TOLERANCE STUDY ON DENZIMOL, A NEW ANTICONVULSANT AGENT, COMPARED TO PHENOBARBITONE AND CARBAMAZEPINE IN MICE

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It is well known that the anticonvulsant effect of antiepileptic drugs is reduced by repeated administration in both experimental animals and man (Frey and Kampmann, 1965; Masuda et al., 1979; Rawling et al., 1975).
The purpose of this investigation was to verify if denzimol, an arylalkylimidazole anticonvulsant agent (Nardi et al., 1981; Graziani et al., 1983) induces tolerance after repeated treatment in mice. Phenobarbitone (PB) and carbamazepine (CB) were used as reference drugs.
We have investigated the antielectroshock activity (MES) and the brain levels of denzimol, after acute and repeated treatment, to verify whether the development of denzimol tolerance involves mechanisms of adaptation of the central nervous system or an induction of hepatic microsomial enzymes.

Three groups of 20 mice each were treated p.o. for 14 days with denzimol (30 mg/kg), PB (25 mg/kg) or CB (30 mg/kg). Three control groups of mice received the vehicle (0.5% methocel). 24 hours after the end of the prolonged treatment, the respective drugs and vehicle, at the same dose as above, were given to the treated and control groups of animals.
The anticonvulsant effect of each drug against MES was examined at 1,2,4,6,8 hrs after the last administration. The protection against the tonic extension of forelimb (TFP) and hindlimb (THP) induced by MES was the criterium used for the assessment of positive response.
After MES, animals treated with denzimol were sacrificed and brains were removed for tissue drug levels evaluation (Abbiati et al. 1985).

The present study shows that repeated administrations of PB, CB and denzimol resulted in a development of tolerance to their anticonvulsant action.
Tolerance induced by denzimol against TFP and THP extension, resulted lower than that of PB and probably of CB. When denzimol was administered in a single dose to denzimol tolerant mice, its brain concentrations was markedly reduced if compared to non-tolerant mice. Moreover the disappearance of brain denzimol concentrations, after a single dose of drug, was alterated by repeated treatment of mice with denzimol. These findings seem to suggest that decreased anticonvulsant activity of denzimol, following repeated administrations, might be due to a development of pharmacokinetic tolerance.

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CROP-SAC STIMULATORY EFFECTS OF CHOLINERGIC MECHANISM IN PIGEONS

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In previous experiments we have shown that prolactin (PRL) secretion in avian species is under a tonic inhibitory control of dopamine (Nisticò et al., 1979). The present study was carried out in order to assess the role played by cholinergic mechanisms in the control of PRL secretion in pigeons (Columba livia). In particular, we have assessed the effects of drugs enhancing by different mechanisms cholinergic transmission, i.e. carbachol, muscarine and physostigmine, on morphology (studied by SEM and TEM) of crop-sac mucosa and pituitary lactotrophs.

Carbachol, microinfused into the III cerebral ventricle (6 nmol) for 3 consecutive days, produced intense crop-sac stimulation with milk-like secretion and a marked activation of pituitary lactotrophs (enlargement of lysosomes, presence of secretory granules and activation of Golgi apparatus and rough endoplasmic reticulum). Similar phenomena at crop-sac level and pituitary lactotrophs were also observed 30 min after last intraventricular (0.005 μmol each day x 3 consecutive days) administration of muscarine or physostigmine (0.5 nmol each day).

Crop-sac and pituitary lactotrophs stimulatory effects evoked by direct and indirect cholinergic drugs were prevented by previous administration of atropine (10 μmol/Kg i.p.) and antagonist at Ach muscarine receptors, whereas mecamylamine (50 μmol/Kg i.p.) and pempidine (100 μmol/Kg i.p.) were ineffective.

In conclusion, the present experiments show that drugs enhancing cholinergic transmission given at very low doses into the III cerebral ventricle produce a marked stimulation of pituitary lactotrophs and crop-sac mucosa suggesting that muscarinic receptors at the hypothalamic and/or pituitary level are involved in avian species in the control of prolactin secretion.

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EFFECT OF SCOPOLAMINE ON A ONE-TRIAL PASSIVE AVOIDANCE TASK IN THE MOUSE

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Scopolamine has been long known to induce amnesia in animals and humans in a variety of experimental paradigms (Bartus et al, 1982; Spencer & Harbans, 1983). However, some controvery persists about its ability to interfere with one-trial passive avoidance learning (Spencer & Harbans, 1983). The aim of the present study was to systematically explore the effects of scopolamine on acquisition of a passive avoidance response.

Male CD1 mice (24-28g) from Charles River were housed under standard laboratory conditions, with light from 7 a.m. to 7 p.m. and free access to food and water. After a 7-12 days habituation period they were grouped ramdomly. A "step-through" apparatus consisting of two chambers separated by a guillotine door was used. In the training trial, each mouse was placed in the lighted chamber (start box, 10x10x12cm) and, after a 5 sec orientation period, the door (5x5 cm) was raised to allow it to enter the second dark chamber (22x16x12cm). The mouse that instinctively entered the darker box was given a foot shock (FS; 1mAx1sec). Retention testing was done about 24h after training. The time the animal need to re-enter the shock box (the latency time) was measured in second (cut-off time = 180 sec), as a measure of retention. Long latencies in mice subjected to FS only demonstrate that they have learned to avoid the shock chamber, while shorter latencies in animals given FS plus scopolamine would indicate that amnesia was induced.

The mean latency times (sec) after several i.p. doses of scopolamine (HBr) or vehicle (saline; 10 ml/kg) given at various times before and after training, are shown in table 1

Table 1 - Scopolamine-induced memory deficit in mice (latency times)

Dose	Interval in minutes between scopolamine treatment and training						
mg/kg	-60	-30	-15	-5	0*	+5	+15
Vehicle	179 (6)	-	180 (10)	176 (12)	180 (6)	180 (6)	180 (6)
0.32	129 (14)	114 (8)	97 (20)	138 (6)	-	-	-
0.63	111 (8)	91 (8)	59 (30)	106 (12)	180 (6)	-	-
1.25	77 (14)	41 (14)	39 (18)	55 (20)	180 (12)	180 (6)	180 (6)
2.50	-	-	-	32 (24)	180 (6)	-	-
10.00	-	-	-	25 (12)	180 (20)	180 (6)	180 (6)

* about 10 sec after training ( ) N° of animals

As have previous studies, our results indicate that pre-training administration of scopolamine markedly affects retention. Our data reveal good doses-and time-related effects with a peak of activity aroud the 15th min, and serve for knowing the optimal schedule of treatment for the use of scopolamine as an amnesia agent in a one-trial passive avoidance task in mice. Post-training administration of scopolamine has been reported to induce retrograde amnesia at high doses (10 mg/kg; i.p.) but not at low (1 mg/kg) (see Spencer & Harbans for references). Our results clearly indicate that the lack of amnesic effect is imputable to the time of treatment rather than to the dose. It is possible that, when scopolamine is given after training, its mnemonic action is not rapid enough to interfere with the consolidation process and to produce retrograde amnesia.

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INVOLVEMENT OF ACETYLCHOLINE AND NORADRENALINE IN STRESS INDUCED ANTINOCICEPTION

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Following exposure to an acute stress, the reaction time to a noxious stimulus is usually prolonged and, depending upon the type and duration of the stress, the antinociception produced is sensitive to opioid antagonists. We describe the investigation of the involvement of acetylcholine(ACh) and noradrenaline(NA) in a model of stress induced antinociception(SIA) which is sensitive to both μ- and δ-opioid receptor antagonists(Hart et al.1985).

Male LACA mice(25-35g) were allowed to acclimatise for 2h prior to experimentation which occurred between 1100-1300h. Mice were stressed by being placed in a water-bath(57x30x19cm) at 20°C for 3min. Antinociception was assessed by measurement of the reaction time of the mouse on a hot-plate(54°C) with a maximum reaction time of 45s. On removal from the water, each mouse was warmed by lamps for 2min: unstressed mice were treated in a similar manner. Reaction times for each mouse were determined three times; 30min prior to the i.p. injection of vehicle or drug, 15min after injection(immediately pre-swim) and 20min after injection(post-swim). Differences between the first and second measurements indicated changes due to drug administration and SIA was expressed as the difference in reaction times at 20min and 15min after injection. Each treatment included at least 6 mice and reaction time values were analysed by the Mann-Whitney U-test.

There were no significant changes in the reaction times of unstressed mice whether treated with vehicle or drug. In vehicle treated mice the median difference between post-swim and pre-swim reaction times was +17s which is presented as SIA in Table 1. The other results in Table 1 show that pre-treatment with hyoscine, with the α_1-antagonist prazosin and with the α_2-antagonists yohimbine and idazoxan produced dose-related reductions in SIA. That muscarinic receptors are involved in a naloxone-sensitive model of SIA is not unexpected for antinociception produced by morphine is atropine sensitive and that produced by organophosphorus anticholinesterases is naloxone-sensitive(Clement & Copeman 1984). Our results demonstrate the involvement of ACh and NA in this model of SIA and support the conclusion of Hayes et al.(1984) that α-adrenoceptor agonists can mediate antinociception via α_1- and α_2-adrenoceptors.

Table 1. Median differences between post-swim and pre-swim reaction times (measured in s) in mice receiving vehicle or drug. * $p<0.05$

	Vehicle	+17			
Hyoscine	0.5mg/kg	8.5*	Yohimbine	0.1 mg/kg	6.0*
	1	5.2*		0.25	3.0*
	5	2.7*		0.50	1.0*
Prazosin	0.25	11	Idazoxan	0.25	11.5
	0.50	4*		0.50	7
	1	2*		1	3.5*

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INHIBITORY EFFECTS OF CATECHOL ON SYNAPTIC TRANSMISSION IN THE RAT OLFACTORY CORTEX SLICE

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In slices of rat olfactory cortex in which GABA-mediated inhibition has been abolished by picrotoxin (25μM), the proconvulsant drug catechol (1,2-dihydroxybenzene) potentiates excitatory transmission (Dewhurst & Collins, 1985). The present study was designed to identify possible effects of catechol on inhibitory transmission by monitoring its actions on slices in the absence of picrotoxin.

Rat olfactory cortex slices (500μm thick) were preincubated and perfused at room temperature (Pickles & Simmonds, 1976) and the surface fields evoked by supramaximal electrical stimulation of the lateral olfactory tract (50μsec pulse width; 0.0033 Hz) recorded using a chlorided silver ball electrode.

Dropwise application of catechol (0.05 to 2mM) significantly ($P < 0.02$) increased the peak amplitudes of all fields in a dose-dependent and partially reversible manner. For example, the maximum increase in N-wave amplitude was from 2.58 ± 0.27 to 3.83 ± 0.43 (0.5mM catechol), the late N-wave from 2.18 ± 0.23 to 2.49 ± 0.29 (0.25mM catechol) and the I-wave from 0.9 ± 0.19 to 1.80 ± 0.26 (1mM catechol) (mean amplitudes in mV± s.e. mean, n = 5).

The evoked fields reflect excitatory transmission at the lateral olfactory tract-superficial pyramidal cell synapse (N-wave) and GABA-mediated recurrent pre- (late N-wave) and postsynaptic (I-wave) inhibition (Pickles & Simmonds, 1976, 1978). The present results suggest that catechol potentiates all three synaptic events. Drug effects on postsynaptic inhibition were investigated further using the conditioning procedure described by Pickles & Simmonds (1978). Over conditioning intervals of approximately 200 to 8000msec, catechol (1mM) increased the latency and decreased the amplitude of the population spike evoked by the test stimulus, thereby confirming a potentiation of postsynaptic inhibition. However, this action of catechol was only partially antagonized by picrotoxin (25 and 75μM). For example, in slices perfused with picrotoxin (25μM) and using a fixed conditioning interval of 100msec, the population spike latency evoked by the test stimulus increased from 7.60 ± 0.12 (zero catechol) to 9.41 ± 0.27 (1mM catechol) (mean latencies in msec ± s.e. mean, n = 3; significant difference, $P < 0.001$).

Thus, in addition to potentiating excitatory transmission in the olfactory cortex (Dewhurst & Collins, 1985), catechol also increases GABA-mediated inhibition but has an added inhibitory action which is GABA-independent. At present it is unclear whether these two effects on inhibition are a consequence of a catechol-induced increased excitatory input to inhibitory interneurones or whether they reflect direct actions of the drug on inhibitory activity.

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OPPOSITE EFFECTS OF 5-HT ON THE EFFLUX OF ^{3}H CHOLINE FROM GUINEA-PIG STRIATAL SLICES

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5-hydroxytryptamine (5-HT) causes either increase or reduction of neuronal firing rate (Jones & Dourish, 1982), and variably affects the transmitter release (De Belleroche & Bradford, 1982; Cerrito & Raiteri, 1979). Recent studies show that 5-HT inhibits the striatal cholinergic interneurones (Vizi et al.,1981), while biphasic effects have been reported on the hippocampal cells (Anwyl & Rowan,1984). In view of this complex pattern of action, we decided to check whether a facilitatory effect of 5-HT could be detected also in the cholinergic striatal cells.

