aliquots (1ml) were filtered through GF/C filters (Whatman) under vacuum and the tritium bound to the particulate material was measured using liquid scintillation counting. Results were expressed as % inhibition of total binding.

Diazepam produced a dose-related inhibition of ^{3}H-flunitrazepam binding. The doses inhibiting binding by 25% (ID_{25}) and 50% (ID_{50}) of total were approximately 1 and 4mg/kg respectively in both cerebellum and cortex. In contrast, the inhibition obtained with CL 218,872 was significantly greater in the cerebellum, the ID_{25} and ID_{50} values being 10 and 39mg/kg in cerebellum and 15 and 80mg/kg in cortex. Similarly, propyl β-carboline-3-carboxylate gave ID_{25} and ID_{50} values of 0.75 and 3.0mg/kg (iv) in cerebellum and 1.2 and 5.0mg/kg (iv) in cortex. Similar differentiations were obtained with methyl and ethyl β-carboline-3-carboxylates.

Thus, the BZ_1, selective agents showed the regional selectivity in vivo that might be predicted from the published data obtained in vitro. The in vivo ^{3}H-flunitrazepam binding assay is a useful tool for studying drug action at benzodiazepine receptors; we hope to be able to use it further to obtain meaningful correlations between pharmacological activity and receptor occupancy.

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Muller, W.E. (1981) Pharmacology 22; 153-161
Nielsen, M. & Braestrup, C. (1980) Nature 286; 606-607.
Squires, R.F. et al (1979) Pharmac. Biochem. Behav. 10; 825-830.