5-HT_{1A} LIGAND EFFECTS IN THE X-MAZE ANXIETY TEST

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We have shown that 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) causes an anxiogenic-like fall in the ratio of open/total arm entries in the elevated X-maze (Critchley & Handley, 1986). We now report the effects of ipsapirone and buspirone, which, like 8-OHDPAT, show selective binding to the 5-HT1a receptor.

Male PVG rats (Bantin & Kingman) were tested as previously described (Handley & Mithani, 1984). Buspirone had no effect from .025 - .1 mg/kg but depressed both ratio and total entries from .25 - 5.0 mg/kg. Ipsapirone dose-dependently increased the ratio from .25 - 2.5 mg/kg with no effect on total entries. The activity of 8-OHDPAT and ipsapirone disagrees with the finding of File et al (1987) in a similar test. Similarly 'anxiolytic' doses (mg/kg) of ipsapirone (1.0); ritanserin (.1); diazepam (1.0) and p-chlorophenylalanine (pCPA 3 daily treatments of 300, last 24 h before testing, mean forebrain 5-HT depletion 79% in parallel group N=6) were tested for interaction with 8-OHDPAT .03 mg/kg (Table 1). Diazepam, ritanserin and buspirone were without significant effect but both ipsapirone and p-CPA significantly reduced the 8-OHDPAT effect (Anova interaction term, AxB).

Table 1. Effects on OPEN/TOTAL ratio ± s.e.m.

A:	vehicle	vehicle	8-OHDPAT	8-OHDPAT	F(AxB)	df	p
B:	vehicle	drug	vehicle	drug			
buspirone	.325 .01	.328 .02	.113 .01**	.063 .03**	.86	1/10	NS
diazepam	.325 .02	.422 .01**	.093 .01**	.070 .03**	1.1	1/10	NS
ipsapirone	.328 .02	.466 .02**	.206 .02**	.358 .02	8.3	1/10	<.01
pCPA	.313 .02	.384 .02*	.245 .02*	.346 .01	4.6	1/18	<.05

* $p<0.05$, ** $p<0.01$ versus vehicle/vehicle ANOVA

It is likely that 8-OHDPAT acts as a 5-HT1A receptor agonist in this test while ipsapirone behaved as an antagonist and buspirone had no clear-cut effect. The abolition of the 8-OHDPAT effect by 5-HT depletion may indicate a presynaptic site of action.

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PUTATIVE ALCOHOL DEPENDENCE IN ADRENAL CELL CULTURES: RELATION TO Ca^{2+} CHANNEL ACTIVITY

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Experiments on intact animals indicate an increase in functional dihydropyridine (DHP) sensitive Ca^{2+} channels on central neurones associated with alcohol physical dependence (Dolin et al, 1987). This increase in Ca^{2+} channels may be causally related to dependence since Ca^{2+} antagonists of the DHP type prevent the alcohol withdrawal syndrome (Little et al, 1986). Similar increases in DHP binding sites occur in the adrenal-derived PC 12 cells grown in medium containing ethanol (Messing et al, 1986) suggesting that these cells may provide a model for dependence. We have previously reported that the inhibitory effect of ethanol on catecholamine release from adrenal chromaffin cells is lost if these cells are grown in ethanol (Harper et al, 1987). We therefore now examined the effects of DHP's on catecholamine release from chromaffin cell cultures grown in medium containing ethanol.

Bovine adrenal chromaffin cells for culture were prepared as described previously (Harper et al, 1987). Three days after plating, cultures were fed with ethanol-free medium or medium containing 200mM ethanol for 6 days. Evaporation of ethanol was prevented by enclosing the culture plates in sealed perspex containers. For experimentation, the medium was removed and replaced with Lockes solution for 10 minutes. The cells were then exposed to "stimulating" medium for 10 minutes and the catecholamines released into the supernatant were measured fluorimetrically. Catecholamine release was expressed as a percentage of the total catecholamine content of the cells. All experiments described were carried out in the absence of ethanol.

The characteristics of ethanol release was greatly altered after growth in ethanol for 6 days. Spontaneous release was increased by 200% in ethanol-treated cells. The spontaneous release was inhibited by Cd^{2+} but not by the DHP antagonists. The release of catecholamines induced by high K^+ was also enhanced in these cutures, higher levels of stimulation causing markedly greater enhancement. K^+-induced release was very sensitive to inhibition by DHP Ca^{2+} antagonists. Paradoxically, the DHP Ca^{2+} agonist, BAY K 8644, which caused the expected enhancement of release in control cultures, inhibited the release from ethanol-treated cultures at very low concentrations.

The results strongly suggest that alterations in DHP binding sites in adrenal cells grown in the presence of ethanol confer alterations in the characteristics of catecholamine release from these cells. Since ethanol is a relatively potent inhibitor of Ca^{2+} flux from PC 12 cells (Messing et al, 1986) and catecholamine release from chromaffin cells (Harper et al, 1987), this alteration may be adaptive in nature and shows the "oppositional" mechanism characteristic of dependence (Littleton, 1980).

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We have previously reported an increase in [^{3}H] Nimodipine binding in cerebral cortical membranes of rats made physically dependent on ethanol (Liitle, H.J. et al,1986) and have also reported alterations in Ca^{2+} sensitivity of isolated vasa deferentia from these animals which could also be a consequence of increased dihydropyridine(DHP) sensitive Ca^{2+} channels (Hudspith et al,1987). This series of experiments investigates [^{3}H] DHP binding in various tissues from alcohol dependent rats.

Male Sprague-Dawley rats were made ethanol tolerant by inhalation of ethanol vapour for 6-10 days, as previously described (Lynch et al,1985). Control groups were kept under identical conditions except for the absence of ethanol. Animals were killed by stunning and decapitation. The brain, heart and vasa deferentia were rapidly removed and cleaned of fat and connective tissue. Crude membrane preparations were made according to previously published methods(Gould et al,1982) with modifications. Binding assays were performed in duplicate using [^{3}H] Nitrendipine and cold Nimodipine (nonspecific binding) to characterise the high affinity DHP binding sites.

The results reported here for brain membranes are consistent with those previously reported showing an increase in [^{3}H] DHP binding sites in ethanol dependent rats (B_{max} increased from 144.10±1.68 to 202.20±23.60 fmol/mg of protein) with no significant change in binding affinity. We now report here a significantly greater increase in B_{max} in cardiac membranes compared to that in brain of 184.00 ±12.90 to 413.70±84.5 fmol/mg of protein in ethanol dependent rats. The K_d for cardiac membranes from control rats was 1.74±0.52 and for ethanol dependent rats, 4.18±1.39, showing no significant difference in binding affinity. [^{3}H]DHP binding sites on the smooth muscle of rat aorta was also investigated, but no significant results can be reported here, principally due to the sparsity of available tissue to perform adequate binding studies. However, studies on vasa deferentia membranes indicate an increase in B_{max} from 215.10±13.60 in control preparations to 321.03±29.05.fmol/mg of protein in ethanol dependent animals. K_d for control vasa deferentia membranes is 4.60±0.09 compared to 4.80±0.15 in ethanol dependent preparations. There was no significant change in binding affinity.

These results strongly suggest generalised changes in these tissues, as a direct consequence of chronic alcohol administration. These changes are possibly adaptive ones occurring in the DHP sensitive "L" type Ca^{2+} channels. Recent evidence suggests that increased numbers of DHP sensitive Ca^{2+} channels play a role in both hypertension and cardiomyopathy (Wagner et al,1986) indicating that alcohol related cardiovascular disease may be a consequence of the apparent "upregulation" of Ca^{2+} channels.

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DIHYDROPYRIDINE AND THE ETHANOL WITHDRAWAL SYNDROME; STEREOSPECIFICITY AND THE EFFECTS OF BAY K 8644

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We have shown previously that the calcium channel antagonists, nitrendipine and nimodipine, were effective against ethanol withdrawal convulsions, at doses which did not have overt sedative actions in normal animals and which gave only slight protection against other types of convulsions ((Little et al, 1986; Dolin & Little, 1986). The number of central dihydropyridine binding sites was increased by chronic ethanol administration (Dolin et al, 1987) and the effects of the dihydropyridines on neurotransmitter release and on inositol phospholipid turnover were also increased (Hudspith & Littleton, 1986; Littleton et al, 1987). We have put forward the hypothesis that a functional increase in the number of neuronal dihydropyridine binding sites is responsible for the effects of chronic ethanol treatment (Littleton et al, 1987). This theory predicts that the calcium channel antagonist PN 200-110 would protect against the ethanol withdrawal syndrome in a stereospecific manner and that the calcium channel agonist, BAY K 8644, would prevent the actions of the antagonists in this syndrome. The present results verify both these predictions.

Ethanol vapour (8→20 mg L^{-1}) was administered to male TO mice (25 - 30g) for ten days. On withdrawal from the ethanol, the drugs were injected, i.p., and ratings of handling convulsion scores (Goldstein & Pal, 1971) made every 60 min, by an observer who did not know the prior drug treatment. All drugs were suspended in DMSO (20%), and given at 0h and 4h after withdrawal. The results are expressed as the mean and s.e.m. of the convulsion scores for each group, between 2h and 6h after withdrawal.

Drug (dose)	Mean ± s.e.m.	P < 0.05	n
Vehicle controls	1.9 ± 0.10		25
Nimodipine (12.5 mg kg^{-1})	0.8 ± 0.22	* a	6
Nimodipine (50 mg kg^{-1})	1.3 ± 0.25	* a	11
BAY K 8644 (2 mg kg^{-1})	1.6 ± 0.24		5
Nim (50 mg kg^{-1}) + BAY K (2 mg kg^{-1})	2.8 ± 0.14	* a, * b	6
(-) PN 200-110 (10 mg kg^{-1})	1.9 ± 0.13		8
(+) PN 200-110 (10 mg kg^{-1})	0.75 ± 0.12	* a, * c	8

a: comparison with controls, b: comparison with nimodipine alone, c: comparison between isomers. * P < 0.05, nonparametric analysis of variance (Meddis, 1984).

Nimodipine (12.5 & 50 mg kg^{-1}) significantly decreased the convulsions. This effect was prevented by the agonist BAY K 8644. The latter compound did not potentiate the syndrome when given alone, but the convulsion scores after BAY K 8644 plus nimodipine were significantly higher than for the vehicle treated animals. The (+) isomer of PN 200-110 was effective against the syndrome but the (-) isomer was not. This follows the same pattern as the effects of the isomers of PN 200-110 on calcium channels (Hof et al, 1986). The results support our hypothesis that neuronal calcium channels are involved in the adaptation to chronic ethanol treatment.

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κ-RECEPTOR INVOLVEMENT IN OPIOID INDUCED HYPERACTIVITY IN NEONATAL RATS

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We have previously demonstrated that the opioid agonist U50,488H (trans-3,4-dichloro-N-methyl-N- [2-(1-pyrrolidinyl)-cyclohexyl]-benzene acetamide) produces a naltrexone-reversible increase in activity in neonatal rats (Jackson & Kitchen, 1987). U50,488H acts selectively at κ-opioid receptors and we have extended our investigations firstly: by comparing the behavioural effects of U50,488H in 5 day old rat pups with those of μ- and δ-opioid agonists; secondly, by confirming the involvement of κ-receptors in the hyperactive response using another κ-agonist tifluadom and also M8008 (16-methyl-cyprenorphine), an opioid antagonist which has low affinity for κ-receptors (Smith, 1987); and thirdly, by employing the dopamine receptor antagonist haloperidol to explore the role of the dopaminergic system in U50,488H-induced hyperactivity.

Activity was measured using a time-sampling procedure (Reinstein & Isaacson, 1977) whereby the behaviours of individually-housed Wistar rat pups (male and female) were scored for 5 s every 60 s over a 60 min testing period. Activity levels represented a composite of the forward locomotion, wall-climbing, paddling and grooming behavioural scores. Drugs were administered intraperitoneally in a dose volume of 0.1ml/20g body weight using a blind protocol and mean treatment group scores (n=8) were statistically compared using non-parametric oneway analysis of variance (Kruskal-Wallis) followed by the Mann-Whitney U test.

Activity levels in 5 day old rat pups were increased 10- and 30-fold by 1 and 10mg/kg doses of U50,488H respectively but not by 0.1 - 10mg/kg of either the μ-agonist D-Ala^2,NMe-Phe^4,Glyol^5-enkephalin (DAGO) or the δ-agonist D-Pen^2, D-Pen^5-enkephalin (DPDPE). Indeed, the highest dose of DAGO produced significant behavioural depression. The absence of any behavioural effects of DPDPE in 5-day old rats is likely to reflect the delayed emergence of δ-compared to μ- and κ-receptors in the rat CNS (Spain et al., 1985) though the possibility of rapid metabolism of this compound cannot be excluded. (+)-Tifluadom, but not its inactive (-)-isomer, produced hyperactivity. Moreover, U50,488H-hyperactivity was resistant to doses of M8008 (1 and 10mg/kg) that effectively block μ-receptor effects in vivo (Birch & Hayes, 1987). These results indicate the stereoselective nature of the response and substantiate its mediation via κ-receptors. Finally, haloperidol (0.1mg/kg), which had no overt behavioural effects per se, attenuated the activity induced by U50,488H (10mg/kg) in the neonatal rats, hence suggesting that dopaminergic mechanisms may be involved in this opioid response.

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EFFECTS OF CARBAMAZEPINE ON SOME BEHAVIOURAL MODELS OF MONOAMINE FUNCTION

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Carbamazepine (CBZ), an established anticonvulsant, is also effective in the treatment of manic depressive disorders (Post and Uhde 1986). Because of hypotheses implicating brain monoamines in the actions of psychotropic drugs, the effects of chronic CBZ on various monoamine related behavioural functions were examined. Rodents were pretreated for 14-21 days with CBZ suspended in propylene glycol (PG) and added to their diet (0.25 g% for 3 days followed by 0.5 g%). Controls had PG only added to their food. All other drugs were dissolved in 0.9% NaCl. Activity of pairs of rats was measured using two Animex monitors. Other tests used randomised groups and blind ratings.