Guinea-pig caudatal slices were preloaded with ^{3}H Choline 0.1 μM and the tritium efflux,taken as an index of Acetylcholine (ACh) release, was measured in the presence of hemicholinium-3 during rest and during electrical stimulation. The stimulation (for 2 min, at 0.2 Hz) was performed at the 45th (St_1) and 85th (St_2) min (for details see Beani et al., 1984). 5-HT was applied: i) during rest, as pulses of two min or as continous perfusion starting from the 60th min of the washout curve, ii) during St_2, as pulses of two min or as continous perfusion starting from the 65th min (i.e. twenty minutes before St_2).

5-HT 10-100 μM temporarely (2-3 min) increased in a concentration related manner the spontaneous tritium efflux. This effect was prevented by Tetrodotoxin 0.5 μM and Methysergide 0.1 μM but not by Methiotepin.The amine applied for 2 min during St_2 did not change the St_2/St_1 ratio, but, when added 20 min before St_2 significantly reduced this ratio. Such inhibition was prevented by Methysergide 1 μM.

Our findings confirm the 5-HT-mediated inhibition of stimulus-induced ACh release (Vizi et al.,1981). In addition they demonstrate for the first time that the amine is able to transiently increase tritium efflux in rest conditions.Since: i) the resting ^{3}H choline release in caudatal slices partly depends on the spontaneous firing of cholinergic cells (Beani et al.,1984) and ii) the 5-HT facilitation is prevented by Tetrodotoxin, one can infer that the amine increases the neuronal firing rate through 5-HT receptors, at the moment of its first contact with the tissue. Taking into account that the excitation vanishes within a few minutes and is followed by inhibition, the physiological 5-HT signal may activate or inhibit the target cell depending on its phasic or tonic pattern.

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EFFECTS OF KETAMINE ON THE FROG NODE OF RANVIER

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Ketamine, a structural analogue of phencyclidine, is used clinically to induce rapid anesthesia and analgesia. The state of unconsciousness is described as "dissociate anaesthesia", characterized by intense analgesia and amnesia, associated with only mild sedation (Corssen & Domino, 1966). In addition to amnesic, analgesic, cataleptic and anesthetic properties, it is endowed with epileptogenic properties (Domino et al., 1965). For these reasons we have investigated its effects on the frog myelinated nerve fiber. The experiments were carried out on current and voltage clamped node of Ranvier. The action potential was reversibly blocked by external ketamine (0.5-1mM). After wash, spontaneous activities appeared when potassium channels were blocked by external tetraethylamonium. Sodium and potassium currents were blocked with apparent dissociation constants of 0.6 and 0.25 mM respectively. After several minutes of drug application, a small maintained sodium current (5 to 10% of the peak Na current) was observed during long lasting depolarizations (Figure 1). This effect was slowly reversible and, in addition, the maintained sodium current was increased during the first minutes of wash. These observations can be explained if one assumes that ketamine exerts two different effects on the sodium current : block of Na current and partial removal of Na inactivation. The appearance of the maintained sodium current could account for the spontaneous activity.

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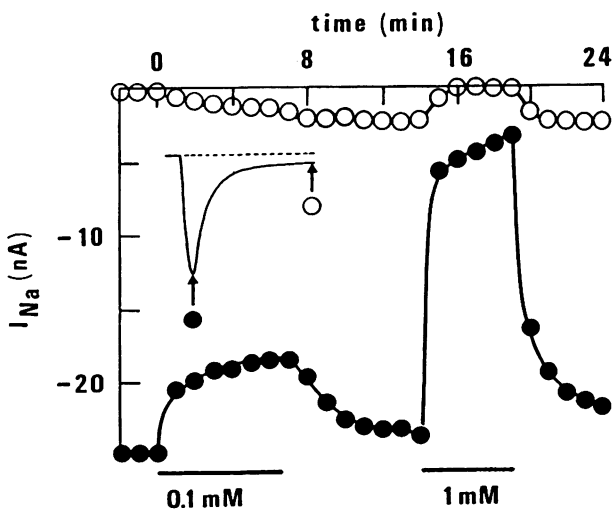

Figure 1 Kinetics of ketamine (0.1 and 1 mM) effects on peak (●) and maintained (○) Na currents. The current was recorded during depolarizations to 0 mV preceeded by 50 ms hyperpolarizations to -120 mV.

A STEREOCHEMICAL MODEL ACCOUNTING FOR RECEPTOR BINDING AFFINITY AND INTRINSIC ACTIVITY OF β-CARBOLINES

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Recently several new drugs, chemically unrelated to benzodiazepines (BDZs), have been discovered which exert their pharmacological action by interacting with the CNS BDZ receptors. These drugs can be classified, according to their spectrum of biological activities, as agonists (AG) (which are, *inter alia*, anxiolytic), inverse agonists (IAG) (anxiogenic)and antagonists (ANT) (without any *per se* biological effect but preventing the interactions of agonists and inverse agonists with the receptor).

β-carbolines interact at various affinity levels with BDZ receptors displaying the full spectrum of AG-ANT-IAG properties according to the molecular substituents.

We propose here a general stereochemical model accounting for both receptor binding affinities (RBAs) and nature of intrinsic activity (IA) in this class of compounds.

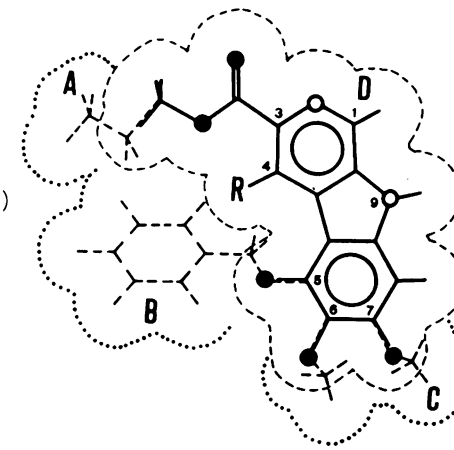

Figure 1 Stereochemical requirements for receptor binding affinity and intrinsic activity in β-carbolines.

Receptor Binding Affinity. β-Carbolines (see Figure 1) can display high RBAs for BDZ receptor only if 3-substituted by an ester (or amide) function, if the molecule is planar (aromaticity of the six-membered rings) and if not 1-substituted (Zone D), suggesting that the recognition site is a planar cleft where the main drug-receptor interaction is mediated by the C=O group of the ester or amide function, which is a typical hydrogen bonding acceptor, and possibly strengthened by the two ring nitrogens.

Intrinsic Activity. In Figure 1 the heavy lined sketch represents β-CCM (Bertolasi *et al.*, 1984), a typical IAG, the surrounding space having been divided in three A-C subdomains. Substitution in Zone A by groups of increasing steric hindrance shifts the pharmacological profile from full IAG (β-CCM) to partial IAG (β-CCE) to ANT (Pr-CC). Substitution in Zone B causes dramatic IA changes, as shown by the comparison of β-CCE (partial IAG) with its 4,5-substituted derivatives, ZK 93426 (4-methyl-5-*i*-propyloxy-β-CCE) being an ANT and ZK 91296 (4-methoxymethyl-5-benzyloxy-β-CCE), substituted by bulkier groups, displaying partial AG properties (Braestrup *et al.*, 1984). Substitution in Zone C seems to increase IAG properties of β-carbolines (compare DMCM with β-CCM).

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REGIONAL DIFFERENCES IN AGONIST AND DEPOLARISATION-INDUCED INOSITOL PHOSPHOLIPID HYDROLYSIS IN RAT BRAIN

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There is now evidence that several neurotransmitter receptors, including muscarinic and alpha$_1$ adrenoceptors, are closely coupled to inositol phospholipid hydrolysis in rat cerebral cortical slices (Brown et al. 1984; Jacobson et al. 1984). Furthermore, depolarisation of such slices also evokes similar phospholipid responses which can be greatly potentiated by the cholinesterase inhibitor physostigmine (Batty et al. 1985) or the Ca^{2+} channel activator BAY-K-8644 (Kendall & Nahorski, 1985). In the present study we examined the responsiveness of slices prepared from several cerebral regions to these stimuli in an attempt to relate them to receptor and/or appropriate nerve terminal localisation.

Brain slices were prepared from the cortex, hippocampus, striatum, hypothalamus, medulla-pons and cerebellum. The slices were preincubated with ^{3}H-inositol in the presence of 5 mM Li^+ and water soluble ^{3}H-inositol phosphates (^{3}H-IP) separated by anion-exchange chromatography on columns (Brown et al. 1984).

Carbachol and noradrenaline evoked an increase in ^{3}H-IP accumulation in all cerebral regions, though there were marked topographical differences in maximal responsiveness. Thus, large muscarinic responses were observed in cortex (7-fold), hippocampus (5.3-fold) and striatum (4.6-fold) but smaller accumulations of ^{3}H-IP were observed in hypothalamus (3-fold), medulla-pons (2-fold), and the cerebellum produced little or no response to carbachol. Noradrenaline responses were largest in hippocampus (7-fold), cortex (5.3-fold), medulla-pons (3.7-fold), striatum (3.5-fold), with the hypothalamus and cerebellum being much less responsive.

Elevation of extracellular K^+ to 18 mM resulted in a 2-fold increase in the production of ^{3}H-IP in the cortex, hippocampus, striatum and hypothalamus but a very weak and no response in the medulla-pons and cerebellum, respectively. The cholinesterase inhibitor physostigmine (50 μM) enhanced (about 3-fold) the response produced by elevated K^+ in the cortex, hippocampus and striatum but had markedly weaker effects in the hypothalamus and medulla-pons, and no evident effect in the cerebellum. Similarly, the dihydropyridine Ca^{2+} channel activator BAY-K-8644 (1 μM) greatly enhanced the 18 mM K^+ response in the forebrain regions (4.6, 3.8 and 2.5 fold in cortex, hippocampus and striatum) but more modestly in hypothalamus and medulla-pons and not at all in cerebellum.

Differences in the potentiation of the K^+ response by physostigmine in comparison to the carbachol response probably reflects a differential distribution of muscarinic receptor and cholinergic terminals. It is not yet known whether the response to K^+ alone that is enhanced by BAY-K-8644 is mediated directly or indirectly (Kendall & Nahorski, 1985). Thus, the topographical responsiveness of this agent may reflect an uneven distribution of an unknown mediator and/or the distribution of dihydropyridine-sensitive Ca^{2+} channels.

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EFFECTS OF ACETYLCHOLINE ON SYNAPTIC TRANSMISSION IN THE FROG OPTIC TECTUM

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The frog optic tectum contains the highest concentration of acetylcholine (ACh) in the frog brain (Nistri et al., 1975). The ACh distribution, synthesis and binding sites support its neurotransmitter role in the optic nerve of lower vertebrates (Freeman & Norden, 1984). Since both nicotinic antagonists and agonists only depress optic nerve-evoked tectal potentials in vivo (Freeman & Norden, 1984), we explored whether any enhancement by ACh of optic nerve-evoked responses might be observed under more advantageous conditions, namely fast drug superfusion of the in vitro tectum. As optic nerve-induced synaptic transmission is thought to be nicotinic (Freeman & Norden, 1984) in spite of abundant muscarinic binding sites (Birdsall et al., 1980), we also examined the action of muscarinic agents on tectal synaptic transmission in vitro.

Experiments were carried out on the optic tectum of in vitro brain preparations of frogs (R. temporaria) according to the method of Sivilotti (1985). Preparations were superfused (10-20 ml min^{-1}) with Ringer solution at 7°C. Electrical field potentials elicited by square pulse stimulation of the controlateral optic nerve were recorded with a microelectrode placed at about 100 μM depth in the tectum, digitized and displayed on a pen recorder.

Tectal field potentials consisted of monosynaptic negative waves (U_1 and U_2; Chung et al., 1974) representing summated EPSPs and spikes. Supramaximal U_1 and U_2 waveforms were usually depressed by ACh (25-250 μM) or carbachol (25-250 μM). When waveforms approximately half of their maximal responses were elicited, ACh enhanced (by up to 47%) U_1 and U_2 amplitudes and subsequently depressed them by 30% (28/31 preparations; apparent ED_{50} value = 25 μM). In 6 preparations neostigmine (100 μM) potentiated the enhancing effect of ACh while fully inhibiting cholinesterase activity assayed according to Ellman et al. (1961). Carbachol was more potent than ACh in enhancing the U_1 and U_2 waves although it had a slower depressant effect on them. The enhancing activity of bethanechol was intermediate between that of ACh and carbachol. D-Tubocurarine (10 μm) depressed U_1 and U_2 amplitudes elicited by low stimulus strength but enhanced their maximal responses. Atropine (1 μM) shifted upwards the strength/amplitude plots of these field potentials. Mixtures of tubocurarine and atropine increased the U_1 and U_2 waves and attenuated the enhancing action of ACh or carbachol.

These data show a biphasic action of ACh on tectal synaptic transmission. The enhancing effect of cholinergic antagonists on field potentials is reminiscent of the one reported by Brown et al. (1984) for rat brain interpenduncular slices and raises the possibility that endogenously-released ACh may partly act on the presynaptic terminals of the optic nerve.

[1]Holder of a Royal Society/Accademia dei Lincei Fellowship. The financial support of the Peel Medical Research Trust is gratefully acknowledged.