Tests of dopamine function (Strombom, 1975)

Chronic CBZ fed to rats did not affect:

1. Hyperactivity in response to apomorphine (1.0 mg/KG sc)
2. Hyperactivity in response to methylamphetamine (0.75 mg/kg sc)
3. The increase in methylamphetamine hyperactivity produced by haloperidol (2 mg/kg sc daily for 14 days)

However CBZ did cause:

1. Increased exploratory activity on withdrawal ($P<0.01$, sign rank test).
2. A reduction in the hypoactivity syndrome in response to apomorphine (0.08 mg/kg sc)($P<0.05$ sign rank test)

Tests of 5-hydroxytryptamine(5HT) function (Goodwin & Green, 1985)

Chronic CBZ fed to mice increased head twitch behaviour in response to carbidopa (25 mg/kg sc) followed by 5-Hydroxy-l-tryptophan (100 mg/kg sc) ($P<0.01$ Mann-Whitney 'U' test).

Chronic CBZ had no effect on:

1. Head twitches in response to 5-Methoxy-N,N-dimethyltryptamine (5 mg/Kg sc) in mice.
2. Hypothermia in response to ±-8-Hydroxydipropylaminotetraline (0.5 mg/kg sc) (8OHDPAT) in rats.
3. The behavioural syndrome in response to 8OH-DPAT (1.0 mg/kg sc) in rats.
4. B_{max} of [^{3}H] ketanserin binding to preparations of rat frontal cortex (Leysen et al 1982).

These experiments suggest that chronic CBZ increases 5HT functions through presynaptic mechanisms and also attenuates presynaptic inhibitory dopamine function without affecting postsynaptic sensitivity to either dopamine or 5HT.

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EFFECT OF LITHIUM ON RELEASE OF CORTICAL 5-HT INDUCED BY MAO INHIBITION: A BRAIN DIALYSIS STUDY

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Administration of MAO inhibitors to rats pretreated over several days with lithium induces the '5HT behavioural syndrome' (Grahame-Smith and Green, 1974). Originally this effect was thought to be due to increased release of brain 5HT, since then lithium has been shown to enhance the postsynaptic responsiveness to directly acting 5HT agonists (Goodwin et al, 1986). Here we report the use of brain microdialysis to determine more directly the effect of lithium pretreatment on the release of 5HT induced by the MAO inhibitor tranylcypromine (TCP).

Male Sprague-Dawley rats (250-300g), treated twice daily for 3 days with lithium chloride (3 mmol/kg sc) or saline, were anaesthetized with chloral hydrate (400 mg/kg ip) and a 'short-loop' microdialysis probe was stereotaxically implanted into frontal cortex (R+2.5,L-0.6,V-3.3mm) as previously described (Zetterstrom et al, 1983). The dialysis probe was perfused at 1µl/min with artificial CSF and perfusates were collected every 20 min and then analysed immediately for 5HT and 5HIAA using HPLC-EC. After a 3hr control period TCP (20mg/kg) was injected s.c. and perfusates were collected for a further 2 hr.

Mean (±SEM) basal levels of 5HT and 5HIAA in cortical perfusates of saline-treated rats immediately before TCP injection 0.15±0.06 (6) and 9.14±1.30 (6) pmols/20min perfusate, respectively, were not statistically different from those of the lithium-treated group 0.11±0.04 (6) and 14.15±1.30 (6) pmols/20 min perfusate. TCP induced marked but similar increases of 5HT in the cortical perfusates in saline (maximum +185%, 80-100 min post drug) and lithium-pretreated rats (+219%). The decrease of 5HIAA in the perfusates observed after administration of TCP was also similar in degree and timing in both groups. In untreated rats, tryptophan (50 mg/kg i.p.) markedly potentiated the TCP-induced rise in perfusate 5HT showing that the method is capable of detecting enhanced 5HT synthesis.

These findings do not lend credence to the hypothesis that the 5HT-mediated behavioural response to TCP in lithium-treated rats is due to the enhancement of 5HT release by lithium and make it more likely that lithium greatly enhances postsynaptic 5HT function in some way.

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EFFECTS OF POTASSIUM-CHANNEL BLOCKING AGENTS ON THE RELEASE OF ENDOGENOUS GLUTAMATE FROM CEREBELLAR SLICES

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Tetrapentylammonium (TPA), 4-aminopyridine (4-AP) and quinidine have been shown to block potassium channels in a variety of neuronal preparations (see Hille, 1984). In this present study we have examined the ability of these agents to modulate endogenous glutamate release from rat cerebellar slices.

Parasaggital slices (250 um) were superfused (0.5 ml/min) with artificial CSF gassed with 95% O_2/5% CO_2 at 37°C. Two minute aliquots of CSF were collected and assayed for glutamate by HPLC with fluorescence detection.

TPA, 4-AP and quinidine (1×10^{-3} M) significantly elevated glutamate release above basal ($p < 0.01$). However, when the calcium dependency of these increases was assessed, it was found that the increases persisted under calcium-free conditions and furthermore, the response to TPA and 4-AP was potentiated. Further experiments, using TPA in calcium-free CSF showed that three, two-minute pulses of TPA exhibited no decay, over the three pulses, in the amount of glutamate released (100%, 98% and 99% respectively). When calcium was included in the second pulse the release was significantly decreased ($p < 0.01$), this effect was inhibited by including verapamil (1×10^{-4} M), a calcium channel antagonist, in the second pulse. Further experiments with tetrodotoxin (TTX, 5×10^{-7} M) and ruthenium red (1×10^{-5} M, an agent postulated to prevent calcium transport) decreased glutamate release under calcium-free conditions.

The effects of potassium channel blockade (in normal CSF) in releasing glutamate is consistent with a prolongation of the action potential. However, in calcium-free CSF an additional mechanism must be postulated which has to involve intracellular calcium mobilization in order to explain the transmitter release. Sandoval (1980) has shown that elevation of sodium levels in the neurone causes mitochondrial calcium release. Thus blockade of the potassium rectifying current, by TPA, would allow the inward sodium current, associated with action potential generation, to be prolonged and possibly bring about a release of mitochondrial calcium and subsequently glutamate release. The results with TTX would tend to confirm this, as would the fact that ruthenium red also decreased glutamate release in calcium-free conditions by preventing calcium transport within the terminal.

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THE INFLUENCE OF DAILY FOCAL INJECTIONS OF EXCITATORY AMINO ACID ANTAGONISTS ON ELECTRICAL KINDLING OF THE DPC

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A site located in the deep prepyriform cortex (DPC) has been shown to be unusually responsive to electrical kindling (Morimoto et al, 1986; Croucher and Bradford, 1987). We report here the effects on DPC kindling of daily focal injections of excitatory amino acid (EAA) antagonists.

Male Sprague-Dawley rats (360-410g) were implanted with bipolar electrodes to which stainless steel guide cannulae had been cemented for location of injection cannulae in the DPC (for co-ordinates see Piredda and Gale, 1986). After-discharge (AD) threshold was estimated for each animal and behavioural responses to electrical stimulation were rated (0-5) as previously described (Croucher and Bradford, 1987). Drug solutions buffered to pH 7.4 were injected (0.5µl over 2 min) daily, 15 min before electrical stimulation.

In an initial study (n=4) 0.1nmols AP7 completely inhibited the electrically-evoked AD in 2/4 animals. A dose of 10nmols AP7 was similarly effective in a 3rd animal. Focal buffer injections did not affect the occurrence, duration or amplitude of evoked ADs.

Groups of 5 animals were subsequently given 10 consecutive daily injections of AP7 (10nmols) or GAMS (10nmols) prior to electrical stimulation. The results are summarized in Table 1.

Table 1 Mean kindled AD durations (± s.e.m.; sec) and seizure scores (SS) following focal injections of buffer, AP7 (2-amino-7-phosphonoheptanoic acid; 10nmols) or GAMS γ-D-glutamylaminomethylsulphonic acid; 10nmols) into the DPC.

	Buffer (n=6)		AP7 (n=5)		GAMS (n=5)	
Day	AD Duration	SS	AD Duration	SS	AD Duration	SS
1	15.7 ± 2.1	1.2	7.8 ± 5.3	0.4	15.2 ± 0.4	0.4
2	19.8 ± 4.2	1.8	7.2 ± 3.0*	0.2**	15.0 ± 4.2	0.8*
3	27.2 ± 5.4	2.0	14.0 ± 4.6*	0.8*	20.2 ± 3.0	1.3
4	37.2 ± 3.1	2.2	17.8 ± 6.0**	0.7**	26.6 ± 5.9	1.7
5	42.5 ± 7.5	2.3	13.6 ± 7.3*	0.5**	26.4 ± 9.3	1.9
6	44.7 ± 6.7	2.5	30.6 ± 5.9	3.0	25.6 ± 8.3	2.0
7	48.3 ± 6.1	2.8	42.4 ± 14.4	3.4	22.0 ± 8.0*	1.8
8	56.8 ± 7.2	3.4	40.0 ± 16.3	3.2	35.0 ± 8.8*	2.7
9	57.0 ± 5.5	3.8	45.0 ± 14.0	3.7	35.4 ± 8.1*	3.0
10	70.0 ± 4.7	4.3	45.2 ± 14.0	3.4	38.4 ± 8.0**	3.6

* P<0.05; ** P<0.01; compared with buffer (Student's 1-tailed t-test).

Both AP7 and GAMS reduced the AD durations and retarded the development of DPC kindling responses. During AP7 treatment all 5 animals showed very low seizure responses at day 5 (0-1, cf buffer, 2-3) with 3/5 animals subsequently showing more severe responses (2-5).

These results demonstrate that focally adminstered EAA antagonists can significantly inhibit the duration of electrically kindled ADs and the time course of kindling in the DPC. EAA receptors may therefore, play a key role in the generation or expression of electrically kindled responses.

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COMPARISON OF THE ANTICONVULSANT ACTIONS OF ALPIDEM AND DIAZEPAM AFTER REPEATED TREATMENT

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Alpidem (chloro-6(chloro-4-phenyl)-2N,N-di-n-propyl imidazo[1,2-a] pyridine-3-acetamide) is a novel anxiolytic (Saletu et al, 1986) which in animal experiments also shows a broad spectrum of anticonvulsant activity. This compound binds to a specific site within the $GABA_A$-chloride ionophore-supramolecular complex identified as BZ-1 and also posseses a high affinity for the peripheral benzodiazepine recognition site (Langer et al, 1987). As a similar molecular mechanism may be involved in the mode of action of alpidem and benzodiazepines, it was of interest to investigate whether tolerance develops to the anticonvulsant action of alpidem as is the case with benzodiazepines.

Mice (CD_1, Charles River, France) received alpidem (100 mg/kg, p.o., b.i.d.), diazepam (5 mg/kg, p.o., b.i.d.) or vehicle (20 ml/kg, p.o., b.i.d.) for 10 consecutive days. Anticonvulsant effects were evaluated 36 h after the last administration using as convulsive challenges: supramaximal electroshock (MES, 50 Hz, 60 mA, 0.4 ms); pentetrazole (PTZ, 125 mg/kg, s.c.); bicuculline (BIC, 0.9 mg/kg, i.v.) or isoniazid (ISO, 800 mg/kg, s.c.). For PTZ and MES, protection against tonic extensions of the hindlegs was observed, whereas in ISO and BIC models the onset of convulsions and protection against death, respectively, were noted.

Drugs	Alpidem*		Diazepam*	
Chronic treatment Models	Vehicle	Drug	Vehicle	Drug
Pentetrazole	11 (5 - 19)	17 (8 - 31)	0.2 (0.1 - 0.3)	0.6 (0.02 - 19)
Electroshock	37 (25 - 52)	100 (64 - 200)	0.86 (0.6 - 1.2)	2.2 (1.2 - 4.9)
Bicuculline	13 (8 - 20)	19 (14 - 33)	0.063 (0.042 - 0.086)	0.40 (0.27 - 0.60)
Isoniazid	14	20	1	3

* ED_{50} (mg/kg ip) with 95 % confidence limits.

As shown in the table, the potencies of alpidem in antagonizing BIC-mortality or ISO-and PTZ-induced convulsions were similar in mice repeatedly treated with vehicle and with alpidem. After its repeated administration alpidem was about 3 times less active against MES. In contrast, diazepam was 2.6 - 6.7 times less potent after its repeated administration, depending on the convulsion model. Repeated treatment with diazepam decreased the efficiency of alpidem against ISO-convulsions (ED_{50}= 30 mg/kg i.p.) in comparison with control mice (ED_{50}= 10 mg/kg i.p.), whereas repeated treatment with alpidem did not change the potency of diazepam in antagonizing convulsions induced by ISO.

These results indicate that less tolerance develops to the anticonvulsant action of alpidem than to that of diazepam and suggest that these drugs interact with the supramolecular complex of the GABA receptor in different manners.

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SEROTONERGIC INVOLVEMENT IN THE BEHAVIOURS PRODUCED BY INTRATHECAL TRH ANALOGUE (CG 3509)

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Thyrotrophin-releasing hormone (TRH) is present in a high concentration in the ventral horn of the spinal cord where it co-exists with 5-hydroxytryptamine (5HT) in bulbospinal neurones which appear to regulate somatic motoneurone output (Barbeau & Bedard, 1981). In the conscious rat, intrathecal injection of the TRH analogue CG 3509 (L-orotyl-L-histidyl-prolineamide) produces dose-related wet-dog shakes (WDS) and a marked forepaw-licking (FPL) behaviour (Fone et al., 1987). The present study investigates whether these behaviours are mediated by bulbospinal serotonergic neurones by using the selective $5HT_2$ antagonist ritanserin and intrathecal (i.t.) administration of the serotonergic neurotoxin 5,7-dihydroxytryptamine (57DHT).

An i.t. cannula was implanted in male Wistar rats under sodium methohexitone anaesthesia (60 $mgkg^{-1}$ i.p.) as previously described (Fone et al., 1987). Following 7 days recovery, the number of WDS and the time spent FPL induced in 30 min following CG 3509 (0.5 µg i.t., n=7) were recorded at 4 day intervals; 30 min after vehicle i.p. (day 7), ritanserin 1 $mgkg^{-1}$ i.p. (day 11), ritanserin 5 µg i.t. (day 19) and vehicle i.t. (day 23), and ritanserin 5 µg i.t. was also tested in combination with CG 3509 (0.5 µg) on day 15. In separate rats the same behavioural responses to 0.5 µg CG 3509 i.t. were compared before and after (9 and 23 days after surgery, respectively) vehicle 10 µl i.t. (5.68 mM ascorbate in 0.154 M saline, n=8) or 57DHT (2x150 µg i.t. on days 11 and 13, n=8) given 1 h after desipramine 25 $mgkg^{-1}$ i.p. At the end of the 57DHT study the thoraco-lumbar spinal cord and brainstem levels of 5HT were determined using high performance liquid chromatography with electrochemical detection and TRH and substance P levels were measured by radioimmunoassay. Values given are mean±s.e.mean and Students' t-test was used for statistical analysis.

The WDS (102±15 in 30 min) and FPL (250±42 s in 30 min) behaviours induced by CG 3509 were attenuated following pretreatment with ritanserin i.p. (55±11; $P<0.01$ and 149±33 s; not significant, respectively) or i.t. (49±15; $P<0.02$ and 57±17 s; $P<0.01$), while neither FPL (188±53 s) nor WDS (89±13 in 30 min) behaviours were significantly reduced when ritanserin was given i.t. at the same time as CG 3509. Both the WDS (81±11 in 30 min) and FPL behaviours (154±41 s in 30 min) induced by CG 3509 were unaltered following i.t. 57DHT (65±19 and 134±40 s in 30 min, respectively) although this treatment markedly depleted 5HT, TRH and substance P in the thoraco-lumbar ventral horn (by 84%; $P<0.001$, 96%; $P<0.001$ and 59%; $P<0.05$ below values in vehicle treated controls, respectively), without reducing levels in the brainstem.

These results suggest that the behaviours induced by i.t. CG 3509 are not mediated by bulbospinal serotonergic neurones as destruction of this pathway had no effect on either behaviour and the CG 3509-induced behaviours were unaffected by combined i.t. administration with ritanserin. The reduction in behaviour following systemic or i.t. pretreatment with ritanserin, however, implies that $5HT_2$ receptors mediate the CG 3509-induced behaviours but that these receptors are not in the spinal cord.

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SECRETION OF TSH AND THYROID HORMONES FOLLOWING TREATMENT OF RATS WITH DESIPRAMINE OR ECS

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We have addressed whether different antidepressant treatments alter the function of the thyroid axis in view of the following information: 1) there is monoaminergic control of TRH at the hypothalamic level 2) there are various inter-relationships between depression and thyroid status (Whybrow & Prange, 1981); 3) at the anterior pituitary level there is a decreased sensitivity to TRH stimulation of TSH secretion in depression and 4) the thyroid gland is innervated by adrenergic nerves (Ahren, 1986). We have now compared the effects of single and repeated treatments with a tricyclic-antidepressant (DMI) and ECS on the plasma concentrations of TSH and thyroid hormones.

Male rats were treated twice daily with DMI (5 mg/kg, i.p.) for 14 days or once on one day only. Controls received saline. Other groups were given ECS x 5 (biphasic, 110V x 1sec) under light halothane anaesthesia on alternate days, or once only. Control rats received halothane only. 3 or 24 Hours following the last treatment blood samples were taken from a lateral tail vein and plasma TSH and thyroid hormones measured by RIA.

Chronic treatment with DMI produced a 30% reduction in TSH ($P < 0.005$) at both 3 and 24h following treatment, with one dose causing a reduction at 24h only. Small (10%) reductions in circulating free and total T_3 and T_4 were also found at certain time points. Compared with their respective halothanized controls ECS x 5 produced an increase in circulating TSH (approx. 30%, $P < 0.01$) at the 3h time-point only with no concomitant changes in thyroid hormones. A single ECS treatment caused very little change in hormone levels. Our results demonstrate, therefore, that DMI and ECS can produce small and time-dependent, but opposing changes in circulating TSH. However, halothane alone reduced TSH by approximately 30% and thus any ECS change was superimposed on a reduced background secretion. Indeed, Duque et al (1983) have shown that repeated ECS without anaesthesia produced no immediate changes but a delayed fall in serum TSH was observed 48 hours following the final ECS treatment. Both ECS and DMI may be acting oppositely on the 'tonic' hypothalamic control of TSH secretion via monoamine systems since it has already been shown, for example, that in mouse brain these treatments have opposite effects on 5-HT function (Goodwin et al, 1984). A direct effect on hypothalamic TRH neurones (Lighton et al, 1984) or the TRH 'sensitivity' of pituitary thyrotrophs seems unlikely since TRH-induced TSH release from isolated superfused pituitary glands in vitro from rats treated chronically with ECS or DMI was not changed. In agreement with Duque et al (1983) we observed no consistent changes in circulating T_3 and T_4 following ECS treatment. These findings, including the lack of effect of halothane, indicate the 'adaptive' ability of the thyroid axis to normalize hormone levels despite persistent changes in TSH output. The small effects on thyroid hormone secretion seen after DMI may be due to additional direct effects on thyroid gland adrenergic control.

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REPEATED T_3 ADMINISTRATION POTENTIATES THE EFFECTS OF ECS ON 5-HT_2 BUT NOT 5-HT_{1A} MEDIATED BEHAVIOURAL CHANGES IN MICE

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We have recently shown that L-triiodothyronine (T_3) alters both 5-HT_{1A} and 5-HT_2 behavioural responses in mice. 5HT_{1A} mediated hypothermia was attenuated by repeated, but not acute T_3 injection, while conversely, 5-HT_2 mediated head-twitches were enhanced by acute, but not repeated T_3 treatment (Smith and Heal, 1987). Repeated administration of electroconvulsive shock (ECS) produces changes in 5-HT_{1A} and 5-HT_2 function (Green et al, 1986) which are similar to those induced by T_3 injection. Since T_3 has previously been found to potentiate the effects of ECS on presynaptic α_2-adrenoceptor function (Heal et al, 1987), we have now studied the interactions between repeated administration of T_3 and ECS on 5-HT_{1A} and 5-HT_2 mediated behaviours in mice.

Adult male C57/Bl/601a mice (25 - 30g) were used throughout. T_3 (100 µg/kg in 0.02M NaOH) or NaOH vehicle (10 µl) were injected subcutaneously daily for up to 10 days. T_3 and vehicle treated groups were further subdivided and were also given either a single ECS (110V,1s) or halothane anaesthesia, again once daily for up to 10 days. 5-HT_{1A} and 5-HT_2 mediated responses were respectively assessed by the hypothermia measured 20 min after 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, 0.5 mg/kg sc) and the head-twitches counted in 6 min after injection of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT, 2 mg/kg ip). Results were analysed using 2 way analysis of variance for 2 independent variables combined with Tukey's test.

8-OH-DPAT hypothermia was unaltered by T_3 x 1, but was reduced by T_3 x 5 (42%, $P<0.01$) and T_3 x 10 (40%, $P<0.01$). This response was also decreased by ECS x 5 (30%, $P<0.01$) and ECS x 10 (24%, $P<0.01$), but not ECS x 1. Head-twitches were increased after T_3 x 1 (50%, $P<0.01$) and T_3 x 5 (50%, $P<0.01$), but not after T_3 x 10. ECS also increased head-twitches after a single shock (31%, $P<0.05$), ECS x 5 (78%, $P<0.01$) and ECS x 10 (46%, $P<0.01$). When T_3 was co-administered with ECS, this hormone markedly ($P<0.01$) potentiated the effects of ECS x 5 and ECS x 10 on head-twitch responses. This was almost certainly not an additive effect because T_3 x 10 alone had no effect on head-twitches. No co-operativity was found for the interaction between T_3 and ECS on 8-OH-DPAT hypothermia.

The results obtained confirm our earlier findings with T_3 (Smith and Heal, 1987) and the enhancement of 5-MeODMT head-twitches after a single ECS (Metz & Heal, 1986). T_3 administration was also shown to produce similar changes in 5-HT_{1A} and 5-HT_2 function to ECS, the exception being that head-twitches were not enhanced by T_3 x 10. T_3 also markedly potentiated the effects of ECS on 5-HT_2 mediated responses. Since repeated ECS probably enhances head-twitches via increased 5-HT_2 receptors (Metz and Heal, 1986), while T_3 does this indirectly and actually reduces 5-HT_2 receptor number (Smith and Heal, 1987), then the data, therefore, suggest that these 2 mechanisms are mutually co-operative. In conclusion, these data show that T_3 potentiates some actions of ECS on 5-HT function, as well as on noradrenergic function.

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ACTIONS OF AVERMECTIN MK 936 (AVM) ON γ-AMINOBUTYRIC ACID (GABA) INHIBITION AND BIOELECTRIC POTENTIALS OF ASCARIS MUSCLE

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The avermectins are commonly used anthelmintics which paralyse Ascaris without causing flaccid paralysis or hypercontraction (Kass et al 1982). Using a range of preparations they have been reported to act at the level of the chloride ionophore to increase chloride conductance and hyperpolarize the membrane potential (Duce & Scott 1985; Tanaka & Matsumura 1985; Bokisch & Walker 1986; Lees & Beadle 1986). Reversible actions of dihydroAVM may involve the GABA receptor ionophore complex but other actions including irreversible effects appear to act at a non-GABA site. In the present study the effects of AVM MK 936, an 80:20 mixture of AVM B_{1a} and AVM B_{1b}, have been examined on Ascaris muscle where there is a resting chloride conductance and GABA increases chloride conductance (Martin 1980).

A 2cm section, just anterior to the genital ring, of Ascaris body wall was excised, cut along one of the lateral lines and pinned onto the Sylgard base of a tissue bath, 5 ml volume. Muscle cell bellies were impaled with two microelectrodes filled with 4M K-acetate, resistance 10-30MΩ. Current pulse parameters for input conductance measurements were 20 nA, 500 ms, 0.2 Hz. Conventional electrophysiological recording techniques were used to amplify and display the results. The bath was continually perfused, 7ml/min, with Artificial Perienteric Fluid (APF) of composition: NaCl 67mM; Na-acetate 67mM; $MgCl_2$ 15mM; KCl 3mM; $CaCl_2$ 3mM; Tris 5mM; pH 7.6 at 21±1°C. Acetate replaced chloride in chloride free experiments. GABA was diluted in 20ml volumes of APF and superfused for 2.5 min, followed by normal APF. AVM was dissolved in DMSO and diluted in APF to a final DMSO concentration of 0.02%. Control experiments were performed using 0.2% DMSO diluted in APF.

AVM, 1μM, had no direct effect on the membrane potential or conductance of either quiescent or spontaneously active muscle cells. When GABA was pressure ejected for 1-10 seconds from an electrode filled with 1mM GABA during AVM superfusion, the GABA hyperpolarization was reduced from control value of 7.02±0.82mV to 2.71± 0.84mV, n=17. This reduction was significantly greater (p=0.007) than the reduction seen in control experiments. Thus AVM partially antagonized the GABA hyperpolarization and this effect was reversible. On spontaneously active cells, AVM 1μM, irreversibly blocked activity after 26±6 min (n=7). GABA, 10μM, still produced some hyperpolarization when applied during AVM superfusion or when cell activity ceased. Chloride free APF depolarized cells and induced bursting activity. The cell activity was not altered following AVM exposure for 45 min demonstrating that chloride is required for AVM effects, n=6.

In this tissue AVM does not produce the hyperpolarization and chloride conductance increase reported in other systems. On quiescent cells it partially antagonises GABA hyperpolarizations. AVM blocks spontaneous spiking activity recorded from muscle cells but not through a membrane hyperpolarization as observed with GABA. It is possible that AVM is acting on a central site.

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BECLAMIDE INDUCED LOCOMOTOR AND BEHAVIOURAL EFFECTS

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Behavioural disorders are prevalent in mentally handicapped patients with epilepsy and beclamide, an anticonvulsant, is of benefit in stabilizing mood and reducing aggressive and antisocial behaviour (Peuch et al. 1962). Such abnormal patterns of behaviour are usually associated with changes in monoamine function in the CNS (Wise et al. 1972). While beclamide in vitro has no affinity for D_2, $5HT_1$ and $5HT_2$ receptors (Darmani et al. 1987), in the rat striatum it causes a 3-fold reduction in turnover and steady state levels of dopamine (DA) and a 3-fold increase in the steady state levels of dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) (Darmani et al. 1986). The drug also causes a marked reduction in striatal levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA). The present investigation evaluates the acute and chronic effects of beclamide on locomotor activity and behaviour and also changes in apomorphine-induced stereotypies after chronic beclamide treatment in rats.

One hour after habituation to test environment, male Wistar rats (150-300g) were given oral doses of either beclamide (250mg/kg suspended in 0.75% sodium carboxymethyl cellulose) or vehicle. Fifteen minutes after treatment, cumulative locomotor activity was measured using photocell cages and behaviour was observed simultaneously for 2 hrs. The above procedure was repeated on the fifth day after chronic daily administration of beclamide (250mg/kg) or vehicle. In the third experiment, rats were simultaneously dosed for five days and treatment was withdrawn for a period of 36 hrs, after which time animals received apomorphine (0.5 mg/kg i.p.). The cumulative locomotor activity and stereotypies were assessed using the scoring system employed by Randall (1985).

In acute studies, beclamide caused a significant increase (190%, $P<0.05$) in motor activity 75 min after treatment compared to controls. No such increase occurred in chronically-dosed animals. However, apomorphine-induced locomotor activity was significantly reduced by 601% ($P<0.05$) compared to chronically vehicle-treated animals within 30 min of treatment. Similarly, stereotyped behaviour induced by apomorphine was significantly ($P<0.05$) decreased in chronically beclamide-treated rats. However, in both acute and chronic studies no stereotypies were produced by beclamide alone.

The increased locomotor activity of a single dose of beclamide may reflect the acute increase in DA turnover previously reported (Darmani et al. 1986). The loss of this stimulant action following chronic administration of the drug may signify DA receptor down-regulation. This is supported by the reduction in both apomorphine-induced locomotor activity and stereotyped behaviour observed in the animals chronically-treated with beclamide. Whether such an effect is related to the clinical efficacy of beclamide remains to be established.