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THE EFFECT OF UNILATERAL OCCLUSION ON THE ELECTROENCEPHALOGRAPH OF THE MONGOLIAN GERBIL

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The finding that the brain of the Mongolian gerbil (Meriones unguiculatus) is unique in lacking connecting arteries between the basilar and internal carotid circulation has made it a suitable model for the study of stroke (Kahn, 1972). As only 30 - 50% of gerbils suffer a stroke following unilateral occlusion of the common carotid artery, an assessment of the integrity of the cerebrovascular anatomy of the circulus arteriosus in all animals must be undertaken. We have previously reported that a good correlation exists between neurovascular deficits and deficiencies in ipsilateral striatal dopamine levels following unilateral occlusion of the right common carotid artery (Alps et al, 1984). This study was to determine if a similar correlation exists between inhibition of the electroencephalograph (EEG) activity and cerebrovascular deficit.

Mongolian gerbils weighing 60 - 80 g, were anaesthetised with 5% halothane in a 70% nitrous oxide/30% oxygen mixture. After induction the halothane concentration was decreased to 1.5% and maintained at this level throughout the remainder of the experiment. The EEG was recorded by subcutaneous needle electrodes between the left and right temporal region. Following recording of a control trace the right common carotid artery was exposed through a paratracheal incision and ligated as previously described (Alps et al, 1984). At termination of the experiment, the brain was perfuse fixed with 10% formal saline with added Indian ink by left ventricular injection. The circulus arteriosus was examined microscopically for anatomical classification.

Animals showing isoelectric EEGs within 10 s of unilateral occlusion of the right common carotid artery were designated "stroke prone". Anatomical investigation of the cerebral vasculature of these animals indicated an incomplete circulus arteriosus and poor collateral circulation. This suggested that a correlation existed between neurovascular anatomical deficiency and the occurance of an isoelectric EEG. However, a subpopulation of animals described as "stroke prone" in anatomical studies did not develop isoelectric EEGs. On further detailed examination, a well-developed collateral bed was identified in these animals, which may sustain blood flow sufficiently to support electrical activity.

"Stroke resistant" animals having a complete circulus arteriosus were not susceptible to unilateral occlusion and showed no EEG flattening.

The results of this study show that EEG recordings can be used to identify a subpopulation of unquestionably "stroke prone" gerbils having an incomplete circulus arteriosus.

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EFFECT OF ERGOLINE DERIVATIVES ON DOPAMINE TURNOVER IN RAT BRAIN

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We have previously reported that chronic oral nicergoline to rats enhances dopamine (DA) turnover preferentially in mesolimbic areas as indicated by the increase of homovanillic acid (HVA) and, to a lesser extent, of 3,4-dihydroxyphenylacetic acid (DOPAC) (Carfagna and Moretti, 1984). Oral dihydroergotoxine caused a slight, non significant decrease of mesolimbic HVA. The effect of nicergoline was not related to an inhibition of D-2 receptors labelled by ^{3}H-butyrophenones (Moretti, Carfagna and Caccia, 1983).

In the present study the drug's effect on DA turnover was explored in rats of various ages (5, 13, 24 months). DA, HVA and DOPAC were assayed by HPLC with electrochemical detection (Sperk, 1982). Oral nicergoline (5 mg/kg b.i.d. for 45 days) raised the level of both DA metabolites in mesolimbic areas (but not in striatum) either of young adult (HVA +36%; DOPAC +17%), or mature (HVA +66%; DOPAC +25%) or old rats (HVA +50%; DOPAC +27%) without affecting DA. Oral dihydroergotoxine (0.5 mg/kg b.i.d.) did not significantly affect these parameters.

In another experiment in young adult rats, nicergoline (20 mg/kg s.c.) enhanced the disappearance of DA from striatum and mesolimbic areas of rats treated with **α**-methyltyrosine (**α**-MT), 250 mg/kg i.p. As expected pergolide (0.5 mg/kg s.c.) and apomorphine (0.2 mg/kg s.c.) reduced this disappearance in striatum.

It may be concluded that nicergoline stimulates DA turnover in specific brain areas of rats of different ages. This effect differentiates nicergoline from most of ergot derivatives which are known to inhibit DA turnover (Burki et al., 1978).

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DOPAMINE RECEPTOR NEUROPLASTICITY CAN BE AFFECTED BY GM_1 GANGLIOSIDE TREATMENT

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The striatal dopamine (DA) receptor supersensitivity which follows a chronic haloperidol treatment can be considered an animal model of tardive dykinesia. The occurrence of this neurological syndrome is one of the major problems of psychiatric therapy with neuroleptics.

We have already reported that the concomitant administration of GM_1 monosialoganglioside (10 mg/kg/day i.p.) is capable of reducing the development of chronic haloperidol-induced receptor supersensitivity (Agnati et al., 1983). We now report that the post-treatment with GM_1 (30 mg/kg/day i.p.) in rats with haloperidol-induced DA receptor supersensitivity is capable of significantly accelerating the recovery of normal receptor sensitivity. The above effect is already evident after 7 days of GM_1 treatment and after 14 days normal DA receptor sensitivity is almost completely restored. At this latter time, the control group post-treated with saline following chronic haloperidol administration still displayed a significantly elevated degree of DA receptor supersensitivity. GM_1 alone did not produce any significant change of striatal DA binding characteristics when added *in vitro* or after treatment *in vivo*.

The above data indicate that GM_1 treatment can effectively not only prevent but also reverse DA receptor supersensitivity induced by haloperidol. The mechanism by which GM_1 produces this effect is still unknown. A possible effect of GM_1 on striatal DA presynaptic activity or on neuropeptides which may determine the modulation of DA receptor function is currently under investigation.

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672P

RELATIONSHIP BETWEEN ELECTRICALLY-INDUCED ADENOSINE RELEASE AND cAMP LEVELS IN RAT STRIATAL PRIMARY CULTURES

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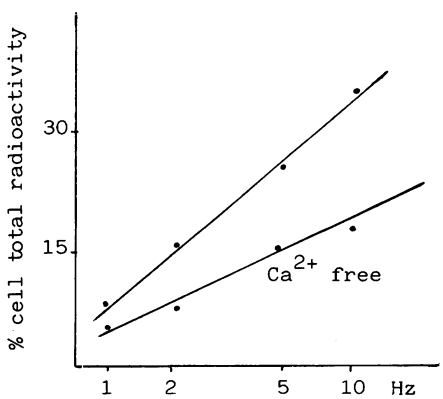
Many Authors agree on the hypothesis that in the nervous tissue Adenosine(A) behaves simultaneously both as a cotransmitter and an activator of metabolic processes. Based on verified interactions between different subtypes of A receptor sites and their catalytic subunits it's possible to assume that both the above mentioned effects almost completely depend on the A interactions with the cAMP system (Olsson R., 1984). Altough these effects can derive from the simultaneous A activity on different molecular mechanisms of the same cell, it's likely to suppose that A, for the most part, exerts different effects on functionally different cells. To support this hypothesis, the present study purposes to verify if the interactions between A release and cAMP system activity differ in neuronal and glial primary cultures.

The striata,dissected out newborn rats and mechanically dissociated in DMEM plus horse serum, were forced through a 14-inch gauge cannulae to obtain a cell suspension. The cells were seeded onto poly-L-lysine coated coverslips. To select the neuronal growth,when it was necessary, Cytosine Arabinoside was added to the cultures after 5-6 days. The amount of neuronal cells was estimated by using the tetanus toxin indirect immunofluorescence of Yavin et al.(1982). At the end of established period of culture the coverslips were placed in superfused microchambers. A field electrical stimulation(trains of pulses of alternating polarity, 30 mA/cm^2, 5msec)was delivered at different frequencies. The changes of cAMP levels were radioenzymatically assayed. ^{3}H-A release was measured according to Lloyd & Stone method(1981),suitably modified.

Our results show a specific and appreciable ^{3}H-A release, electrically evoked, in neuronal cells only after the 8th day of culture. At the 14-16th day ^{3}H-A release was frequency and Ca^{2+} dependent(see Fig.1) and almost completely counteracted by pretreatment with TTX(5×10^{-7} M). In glial cultures ^{3}H-A release,partially frequency related,was neither TTX sensitive nor Ca^{2+} dependent. During the first 5-7 days, it was not possible to evoke a specific ^{3}H-A release,even if the cellular ability to take up and accumulate the exogenous labeled A was recovered. However,in this period the basal release of ^{3}H-A was significantly higher (about 3 times) than that found in 14-16 days old cultures. Likewise,the cAMP basal levels were elevated in the cultured cells at the beginning and resulted significantly diminished when higher degree of cell maturation was achieved. In any case,significant changes of cAMP levels were found when the electrical stimulation evokes a detectable ^{3}H-A release.



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THE EFFECT OF 5,7-DIHYDROXYTRYPTAMINE ON AMITRIPTYLINE-INDUCED INCREASES IN BRAIN AND SPINAL CORD LEVELS OF TRH

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Treatment with 5,7-dihydroxytryptamine (5,7-DHT) causes a marked reduction in the levels of thyrotrophin-releasing hormone (TRH), as well as 5-hydroxytryptamine (5-HT), in rat nucleus accumbens and ventral spinal cord (Lighton et al, 1984a), suggesting a close peptide-indoleamine interaction in these two CNS regions. Following chronic treatment with antidepressant drugs, or the 5-HT antagonist metergoline, levels and ex vivo release of TRH in the same two regions are increased (Lighton et al, 1985). In contrast repeated electroconvulsive shock results in a decrease in the levels of TRH (Lighton et al, 1984b). These results, together with the well-documented reciprocal effects of the two antidepressant treatments upon the 5-HT_2 receptor, may further suggest an interaction between TRH and 5-HT_2 receptors.

In the present study, the effect of 5,7-DHT pretreatment has been assessed on the regional increase in TRH induced by chronic amitriptyline administration.

Male Wistar rats were anaesthetized with sodium pentobarbitone (60 mg/kg i.p.). 5,7-DHT (200 µg) was dissolved in 0.9% saline containing 0.1% ascorbic acid and injected bilaterally (2 x 0.5 µl) into the lateral ventricles (co-ordinates taken from bregma were A/P 0; L/R ±1.5; V -3.2 mm with the incisor bar set 5 mm above the interaural line). Control animals received an equivalent volume of vehicle alone. All animals were pretreated 60 min prior to 5,7-DHT with desmethylimipramine (25 mg/kg i.p.). Following recovery, both 5,7-DHT and sham lesioned animals were chronically injected with amitriptyline (15 mg/kg twice daily, i.p. for 14 days). A third group of non-operated animals were chronically injected with an equivalent volume of 0.9% saline.

Four hours following the final injection, animals were killed and TRH measured in selected CNS regions by radioimmunoassay (Lighton et al, 1984a). TRH levels (pg/µg protein, mean ± SE, n=6) were 2.85±0.45 (lumbar spinal cord), 3.60±0.50 (nucleus accumbens) and 4.05±0.40 (suprachiasmatic nuclei). In sham-lesioned animals, administration of amitriptyline resulted in a significant ($p<0.01$) increase in TRH content of the lumbar spinal cord (+209%), nucleus accumbens (+287%) and suprachiasmatic nuclei (+150%) compared to saline-injected control animals. However, in animals pretreated with 5,7-DHT and chronically injected with amitriptyline the increase in TRH levels was abolished and, furthermore, there was a significantly ($p<0.05$) lower level of TRH in the accumbens and spinal cord when compared with the saline-treated controls. Amitriptyline with and without 5,7-DHT pretreatment had no effect on peptide levels in median eminence or septal nuclei.

The results further demonstrate the effect of 5,7-DHT on TRH in the nucleus accumbens and spinal cord and indicate that an intact 5-HT system is essential for the amitriptyline-induced increase in TRH to occur.

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DEOXYGLUCOSE UPTAKE IN RAT CEREBRAL CORTEX FOLLOWING IBOTENIC ACID LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS

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The cholinergic systems which originate in the basal forebrain and innervate the cerebral cortex degenerate in Alzheimer-type Dementia (ATD) (Arendt, Bigl, Tennstedt et al., 1985; Davies, 1979). Glucose metabolism in the neocortex is markedly reduced in patients with ATD (Foster, Chase, Fedio et al., 1983). To determine whether these observations might be causally related we have examined the effects of destroying cell bodies in the nucleus basalis magnocellularis (nBM) on choline acetyltransferase (CAT) activity and glucose metabolism in rat brain.

Thirteen female Sprague-Dawley rats (wt. 220-230g) were anaesthetized with Equithesin and placed in a stereotaxic frame with the incisor bar set 5mm above the interaural line. Bilateral lesions of the nBM were produced in 7 rats by injection of 4μg ibotenic acid in a volume of 0.4μl; 6 sham controls had buffer only injected. The co-ordinates used were 0.8mm anterior to bregma; 3mm either side of the midline; 7.2mm down from dura. Two weeks later [^{14}C] 2-deoxyglucose (2DG) uptake was measured in discrete regions throughout the brain using the quantitative autoradiographic technique of Sutherland et al (1983). CAT activity was measured in homogenised cryostat sections of frontal, parietal and occipital cortex by the method of Fonnum (1975) and expressed as nmoles/h/mg protein.