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TPA INCREASES 5-HYDROXYTRYPTAMINE SYNTHESIS IN RAT HIPPOCAMPAL AND HYPOTHALAMIC SYNAPTOSOMES

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There is evidence that tryptophan hydroxylase, the rate limiting enzyme in 5-hydroxytryptamine (5-HT) biosynthesis, is activated by a calcium-calmodulin (CaM) dependent protein kinase (Kuhn & Lovenberg, 1982; Fujisawa et al., 1984). Whether it can be regulated by other mechanisms is at present not clear. Protein kinase C (PKC), a calcium/diacylglycerol/phospholipid dependent protein kinase, is highly concentrated in nerve terminals of rat brain (Girard et al., 1985). When PKC was activated by phorbol esters, it enhanced 5-HT release in rat synaptosomal preparations from various brain regions (Nichols et al., 1987).

We have carried out experiments to see whether PKC also has an effect on the rate of 5-HT synthesis. Synaptosomes were prepared from hippocampus and hypothalamus of male Sprague Dawley rats. The synaptosomal preparations were incubated in oxygenated HEPES-buffered incubation medium containing the monoamine oxidase inhibitor, pargyline. 5-HT content was measured by HPLC with electrochemical detection. The synthesis rate was calculated from the difference in 5-HT content of samples incubated for 5 and for 25 min and expressed as pmoles 5-HT formed per min per mg protein. The synthesis rate of 5-HT in the absence of drugs is 0.27 ± 0.01 pmole/min/mg protein in hippocampal synaptosomes and 0.59 ± 0.03 pmole/min/mg protein in hypothalamic synaptosomes.

Addition of a phorbol ester, TPA (1μM), a stimulator of PKC, increased the 5-HT synthesis rate by 46% in hippocampal synaptosomes and 41% in hypothalamic synaptosomes. This effect is completely blocked by a specific PKC inhibitor, polymyxin B (10μM).

When K^+ concentration in the incubation medium was raised to 50mM by partial replacement of Na^+, 5-HT synthesis rate was increased by 45% in hippocampal synaptosomes and 38% in hypothalamic synaptosomes. The combined effect of 1μM TPA and 50mM K^+ was a 75% increase in synthesis rate in hippocampal synaptosomes and a 66% increase in 5-HT synthesis rate in hypothalamic synaptosomes.

These results suggest that in addition to the Ca-CaM dependent protein kinase, the Ca/phospholipid-dependent protein kinase C also plays a role in the regulation of the activity of tryptophan hydroxylase and that the two mechanisms are independent of each other, since their combined effects are additive.

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CAESIUM IONS DIMINISH THE DOPAMINE INDUCED HYPERPOLARISATIONS IN IDENTIFIED HELIX ASPERSA NEURONS

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Snail neurons identified as F1,F5 and F6 respond to dopamine with a hyperpolarisation (Bokisch & Walker,1986).The reversal potential for the dopamine induced hyperpolarisation and the use of channel blockers indicate that the response may involve an increase in potassium conductance (Cox & Walker,1986). Here we report on the effect of caesium ,a potassium channel blocker , on the dopamine induced hyperpolarisation in Helix aspersa neurons.

The suboesophageal ganglia of snails were mounted on a glass slide and placed in a 10ml perfusion chamber.Cells F1,F5 or F6 were impaled with KCl (1M) filled glass microelectrodes (10-30 MEGOHMS).Dopamine was freshly prepared at 15 min. intervals from a stock (1 mM) diluted in 5 mM tartaric acid .Dopamine or ringer was applied to the bath by a rapid microperfusion technique (Slater & Carpenter,1984) for 30 secs. followed by 1 min.washout. Transmembrane potentials were monitored. Membrane conductance changes could be estimated by the injection of current pulses (0.5-1 nA,750 msec duration ,0.1 Hz) through the recording electrode.The effect of caesium on the dopamine response was investigated by either re-entering the cell with a caesium chloride filled electrode (1M) or by substituting the potassium ions in the perfusing medium for caesium.Responses to dopamine were monitored for up to 1 hr.

Cells responded in a dose dependent fashion to dopamine EC50 3.8 +/- 1.14 uM (n=8) accompanied by an increase in membrane conductance.Resting membrane potential was around -45 mV and varied slightly (+/- 5 mV) during 2 hr. experiment.The response to dopamine ,apart from an initial desensitisation, was stable for as long as recordings could be made from the cell.The response of the cells to dopamine was attenuated by both externally applied and internally applied caesium at all concentrations of dopamine studied.(Table 1.)

DOPAMINE (- LOG M)	Cs OUT (n=3)	Cs IN (n=5)
6	0	0.51 +/- 0.08
6.5	0.25 +/- 0.24	0.62 +/- 0.10
5	0.49 +/- 0.06	0.71 +/- 0.10
5.5	0.51 +/- 0.11	0.64 +/- 0.08

Table 1. The ratio (+/- SEM) of the hyperpolarising responses to dopamine after and prior to exposure to caesium.

Inhibition of the dopamine response by caesium was accompanied by a notable increase in the apparent duration of the action potential, a dimunition of the after-hyperpolarisation and occasionally a decrease membrane conductance.Such observations are consistent with a blockade of membrane potassium channels and support the hypothesis that the dopamine induced hyperpolarisation in Helix neurons is a consequence of an increase in potassium conductance.

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MEASURING MUSCARINIC ANTAGONIST POTENCY USING STIMULATED PHOSPHOINOSITIDE BREAKDOWN IN RAT CORTEX SLICES

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Antagonist potencies are frequently estimated in functional neurochemical studies by inhibition of a fixed agonist effect with various concentrations of antagonist. The IC_{50} value is then transformed into a Ki value using the equation of Cheng and Prusoff (1973) - a procedure which is inappropriate in functional studies due to unknown receptor reserve. We have assessed the effects of muscarinic antagonists on muscarinic agonist stimulated phosphoinositide breakdown in rat cortex slices using both the method of Arunlakshana & Schild (1959) and the inhibition curve design followed by dose-ratio analysis.

The general procedure was that of Brown et al. (1984). Briefly, 50µl (14mg wet weight) of slices, prelabelled with ^{3}H-inositol, were incubated at 37°C with agonist for 45 min in a total volume of 330µl Krebs buffer containing ^{3}H-inositol and LiCl (5mM). Antagonists were added 30 min before the agonist. ^{3}H-inositol phosphates were recovered using anion exchange chromatography.

Concentration-effect curves were constructed for muscarine alone and in the presence of 3 or 4 concentrations of antagonist. The following pA_2 (and Schild slope) values were obtained:- atropine 8.4±0.1 (1.6±0.1) n=3; pirenzepine 7.4±0.2 (0.8±0.1) n=3; propylbenzilyl choline (PrBCh) 7.7±0.2 (1.0±0.1) n=3. In other experiments responses to a fixed concentration, 10^{-3}M, of carbachol or muscarine were measured in the presence of five or more concentrations of antagonist and a concentration-effect curve was also constructed for the agonist in each assay. Since the effect of 10^{-3}M agonist in the presence of the IC_{50} of antagonist is the same as the effect of agonist at its EC_{50} (taking the effect of the fixed agonist concentration as the Emax) the antagonist K_B was estimated from its IC_{50} using the following form of the Schild-Gaddum equation (Barlow, 1980):- $pK_B = -\log IC_{50}/(10^{-3}/EC_{50}-1)$. The following pK_B (and Hill slope) values were obtained:- atropine 8.8±0.1 (0.98±0.05) n=6; pirenzepine 7.5±0.1 (0.70±0.04) n=11; PrBCh 7.7 (0.94±0.13) n=3.

The shallow Schild and Hill slopes observed with pirenzepine may reflect the involvement of both M1 and M2 receptors in the response (Lazareno et al., 1985). The steep Schild slope found with atropine may reflect a reduction in concentration of free atropine by binding to the high receptor concentration in this assay (≧5nM).

We conclude that, under the conditions of this assay, traditional dose-ratio methods can lead to problems in the study of antagonists with nanomolar potency. The inhibition curve design does not have these problems, and K_B estimates can be made from IC_{50} values as described above, without the inappropriate use of the Cheng-Prusoff equation.

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SELECTIVE EFFECTS OF LITHIUM ON INOSITOL POLYPHOSPHATE FORMATION IN MOUSE CEREBRAL CORTICAL SLICES IN VITRO

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In the treatment of affective illnesses the mechanism of action of lithium remains unresolved. Recently attention has focussed on the effects of Li^+ on calcium mobilizing receptors. Since both inositol 1,4,5-trisphosphate (1,4,5-IP_3) and inositol 1,3,4,5-tetrakisphosphate (IP_4) have second messenger functions (Streb et al., 1983; Irvine & Moor, 1986) we have examined the in vitro effects of Li^+ on inositol polyphosphate generation in mouse cerebral cortex slices.

Mouse (C57 black) cerebral cortical slices (350 µm x 350 µm) were washed and preincubated in a Krebs Henseleit buffer, pH 7.5, for 60 mins at 37°C. 25 µl aliquots of gravity packed slices were then preincubated with ^3H myo-inositol (120 KBq) in Krebs medium for 60 mins before the addition of agonist (10 µl). LiCl (5 mM) was added 10 mins before agonists. Incubations were terminated by the addition of 100 µl perchloric acid (10% w/v). Inositol phosphates were extracted into a freon:tri-n-octylamine mixture and the individual phosphates were then separated by anion exchange chromatography or by high performance liquid chromatography (Batty et al., 1985).

After 10 mins Li^+ (5 mM) enhanced the accumulation of ^3H-inositol monophosphate (^3H-IP_1) and ^3H-inositol bisphosphate (^3H-IP_2) in the absence of stimulation and in the presence of the muscarinic agonist carbachol (1 mM), noradrenaline (100 µM), histamine (1 mM), 5HT ($3x10^{-4}$M) and elevated KCl (31 mM). Increases in ^3H-IP_1 accumulation due to Li^+ (expressed as percentage of mean accumulation ±s.e., n=3, in the absence of Li^+) were: control (244±3%), carbachol (300±5%), histamine (471±14%), noradrenaline (528±8%), 5HT (423±24%) and KCl (345±51%). Accumulations of ^3H-IP_2 were: control (146±5%), carbachol (289±2%), histamine (300±4%), noradrenaline (549±22%), 5HT (336±10%) and KCl (314±50%). Accumulation of the ^3H-IP_3 fraction (containing both 1,3,4-IP_3 + 1,4,5-IP_3) was also enhanced by lithium when the tissue was stimulated with histamine (288±37%), noradrenaline (283±18%) and KCl (152±33%) but not with 5HT (125±13%) or carbachol (105±4%).

In the absence of Li^+ the accumulation of ^3H-IP_4 was enhanced ($P<0.05$) over basal only by KCl and carbachol stimulations with the level of carbachol-stimulated ^3H-IP_4 being 10-fold greater than that due to KCl. Li^+ produced a relatively small enhancement of ^3H-IP_4 production due to histamine (139±11%), noradrenaline (191±9%), 5HT (114±3%) and KCl (171±44%) but, uniquely, reduced carbachol-stimulated ^3H-IP_4 accumulation (43±4%) ($P<0.05$).

Time course studies showed that the reduction in ^3H-IP_4 was evident only after 5 min of carbachol stimulation with earlier time points unaffected. HPLC separation revealed that the increase in the ^3H-IP_3 fraction due to Li^+ was entirely accounted for by an increase in the ^3H-1,3,4-IP_3 isomer.

In conclusion, muscarinic receptor stimulation in mouse cerebral cortical slices produced a much greater increase in ^3H-IP_4 compared with the other stimuli examined and this increase was selectively reduced by Li^+. This suggests that the muscarinic post receptor mechanism might be a therapeutically-relevant target for Li^+ action.

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A STUDY OF THE RESPONSES OF MOTONEURONES TO 5-HYDROXYTRYPTAMINE (5-HT) IN THE HEMISECTED SPINAL CORD OF THE NEONATE RAT

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Rats aged between 3 and 8 days were anaesthetized with ether and decapitated. The lumbar spinal cord was dissected out, hemisected and maintained by superfusion with oxygenated modified Krebs solution at a rate of 2.5 ml/min and a temperature of 18 - 22^{o} C. Recordings were made of the potential between a ventral root and the surface of the cord using a pair of non-polarizable Ag/AgCl wick electrodes. Superfusion with 5-HT at 10^{-6}, 10^{-5} and 10^{-4} M was found to produce concentration-related depolarization of motoneurones. The EC_{50} was 20.5 uM ($\pm$ 1.2, n = 20) and the amplitude of the maximal response was 1.0 $\pm$ 0.1 mV (mean $\pm$ s.e.mean, n = 24).

A study was made of the relative potencies of 5-HT and some selective 5-HT receptor agonists. 5-carboxamidotryptamine ($5\text{-}HT_1$ selective) produced concentration-related depolarizations and had a potency not significantly different from 5-HT (EC_{50} = 33.3 $\pm$ 6.9 uM, n = 12, mean amplitude of the maximum response = 0.84 $\pm$ 0.07 mV, n = 15). Similar results were obtained with αmethyl-5-HT ($5\text{-}HT_1$, $5\text{-}HT_2$ selective, EC_{50} = 31.2 $\pm$ 5.6 uM, n = 8, mean amplitude of the maximum response = 0.87 $\pm$ 0.14 mV, n = 8). 8-OH-DPAT ($5\text{-}HT_{1A}$ selective) and RU 24969 ($5\text{-}HT_{1A,1B}$, $5\text{-}HT_2$? selective) failed to depolarize motoneurones as did 2-methyl-5-HT ($5\text{-}HT_3$ selective).

Concentration-response curves to 5-HT were constructed before and in the presence of antagonists. Methysergide ($5\text{-}HT_1$,$5\text{-}HT_2$ selective) caused a non-surmountable antagonism of the depolarization of motoneurones at 10^{-8} and 10^{-7} M. Methiothepin ($5\text{-}HT_1$,$5\text{-}HT_2$ selective) was completely ineffective at the same concentrations. Ketanserin ($5\text{-}HT_2$ selective) had only a weak blocking action.

The lack of effect of 2-methyl-5-HT and the low potency of ketanserin indicate that the receptor is not of the $5\text{-}HT_3$ or $5\text{-}HT_2$ type (Bradley et al, 1986). If the receptor is $5\text{-}HT_1$-like, albeit insensitive to methiothepin, it cannot be readily equated with $5\text{-}HT_{1A}$, $_{1B}$ or $_{1C}$ subtypes.

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THE SELECTIVE INHIBITION OF A LOW K_M CYCLIC AMP PHOSPHODIESTERASE FROM RAT CEREBRAL CORTEX BY DENBUFYLLINE

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Denbufylline [1,3-di-n-butyl-7-(2'-oxopropyl)-xanthine; BRL 30892] is a novel agent shown to be of value for the treatment of multi-infarct dementia (O'Connolly et al., 1986). This compound potently inhibits cyclic nucleotide phosphodiesterase (PDE) in rat erythrocytes and skeletal muscle but is a relatively ineffective inhibitor in cardiac and smooth muscle (Nicholson and Wilke, 1986). The ability of denbufylline and, for comparison, 3-isobutyl-1-methylxanthine (IBMX) to inhibit the hydrolysis of cyclic AMP by PDE from rat cerebral cortex has now been examined.

In an initial series of experiments, PDE was assayed in the presence of 0.3 μM cyclic (8-^{3}H) AMP in homogenates of rat cerebrum as described by Arch and Newsholme (1976). Denbufylline potently inhibited only a part of the total PDE activity. After addition of the Ca^{2+} chelator EDTA (2 mM) to the assay medium denbufylline, but not IBMX, more effectively inhibited that fraction of PDE which retained activity (Fig. 1).

In a further series of experiments, PDE isoenzymes were separated using a Q Sepharose Fast Flow column. Three fractions capable of hydrolysing cyclic nucleotides were identified. Peak I, Ca^{2+}/calmodulin dependent PDE, was not inhibited by denbufylline. Peak III, cyclic AMP PDE, was potently inhibited by denbufylline (IC_{50} 1 μM; Fig. 1). Peak II, cyclic GMP PDE, was weakly inhibited by denbufylline (IC_{50} 100 μM; not shown).

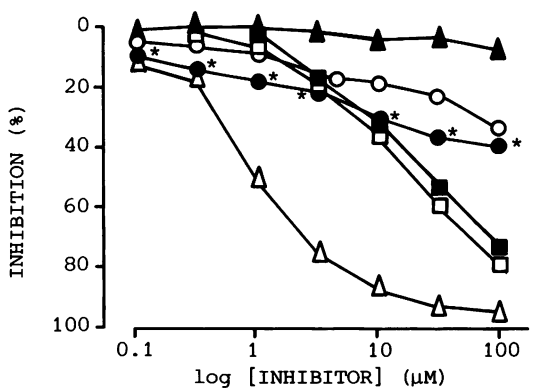

Fig. 1:
The inhibition ($n \geqslant 3$) of PDE in rat cerebral cortex homogenates by denbufylline (circles) and IBMX (squares) in the absence (open symbols) and presence (closed symbols) of EDTA (* ● > o, $p < 0.05$), and the inhibition of Ca^{2+}/calmodulin dependent PDE (▲) and cyclic AMP low K_m PDE (Δ) fractions, obtained from cortical homogenates, by denbufylline; s.e.mean within symbols.

The major proportion of PDE present in rat cerebrum has a high K_m for cyclic AMP and is Ca^{2+}/calmodulin dependent (Strada et al., 1984). This investigation has shown denbufylline to be a weak inhibitor of this isoenzyme. In the cerebral cortex denbufylline appears to be a selective inhibitor of a PDE which has a low K_m for cyclic AMP and which is not Ca^{2+}/calmodulin dependent. The tissue selective inhibition of PDE by denbufylline (Nicholson and Wilke, 1986) may be due to differences in the tissue distribution of the low K_m cyclic AMP PDE selectively inhibited by denbufylline.

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MONOAMINE OXIDASE B DISTRIBUTION IN THE HUMAN, MARMOSET AND RAT BRAIN

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An understanding of monoamine oxidase (MAO) distribution in the brain is clearly relevant to interpreting the action of selective MAO substrates and inhibitors *in vivo*. The distribution of MAO B is of particular interest with respect to the activity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes a parkinsonian syndrome in man and primates with a selective degeneration of the substantia nigra. Rats are less susceptible to its action.

In this study the histochemical localisation of MAO B in the human, marmoset and rat brain was examined. The technique used unfixed frozen sections which were stained using a coupled peroxidatic technique employing benzylamine as a substrate and (-)-deprenyl as a selective inhibitor (Ryder *et al.*, 1979).

In the rat there was a low background distribution of MAO B together with areas of high intensity staining (see Table 1). These were the 5-hydroxytryptamine (5HT) rich areas i.e. pineal gland, the interpeduncular nucleus and the raphe nucleus. No staining above background was apparent in the striatum and substantia nigra.

Table 1 Areas of High Intensity MAO B Staining in the Rat, Marmoset and Human Brain

Region	Intensity of Staining		
	Rat	Marmoset	Human
Pineal gland	++++	ND	ND
Lining of ventricles	++++	-	+++
Interpeduncular nucleus	+++	++++	ND
Raphe nucleus (dorsal)	+++	+++	+++
Hypothalamus	++	++/+++	ND
Ventral tegmental area	++	++	ND
Substantia nigra	-	+++	++++
Striatum	-	+++	+++/++
Nucleus accumbens	-	++++	ND

ND = not determined

In contrast the main areas of MAO B staining in the marmoset brain included the dopamine rich regions, the substantia nigra and the striatum, as well as the raphe nucleus and interpeduncular nucleus. A preliminary mapping of MAO B in the human brain has shown in general a similar pattern to the marmoset, the main areas of staining including the raphe nucleus, the substantia nigra and the striatum. However unlike the marmoset, but similar to the rat, the lining of the ventricles showed strong staining. These results demonstrate how different the distribution of MAO can be between species and indicate that in the marmoset where MPTP causes nigrostriatal degradation more MAO B is available in the immediate vicinity. Langston and Irwin (1986) suggest that a close extraneuronal source of MAO B is necessary to express MPTP neurotoxicity. However the high level of MAO B may be necessary but not sufficient to explain the difference in susceptibility as other dopaminergic areas in the marmoset i.e. the ventral tegmental area and the nucleus accumbens, were also rich in MAO B, although less affected by MPTP.

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GRAFTING OF HUMAN FETAL DOPAMINE NEURONS IN A RAT MODEL OF PARKINSONS DISEASE:A TYROSINE HYDROXYLASE IMMUNOCYTOCHEMICAL STUDY

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The clinical disorder, Parkinson's Disease (PD), involves the degeneration of the mesostriatal dopaminergic (DA) pathway. An animal model of PD is achieved by destruction of this DA pathway with 6-hydroxydopamine. Experiments (Brundin et al 1987a: Dunnett et al 1985) involving the syngeneric transplantation of fetal rat ventral mesencephalon directly into the lesioned neostriatum in this model have shown that such fetal grafts can ameliorate behavioural impairments. Anatomical studies have shown that these grafted DA neurons integrate with the host's neuronal circuitry and new synaptic connections are formed (Freund et al 1985).

The present experiments examined whether human fetal DA neurons exhibit these same properties of growth and plasticity when xeno-grafted to rat striatum, thus providing a basis for clinical trials leading to a novel therapy for PD. The human fetal DA neurons were obtained in Sweden from 6½-9½ weeks old aborted fetuses (with full permission of the research ethical committee at Lund University) and implanted intrastriatally, using the cell suspension grafting technique (Bjorklund et al 1983), into rats immunosuppressed using cyclosporin A. Five months after grafting the rats' rotational behaviour was tested following a challenge with apomorphine and amphetamine, and the DA release from surviving grafted human ventral mesencephalic neurons was measured by in vivo dialysis (Brundin et al 1987b).

The rats were then perfused with a buffered fixative containing 2% paraformaldehyde and 0.1% glutaraldehyde and 70μm sections of the striatum processed for immunocytochemistry using antibodies to tyrosine hydroxylase (TH), the synthetic DA enzyme. In the light microscope, surviving grafts were readily located in all animals. They contained between 500 and 700 TH-immunoreactive neurons of characteristic morphology; being large, multipolar neurons with long dendrites which extended several microns into the host brain. Within the rat neostriatum, dense terminal networks of fine-calibre TH-positive fibres were seen which extended the whole rostro-caudal extent of the striatal complex and, to some extent, also into nucleus accumbens and globus pallidus.

At the ultrastructural level, the TH-immunoreactive terminals made symmetrical synaptic contacts with the rats' neuronal elements in a pattern of termination similar to that observed in the control hemisphere. The predominant targets were dendritic elements, both spines and shafts, although some contacts were observed onto neuronal perikarya, mainly of the medium-sized densely spiny class. The immunoreactive dendrites of the graft DA neurons, which extended into the host brain, also received afferent input presumably of rat host origin.

Thus, these anatomical findings, together with the previous behavioural and physiological data, suggest that intracerbral grafting of fetal DA neurons could provide a viable approach to ameliorate neurological symptoms in patients with PD.

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WIDESPREAD DISTRIBUTION OF GABA IMMUNOREACTIVE SYNAPTIC BOUTONS ON DENDRITES OF RAT SYMPATHETIC PREGANGLIONIC NEURONS

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Although there is evidence that central control of autonomic function can be influenced by drugs that act on GABAergic neurotransmission (DeFeudis, 1982), there is relatively little information about the precise sites of action of these drugs. Infusion of GABA agonist (muscimol) into the spinal cord causes a fall in blood pressure (Gordon, 1985) and iontophoretic application of GABA to sympathetic preganglionic neurons inhibits their firing (Backman & Henry, 1983). We have examined identified sympathetic preganglionic neurons to see whether they receive synaptic boutons that are immunoreactive for GABA.

Sympathetic preganglionic neurons that project to the rat adrenal gland were identified by retrograde tracing using a conjugate of cholera toxin B-chain to horseradish peroxidase (CTB-HRP) which is avidly transported in the peripheral nervous system (Trojanowski et al., 1981). Injections were made into the adrenal medulla and after 48h the animals perfused with fixative and tissue from spinal cord segments T7-T12 removed and sectioned. The retrogradely transported CTB-HRP was visualised using a sensitive chromogen o-tolidine, then made electron dense by stabilisation with 3'3'-diaminobenzidine. This method reveals the extensive dendritic arbors of neurons which can be studied in the electron microscope. Dendrites from the lateral funiculus, intercalated region, central canal and the soma were photographed, re-embedded, ultrathin sectioned, then processed by the post-embedding immuno-gold method for GABA immunoreactivity (Somogyi & Hodgson, 1985) and the distribution of GABA immunoreactive synaptic inputs to these identified structures studied in the electron microscope. Immunoreactive boutons were characterised by a high density of gold particles overlying their profiles, which could be observed in serial sections.

GABA immunoreactive boutons made symmetrical synaptic specialisation with all the regions of the sympathoadrenal neurons studied. In a random analysis of 72 dendritic profiles from sympathoadrenal neurons containing CTB-HRP, 37% of the 93 boutons which made contact with the profiles were GABA immunoreactive. In a similar analysis of 14 CTB-HRP labelled cell bodies, 64 synaptic boutons were counted of which 32% were GABA immunoreactive. Many GABA immunoreactive myelinated axons were also observed in the sections, suggesting the input may, at least partially, arise from more distant areas, possibly the brainstem (Fuji et al., 1985) as well as from cell bodies in the spinal cord (McLaughlin et al., 1974).

This study demonstrates the existence of a major GABAergic input to all parts of the sympathetic preganglionic neuron including the distal dendrites and adds to the evidence that GABA may be involved in the control of autonomic function, such as the regulation of blood glucose levels and blood pressure.

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HIPPOCAMPAL AND DOPAMINERGIC INPUTS TO THE NUCLEUS ACCUMBENS CONVERGE ONTO THE SAME NEURONS

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It has been shown in rat, by a combination of retrograde labelling and Golgi-impregnation, that the hippocampal-accumbens projection originates mainly from pyramidal neurons lying in the ventral subiculum (Totterdell & Smith, 1986). The nucleus accumbens also receives, from neurons in the ventral tegmental area (VTA), a large dopaminergic input which is distributed over the whole nucleus, with some patchiness (Voorn et al., 1986). The hippocampal input is mainly to the antero-medial region (Phillipson & Griffiths, 1985). The anatomical relationship between these two afferents within the nucleus accumbens is important when considering their interactions, which may be perturbed in schizophrenia.

The hippocampal input to the nucleus accumbens was interrupted by an electrolytic lesion of the fimbria/fornix. After perfusion sections of n. accumbens were incubated with an antibody to tyrosine hydroxylase, the synthetic enzyme for dopamine, using the peroxidase-antiperoxidase technique to reveal dopaminergic fibres and terminals, and then Golgi-impregnated by the section Golgi technique (Izzo et al., 1987). Golgi-impregnated neurons in the medial n.accumbens of rats in which lesions had been placed and in which immunocytochemistry was successful, were drawn, photographed and re-embedded for ultramicrotomy.

Examination of serial sections through identified neurons reveals that boutons of hippocampal origin, degenerating as a result of the lesion, are in asymmetrical synaptic contact with spines and occasionally shafts of medium-size densely spiny neurons. Degenerating boutons also contact dendrites of non-spiny neurons. Immunostained boutons are found in symmetrical synaptic contact with dendrites of medium-size densely spiny neurons, sometimes onto a spine neck, as reported by Freund et al., (1984). It was possible to show that Golgi-impregnated dendrites of an individual neuron were postsynaptic both to degenerating boutons and boutons immunoreactive for tyrosine hydroxylase.

This observation not only indicates a potential site of interaction between the dopaminergic and hippocampal inputs to the n. accumbens, but also a possible explanation of the role of cholecystokinin (CCK) in schizophrenia. Levels of CCK are reduced post mortem in the hippocampus of schizophrenics (Roberts et al., 1983) We have demonstrated CCK-immunoreactive boutons in synaptic contact with hippocampal pyramidal neurons which project to the n. accumbens. This hippocampal projection is, in part at least, in synaptic contact with the same accumbens neurons that receive dopaminergic input from the VTA. Hence a dysfunction of hippocampal CCK could be compensated for by neuroleptics acting on dopamine synapses in the nucleus accumbens.