The levels of CAT activity (mean ± s.e.m., n = 5) in the sham controls were : frontal 37.8 ± 4.3; parietal 27.6 ± 1.3; occipital 27.7 ± 0.7. In the lesioned animals (n = 5) these were significantly reduced (student's 't' test) in the frontal 23.7 ± 1.1 ($P < 0.02$) and parietal cortex 21.4 ± 2.0 ($P < 0.05$) but unchanged in occipital cortex (26.8 ± 2.5). These values are consistent with substantial destruction of cell bodies in the nBM. There were no significant differences between lesioned and control animals in 2DG uptake measured in 42 brain areas including those to which cholinergic cell bodies of the nBM project. Autoradiograms from lesioned brains were indistinguishable from controls with a normal laminar distribution of label in the cerebral cortex. These results confirm the biochemical findings of Lamarca and Fibiger (1984) and suggest that destruction of cholinergic cell bodies in the nBM do not directly affect cortical glucose utilization in the rat

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CHANGES IN QUANTAL CONTENT PRODUCED BY ENDPLATE CHANNEL BLOCKING DRUGS AS ASSESSED BY ANALYSIS OF DRIVING FUNCTION

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Several substances which block endplate ion channels have also been claimed to produce changes in quantal content (Durant & Lambert, 1981; Fiekers et al, 1983). These conclusions are based on analysis of the amplitudes of evoked endplate currents (EPCs). Such analysis is complicated by the possibility of rapid channel block occurring during the relatively slow rising phase of the EPC, resulting in truncation of peak amplitude. We have used the driving function (a measure of the rate of transmitter release) to obtain an estimate of quantal content which is independent of this effect of ion channel block. We have thus used the driving function to separate out the quantal content and ion channel block features of one of a series of chloramphenicol isomers; L-erythro chloramphenicol. Experiments were performed on the voltage-clamped costocutaneous muscle of the garter snake. Driving functions were derived by a Fast Fourier Transform method from EPCs and MEPCs as described by Connor et al (1983).

Studies of the raw recorded EPCs and MEPCs showed that L-erythro chloramphenicol increased EPC amplitude by 17 $\pm$ 6% (mean $\pm$ S.E., n=6), but decreased MEPC amplitude to 94 $\pm$ 7% (n=6) of control. By using the direct method of quantal content measurement, i.e. EPC amplitude/MEPC amplitude it was calculated that the drug caused an approximately 25% increase in quantal content. As expected the driving function for MEPCs was much smaller and shorter than that for EPCs, reflecting the temporal dispersion of transmitter release during nerve stimulation. L-erythro chloramphenicol had no major effects on MEPC driving function but increased both the amplitude and area of EPC driving function. This was taken to indicate an increase in quantal content. In order to study the effect of the drug in the absence of channel block, the drug-treated driving function, which was derived semi-qualitatively from a double exponentially decaying EPC, was used to generate a simulated single exponential decay EPC with the same decay time constant as the control EPC. This simulated EPC was 72% larger than control. In contrast, the measured drug-treated EPC amplitude was only 13% greater than control. It is concluded that the direct method of quantal content determination by comparison of EPC amplitudes is likely to be inaccurate in the presence of an endplate channel blocking drug.

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LOCUS COERULEUS: SITE OF SEDATION AND SLEEP INDUCED BY CLONIDINE

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The central effects of clonidine and clonidine-like compounds are mediated by activation of $\alpha_2$subtype adrenoceptors (Drew et al., 1979; Timmermans et al, 1981; Marley and Nisticò, 1975; Rotiroti et al., 1983).However, little is known about CNS sites at which clonidine and related compounds exert their sedative effects. In order to clarify this point we have studied behavioural and electrocortical spectrum power effects of clonidine and drugs acting at $\alpha_2$adrenoceptors after their microinjection in several areas of the rat brain.

The implantation of chronic cannulae was performed under general anaestesia according to Paxinos and Watson (1982) atlas. Electrocortical activity was recorded by means of 8 channel machine or quantified by means of a Berg-Fourier analyzer.

In freely moving rats (at least 48 h after cannula implantation) clonidine given into the third cerebral ventricle (9.4, 18.8 and 37.6 nmol) produced behavioural and electrocortical slow-wave sleep with a significant increase in total and predominantly 3-6 and 6-9 Hz voltage power.These phenomena lasted 90-180 min depending on the dose.Much lower doses of clonidine (0.19,0.28 and 0.36 nmol) were required to produce behavioural and electrocortical slow-wave sleep after microinfusion into the locus coeruleus (LC). In addition, from this site atonia was evoked. On the contrary, equimolar doses of clonidine given into the hippocampus were ineffective whereas larger doses (33-99 times higher) produced only behavioural and ECoG sedation. Behavioural and electrocortical effects of clonidine infused into the LC (0.28 nmol) were selectively prevented by a previous microinfusion (15 or 30 min before) into the same site of phentolamine (10 nmol) or yohimbine (1.3 nmol). In addition, a pretreatment with yohimbine (1 mg/Kg i.p. 30 min before) was able to antagonize the effects of clonidine (0.28 and 0.56 nmol) injected into LC. Yohimbine (1.3 and 2.6 nmol) or phentolamine (10 and 20 nmol) given alone into the LC induced arousal, increase in locomotor activity, behavioural stimulation and ECoG desynchronization with a significant fall in total and 3-6, 6-9 and 9-12 Hz voltage power lasting approx 2 or 3 hours depending on the dose.

The present findings show that the locus coeruleus is a very sensitive site from which behavioural and electrocortical sleep by clonidine is evoked and seem to suggest that cortical areas receive a tonic inhibitory input from LC catecholaminergic neurons.

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NEFOPAM ANALGESIA AND CEREBRAL MONOAMINES

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In the present study we investigated the effect of nefopam, a tricyclic compound with analgesic activity, on cerebral monoamines. In vitro studies with brain synaptosomes have shown that nefopam inhibits the reuptake of monoamines and increases ^{3}H-dopamine overflow with little or no effect on ^{3}H-serotonin or ^{3}H-norepinephrine release. We then investigated whether nefopam antagonized the decrease of cathecolamine and serotonin (5-HT) levels produced respectively by 6-hydroxydopamine (6-OHDA) and fenfluramine; this method may provide information on whether a drug inhibits the reuptake of monoamines in vivo.

Nefopam (40 mg/kg, a dose found analgesic in preliminary experiments) was injected i.p. 30 min before the intracerebroventricular (ICV) administration of 6-OHDA (200 µg/20 µl) or i.p. fenfluramine (15 mg/kg); one week after 6-OHDA and 2 h after fenfluramine rats were killed and monoamines were determined in the whole brain using HPLC. Nefopam significantly inhibited the reduction of norepinephrine (NE) and 5-HT levels; however, it did not prevent the reduction of dopamine (DA) levels induced by 6-OHDA. Together with in vitro studies, these results suggest that, at the dose used, nefopam inhibits the reuptake of 5-HT and NE, while its main action on DA may be an enhancement of release from nerve terminals. In support of this hypothesis, we found that at the time of its peak analgesic effects (30 min after injection) nefopam raised 3-methoxytyramine concentrations in striatum and nucleus accumbens: this extraneuronal metabolite of dopamine has been suggested to be a good index od DA release. Values in ng/g ± S.E. were: in striatum, controls 17.5 ± 0.3 and nefopam 26.5 ± 1.1, $p<0.01$; in nucleus accumbens, controls 7.0 ± 0.5 and nefopam 12.0 ± 0.9, $p<0.01$ (Student's t test).

The role of monoamine-containing neurons for the analgesic effect of nefopam was investigated in rats with modifications of brain monoamines induced by reserpine or selective neurotoxins. The antinociceptive effect of 40 mg/kg nefopam in the hot plate test was significantly antagonized by reserpine (2 mg/kg i.p., given 4 h before), ICV 6-OHDA (200 µg/20 µl,given one week before) and 6-OHDA plus desipramine (25 mg/kg),a procedure that reduces selectively DA levels.Nefopam antinociceptive effect was not modified in rats injected ICV with 5,7-dihydroxytryptamine (150 µg/20 µl, ten days before), which produced a selective reduction of brain 5-HT levels. Moreover pretreatment with DSP-4 (50 mg/kg i.p.,ten days before) and FLA-63 (30 mg/kg i.p.,3 h before) two agents which selectively depleted brain NA, did not affect nefopam analgesia.

In conclusion, the results of the present study suggest an important role for brain dopamine in the mechanism by which nefopam inhibits nociceptive responses in rats.

A UTERINE-TYPE OXYTOCIN RECEPTOR IN THE MALE MOUSE ANOCOCCYGEUS

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Anococcygeus muscles, isolated from male mice, contract in response to neurohypophyseal peptides, being more sensitive to oxytocin than to vasopressin (Gibson et al., 1984). Indeed, the tissue provides a useful oxytocin bioassay preparation (Botting & Gibson, 1985). This study investigates the nature of the receptors involved.

Anococcygeus muscles were dissected from male mice (25 - 35 g ; LACA strain) and were set up for the recording of isometric tension responses as described previously (Gibson & Wedmore, 1981). Agonists were left in contact with the tissue for 5 min or until any consequent rise in tone had reached a peak. Following washout, further doses were not added until tone had returned to baseline. Antagonist potency was calculated from Schild plots (Arunlakshana & Schild, 1959), the antagonist being in contact with the tissue for 30 min prior to testing its effect on oxytocin sensitivity.

The naturally-occurring peptides oxytocin (OT), Arg-vasotocin (AVT), Arg-vasopressin (AVP), and Lys-vasopressin (LVP) all produced dose-related contractions of the mouse anococcygeus. The maximum responses and slopes of the dose-response curves were similar for the four peptides, those for OT being (mean ± se) 414 ± 22 mg and 250 ± 14 respectively. The order of potency, in terms of the pD_2 values, was OT (9.16 ± 0.03) > AVT (8.68 ± 0.05) > AVP (8.46 ± 0.09) > LVP (7.52 ± 0.05). This resembles the order of potency found in the uterus and the mammary myoepithelium.

Four selective OT agonists (Manning & Sawyer, 1981) also produced contractions in low concentrations. The pD_2 values were : 4-Thr-OT (8.41 ± 0.08) ; 1-hydroxy-4-Thr-OT (8.98 ± 0.07) ; 7-Gly-OT (8.08 ± 0.12) ; and 4-Thr-7-Gly-OT (8.61 ± 0.05). The maximum responses and slopes of the dose-response curves were similar to those for OT.

The OT antagonist, 1-deaminopenicillamine-8-Orn-VT (Sawyer et al., 1980), produced competitive antagonism of responses to OT (slope of Schild plot = 1.04 ± 0.05 ; pA_2 value = 7.52 ± 0.06).

The order of potency of the naturally-occurring peptides and the effectiveness of the selective OT agonists suggests that the neurohypophyseal peptide receptor of the mouse anococcygeus is of the OT type. Since 1-deaminopenicillamine-8-Orn-VT is an agonist in milk ejection assays (Sawyer et al., 1980), the OT receptor of the anococcygeus shows greater similarity to that of the uterus.

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HUMAN AND RAT CALCITONIN GENE RELATED PEPTIDES (CGRP) ACT POST-JUNCTIONALLY IN THE MOUSE VAS DEFERENS

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Analysis of the calcitonin gene led to the identification of the neuropeptides rat and human calcitonin gene related peptides (CGRP; Rosenfeld et al, 1983; Edbrooke et al, 1985). When given intracerebroventricularly rat CGRP increased the blood pressure, heart rate and plasma noradrenaline levels in rats (Fisher et al, 1983). These results suggested that CGRP might increase sympathetic tone. The present experiments have studied the effects of rat and human CGRP and their relationship to adrenergic mechanisms using as a model the isolated vas deferens of the mouse.

The vas deferens was set up as previously described (Marshall et al, 1978) and field stimulated at 0.2 Hz, 2 ms pulse width. The two peptides produced a concentration dependent inhibition of twitch responses (IC_{50} 12±4 nM, mean±s.e.mean, and 15±3 nM, rat and human CGRP respectively). Rat CGRP was less potent at inhibiting responses to 10 Hz stimulation than to those at either 0.2 Hz or 1.0 Hz. The potency of rat CGRP at 1.0 Hz was unaltered by halving the calcium concentration of the Krebs solution to 1.25 mM. The inhibitory effect of human CGRP on twitches elicited by 0.2 Hz was not antagonised by either propranolol (300 nM) or idazoxan (300 nM) although in the same tissues the latter drugs antagonised twitch inhibition elicited by isoprenaline and clonidine respectively.

The uptake of 1-(7,8-^{3}H)-noradrenaline, 30 nM (Sp.Act.1.33TBq.mmol^{-1}) by isolated vasa over 10 mins was unaltered by either rat CGRP (100 nM) or human CGRP (1.0 μM). The release of tritium was measured from vasa preloaded with (^{3}H)-noradrenaline, 590 nM, for 30 min (Marshall, 1983). The control fractional release per pulse of tritium (1.0 Hz, 100 pulses; 1.17±0.12 x 10^{-5}) was not significantly altered by rat CGRP, 3-100 nM, although in the same tissues the peptide inhibited the responses of the smooth muscle to stimulation.

The vas deferens was contracted by acetylcholine, 30 μM (284±19mg tension), a concentration producing about half the maximal response. Rat and human CGRP (3-300 nM) inhibited contractions when given 1 min before acetylcholine. The peptides were approximately equi-potent but were less potent than against twitch responses induced by field stimulation at 0.2 or 1.0 Hz.

These results suggest that CGRP acts independently of adrenergic mechanisms in the vas deferens. It is likely that CGRP acts post-junctionally to inhibit contractions of the vas deferens evoked either by released transmitter or by exogenous acetylcholine. This post-junctional action of the peptides suggests a mechanism by which CGRP could modulate the actions of neurotransmitters and hormones in other tissues.