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EXAMINATION OF THE SIGMA OPIOID-MEDIATED RELEASE OF [^{3}H]-NORADRENALINE FROM RAT AMYGDALA SLICES IN VITRO

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The effect of phencyclidine (PCP) and + n-allylnormetazocine (+NANM), also known as +SKF10,047 the sigma opioid ligand, on the potassium stimulated, calcium dependent release of preloaded [^{3}H] noradrenaline (NA) has been investigated using superfused slices of the rat amygdala in vitro. Two pulses of 25mM K^+ were given 28 minutes apart and the ratio of the [^{3}H] NA released by the second stimulus to the [^{3}H] NA released by the first stimulus was calculated (S_2/S_1). Under control conditions this ratio was 0.94 ± 0.04 (n=21). To investigate the action of the various drugs on [^{3}H]NA release the drug was included in the superfusion during the second K^+ stimulation and the ratio of S_2/S_1 was compared to the control values. PCP at doses of 2.5μM and 5μM produced a significant increase ($p < 0.05$) in release of [^{3}H]NA to 1.97 ± 0.29 (n=5) and 2.51 ± 0.51 (n=5) respectively, whilst +NANM at 5μM and 10μM also produced a significant increase ($p < 0.05$) in release to 1.42 ± 0.15 (n=12) and 1.39 ± 0.14 (n=6) respectively. It has been suggested that some effects of PCP and +NANM may be mediated through two different receptors (Gundlach et al, 1986) and that the drug haloperidol may show a higher affinity for the high affinity +NANM receptor than the low affinity PCP receptor (Tam & Cook, 1984). Consequently we examined the effects of 5μM haloperidol on the [^{3}H] NA release evoked by the two drugs. 5μM haloperidol on its own had no significant effect on K^+ stimulated release, (S_2/S_1 = 0.91 ± 0.10, n=5) and similarly produced no significant change in the effect of 5μM +NANM (S_2/S_1 = 1.34 ± 0.07, n=6) or 2.5μM PCP (S_2/S_1 = 1.84 ± 0.16, n=5). This lack of effect of haloperidol on the stimulated release of [^{3}H]NA by PCP and +NANM would indicate that the majority of the effect seen in the rat amygdala was mediated by the "PCP" receptor rather than the "sigma opioid" receptor in this preparation.

It has recently been reported (Jones et al., 1987) that PCP can inhibit N-methyl-aspartate (NMDA) evoked release of [^{3}H]NA from the nucleus accumbens. We have examined the effects of +NANM and PCP on the release of [^{3}H]NA from slices of rat amygdala evoked by 2 minute pulses of NMDA. Preliminary experiments indicated that 0.2mM NMDA produced very little release of [^{3}H]NA; whilst 0.4mM NMDA produced a larger effect it was still not a clear release, and so a stimulating pulse of 1mM NMDA, which raised the fractional release constant from about 0.8% to 1.4%, was utilised in all the subsequent investigations. Two pulses of NMDA were used, 28 minutes apart, and the ratio of the [^{3}H]NA released by the second pulse to the first pulse (S_2/S_1) was measured and subsequent drug applications were made in the second stimulation period. In these experiments magnesium ions were omitted from the perfusion medium since Mg^{++} ions have been shown to be powerful inhibitors of NMDA effects. If Mg^{++} ions were included during the second period of stimulation the evoked release of [^{3}H]NA by NMDA was completely abolished. Control experiments using two pulses of 1mM NMDA gave a S_2/S_1 ratio of 0.89 ± 0.09 (n=12), if 5μM +NANM was present during the second stimulation the ratio fell to 0.27 ± 0.10 (n=6) ($p < 0.05$) and 2.5μM PCP caused the ratio to fall to 0.18 ± 0.08 (n=6) ($p < 0.05$). The presence of 30μM 2 amino-5-phosphonovalerate (APV) completely abolished the effect of the second NMDA stimulus.

Thus whilst +NANM and PCP can cause a stimulation of K^+-evoked [^{3}H]NA release they cause an inhibition of NMDA-evoked [^{3}H]NA release in a similar manner to that described by Jones et al. (1987) in the nucleus accumbens.

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AUTORADIOGRAPHIC EVIDENCE FOR THE LOCALIZATION OF CCK RECEPTORS ON INTRINSIC STRIATAL NEURONES

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Considerable evidence supports the existence of modulatory interactions between cholecystokinin (CCK) and dopamine (DA) in the striatum (Fuxe et al., 1980; Meyer & Krauss, 1983). In order to explore further the mechanism of such CCK-DA interactions, the precise anatomical localization of CCK and DA receptors in rat striatum has been investigated autoradiographically following selective lesions.

Male Wistar rats were anaesthetized with sodium pentobarbital and then unilaterally decorticated or infused with ibotenic acid (10μg in 1μl; 1μl/min; x 2 injection sites) into the striatum, 6-OH-DA (8μg in 4μl; 1μl/min) into the medial forebrain bundle (MFB), or 1-methyl-4-phenyl pyridinium (MPP^+) iodide (10μg/day for 10 days via osmotic minipumps) into the nigrostriatal tract. Animals were left 21 days to recover and to allow for neuronal degeneration, then CCK receptors were mapped autoradiographically using the probe [125-I]CCK-8. Tyrosine hydroxylase (TH) immunoreactivity was visualized as a marker for DA content. In addition, striata were removed for measurement of DA content using HPLC with electrochemical detection and D-2 receptor number was determined by saturation analysis of [3-H]spiperone binding. The presence of unilateral lesions was verified both by behavioural tests and histological examination.

Infusion of 6-OH-DA or the more selective neurotoxin MPP^+ into the MFB resulted in denervation supersensitivity as evidenced by >10 contralateral rotations per min following injection of apomorphine (0.5mg/kg) and increased (~30%) dopamine D-2 receptor number. In addition these lesions were shown by TH immunoreactivity and HPLC analysis to reduce striatal DA content by >95%. However, striatal CCK content and CCK receptors were unchanged by these lesions when compared to sham-lesioned controls.

In contrast, destruction of striatal cell bodies with ibotenic acid resulted in a large decrease in the number of both striatal CCK (~65%) and D-2 (~45%) receptors, with no detectable effect on CCK or DA content. Behavioural verification of this lesion was shown both by ipsilateral bias and hyperactivity 1-2 days post drug infusion and extensive striatal cell loss and gliosis upon histological examination.

Unilateral decortication to destroy corticostriatal afferent fibres was found to have no effect on either striatal CCK receptors or DA content.

These lesion studies suggest that CCK receptors are located primarily on intrinsic striatal neurones and not on nigrostriatal or corticostriatal afferent fibres. Further studies are in progress to evaluate a functional role for these striatal CCK receptors.

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ELECTROPHYSIOLOGICAL EVIDENCE FOR A D_2 RECEPTOR AGONIST EFFECT OF SKF82526 IN SUBSTANTIA NIGRA IN VITRO

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Dopamine causes a concentration-related reduction in firing rate of substantia nigra zona compacta cells in slice preparations (Pinnock 1982). In order to characterise the receptor responsible for this effect we have investigated the actions of two selective D_1 receptor agonists, SKF82526 (Hahn et al., 1982) and SKF38393 (Pendleton et al., 1978), on dopamine-sensitive zona compacta cells.

Rats (male, AHA Wistar-derived 100-140g) were killed by cervical dislocation and brain tissue containing the two substantia nigra nuclei was sectioned using an Oxford vibratome. A single 350μm slice was superfused with oxygenated artificial cerebrospinal fluid (ACSF) for at least 1.5hr before recording began. Extracellular recordings of spontaneously active single units were made by conventional methods.

There appeared to be two classes of dopamine-sensitive cells, one in which dopamine (1-100μM) produced complete cessation of firing (which will be termed type 1A) and one in which no more than 40-60% reduction could be obtained (type 1B). These two classes of cells could not be distinguished by firing rate or the action potential shape and duration. Reproducible dopamine concentration-response curves (1-100μM) could be obtained from a single type 1A cell. This response was antagonized by the D_2 receptor antagonist haloperidol (0.03, 0.1 and 0.3μM) in a concentration-related manner. Haloperidol (0.03-0.3μM) also caused a reduction in the dopamine maximum response and as such only an approximate pA2 value of 7.75 with a slope of 1.29 could be calculated. Haloperidol (0.01 and 0.3μM) did not affect concentration response curves to GABA.

SKF38393, (1-1000μM), produced no change in firing rate in eight of nine type 1A cells studied and a small increase in one. However, SKF82526, (3-100μM), produced a concentration-related reduction in firing rate in a manner similar to dopamine on type 1A and 1B cells. This effect of SKF82526 could not be washed out with ACSF or reversed by the D_1 receptor antagonist SCH23390 (0.03μM), but complete reversal was achieved with haloperidol (0.3 and 1.0μM). An estimated pA_2 of 7.44 was calculated from haloperidol-induced rightward shifts of the agonist-curve.

These data suggest that the selective D_1 receptor agonist, SKF82526, produces the effects described here in nigral slices via D_2 receptors. This apparently unusual finding is consistent with those of Dallas et al (1986) who found the prejunctional effects of SKF82526 in cat nictitating membrane were reversed by haloperidol.

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EFFECTS OF TETRAETHYLAMMONIUM (TEA) ON THE PRESYNAPTIC AUTOINHIBITION OF DOPAMINE RELEASE FROM THE RAT NEUROINTERMEDIATE LOBE

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It has been demonstrated on different dopamine systems and in several species that the release of dopamine (DA) is under inhibitory control of presynaptic autoreceptors (see Starke, 1981). Nevertheless, the mechanism is still unknown by which autoreceptor activation causes the inhibition of transmitter release. On neurons in the substantia nigra DA can induce an increase in potassium conductance (Lacey et al., 1986). It appears possible that autoreceptors at the nerve terminals mediate a hyperpolarization which reduces the efficiency of stimulus-secretion coupling. In the present experiments the effects of the potassium channel blocker TEA on the autoreceptor mediated modulation of dopamine release was tested.

Isolated NILs with their stalks held in a platinum wire electrode were incubated in 80 µl Krebs-HEPES solution which contained also pargyline (10 µM) and the DA uptake inhibitor GBR 12909 (1-(2-(bis(4-fluorophenyl)methoxy)ethyl)-4-(3-phenylpropyl)-piperazine, 200 nM). The medium was changed every 10 min and DA determined by HPLC with electrochemical detection. The pituitary stalks were stimulated after 50 min (S1) and after 90 min (S2) of incubation with pulses of 0.2 ms; 10 V; 3, 5 or 15 Hz; 3 times for 1 min with 1 min intervals. TEA (10 or 30 mM) was added to the medium from the onset of incubation and (-)-sulpiride 20 min before S2. In some experiments, calcium was changed from 1.2 mM to either 3 or 0.3 mM.

In previous experiments it was shown that under the present incubation conditions endogenous dopamine activates the autoinhibiton in the NIL already maximally (Großhans et al., 1987; Racké et al., 1987). At all frequencies tested, (-)-Sulpiride increased the electrically evoked release of dopamine maximally at a concentration of 1 µM. Table 1 summarizes the release of dopamine evoked by S1 and the effect of 1 µM (-)-sulpiride observed under the various conditions.

Table 1:

Stimulation conditions	Dopamine release during S1 (fmol)	% increase by 1 µM (-)-sulpiride
15 Hz	922 ± 63 (7)	117 ± 30 (3)**
5 Hz	418 ± 40 (7)	201 ± 12 (3)**
3 Hz	221 ± 15 (15)	226 ± 30 (7)**
3 Hz + 10 mM TEA	992 ± 130 (9)+	77 ± 16 (4)*+
3 Hz + 30 mM TEA	1038 ± 102 (10)+	28 ± 9 (5)+
1 Hz + 30 mM TEA	1080 ± 69 (8)	16 ± 19 (4)
1 Hz + 30 mM TEA + calcium 0.3 mM	396 ± 39 (11)	32 ± 14 (6)
3 Hz + calcium 3 mM	675 ± 45 (10)+	66 ± 19 (5)*+

Means ± SEM of (n) experiments. Significance of difference: from the corresponding value in the absence of (-)-sulpiride: * $P < 0,05$; ** $P < 0,01$; from the corresponding value of the controls experiments 3 Hz: + $P < 0,01$.

In conclusion, blockade of potassium channels reduces the inhibitory effect of presynaptic DA autoreceptor activation, both in the presence of normal and low calcium concentrations. However, increasing the extracellular calcium concentration to 3 mM can also surmount the inhibitory effect of autoreceptor activation.

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EX-VIVO BINDING: A MEASURE OF DRUG PENETRATION INTO THE CNS

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The passage of a drug across the blood brain barrier is dependent on factors such as a lipophilicity and ionic charge. A direct measure of central nervous system (CNS) penetration can be obtained by using an ex-vivo binding technique to assess the brain levels achieved following systemic administration. The sensitivity of this method is dependent on the affinity of the compounds under study for the receptor system used in the binding assay. It has advantages over the more usual methods, using radiolabelled drugs, in that a larger range of compounds can be tested and problems due to radiolabelled metabolites are avoided.

In this study the opioid ligand [^{3}H]bremazocine, under conditions selective for the kappa receptor, has been used to measure the levels of a range of opioid standards obtained in mouse brain following systemic administration. The relative CNS penetration of these drugs has been evaluated, and compared with their lipophilicity, as measured by the log of compounds distribution between octan-1-ol and an aqueous buffer system at pH 7.4 (log D value).

Groups of 6 mice (Alderley Park, female) were dosed s.c. with saline or test compound, and killed 30 mins later. Homogenates were prepared using pooled saline or drug treated brains in HEPES buffer at pH 7.4. [^{3}H]bremazocine (0.2nM) binding assays were run for 40 min. at 25°C. Binding at the μ and δ receptors was suppressed using 3uM DADLE ([D-Ala^2,D-Leu^5]enkephalin). Brain levels were measured by comparison with calibration curves obtained to the test compounds added in vitro. All values were drawn from dose response curves of brain levels/dose for each compound and were the result of 2-4 separate determinations.

The brain levels obtained for these compounds at equianalgesic doses are shown in the table below, together with a measure of their relative CNS penetration, defined as the ratio of the (brain level/dose)/1000, and their log D values.

COMPOUND	DOSE (mg/kg)	BRAIN LEVEL (pg/mg tissue)	RATIO (brain level/dose)1000	LOG D
U20488	3.20	1050	0.33	1.7
LEVALLORPHAN	2.44	640	0.26	2.4
CYCLAZOCINE	0.56	63	0.11	1.3
NALORPHINE	1.54	58	0.04	1.5
Q.TIFLUADOM*	75	265	0.002	0.2

* 4-methyl quaternary salt of tifluadom.

All the standards were detectable at doses close to their analgesic IC_{50} values (0.4% acetic acid induced abdominal constriction test). There was a wide variation in the brain levels achieved by the compounds tested. However a good correlation was obtained between relative CNS penetration as measured by the brain level/dose ratios, and lipophilicity as defined by the log D values. The results confirm both the importance of the fat solubility of a drug in determining its passage across the blood-brain barrier, and the relevance of ex-vivo binding as a technique for the direct measurement of drug levels in brain tissue.

ALTERATIONS IN INDICES OF LIPID PEROXIDATION IN PARKINSONIAN SUBSTANTIA NIGRA

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In parkinsonian (PD) substantia nigra a decrease in reduced glutathione and glutathione peroxidase occurs suggesting that excessive lipid peroxidation provoked by free radicals may kill dopamine neurones (Perry et al 1982; Kish et al 1985). Recently, we demonstrated an increase in total iron content of PD nigra (Dexter et al 1987). Free iron may catalyse free radical production and lipid peroxidation so we have measured an index of lipid peroxidation in PD and age-matched control brains.

Nigral tissue was obtained from 14 patients with PD (Mean age 75.7 years $\pm$ 1.6) and from 19 control patients (Mean age 72.4 $\pm$ 4.7). Average time between death and removal of the brain tissue was 18.3 $\pm$ 2.4 h for PD patients and 16.2 $\pm$ 2.0 h for control patients. Polyunsaturated fatty acid (PUFA) and malondialdehyde (MDA) content of the tissue homogenates were measured by spectrometric techniques (Dexter et al 1987). Both basal levels of MDA and stimulated production of MDA by incubation of the tissue homogenates at 37°C for 90 mins in air alone or following addition of 0.01 mM $FeSo_4$ plus 0.25 mM ascorbic acid or 200 mM H_2O_2 plus 2 mM $FeSo_4$ were measured.

The basal content of MDA was higher in the PD samples (Table 1). However, PUFA content of the PD nigral tissue was lower than in the control tissue. Consequently, when expressed as a ratio in terms of PUFA content, the differences in basal MDA content of PD nigral tissue and control is more pronounced (Table 1). Incubation of tissue homogenates in air, or with $FeSo_4$ plus ascorbic acid or H_2O_2 plus $FeSo_4$ produced lower yields of MDA in PD nigral tissue when compared to the controls, reflecting the lower lipid content. No differences between PD and control cerebellum, cortex (Brodmann 10) or caudate tissues in PUFA or basal or stimulated MDA levels were observed.

Table 1 Levels of MDA formation and PUFA's in substantia nigra

Patient	PUFA's (nmol linoleic acid/mg protein)	Basal MDA levels (nmol MDA/ mg protein)	Ratio Basal MDA/ PUFA ($\times 10^{-3}$)	Stimulated lipid peroxidation (nmol MDA/mg protein above basal levels)		
				Air	0.01 mM $FeSo_4$ + 0.25 mM ascorbic acid	200 mM H_2O_2 + 2 mM $FeSo_4$
Control	298$\pm$12	2.0$\pm$0.1	7.0$\pm$0.5	1.8$\pm$0.1	9.9$\pm$1.0	17.4$\pm$1.2
PD	254$\pm$14*	2.7$\pm$0.3*	11.0$\pm$1.2*	1.0$\pm$0.1*	5.5$\pm$0.7*	13.1$\pm$0.6*

Value expressed as Mean $\pm$ SEM: * $p < 0.05$ compared to control tissue

Basal lipid peroxidation may be increased in PD nigra compared to control subjects. This may reflect the accumulation of iron in the PD nigra, a reduction in protective enzyme mechanisms or an increase in neurotoxic species.

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INTERACTIVE EFFECTS OF α_2-ADRENOCEPTORS AND κ-OPIATE RECEPTOR AGONISTS ON INTRASYNAPTOSOMAL FREE Ca^{2+}

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We have previously demonstrated a reduction in intrasynaptosomal free $[Ca^{2+}]$ following incubation with the alpha$_2$-adrenergic agonist clonidine (Adamson et al 1987). In this study we have investigated the effects of some opiate ± alpha$_2$-agonists on basal intrasynaptosomal free $[Ca^{2+}]$; using the intracellular fluorescent Ca^{2+}-sensitive probe quin2 (Ashley et al 1984). Synaptosomes were prepared on percoll discontinuous density gradients (Dunkley et al 1986) and resuspended in Krebs buffer containing 1.0 mM $CaCl_2$. Loading of synaptosomes with quin2 was achieved by incubation for 40 min at 37°C with 50 uM quin2 acetoxymethylester.

TABLE 1

Ligand(s)	Number of observations.	% change ± S.E.M.	Significance (paired t test)
Dynorphin A (1-13)(1uM)	7	-19.98 ± 2.50	P < 0.004
Clonidine (1uM)	14	-26.5 ± 3.12	P < 0.001
Morphine (1uM)	12	+ 7.61 ± 6.35	NS
[D-Ala-D-Leu]-enkephalin	11	+ 0.79 ± 8.06	NS
Dynorphin A(1-13)(1uM)) + clonidine (1uM))	7	+18.18 ± 4.65	P < 0.05
Dynorphin A(1-13)(1uM)) + naloxone (20uM))	4	+21.45 ± 11.81	P < 0.05
Dynorphin A(1-13)(1uM)) + clonidine (1uM)) + naloxone (20uM))	9	-19.31 ± 2.68	P < 0.001
Dynorphin A(1-13)(1uM)) + clonidine (1uM)) + idazoxan (2uM))	8	-22.64 ± 1.61	P < 0.001
Clonidine + Idazoxan	-	Previously determined[1]	

It can be seen (Table 1) that the effects of both clonidine and dynorphin A (1-13) are antagonised by idazoxan and naloxone respectively and that only the preferential opioid agonist has any significant effect on basal intrasynaptosomal free $[Ca^{2+}]$. Therefore the Kappa-opiate receptor may function in a manner analagous to that proposed for the alpha$_2$-adrenoceptor i.e. by increasing intracellular sequestration of Ca^{2+} (Schoffelmeer et al 1983). There is no Ca^{2+}-entry via voltage sensitive Ca^{2+}- channels since in these experiments the synaptosomes were not depolarised. Results from this study also show a mutual anatagonism between the effects of dynorphin A (1-13) and clonidine when they are co-incubated. We propose this may be a mechanism which allows an "escape" from excessive inhibitory responses.

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THE RELEASE OF ENDOGENOUS ACETYLCHOLINE AND [^{3}H]-ACETYLCHOLINE FROM CORTICAL SLICES OF AGING RATS

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The purpose of this work was to study the age-induced changes in cortical cholinergic mechanisms. Endogenous acetylcholine (ACh) release was therefore investigated in cortical slices taken from 3 to 28 month-old male Wistar rats. The slices were superfused with Krebs solution containing physostigmine 3.8 uM and electrically stimulated as described by Pedata et al.(1985). Stimulation periods of 5 min at 1, 2 and 5 Hz were followed by 10 min rest. ACh content of the superfusate was quantified by bioassay. In some experiments ACh content of the cortical slices was measured at the end of the superfusion.

ACh release from the cortical slices showed a frequency-dependent increase at all ages tested. However, while no difference was detected in ACh release at rest, the evoked ACh release showed approximately a 50% decrease between 11 and 14 months of age. The release at 5 Hz stimulation frequency in the 11 month-old rats was 124.1$\pm$18.0 ng/g min, while that in the 14 month-old rats was 59.8 $\pm$ 8.9. No further decrease was observed up to 28 months of age. Similar differences were found with the other frequencies used. ACh content in the stimulated cortical slices of 16 month-old rats was 40% smaller than in the stimulated cortical slices of 3 month-old rats.

In order to clarify the reasons for reduced ACh release and content in more than 14 month-old rats, the release of newly formed (^{3}H)ACh was investigated in cortical slices prepared from 3 amd 16 month-old rats incubated for 30 min with 0.1 uM (3H)choline chloride (Amersham, specific activity 80 ci/mmol) according to Hertting et al. (1980). After a 60 min wash-out period the slices were stimulated at 2, 5 and 10 Hz and the tritium efflux was counted in a scintillation spectrometer. If the slices were incubated in calcium-free Ringer solution the electrical stimulation evoked no increase in tritium efflux.On the contrary, during incubation in Ringer solution containing physostigmine there was a frequency-dependent increase in tritium efflux but no significant difference between the 3 and 16 month-old rats was detected. Therefore under our experimental conditions age does not appear to affect choline uptake and de-novo ACh synthesis.

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EFFECTS OF γ-VINYL GABA ON NEURONAL AND GLIAL GABA RELEASE FROM THE RETINA

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Gamma-vinylGABA (GVG) is an irreversible inhibitor of GABA-transaminase (GABA-T)(4-aminobutyrate:2-oxoglutarate aminotransferase), the main enzyme responsible for GABA catabolism. GVG has anticonvulsant properties in animals and is under investigation as an anti-epileptic in man. The retina, like other areas of the central nervous system, possesses GABAergic neurones and in the rat, about one third of the amacrine cells and a few interplexiform cells are GABAergic. As a first step in studying the effects of GABA-T inhibition on retinal function, we have examined the effects of GVG on GABA content and on the release of both endogenous and ^{3}H-GABA from the rat retina.

GVG (250mg/kg.ip.) was administered 18 hours before the rats were killed and the retinas removed. Endogenous GABA and ^{3}H-GABA release from the isolated retinas were measured as described previously (Yazulla et al 1985; Cunningham & Neal, 1985).

GVG treatment significantly increased retinal GABA content. Thus, the retinal GABA content of GVG treated rats was 5.1+0.66umoles/g wet weight, whereas in uninjected and saline injected rats it was 1.7+0.16 and 2.4+0.30 umoles/g wet weight respectively.

Glutamate (10mM) aspartate (10mM) and KCl (50mM) had little or no effect on the release of ^{3}H-GABA (presumed to originate mainly from the glial Muller fibres since these cells are the predominant site of GABA uptake) from control retinas in GVG treated animals, the glutamate but not the aspartate or KCl evoked release of ^{3}H-GABA was strikingly increased. The glutamate evoked release of ^{3}H-GABA was highly sodium and calcium dependent.

The spontaneous resting release of endogenous GABA (mainly from amacrine cells) was increased 3-fold by GVG. In untreated rats, KCl depolarisation had no significant effect on GABA release, but in GVG treated animals, the GABA release was increased 2.5-fold. Similarly, the glutamate evoked release of GABA from the retina was increased 8-fold in GVG treated animals. The glutamate evoked release was calcium dependent. Aspartate did not significantly increase GABA release from the retina of GVG treated animals.

The results suggest that GVG may increase synaptically evoked GABA release from amacrine cells because they receive major inputs from bipolar cells which are believedto use glutamate as their transmitter. In addition, inhibition of GABA-T in glial cells may result in them accumulating GABA which could subsequently be released by glutamate in the extracellular space.

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THE STABILITY OF ETHYLCHOLINE MUSTARD AZIRIDINIUM (ECMA) IN CEREBROSPINAL FLUID AND CULTURE MEDIA

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ECMA is used in attempts to cause selective lesions of rat brain cholinergic neurones in vivo. Focal injections risk at least transiently high local concentrations and the possibility of non-specific cytotoxic effects (Pillar et al, 1987). Intracerebroventricular (icv) injections require diffusion in CSF. Since ECMA is susceptible to nucleophilic attack and likely to be unstable in biological fluids (Colhoun & Rylett, 1986) its penetration to the different brain tissues is open to question. We have previously noted the instability of ECMA in culture medium (Atterwill et al, 1986). Leventer et al (1985) reported loss of markers for cholinergic neurones in rat hippocampus, but perhaps significantly not in cortex or striatum, as assayed 7 days after icv injection of ECMA (lateral ventricles 3nmol/side, ie, approx 30 μM in CSF). We therefore compared the stability of ECMA in rat CSF with that in Na-phosphate buffer (NaP), serum-supplemented (S+) and chemically defined (S-) culture media.

Male Wistar rats were anaesthetised with pentobarbitone and CSF withdrawn from the cisterna magna. Acetylethylcholine mustard hydrochloride (Salford Ultrafine Chemicals) was stirred in distilled water (20°C, 10 min, 20 mg/ml), held at pH 11.5-11.6 (10 min, 1M NaOH), adjusted to pH 7.4 (1M HCl) then stored on ice. By thiosulphate (0.1N) - iodine (0.1N in 2% KI solution) titration, cyclisations were routinely 76% giving stock solutions of aprox 66mM ECMA. Aliquots were diluted 10-fold into rat CSF, 50mM NaP, S+ or S- culture media (37°C) and aziridinium content monitored by thiosulphate (0.01N) - iodine (0.01N in 2% KI solution) titration allowing 45 min for complete reaction of ECMA with the thiosulphate.

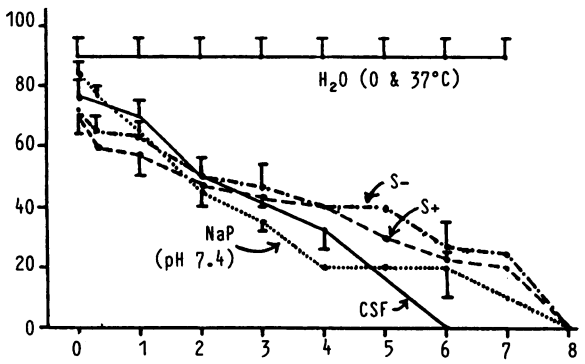

FIG 1 Iodine/KI titre (μ1±SEM, n=2-6) vs time (h)

ECMA concentrations fell below the limits of detection (0.37mM) within 6-8h in CSF, NaP, S+ or S- culture media (pH 7.4). At pH 8.6 ECMA was undetectable after 2 or 4h in S+ medium or NaP. There was no evidence these losses of ECMA were not linear with time (linear regression, coefficients of determination 0.91-0.98) except for those in NaP (pH 7.4, coefficient 0.3, Fig 1). 50μM ECMA was preincubated (37°C, pH 7.4) for up to 3h in S+ medium. The resulting solutions were then used to treat (for 2h) foetal rat brain reaggregate cultures (Pillar et al, 1987). ECMA caused approx 45% inhibition of muscarinic receptor binding within 2h, preincubation for 1 to 1.5h abolished this effect.