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PHARMACOLOGICAL EFFECTS OF Δ-AMINOLAEVULINIC ACID AND Y-AMINOBUTYRIC ACID IN ISOLATED PREPARATIONS OF RABBIT JEJUNUM

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We have previously reported that δ-aminolaevulinic acid (ALA) at concentrations above 0.2 mM inhibits contractile activity in isolated preparations of rabbit small intestine (Cutler et al, 1984) and that these findings might be relevant to the gastrointestinal features of acute intermittent porphyria in which blood concentrations of ALA can rise many fold above normal values (Laiwah et al, 1983) The purpose of the present experiments was to examine the possibility that the observed pharmacological actions of ALA in rabbit small intestine arose from interaction with γ-aminobutyric acid (GABA) receptors modulating acetylcholine release which have been found within the myenteric plexus (Ong & Kerr, 1982). It has been found that ALA can act as an agonist for neuronal GABA receptors (Brennan & Cantrill, 1979).

Thus, in the present studies, effects of ALA in the isolated rabbit jejunum were compared with those of GABA and of $GABA_A$ and $GABA_B$ agonists, muscimol and baclofen. Preparations of rabbit jejunum were bathed in oxygenated Ringer-Locke solution at 37°C and contractions of the preparation were recorded by isotonic transducer and displayed on a calibrated Washington 400 MD2R oscillograph. Response to ALA was examined after preparations had been pretreated with atropine (10 μM) and tetrodotoxin (TTX, 0.3 nM) and with GABA antagonists, picrotoxin (10 μM), bicuculline (10 μM) and δ-aminovaleric acid (1 mM). Likewise, effects of pretreatment with antagonists on response of the preparation to GABA and baclofen were investigated.

The inhibitory effects of ALA upon tone and amplitude of contractions occurred in all preparations and were dose-related. Pretreatment with atropine, TTX, bicuculline or picrotoxin did not alter the response to ALA, although pretreatment with the $GABA_B$ antagonist, δ-aminovaleric acid, did significantly reduce the inhibitory effects of ALA (3.8 mM, $P < 0.05$). Δ-aminovaleric acid, which has a similar chemical structure to ALA, itself caused a small reduction in amplitude of contractions.

Baclofen (20 μM and 30 μM) and GABA (10 μM) significantly reduced the amplitude of contractions for a brief period and decreased tone in 13% of preparations, while having no detectable effect in the remainder. Muscimol (30μM and 60 μM) was without significant effect. In preparations responsive to GABA, pretreatment with atropine or TTX significantly reduced the inhibitory effects of GABA ($P < 0.01$). Pretreatment with TTX also reduced significantly ($P < 0.01$) the inhibitory actions of baclofen (20 μM). Bicuculline and picrotoxin had no significant effects. The results suggest that GABA and baclofen may be inhibiting contractions by reduction of acetylcholine release from nerve terminals, and thus differ from ALA in their mode of action. Overall, despite some qualitative similarities between effects of ALA and GABA in this preparation, there is little evidence to support the hypothesis that inhibitory effects of ALA are due to interaction with $GABA_B$ receptors.

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ALTERATIONS OF 5-HT BULBO SPINAL SYSTEM INDUCED BY 5,7-HT: EFFECTS OF MONOSIALOGANGLIOSIDE GM_1

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Gangliosides are agents capable of enhancing neurite formation in vitro and neuronal repair in vivo after injury to peripheral and central nervous system. The present study was undertaken to assess the potential counteracting effects of monosialoganglioside GM_1 on 5,7-HT induced alterations in the bulbo-spinal serotoninergic pathways. 5,7-HT (50 mg free base/Kg s.c.) was administered few hours after birth and GM_1 (30 mg/Kg s.c.) was injected daily from post-natal days 1 to 4. The biochemical analyses were performed in the pons-medulla, cervical, thoracic and lumbar spinal cord of 2 month-old rats. The neurotoxin caused a marked reduction of 5-HT and 5-HIAA levels in the most distal areas innervated by the bulbo-spinal serotoninergic system (thoracic and lumbar spinal cord, Table I). Conversely in the pons-medulla 5-HT and 5-HIAA contents were increased (+30% "pruning effect"). The 5-HT reduction in the lumbar spinal cord induced by 5,7-HT was accompanied by apparent changes of the binding parameters evaluated for 5-HT_1 receptors (Table II). GM_1 administration was found to counteract the 5-HT and 5-HIAA reduction induced by 5,7-HT in the thoracic and lumbar spinal cord as well as the changes of 5-HT_1 receptors binding parameters in the lumbar region. These data further support the capacity of gangliosides in promoting axonal regeneration after lesions to the nervous system.

Table I 5-HT content (ng/g, mean ± S.E.M.; n=8) in the spinal cord of 2 month-old rats

	THORACIC	LUMBAR
CONTROL	406±50	533±27
GM_1	384±30	527±30
5,7-HT	271±27	237±24
5,7-HT+GM_1	*355±44	409±76

*p<0.05 vs 5,7-HT

Table II Scatchard plot analysis of [^{3}H]-5-HT binding sites in the lumbar spinal cord of 2 month-old rats. Each value is the mean ± S.E.M. obtained from 4 indipendent experiments.

	B_{Max} (pmol./mg prot.)	K_D (nM)
CONTROL	0.185±0.027	7.6±1.2
GM_1	0.198±0.009	6.0±0.75
5,7-HT	*0.255±0.018	7.4±1.1
5,7-HT+GM_1	0.218±0.034	3.5±2.3

*p=0.07 vs control

EFFECTS OF VARIOUS ENZYMATIC TREATMENTS ON GLYCINE-DISPLACEABLE [^{3}H]-STRYCHNINE BINDING TO SYNAPTIC MEMBRANES OF RAT SPINAL CORD

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There is considerable experimental evidence that the specific high affinity [^{3}H]strychnine binding to synaptic membranes of mammalian CNS is associated with glycine receptors (Young & Snyder, 1974; Graham et al.,1983).

To acquire information on the nature of the neurotransmitter receptor site and of its membrane environment, membrane preparations from rat brainstem and spinal cord (Young & Snyder, 1974) were pretreated with various enzymes at 37°C for 30 min in Ringers' solution (120 mM NaCl, 5 mM KCl, 1.24 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM Tris), pH 7.4, before being subjected to binding assay. At the end of enzymatic treatments, membranes were separated from the incubation media by centrifugation (48,000 x g for 20 min) and washed once with 0.05 M Tris-citrate buffer, pH 7.1. Binding assays were carried out in the presence of 2 nM [^{3}H]strychnine, using glass fibre filters to separate bound radioactivity and 10 mM glycine to assess unspecific binding (Galli et al.,1983). Control membranes were subjected to the same procedure as treated membranes, but enzymes were added in the cold after the incubation period.

The results obtained with maximal enzyme activities are shown in Table 1.

Table 1 Effect on [^{3}H]strychnine binding of enzymatic treatments of synaptic membranes (mean ± s.e. mean).

Treatment	Unit/ mg of membrane protein	[^{3}H]Strychnine bound (% of control)
Trypsin (Bovine pancreas)	50	80 ± 6
α-Chymotrypsin (Bovine pancreas)	50	84 ± 5
Neuroaminidase (Cl. perfrigens)	0.43	97 ± 2
β-Galactosidase (Bovine liver)	1	105 ± 2
Arylsulfatase (Helix pomatia)	97	93 ± 3
Phospholipase A_2 (Porcine pancreas)	2	57 ± 14
Phospholipase C (Bacillus cereus)	10	55 ± 12
Phospholipase D (Strept. chromofuscus)	10	81 ± 9

These results indicate that glycine receptor-associated strychnine binding is particularly resistant to enzymatic treatment, only phospholipases A_2 and C causing substantial decrease in the binding capacities (Bmax) of the membranes. This finding suggests that strychnine binding sites may be located in a hydrophobic domain of the receptor-chloride ionophore complex which is inaccessible to the enzymes, as was first proposed by Graham et al. (1983).

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LEARNING AND BRAIN MONOAMINES IN AGING RATS: EFFECT OF CHRONIC TREATMENT WITH ACETYL-L-CARNITINE

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Acetyl-L-Carnitine (ST 200) is a natural component functionally active in several biological systems, found to improve mood and attention in old patients with long term treatment.

In rats, ST 200 modifies spontaneous and evoked elettrocortical activity.

This experiment was aimed at ascertaining the effect of a chronic treatment with ST 200, 50 mg/kg/day in drinking water for 100 days, on the light discrimination learning performance in aged rats, which becomes proportionately impaired with age. Monoamines were determined in cortex, striatum, nigra, hippocampus and hypothalamus.

Male Albino Wistar rats (8 controls, 7 treated) were used, 12 months old at the experiment onset. The animals were trained in a Skinner box to lever pressing for reward. The discrimination test was carried out over 30 alternate reward (light on)-unreward (light off) two-min periods in a single session. Discriminative learning was improved in treated animals. Brain amine levels reported to be reduced in the old rat, were affected by treatment: dopamine was increased in the substantia nigra and in the hippocampus; NE in the striatum, on the contrary, was reduced (Table 1). On these bases, no clear correlation could be established between behavioral and neurochemical effects of ST 200.

Table 1 Levels of Dopamine, 3, 4-Dihydroxyphenylacetic acid, Homovanillic acid and Noradrenaline in the brain of aged rats treated with ST 200. Mean values in nmoles/g ± S.E. (N= 17).

	DA	DOPAC	HVA	NA	
Controls	58.60±4.70	10.10±2.20	4.10±0.29	0.82±0.11	STRIATUM
ST 200	63.00±3.90 (+7.5)	11.40±2.30 (+13.8)	4.75±0.28 (+15.9)	0.45±0.07[a] (-45.1)	
Controls	0.31±0.05	0.15±0.02	0.17±0.05	1.54±0.11	SUBSTANTIA NIGRA
ST 200	0.39±0.11 (+25.8)	0.30±0.10 (+100.0)	0.28±0.08 (+64.7)	1.54±0.10 (0.0)	
Controls	0.19±0.02	0.14±0.02	0.09±0.02	1.89±0.09	HIPPOCAMPUS
ST 200	0.24±0.03 (+26,3)	0.18±0.03 (+28.6)	0.13±0.06 (+44.4)	1.87±0.08 (-1.1)	

In parentheses, percent variations. a = $p \leq 0.05$.

A POSSIBLE LINK BETWEEN Δ-9-TETRAHYDROCANNABINOL AND GABA IN MICE

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As part of a study aimed at identifying the central neurotransmitters mediating the hypothermic effect of delta-9-tetrahydrocannabinol (THC) in mice, experiments were carried out to test for an interaction between THC and the benzodiazepines flurazepam dihydrochloride (FZ) and chlordiazepoxide hydrochloride (CDX). Benzodiazepines are thought to act mainly by enhancing the response to neuronally released gamma-aminobutyric acid (GABA) and it was predicted that if GABA were one of the transmitters mediating the hypothermic response to THC, then this response would be increased in the presence of a benzodiazepine. Experiments were carried out with groups of 6 to 12 unrestrained, adult, male MF1 mice at an ambient temperature of 22°C. THC was mixed with 2 parts of Tween 80 by weight, dispersed in saline and injected intraperitoneally. Other drugs were dissolved in saline and injected subcutaneously. Sodium barbitone, FZ, CDX and saline were given at -30 min and all other treatments at time zero (0.25 ml/25g body weight). Body temperature (Tr) was measured with a rectal thermistor probe.

The results showed that benzodiazepines can indeed enhance THC hypothermia. Thus, for example, the Tr of mice given FZ (30 mg/kg) and THC (5 mg/kg) fell to a minimum value of 33.0±0.6°C (mean±s.e.). This was significantly lower ($P<0.01$; Dunnett's test; Dunnett, 1964) than the minimum values observed following treatment with FZ and Tween 80 or with saline and THC (respectively 35.8±0.4° and 37.1±0.2°C). During these experiments it was noted that THC appeared markedly to reduce the benzodiazepine dosage needed to induce a loss of righting reflex (RR). This observation was investigated further, mice being said to have lost their RR if they remained on their backs without struggling for at least 10 s. After treatment with 10, 30 or 90 mg/kg of FZ followed by THC (20 mg/kg), the proportion of mice losing their RR was respectively 0, 83 and 100%. Similarly, after 10, 30 or 90 mg/kg of CDX followed by THC (20 mg/kg), the percentage of mice losing their RR was 0, 58 and 100%. Almost identical results were obtained with CDX and THC when mice were kept at 34°C to prevent hypothermia. When saline was given instead of FZ or CDX there was no loss of RR. The reflex was also unimpaired in animals receiving FZ or CDX (10, 30, 90 or 360 mg/kg) followed by Tween 80 (40 mg/kg). When given 720 mg/kg of FZ or CDX followed by Tween 80, however, some animals, respectively 100% and 40%, did lose their RR. The question of whether these results reflect a link between THC and GABA requires further study. It is noteworthy however that in animals receiving bicuculline (2 mg/kg) plus THC (20mg/kg), the incidence of loss of RR after pretreatment with 30, 60, 90 or 180 mg/kg CDX was respectively 0, 33.3, 50 and 100%. In contrast, without bicuculline, the incidence of this response after 10, 30 or 90 mg/kg CDX was respectively 0, 50 and 100%. Finally, since barbitone is expected to interact far less selectively than FZ or CDX with GABA-releasing pathways, it is also noteworthy that THC (20 mg/kg) produced at most only a two fold shift to the left in the barbitone log dose-response curve for abolition of the RR. Thus, after treatment with 90, 150 or 300 mg/kg of barbitone followed by THC (20 mg/kg), the proportion of mice losing their RR was respectively 0, 66.7 and 100%. The incidence of RR loss after treatment with 150, 300 or 600 mg/kg of barbitone plus Tween 80 (40 mg/kg) was respectively 20, 70, and 100%.