If the losses of ECMA in the titration range (0.37 to 6mM) are linear with time, the decay is by a zero-order mechanism. At concentrations likely after icv injection therefore (approx 30μM in CSF) ECMA would be completely lost in approx 2 min. It is also possible the decay curves are "shallow" exponentials, reflecting first order decay of ECMA, half life approx 3h (Fig 1). Inhibition of muscarinic receptor binding is detectable down to ECMA concentrations of at least 12.5μM (Pillar et al, 1987). That inhibition was abolished in 1 to 1.5h preincubations therefore indicates the largest the half life of ECMA can be in the pharmacological concentration range is 30-45 min. This is small compared with that suggested by first order kinetics in the titration range, but clearly larger than predicted by the alternative zero order kinetics. The cause of these apparently concentration-dependent kinetics is not clear. However, that ECMA has very similar decay curves for titration in CSF and in S+ medium suggests its half life after icv injection will also be 30-45 min. The IC50 for choline transport or for intraterminal choline acetyltransferase will presumably therefore determine the time over which effective concentrations remain in CSF and therefore the brain tissues susceptible to lesion.

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RAT BRAIN NADPH-DIAPHORASE UTILISES CYTOCHROME C AS SUBSTRATE

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Distinct populations of neurons throughout the central nervous system can be stained specifically by a histochemical method involving the reduction of nitrobluetetrazolium (NBT) to formazan products, that demonstrates NADPH-dependent "diaphorase" activity (Thomas and Pearse, 1964). In the striatum for example, diaphorase positive neurons are few in number (approx 1-2%) and are of the medium aspiny type containing somatostatin and neuropeptide Y (Vincent et al., 1983). The function of NADPH diaphorase is not known and its endogenous substrate has not been identified. It has been claimed that diaphorase-positive cells are spared following intracerebral injections of quinolinic acid (Beal et al., 1986); however, these observations have not been confirmed in our own studies (Davies and Roberts, 1987). Although it has long been assumed to be the case, it is in fact only recently that we have established that the histochemical staining reaction is enzymically mediated. We have now isolated and purified the enzyme, developed an assay system for it, and recently reported its detailed kinetic characteristics (Kuonen et al., 1987).
In this study, we report that cytochrome c is a good substrate for NADPH dependent diaphorase.

Rat brain microsomes were solubilised with 0.1% Triton X-100 in 50 mM Tris-HCl buffer (pH 7.5). Non-denaturing polyacrylamide gel electrophoresis (Kuonen et al., 1986) of this fraction revealed single, coincident bands of activity when nitrobluetetrazolium and cytochrome c were employed as substrates. Aliquots of crude enzyme preparation were incubated at 37°C in a volume of 1 ml 50 mM Tris-HCl buffer (pH 7.5) containing 0.1% Triton X-100 and cytochrome c (3-30 µM). Incubations were initiated by addition of NADPH (0.03 to 0.3 µM) and the rate of formation of reduced cytochrome c (peach colour) monitored at 550nm in a double-beam spectrophotometer.

The data revealed a Km of 1.3 µM for cytochrome c and 1.4 µM for NADPH (as compared with a Km of 17.3 µM using NBT as substrate), with a Vmax of approximately 4.7 nmol µg protein per min. It is thus possible that cytochrome c represents the endogenous substrate for brain NADPH-dependent diaphorase, although it may be functioning purely as an alternative electron acceptor. It seems highly probable that the enzyme isolated in these studies is identical to that observed histochemically. We hope to resolve this issue utilising antibodies we have raised to enzyme electroeluted from preparative gels.

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CAN SOMATOSTATIN BE DETECTED USING *IN VIVO* VOLTAMMETRY?

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The differential pulse voltammetric (DPV) technique for measuring amines and their metabolites has recently been improved. The use of new electrical pretreatment conditions for the carbon fibre electrodes makes it possible simultaneously to monitor *in vivo* extracellular ascorbic acid (Peak 1 at -50 mV), dihydrophenylacetic acid (DOPAC, Peak 2 at +100 mV) and 5-hydroxyindoleacetic acid (5HIAA, Peak 3 at +280/300 mV). Under certain conditions a fourth peak is observed at +450 mV which is probably due to homovanillic acid (Crespi *et al.*, 1984). We have recently found that increasing the potential sweep range to +950 mV allows the detection of a fifth peak (Peak 5) in the striatum at +800 mV. The present study investigates the possible contribution of electroactive neuropeptides to Peak 5.

Neuropeptides containing tyrosine or tryptophan are electroactive *in vitro* at pH 7.4 between +600 and +900 mV using carbon paste electrodes (Bennett *et al.*, 1981). With carbon fibre electrodes only cholecystokinin-8 (CCK-8), α-melanocyte-stimulating hormone (α-MSH) β-endorphin and somatostatin oxidised *in vitro* between +800 and +850 mV. *In vivo*, in rats anaesthetised (chloral hydrate 500 mg/kg i.p.) and implanted with carbon fibre electrodes into the striatum, intrastriatal injections (2 μg/μl in saline) of these peptides increased the height of peak 5, with somatostatin producing the largest increase (+475%) compared to other peptides (+150%) (n=7 in each case). Furthermore intrastriatal administration of bacitracin (10 μg/2 μl in saline) significantly increased the height of Peak 5 (+159% 90 min later; n=4). Bacitracin is a potent peptidase inhibitor; the rise of Peak 5 observed after bacitracin treatment supports the peptide nature of Peak 5. Peripheral administration of cysteamine (100 mg/kg i.p.) markedly decreased the height of Peak 5 and the peak was not measurable 30-40 mins after administration. Cysteamine is reported to selectively decrease brain somatostatin (Bakhit *et al.*, 1983). Local injection into the striatum of somatostatin antisera (2.5 μl), but not control antisera (2.5 μl), also caused the eventual disappearance (120 mins) of Peak 5.

Somatostatin is found in relatively high amounts in the striatum (Beal *et al.*, 1983) and the present results suggest it may be possible to detect striatal somatostatin *in vivo* by using DPV with specifically pretreated carbon fibre electrodes. It remains to be determined why the treated carbon fibre electrodes show relative selectivity for somatostatin and whether tryptophan and tyrosine contribute to Peak 5.

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INTERACTIONS BETWEEN κ-OPIOID AND α_2-ADRENOCEPTORS IN RAT HYPOTHALAMUS

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Alpha$_2$-adrenoceptors on the terminals of adrenergic neurones modulate noradrenaline (NA) release via an autoregulatory inhibitory mechanism. Recently, opioid receptor stimulation has been shown to produce a similar inhibitory effect on NA release from rat cortical nerve endings (Gothert et al 1980). The role of opioid receptors in regulating NA release, or modulating this release during α_2-adrenoceptor stimulation was examined in rat hypothalamus using a superfusion system.

Synaptosomes (P_2) prepared from the medial and anterior hypothalamus of male Wistar rats were incubated with [^{3}H]NA (50nM, 15 min, at 37°C) and then gently drawn onto GF/A glass fibre filters under low pressure. These synaptosome beds were superfused continuously with oxygenated Krebs-bicarbonate buffer at 37°C for 105 min. Two 6 min pulses of K^+ (16mM) (S_1 and S_2) were used to evoke release. [^{3}H] NA release in S_2 was measured in the presence of the opiate receptor agonist morphine (10nM-100μM), the opioid peptide dynorphin 1-13 (D13, 1nM-10μM), or the α_2-adrenoceptor agonists clonidine (10nM-100μM) or Uk-14, 304 (1μM) in the superfusion media.

Clonidine or D13 significantly ($p<.01$) inhibited [^{3}H]NA release, with IC_{50} values of 0.73μM and 0.06μM respectively; morphine was less effective and had an IC_{50} value greater than 5μM. The release inhibiting effect of D13 (0.1μM) was not blocked by naloxone (1.0μM). Interactions between these two systems was investigated by co-administration of an opioid and an α_2-adrenoceptor agonist. When D13 (0.1μM) was present in the Krebs-bicarbonate buffer, the release inhibiting effects of clonidine (1.0μM) or UK 14,304 (1.0μM) were abolished. This effect was not apparent if morphine (1.0μM) replaced D13.

These results suggests that interactions occur between opioid and α_2-adrenoceptors in rat hypothalamus to modulate the release of NA. These interactions probably involve the k-opioid receptor subtype (Chavkin et al 1982).

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THE MEASUREMENT OF κ-OPIOID RECEPTORS IN REGIONS OF RAT BRAIN

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Opioid receptors are a heterogenous group consisting of at least three subtypes, u, d and k. Endogenous opioids active at these receptors include Leu and Met-enkephalins, B endorphins and the recently identified dynorphins, which are particularly selective for the k-opioid receptor subtype (Corbett et al 1982). High concentrations of dynorphins occur in discrete areas of rat brain and may participate in the modulation of catecholamine release from these regions (Mulder et al 1984).

To assess the regional distribution of k-opioid receptors, brains from 20 male Wistar rats were dissected into 5 regions; amygdala, hypothalamus, thalamus, striatum and cerebellum. Tissue was pooled, weighed, and homogenised in 10 vols of 50mM tris-HCl pH 7.4. Tissue preparation included a 30 min incubation at 37°C to assist the dissociation of endogenous dynorphin. Receptor binding assays using [^{3}H] Ethylketocyclazocine (EKC, 0.12-4.0nM) were performed in duplicate, each tube contained 20 mg original tissue, D-Ser D-Leu enkephalin (100nM) and naloxone (30nM), to block the d and u opioid receptor subtypes respectively. Non-specific binding was assessed in the presence of dynorphin 1-13 (10uM). The total incubation volume was 500ul. Samples were incubated at 30°C for 80 min and filtered under reduced pressure through GF/B glass fibre filters, presoaked in tris HCl containing 0.5% BSA for 1 hr, and rinsed with 3 x 4ml 50mM tris HCl. Filters were dried and assayed by liquid scintillation counting. The specific binding of [^{3}H] -EKC was saturable and represented 40-50% of total binding at 1nM; the percentage specific binding averaged 35% if the incubation was conducted at 4°C.

Scatchard analysis of the eight point binding data was not linear; further analysis was made using the Affinity Spectra method for heterogenous receptor populations (Tobler and Engel 1983) and the Direct Linear Plot.

Region	HYPOTHALAMUS	STRIATUM	AMYGDALA	THALAMUS
Affinity Spectra Plot				
Bmax	1.32 ± 0.3	0.68 ± 0.12	0.77 ± 0.4	1.4 ± 0.38
Kd	1.68 ± 0.8	0.66 ± 0.1	2.9 ± 0.3	2.2 ± 0.48
Direct Linear Plot				
Bmax	1.2 ± 0.15	1.1 ± 0.27	1.4 ± 0.2	1.42 ± 0.17
Kd	1.1 ± 0.2	0.8 ± 0.1	1.5 ± 0.3	1.2 ± 0.1

Bmax in fmol/mg original tissue, Kd in nM, n = 4 per group

There was no specific binding in the cerebellum. The effect of acute immobilisation stress on k-opioid receptor binding was also assessed.

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EFFECTS OF 5-HT AGONISTS WITH SELECTIVITY FOR 5-HT RECEPTOR SUBTYPES ON 5-HT TURNOVER IN RAT BRAIN REGIONS

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It is currently believed that the 5HT autoreceptor in the brain is of the $5HT_1$ class, with possibly the autoreceptor at the level of the cell body being of the $5HT_{1A}$ subtype with that on the nerve terminals being $5HT_{1B}$ (Engel et al., 1986; Verge et al., 1985). However, this pharmacological classification has developed in the absence of a selective agonist for the $5HT_2$ receptor. Recently, the compound 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) has been shown to have high selectivity for the $5HT_2$ binding site and have full agonist action in a functional test of central $5HT_2$ receptors (Glennon, 1986).

In this study we have compared the effects of DOI with the putative $5HT_{1A}$ agonists 8-OHDPAT, gepirone and ipsapirone and the putative $5HT_{1B}$ agonist m-chloro-phenylpiperazine (mCPP) on 5HT turnover as assessed by the in vivo 5HTP accumulation method (Carlsson et al., 1972).

Male Sprague-Dawley rats (220-280g) were injected s.c. with drug or saline followed 30 min later by NSD 1015 (100 mg/kg i.p.), 30 min after which the animals were killed. Frontal cortex, hippocampus and raphe region were dissected out and stored at -70°C. Tissues were extracted and analysed for 5-HTP by HPLC-EC (Duda and Moore, 1985).

8-OHDPAT (0.01-1.00 mg/kg), gepirone (2.0-10.0 mg/kg) and ipsapirone (5.0-20.0 mg/kg) caused a dose-related reduction in 5HTP accumulation in both frontal cortex and hippocampus. The 5HTP levels in hippocampus at the highest doses of each drug compared to saline-injected controls were respectively, 11±2%, 41±5% and 58±6%. mCPP (0.05-1.00 mg/kg) also produced a dose-related decrease of 5HTP accumulation in hippocampus, to 77±6% of control values and to 68±5% of control in the frontal cortex. In comparison DOI (0.05-1.00 mg/kg) had no consistent effect on 5HTP accumulation in either hippocampus or frontal cortex. None of the drugs tested altered 5HTP accumulation in the raphe area.

The findings on the effects of several so-called $5HT_{1A}$ ligands on 5HT synthesis emphasizes, by inference, the importance of the $5HT_{1A}$ autoreceptor in regulating 5HT neuronal function. In contrast the $5HT_2$ receptor does not seem to mediate this action.

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