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LOSS OF STRIATAL AND LIMBIC DOPAMINE AFTER A SINGLE TREATMENT OF MICE WITH MPTP

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1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) has been shown to deplete nigrostriatal dopamine of the mouse when given by repeated systemic injection (Heikkila et al, 1984; Bradbury et al, 1985). In the present study we investigate the neurotoxic actions in the mouse of MPTP given as a single injection.

Female albino mice, BKW strain, 30-35g, were treated with a single dose of MPTP (1, 10, 20 or 40 mg/kg i.p.) or its vehicle. Spontaneous locomotor activity was assessed 45 min after the MPTP, then daily for the subsequent 3 days, using individual photocell cages. Animals were killed on the 4th day and the striatum and limbic tissue (tuberculum olfactorium and nucleus accumbens) dissected for determination of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using HPLC with electrochemical detection.

Table 1. Biochemical changes in striatal and limbic tissue following peripheral treatment of mice with a single dose of MPTP 4 days before killing (n=6).

Dose MPTP	STRIATUM			LIMBIC		
mg/kg (i.p.)	DA ng/mg	DOPAC pg/mg	HVA pg/mg	DA ng/mg	DOPAC pg/mg	HVA pg/mg
Veh.	21.36±1.15	1025±108	1290±52	7.42±.4	596±27	391±21
40	5.72±0.30***	361±20***	625±20***	3.41±.25***	276±18***	193±24**
20	16.64±0.47**	855±27	995±41**	5.08±.47*	508±79	369±47
10	20.48±1.60	1071±100	1184±117	6.39±.39	548±50	435±39
1	21.34±0.94	1181±68	1285±151	6.64±.55	632±35	473±42

Reductions significant to *P<0.05, **P<0.01, ***P<0.001 (Student's 't' test)

A single injection of mice with 20 mg/kg i.p. MPTP caused significant loss of striatal DA (-22%) and HVA (-23%), and also caused depletions of limbic DA (-32%). These neurotoxic actions of MPTP were enhanced when a larger single dose was administered, 40 mg/kg i.p. MPTP causing 73% loss of striatal DA associated with depletions in striatal DOPAC (-65%) and HVA (-52%). Again, the neurotoxic action of MPTP extended to limbic tissue where DA, DOPAC and HVA levels were reduced by 54, 54 and 51% respectively. Lower doses of MPTP, 1 and 10 mg/kg i.p., given as single injections did not cause biochemical changes consistent with neurotoxicity on dopamine mechanisms. Although 10, 20 and 40 mg/kg i.p. (but not 1 mg/kg i.p.) MPTP caused inhibition of mouse spontaneous locomotion (by 66 to 86%; however, animals appeared prostrate, and the effect may be non-specific) when measured during the 1-2h period after administration, activity levels had returned to vehicle control values by 24h.

It is concluded that the marked neurotoxic action of MPTP in the mouse following systemic administration can develop after a single challenge. The effect is dose-dependent. However, this neurotoxicity is not specific for the striatum but also extends to limbic areas. Further, the biochemical deficits are without behavioural correlates.

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[^{3}H]-D-ASPARTIC ACID LABELS EXCITATORY AMINO ACID UPTAKE SITES IN HUMAN BRAIN

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The role of excitatory amino acids (EAA) in human neurodegenerative diseases is currently the focus of much interest. It has proved difficult to study EAA-containing neurones in autopsied human brain due to the lack of a reliable marker. Recently, [^{3}H]D-aspartate ([^{3}H]D-Asp), a substrate for the high affinity glutamate uptake system, was proposed as a ligand for labelling the uptake site in rat brain (Parsons & Rainbow, 1983). In this study, we have compared [^{3}H]D-Asp binding with [^{3}H]glutamate ([^{3}H]Glu) uptake in human brain.

For [^{3}H]D-Asp binding, brains obtained at autopsy were frozen at -40°C. Membranes were produced by homogenisation of cerebellar cortex in 100 vol 50 mM Tris HCl, pH 7.4, followed by centrifugation at 15,000 g for 10 min and a single wash. [^{3}H]D-Asp binding was studied using a filtration technique (Cross et al, 1984) using 50 nM ligand in Tris buffer containing 300 mM NaCl. For [^{3}H]Glu uptake, rapidly frozen human cortex was homogenised in 0.25 M sucrose + 50 mM Tris HCl, pH 7.4, and uptake of 50 nM [^{3}H]Glu studied as described previously (Fonnum et al, 1977).

[^{3}H]D-Asp binding to human brain was saturable (K_D = 1 μM) and totally sodium dependant (ED_{50} = 20 mM). The relative potencies of a series of glutamate analogues in inhibiting [^{3}H]D-Asp binding and [^{3}H]Glu uptake are shown in Table 1. A highly significant correlation was found between potency at the two sites.

Table 1 Inhibition of [^{3}H]glutamate uptake and [^{3}H]D-aspartate binding

Compound	IC_{50} (μM)	
	[^{3}H]D-aspartate	[^{3}H]glutamate
Cysteine sulfinate	4.8	1.1
D-Aspartate	8.9	4
L-Aspartate	7.8	8
L-Glutamate	7.6	10
Aspartate-β-hydroxamate	15	45
N-Methyl-D-aspartate	45	250
Aminophosphonopropionate	160	400
N-Acetylaspartate	100	500

The pharmacological profile of [^{3}H]D-Asp binding was quite different to that of [^{3}H]L-aspartate binding (Cross et al, 1985).

These findings suggest that [^{3}H]D-Asp labels a site in human brain with properties similar to the high affinity glutamate uptake site. [^{3}H]D-Asp binding may therefore prove to be a useful marker of EAA neurones in post-mortem human brain.

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CALCITONIN INHIBITION OF PROLACTIN RELEASE INDUCED BY SEROTONERGIC DRUGS

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Data obtained previously in our laboratory showed that salmon calcitonin (sCT) is able to decrease both baseline levels of prolactin (PRL) and physiological lactation hyperprolactinemia in rats. Since suckling-induced PRL release has been reported to be under serotonergic control '(Krulich, 1979; Preziosi, 1983)', we investigated whether the PRL-inhibiting action of sCT works through serotonin pathways. We examined the effects of sCT on PRL secretion induced by drugs that potentiate central serotonergic tone through enhancement of serotonin biosynthesis, release or receptor stimulation. sCT (250 ng/rat) was injected intracerebroventricularly into male Sprague Dawley rats (180-200 g) 15 min before the intracarotid administration of 5-hydroxytryptophan (5 HTP, 25 mg/kg), fenfluramine (Fen, 7,5 mg/kg) or quipazine (10 mg/kg). Bloods for PRL assay by RIA were collected at - 15, 0, 15, 30, 45 and 60 min after treatment. The results showed that sCT inhibited release of PRL by 5 HTP at 45 and 60 min after treatment and the release of PRL by fenfluramine at 30 and 45 min, whereas quipazine-induced PRL release was completely suppressed at all the times.

The present data indicate that sCT can counteract the activating effect of serotonin on PRL release. The time course of the inhibitory effects of sCT on PRL secretion evoked by 5 HTP or Fen and the more pronounced inhibition of quipazine-induced PRL indicate that sCT does not reduce serotonin synthesis or release but probably interferes with post-synaptic mechanisms involved in the facilitation by serotonergic neurons of PRL secretion.

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AN EEG STUDY OF THE INVERSE BENZODIAZEPINE AGONISTS IN THE RABBIT

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Pharmacological attempts to characterize the endogenous ligand of the central benzodiazepine (BDZ) receptors lead to the discovery of a number of β-carboline derivatives which however do not display effects similar to those of BDZ, namely, anxiolytic, anticonvulsant, sedative and myorelaxant effects. These drugs, on the contrary, are convulsants and proconvulsants, and elicit effects opposite to those of the anxiolytic BDZ in several conflict procedures (Pellow and File, 1984). For this reason, these drugs have been called inverse BDZ agonists.

We have studied the effects of these drugs on the electroencephalogram (EEG) in the rabbit. In general, these drugs elicit EEG changes, characteristic enough to be classified in three stages. The first stage consists of a desynchronized record with the presence of trains of 2-4 Hz high voltage waves in the optic cortex. The second stage is characterized by the presence of the EEG changes reported in the first stage plus the presence of 4-6 Hz spike-and-wave complexes at the level of the sensorimotor cortex. These EEG changes are accompanied by a behavioral state of arousal.The third stage consists of repeated EEG and motor "grand-mal" seizures. All these effects are antagonized by administration of the BDZ antagonist Ro 15-1788 (0.4 mg/kg iv). No change of the electrical activity could be recorded at the level of the spinal cord.

In the Table are reported the doses of the drugs which induce the three stages of EEG changes.

INVERSE BDZ AGONISTS: "IN VIVO" VERSUS "IN VITRO" EFFECTS IN RABBITS

Drugs	EEG EFFECTS (mg/kg i.v.) Slow Waves (optic cortex)	Spike-and-Waves complex (sensorimotor cortex)	Grand Mal seizures	GABA SHIFT
1)β-CCM	0.25-1.20	1.2-2.0	>2.0	0.20
DMCM	0.40-1.00	(0.8-1.10)*	>1.0	0.14
2)FG 7142	2.00-10.0	10.0-20.0		0.83
β-CCE	0.20-0.50	0.5-2.00		0.80
3)CGS 8216	2.00-20.0			0.94

*effects occur occasionally

As shown in the table, three distinct groups can be observed. The first includes β-CCM and DMCM which elicit all the three stages of EEG changes. The second includes β-CCE and FG 7142 which produce only the first two stages and finally CGS 8216, which elicits only the first stage of EEG changes. These manifestations resemble to those observed after bicuculline, picrotoxin, pentetrazol and Ro 5-3663 (Longo et al, 1983). As shown in the last column of the table, the ability of these drugs to progress through the three stages of EEG changes seems to be related to the extent of GABA-induced reduction of their binding affinity.

Large doses of FG 7142 and β-CCE never produce convulsions, but elicit synchronization and spikes in the cortical leads accompanied by behavioral sedation and myorelaxation. These unexpected manifestations are not inhibited by Ro 15-1788, even at the dose of 20 mg/kg, and are consistent with the observation that large doses of FG 7142 fail to produce proconvulsant effect (Little et al, 1984).

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THE EFFECT OF ANORETIC AGENTS ON CAFETERIA-DIET INDUCED HYPERPHAGIA AND OVERWEIGHT

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Rats offered a varied and palatable "cafeteria-diet" voluntarily overeat and gain more weight than controls (Sclafani & Springer, 1975). This model is useful for studying potential antiobesity agents (Kirby et al., 1978). We studied the effects of CM 57373, 4-amino-1 (6-bromopyrid-2-yl)piperidine HCl (CM) a new serotoninergic anorectic agent better tolerated than fenfluramine (F) by animals (Bianchetti et al., 1984).

Female Sprague Dawley rats (C. River, Italy), initial weight 254 ± 1 g were housed individually. The animals had ad libitum access to Altromin MT chow pellets (Rieper) and tap water. Controls were given chow pellets only. The experimental group was also given for 25 days (6 h/day) a variety of palatable foods including chocolate, marshmallows, cookies, bologna, cheese, corned beef, pate, lard, sweetened condensed milk (cafeteria-diet). Body weights were significantly increased after 15 days. The following day (day 0) the cafeteria-fed animals were given CM 7.5 mg/kg, F 2.5 mg/kg or saline (S) p.o., 1 h before cafeteria and 6 h food consumption was measured. CM and F groups then were given CM 0.3 mg/ml or F 0.1 mg/ml in the drinking water for 16 days (estimated daily dose/rat : CM:$\sim$3 mg; F:$\sim$1 mg). On day 8 and 16 the development of tolerance to anorexia was determined by measuring again food consumption after CM 7.5 mg/kg, or F 2.5 mg/kg. On the same days a few rats were killed and weights of abdominal fat recorded.

Cafeteria-diet consumption (% of S treated group) was significantly reduced after the first administration of CM (52% ; $p<0.01$). Tolerance to the anorectic effects developed both with CM and F after 16 days (food intake: CM 76% and F 75% of S) although it was initially (day 8) less pronounced with CM (56%) than with F (75%). Body weights (g ± S.E.) of CM and F treated rats decreased immediately after treatment (2nd day) and remained significantly lower than S (on day 16: S 299 ± 5; CM 277 ± 4; F 271 ± 4, $p<0.01$). Weights (g ± S.E.) of abdominal fat of treated rats was reduced more on day 8 (S: 7.5 ± 0.8; CM: 4.9 ± 0.5; F: 4.9 ± 0.6, $p<0.01$) than on day 16 (S: 8.4 ± 0.9; CM: 7.3 ± 0.8; F: 6.2 ± 0.9).

The results suggest that both CM and F inhibit the development of obesity by decreasing the hyperphagia induced by palatable food. In spite of development of tolerance to CM and F anorectic effects, body weights remained low throughout the treatment period. CM, like F (Duhault et al., 1979), might affect body weight also by acting on lipid metabolism. These results indicate that CM 57373 is of prospective therapeutic interest for treatment of obesity and hyperphagia.

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ACUTE AND LONG-TERM CHANGES IN LOCOMOTOR ACTIVITY RELATED TO THE CONTINUITY OF DOPAMINE RECEPTOR STIMULATION

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The infusion of low doses of pergolide of less than 9ng/h can enhance rat locomotor activity both during and after the period of infusion. However, when a single bolus injection of pergolide is given over seconds, even at doses in the order of 10 fold greater than delivered by infusion over 24h, locomotor hyperactivity does not develop (Costall et al, 1985). The data suggest that the continuity of dopamine receptor stimulation may be of considerable importance for securing a response and causing long-lasting changes. In the present study we investigate the acute and long-term consequences on mouse locomotor activity of administering apomorphine or (-)N-n-propylnorapomorphine [(-)NPA], by infusion.

Male ICR mice (25-30g) received apomorphine or (-)NPA acutely (s.c.) or as 13-day infusions from Alzet osmotic minipumps implanted s.c. (n = 6 for each treatment). Locomotor activity was assessed within a 60 min period following an acute injection and for 60 min daily during infusion using individual treadwheels (14cm diameter, 6cm tread). After 13 days the pumps were removed and locomotor activity measured every second day for 44 days.

Doses of apomorphine and (-)NPA required to reduce locomotor activity by 50% following acute injection were 40 and 2.5µg/kg s.c. respectively. In the infusion studies the s.c. implantation of the minipumps did not adversely affect wheel running behaviour (vehicle-infused mice gave consistent responses of 2000-3000 revs/60 min throughout the 13-day infusion period). Animals receiving apomorphine by infusion (0.4 and 4µg/kg/day i.e. 280 and 2800pg/kg/min) exhibited wheel running of 1700-3200 revs/60 min ($P>0.05$). However, following withdrawal of apomorphine levels of wheel running were consistently decreased, particularly in those animals which had received 0.4µg/kg/day apomorphine in which reductions of 23-41% persisted throughout the 44-day post-infusion period ($P<0.01$-$P<0.001$). In contrast, the infusion of a higher dose of apomorphine (347ng/kg/min) significantly elevated wheel running activity throughout the period of infusion (by 31-46%, $P<0.001$). After discontinuing the infusion of this higher dose of apomorphine mice maintained an approximate 2-fold elevation of wheel running behaviour throughout the 44 day post-infusion period ($P<0.001$). The infusion of low doses of (-)NPA, 0.025 and 0.25µg/kg/day, caused modest reductions in wheel running activity (10-46% reductions) which achieved significance ($P<0.05$-$P<0.001$) in mice receiving 0.25µg/kg/day (-)NPA. The reductions in locomotor activity (9-48%) persisted throughout the 44-day drug withdrawal period ($P<0.001$ for mice which had received 0.25µg/kg/day (-)NPA). Mice given 100µg/kg/day (-)NPA by infusion tended to show exaggerated wheel running during infusion: this persisted, indeed increased, post-infusion (increases of up to 52%, $P<0.001$, for 44 days).

These studies therefore show that the infusion of apomorphine and (-)NPA can (a) cause changes in mouse spontaneous wheel running activity at doses that would have no effect on acute challenge and (b) cause long-term changes in wheel running behaviour which persist after discontinuation of infusion.

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THE NEUROTOXIN ACTIONS OF MPP^+ IN THE RAT ARE NOT CONFINED TO DOPAMINE AND THE SUBSTANTIA NIGRA

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1-methyl-4-phenylpyridine (MPP^+) infused into the rat substantia nigra causes motor deficits associated with loss of nigrostriatal dopamine (Bradbury et al, 1985). The present study assesses whether the neurotoxic actions of MPP^+ can be extended to other cell body areas, either dopaminergic (ventral tegmental nucleus, VTN) or non-dopaminergic (medial raphe nucleus, MRN).

Male Sprague-Dawley rats were subject to standard stereotaxic techniques for the implantation of guide cannulae to allow subsequent drug infusion into the MRN (Ant. 0.2 Vert. -4.0 Lat. 0.0) or VTN (Ant. 2.6 Vert. -4.0 Lat. ±1.0, coordinates according to Pellegrino & Cushman). MPP^+ (10µg/24h) or vehicle were bilaterally infused into the MRN or VTN via injection units coupled to Alzet osmotic minipumps located subcutaneously in the back neck region. During infusion the locomotor activity of rats was assessed in individual photocell cages, and difficulties in forelimb movement and rigidity in the limbs and trunk were noted. On day 4 of infusion animals were killed and the caudate-putamen and limbic tissue (tuberculum olfactorium and nucleus accumbens) removed for determination of the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) using HPLC with electrochemical detection.

Table 1. Striatal biochemistry after MPP^+ infusions into VTN and MRN

Infusion	Brain Area	DA ng/mg	DOPAC pg/mg	5-HT pg/mg	5-HIAA pg/mg
Vehicle	VTN	8.54±0.30	749±24	425±38	289±11
MPP^+	VTN	3.93±0.70***	181±17***	433±28	214±10**
Vehicle	MRN	10.75±0.31	823±60	336±19	243±9
MPP^+	MRN	9.52±0.27*	858±52	224±26*	166±23*

n=6. Apart from reductions in limbic DA (from 3.17±0.15 to 1.39±0.21 ng/mg***) and DOPAC (from 477±35 to 131±32 pg/mg***) following MPP^+ infusions into the VTN, there were no significant biochemical changes in limbic tissue.

MPP^+ infused into the dopamine cell body area of the VTN for 4 days caused depletions of striatal DA (-53%), DOPAC (-75%) and 5-HIAA (-26%) and of limbic DA (-56%) and DOPAC (-72%). When infused for the same time period into the serotonin cell body area, the MRN, MPP^+ specifically reduced levels of 5-HT (-33%) and 5-HIAA (-32%) in the caudate-putamen. The loss in forebrain dopamine following the action of MPP^+ in the VTN was correlated with marked motor impairment (60-80% reduction in locomotion associated with marked deficits in front and hind limb movements), whilst the neurotoxic action of MPP^+ in the MRN was not associated with any motor deficit.

It is concluded that MPP^+ is neurotoxic to both the dopamine cell bodies of the mesolimbic system and the serotonergic cell bodies of the raphe system. The neurotoxic actions of MPP^+ are therefore not confined to dopamine or to the substantia nigra of the rat.

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INFLUENCE OF ANEASTHETICS ON STRIATAL DOPAMINE METABOLISM IN VIVO

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The increase in striatal dopamine (DA) metabolism caused by haloperidol can be monitored in vivo using voltammetry (Sharp et al 1984). Most studies are carried out in anaesthetised animals using a variety of anaesthetics. There are, however, few reports on the comparative effects of anaesthetics on DA metabolism in vivo. This study examines whether the choice of anaesthetic influences the DOPAC level changes induced by the DA receptor antagonist haloperidol and the agonist apomorphine.

Male Sprague Dawley rats (270-330 g) were anaesthetised with pentobarbitone (60 mg/kg, i.p., then 10 mg/kg/every h for maintenance), halothane (2-3% in 50:50 O_2/N_2O), α-chloralose (50 mg/kg i.v., then 5 mg/kg/every h with O_2/N_2O), or chloral hydrate (500 mg/kg i.p. then 50 mg/kg/ every h). For α-chloralose administration, the jugular vein was cannulated under halothane/O_2/N_2O. Electrically pretreated (Peak 2) carbon fibre microelectrodes were stereotaxically implanted into the left striatum (Sharp et al, 1984); reference and auxiliary electrodes were positioned beneath the dura. Differential pulse voltammograms were recorded every 4 min. After a minimum stabilisation period of 1 h, haloperidol (0.3 mg/kg, i.p.) was given followed, 2 and 3 h later, by apomorphine (0.5 mg/kg, s.c.).

In the halothane anaesthetised rat, haloperidol caused an increase in the DOPAC peak height which was maximal at 2 h, and was maintained, with little attenuation, for more than 5 h. Apomorphine (0.5 mg/kg) given without haloperidol pretreatment, almost completely abolished the DOPAC peak. After haloperidol administration, this dose produced a partial reversal of the increase in DOPAC (Table 1). Similar pharmacological manipulation in rats anaesthetised with either α-chloralose or chloral hydrate elicited similar responses but of differing magnitude (Table 1). In the case of α-chloralose, the changes in DOPAC peak height resemble most closely in magnitude those reported in the conscious rat using the intracerebral dialysis technique (Imperato & Di Chiara, 1985). Chloral hydrate and, more especially, pentobarbitone caused a continual reduction in Peak 2 height during the control period, making subsequent drug-induced changes difficult to interpret.

Table 1 Effect of anaesthetics on DOPAC peak height following haloperidol at t=0 h and apomorphine at t=2 and 3 h.

	t=1 h	t=2 h	t=3 h	t=4 h
Halothane (4)	145.3± 1.8*	165.3±13.6*	139.8±20.2*	98.0±19.5*
Pentobarbitone (4)	118.8±20.7*	111.5±17.8*	79.8±11.3*	56.3±16.6*
α-Chloralose (4)	268.1±37.5	367.3±48.7	277.4±16.2	218.7±12.7
Chloral hydrate (4)	122.1±14.1*	139.7±17.4*	89.2±19.9*	56.3±14.2*

Results expressed as mean±SEM of % pre-drug peak heights. *$p<0.01$ compared to α-chloralose.

In conclusion, the choice of anaesthetic for similar experiments influences the magnitude of the changes observed, and the effect of the anaesthetic on the responses should be carefully considered.

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HYPERTHERMIA INDUCED IN RABBITS BY AMBD AND ORGANIC CALCIUM ANTAGONISTS: ITS REVERSAL BY TAURINE

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Previous work performed in this laboratory has shown that in rabbits the intracerebroventricular (i.c.v.) injection of taurine produces a dose dependent fall in core and a rise in ear skin temperature, and that this effect is mediated at least in part by central 5-HT systems (Sgaragli et al.,1981). In order to ascertain whether these effects are related to a physiological role of taurine, an experiment was performed to study the consequences of deleting the action of endogenous taurine on body temperature by i.c.v. administration of the taurine antagonist (Yarbrough et al.,1981) AMBD (6-aminomethyl-3-methyl-4H,1,2,4-benzothiadiazine-1,1-dioxide).

Since taurine has been reported to influence calcium ion fluxes in some nervous cells (Okamoto et al.,1976) and calcium metabolism in the posterior hypothalamus has been postulated (Myers & Tytell,1972) to play a role in the mechanisms of thermoregulation a second experiment was designed to evidentiate a possible link between these two observations. The effects on thermoregulation induced by i.c.v. injection of some organic calcium antagonists (verapamil and nifedipine), given either alone or with taurine, have been investigated.

Both calcium antagonists and AMBD induced hyperthermia in rabbits. Hyperthermia induced by verapamil and AMBD was dose-related leading to death at the highest dose tested. Taurine (16 μmol.kg^{-1} b.w.) given 5 hours after either verapamil (180 nmol.kg^{-1} b.w.) or AMBD (71 nmol.kg^{-1} b.w.) transiently reversed the hyperthermia induced by the two compounds. On the other hand muscimol (22 nmol.kg^{-1} b.w.),a GABA agonist which has been shown to induce hypothermia, (Sgaragli et al., 1978) was able to reverse transiently verapamil-but not AMBD-induced hyperthermia, thus suggesting that different mechanisms are involved in hyperthermia caused by the two compounds.

The finding that AMBD induces hyperthermia suggests a physiological role of taurine in the central mechanisms of thermoregulation and the observation that calcium antagonists induce hyperthermia outlines the importance of calcium. Finally, the possible modulation by taurine of calcium metabolism in the nervous cells involved in thermoregulation is an open field of investigation.

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AN INVESTIGATION ON THE ANTICONVULSANT ACTIVITY OF PHENCYCLIDINE AND KETAMINE IN RABBITS

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Phencyclidine (PCP) and its related compound ketamine (KT) defined as "dissociative anaesthetics" are potent anaesthetics that possess also psychotomimetic effects (Chen 1961). In a previous study from our laboratory, an antagonistic effect of PCP has been demonstrated on the convulsions induced by pentylentetrazol (PTZ) in rats and in rabbits (Sagratella et al., 1983).

The aim of the present paper is to extend the study of the anticonvulsant activity of PCP and KT in regard to the motor and EEG effects due to cortical application of penicillin in rabbits. The effects of PCP and KT were compared to the antagonistic influence of diazepam and pentobarbital (PB). In addition, it has been investigated the ability of some drugs affecting various neurotransmitter systems such as clonidine, haloperidol, physostigmine and naloxone to counteract the influence of PCP on the convulsions induced by topical application of penicillin.

The experiments were performed in 50 unanaesthetized not curarized male rabbits, weighing 2.0-2.5 kg. The animals were prepared for EEG recording under local anaesthesia (2% xilocaine): 6 screw electrodes were fixed into the skull over the sensorimotor and optic cortices of both sides according to the technique described elsewhere (Longo 1962). The epileptic focus was produced by intracortical injection of Na penicillin (500 units) dissolved in water. A volume of 10 µl was injected through a 1 mm hole made in the skull, at a depht of 1 mm from the cortical surface, into the sensorimotor cortex of one side with a Hamilton syringe. All drugs were injected intravenously.

Administration of PCP (0.7-1.0 mg/kg), KT (20-40 mg/kg), pentobarbital (10 mg/kg) and diazepam (3 mg/kg) inhibited the generalization of the epileptiform activity induced by penicillin counteracting the EEG and motor patterns of the ictal events, while did not influence the interictal spike and wave complexes. Physostigmine (0.1 mg/kg), clonidine (0.1 mg/kg), haloperidol (1 mg/kg) and naloxone (10 mg/kg) did not affect the inhibitory influence of PCP on epileptiform activity due to cortical application of penicillin. Thus, the mechanism of this anticonvulsant action of PCP seems not to depend on the neurotransmitter systems related to the reported drugs.

The mechanism of action of PCP and KT is discussed in connection with the similarities of the effects of these drugs in respected to sigma opiate agonists and pentobarbital.

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HYPERACTIVITY INDUCED BY DOPAMINE FROM RAT NUCLEUS ACCUMBENS CAN BE MODULATED BY GLYCINE

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It has been suggested that glycine may have a neurotransmitter role in the ventral tegmentum of rat brain to regulate the activity of somatodendritic regions of mesolimbic dopamine neurones (Gundlach & Beart, 1982). We have therefore investigated whether the actions of dopamine in a mesolimbic terminal region, the nucleus accumbens, can be modulated by glycine at the same site.

Female Sprague-Dawley rats having stereotaxically implanted cannulae were subject to bilateral infusions into the nucleus accumbens (ACB, Ant. 9.6, Vert. 0.0, Lat. ±1.6; atlas of De Groot) of glycine, dopamine, strychnine or vehicle, either alone or as combined administrations, via Alzet osmotic minipumps located subcutaneously and infusing at a rate of 0.48 µl/h for 13 days (see Costall et al, 1982, for experimental details; n = minimum of 5 for each treatment). Locomotor activity was assessed daily (for a 1h period between 8.00 and 11.00 a.m.) during the infusion period using individual photocell cages.

The 13-day infusion of 12.5 or 25µg/24h glycine alone into the ACB failed to significantly modify rat spontaneous locomotion on any day of testing (all values in the range 110-160 counts/60 min). However, the infusion of a higher dose of glycine, 50µg/24h, lead to increased spontaneous locomotion (130-206% of vehicle control values, $P<0.05$-$P<0.001$ on days 3 and 8-12). Dopamine infused into the ACB, 25µg/24h, caused a biphasic increase in activity with peaks of hyperactivity occurring between days 3 to 4 and 9 to 12 (173-231% of vehicle control values, $P<0.05$-$P<0.001$). The biphasic hyperactivity caused by intra-ACB dopamine infusion could be modified by glycine infused at the same time, the outcome dependent on the dose of glycine. Thus, whilst 3.13µg/24h glycine infused at the same time as 25µg/24h dopamine failed to significantly modify responding to dopamine, the infusion of 12.5 or 25µg/24h glycine prevented the development of the first peak of hyperactivity to dopamine (counts of 409 ± 91/60 min reduced to 145 ± 15 counts/60 min on day 3, $P<0.001$) with a tendency to exaggerate the second peak during days 8-11 (values elevated by 35-59% by 25µg/24h glycine, but inter-rat variation precluded significance). The infusion of 50µg/24h glycine at the same time as dopamine also lead to modification of the dopamine peaks but the change here appeared to be temporal, with fusion of the first and second peaks. The infusion of strychnine (10µg/24h) alone into the ACB failed to modify locomotor activity (or to induce seizures) and when strychnine was infused with dopamine there was no significant change in the dopamine response. However, strychnine (10µg/24h) was shown to antagonise the inhibitory action of glycine (12.5µg/24h) on the first hyperactivity peak to dopamine (25µg/24h) when the three agents were infused concurrently (counts of 334±45/min for dopamine alone were reduced to 130±13/60 min in the presence of glycine and were returned to 265±38/60 min by strychnine, $P<0.001$ for antagonism by strychnine).

Thus, whilst glycine may not normally exert a tonic modulatory influence on those mechanisms in the ACB which regulate locomotor activity, exogenously infused glycine can differentially modify the two phases of responding to dopamine infused into the ACB to selectively attenuate the first phase via action on glycine (strychnine) receptors.

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CHARACTERISATION OF TACHYKININ RECEPTORS IN THE MARMOSET ILEUM AND COLON

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The majority of pharmacological studies investigating the contractile action of substance P (SP) and related tachykinins on isolated intestinal smooth muscle have been restricted to species such as guinea-pigs and rats. Within the SP-P/SP-E tachykinin receptor classification first proposed by Lee et al., 1982, it is notable that intestinal smooth muscle in the guinea-pig has a predominance of "SP-P" type receptor characteristics whereas, in the rat, it tends to exhibit "SP-E" type properties (Watson 1984). In view of this species variation, it is of interest to investigate the nature of tachykinin receptors in the primate gastrointestinal tract and so we have determined the effects of tachykinins, including neurokinin A and B (NKA, NKB), on the ileum and colon of the marmoset.

Male common marmosets (approx. 300g) were anaesthetised with a mixture of halothane (5%) in nitrous oxide/oxygen (60:40). The ileum and colon were quickly dissected and placed in Krebs saline pre-heated to 37°C. The outer longitudinal muscle layer was obtained from the ileum with the aid of moist cotton wool. In the case of the colon, the outer muscle layers were removed as for the ileum and then discarded, leaving a tubular preparation. Both preparations were suspended in 2ml organ baths containing Krebs Henseleit solution at 37°C bubbled continuously with 95%O_2 and 5%CO_2. Atropine, indomethacin, mepyramine and methysergide were present throughout (each at a concentration of 1μM) to antagonise any indirect effects of agonists or antagonists. Responses of both tissue preparations were recorded isotonically with a resting tension of 1g. Determination of relative activities of agonists and dose ratios was from analysis of log concentration-response curves.

In both preparations, each of the naturally-occurring tachykinins tested produced a fast, concentration-dependent contraction which returned quickly to baseline. SP methyl ester had no effect on either tissue up to 30μM. In the marmoset ileum, the rank order of relative activities for the tachykinins was NKA (171)> kassinin (137) > eledoisin (62) > NKB (27) >> SP(1.0). The EC_{50} value for SP was 2.9 ± 0.4μM. In the marmoset colon, the rank order of relative activities was similar to the marmoset ileum: NKA (102) > eledoisin (38) > NKB (28) >> SP (1.0) = physalaemin (1.0). The EC_{50} value for SP was 1.5 ± 0.2μM. Hence, in these tissues the relative activities of the tachykinins are similar to those in rat ileum and the rat colon muscularis mucosae (RCMM) which are believed to contain a predominance of SP-E receptors (Bailey et al., 1982). The effect of the SP antagonist [Arg^5,D-$Trp^{7,9}$,Nle^{11}]-SP(5-11) (SPA) has been tested against SP and eledoisin in both the marmoset ileum and colon. The pA_2 values for SPA against SP were respectively 5.58 (4.77-6.39) and 5.61 (5.4-5.83), against eledoisin 5.89 (5.4-6.38) and 5.42 (5.23-5.61) and against NKA in the ileum 5.71 (5.07-6.34). Schild slopes were not significantly different from unity. These values are similar to those for this antagonist in the RCMM (Brown et al., 1985).

Thus, it appears that, in the marmoset ileum and colon, the tachykinins are interacting with a type of receptor similar to that in rat ileum and colon muscularis mucosae, which is most likely to be an "SP-E" like receptor.

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CHARACTERISATION OF HIGH-AFFINITY BINDING SITES FOR VASOACTIVE INTESTINAL PEPTIDE(VIP) IN HUMAN LUNG

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Exogenous VIP is a potent bronchodilator in mammals, including man (Morice & Sever 1984). It is possible that the endogenous peptide has an important physiological role in the maintenance of airway patency. In several species high and low affinity VIP binding sites have been demonstrated in lung, using ^{125}I labelled VIP (Robberecht et al., 1982). This has not been possible in human lung because of very high levels of non-specific binding. However, other experiments such as the measurement of VIP-induced cyclic AMP accumulation, suggest that similar receptors are also present in human lung (Taton et al., 1981). We describe a technique which has enabled us to characterise the high-affinity binding site for ^{125}I-VIP.

Normal human lung, obtained at lobectomy, was immediately placed in liquid nitrogen. The lung was later thawed in ice-cold 0.25M sucrose, containing 10mM TRIS, 5mM EDTA, 200μg/ml bacitracin, 10μg/ml leupeptin and 20μg/ml soya bean trypsin inhibitor, phenylmethyl sulphonyl fluoride (PMSF) 0.1mM (pH 7.4). The lung parenchyma was minced with scissors and then homogenised using a Polytron, setting 10, 20 secs x 4. The homogenate was centrifuged at 1000g for 10min. The supernatant was filtered through two layers of gauze and the filtrate centrifuged at 16,000g for 15min. The pellet was resuspended in TRIS/sucrose containing 5mM EDTA and 0.1 mM PMSF. The suspension was layered onto Lymphoprep and centrifuged at 30,000g for 40min. The membranes at the interface were collected and centrifuged at 45,000g for 15min. The pellet was resuspended in TRIS 25mM buffer, containing 5mM $MgCl_2$ and 1mg/ml bacitracin. After further centrifugation at 45,000g for 15min the final pellet was resuspended in the same buffer, at a final protein concentration of 400-600μg/ml.

In the binding assay, ^{125}I-VIP (conc. 0.15-1.5nM) and the membrane suspension was incubated at 37° for 15min, with or without 1μM unlabelled VIP. Incubation buffer was TRIS/$MgCl_2$/Bacitracin with 0.5% BSA. Incubation was terminated by filtration through Whatman GF/C filters pre-soaked in 0.3% polyethyleneimine. The filters were washed with 50mM TRIS buffer, containing 0.5mM EDTA and 0.5% BSA. Saturation binding experiments indicate K_d of 0.21-0.26nM, and a B_{max} of 110-160 fmoles/1mg (n=3). At the K_d specific binding represents 75-80% of total binding. Displacement experiments showed the following order of potency: VIP > PHM > PHI ≫ secretin. Glucagon was completely inactive. The displacement curves were shallow, consistent with the presence of a second, low-affinity binding site. Under these conditions, binding of ^{125}I-VIP in the absence of membranes is 4-7%: with untreated filters it is at least 90%. None of the residual binding is displaceable by excess unlabelled VIP. These preliminary results indicate that the high-affinity ^{125}I-VIP binding site in human lung resembles that in other species, and that a low-affinity site is also present. It is not yet possible to compare receptor number with other species because the methods have only been used with human lung. We believe that this technique makes the assay feasible by removing the carbon particles which are present in most adult human lungs. We also describe a filtration assay rather than the centrifugation methods previously used, which may prove more convenient.

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A NOVEL STIMULATORY ACTION OF SOMATOSTATIN ON PROLACTIN SECRETION

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Several actions of somatostatin on the secretion of anterior pituitary hormones have been described (Reichlin, 1983). Further to the classical inhibition of GH secretion (Brazeau et al, 1973), inhibitory effects on basal - (or more prominently, stimulus-induced) - secretion of TSH and ACTH have been reported. Early experiments observed only weak and inconsistent effects oN prolactin secretion (Vale et al, 1974), but employed long incubation periods, likely to obscure the initial response.

We have investigated the effects of somatostatin analogues on secretion of prolactin and GH, using a rapid superfusion technique which allows the measurement of transient effects.

Anterior pituitary glands from male Wistar rats were chopped into 500μm prisms and incubated for 1 hour at 37°C in oxygenated Krebs bicarbonate medium, pH 7·4 containing 0·1% BSA, 2 g/l glucose and 30 μg/l bacitracin. Tissue was then superfused with medium at 0.5 ml/min. Fractions (2 min) were collected serially and hormone concentrations were measured by radioimmunoassay.

After 90 min, a relatively steady baseline was reached and drugs were added. Somatostatin-14 produced a marked increase in prolactin secretion in experiments where GH secretion was concomitantly inhibited. The response gradually diminished towards baseline over about 1 hour, even in the continued presence of somatostatin. The effect was concentration-dependent from 20-250 nM but declined at higher levels. Somatostatin-28 mimicked this response over a similar concentration range with some 65± 10% increase at 100nM. Neither somatostatin-28_{1-12} (Benoit et al 1982) nor d-Trp^8 somatostatin-14 produced any significant increase in prolactin secretion at concentrations up to 300nM.

The response to 100nM somatostatin-28 was significantly attenuated to 16 ± 4% increase by the putative somatostatin antagonist cyclo [7-aminohepantanoyl- Phe-DTrp-Lys-Thr(Bzl)] (Fries et al, 1982) at 60nM.

These results demonstrate a novel stimulatory effect of somatostatin on prolactin secretion, which contrasts with previously described effects on other anterior ptiuitary hormones in that they are all inhibitory. Both somatostatin-14 and somatostatin-28 but not the fragment somatostatin-28_{1-12}, are active at this site and it is susceptible to the putative somatostatin antagonist. The novel stimulatory influence exerted on lactotrophes suggests that this somatostatin recognition site may have different properties from those on other anterior pituitary cells.

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