CHRONIC IMIPRAMINE TREATMENT INHIBITS EXCITATORY SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS IN VIVO

J.J. O'Connor, M.J. Rowan & R. Anwyl[1], Department of Pharmacology and Therapeutics and [1]Department of Physiology, Trinity College, Dublin 2, Ireland.

The "impulse blocking" hypothesis of antidepressant action (Randall, 1981) recently obtained support from the finding that acute bath-application of the antidepressant imipramine (IMI), at therapeutically relevant concentrations, produced a frequency-dependent block of field potentials in the rat hippocampal slice in vitro (Anwyl & Rowan, 1985). In the present communication we report on the in vivo effects of acute and chronic treatment with IMI on the amplitude of the excitatory postsynaptic potential (e.p.s.p.) evoked at both low and high frequencies of stimulation.

Recordings were taken from male Wistar rats (250-350g) in a restraining hammock, at least one week after implantation of screw electrodes in the skull and fine tungsten wire electrodes in the dorsal hippocampus under pentobarbitone (60 mg/kg i.p.) anaesthesia. Stimulation and recording was carried out in the stratum radiatum of the CA1 region. The effects of a single or daily injection for two weeks with saline or IMI (10 mg/kg, i.p.) were compared, 1-2 hr after the last injection.

Acute doses of IMI had no effect on e.p.s.ps evoked at a low frequency of stimulation (0.05 Hz). Following long-term IMI treatment, the e.p.s.p. amplitude had declined by 57 ± 15% (n = 5, mean ± s.e.mean; $P < 0.05$, compared to a 19 ± 12% reduction in saline controls, n = 5). This effect was observed across a wide range of stimulus intensities. At higher frequencies of stimulation there was a clear frequency-dependent block of the e.p.s.p. after both acute and chronic IMI (Table 1).

Table 1 The effect of acute and chronic IMI on e.p.s.p. trains evoked at different stimulation rates (Amplitude of the 20th e.p.s.p. in the train is expressed as a % of the first potential in the train)

		20 Hz		50 Hz	
		Saline	IMI	Saline	IMI
Acute	(Day 1)	108 ± 6	54 ± 5*	83 ± 7	30 ± 4*
Chronic	(Day 14)	74 ± 4	36 ± 4*	56 ± 6	10 ± 5*

* $P < 0.05$ compared to saline controls (n = 5 for each group)

The observed frequency-dependent block was probably due to a combination of both presynaptic and postsynaptic effects of IMI. Known presynaptic effects of IMI include inhibition of the Na^+-mediated afferent nerve volley (Anwyl & Rowan, 1985) and reduction of depolarization induced Ca^{2+} uptake into synaptosomes (Aronstam & Hoss, 1985). Known postsynaptic effects of IMI include a binding to the activated but non-conducting state of receptor operated ion channels (Aronstam, 1981; Schofield et al, 1981). The novel finding that chronic as opposed to acute treatment with IMI inhibited low frequency e.p.s.ps is of particular interest in view of the two week delay in the onset of antidepressant action of IMI in humans.

Supported by the Health Research Board of Ireland.

Anwyl, R. & Rowan, M.J. (1985). Br. J. Pharmac. 86, 201-208.
Aronstam, R.S. (1981) Life Sci 28, 59-64.
Aronstam, R.S. & Hoss, W. (1985) Biochem. Pharmacol. 34, 902-904.
Randal, P.L. (1981) Med. Hypoth. 7, 251-260.
Schofield, G.G. et al. (1981) Proc. natn. Acad. Sci. U.S.A., 78, 5240-5244.

CHANGES IN STRIATAL DOPAMINE, ASCORBIC ACID AND MOTOR ACTIVITY IN RATS AFTER ADMINISTRATION OF DOPAMINE RECEPTOR AGONISTS

T. Zetterström and M. Fillenz, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, England.

Ascorbic acid (AA) is present in high concentrations with a heterogenous distribution in the mammalian brain and is released from nerve terminal preparations. Extracellular brain AA in rats shows marked changes during the 24 h cycle and following the administration of drugs. It has been suggested that changes in AA lead to modulation of dopamine (DA) function (Rebec et al., 1985).

In the present study striatal AA and DA were monitored in parallel with motor activity following administration of DA receptor agonists. Rats were implanted with a microdialysis probe under chloral hydrate anaesthesia. Following a 24 h recovery period striatal dialysates were collected every 20 min and assayed by HPLC with electrochemical detection for AA and DA content. Changes in motor activity were monitored with a doppler shift device.

80-100 mins after the subcutaneous administration of 10mg/kg SKF 38393 (a selective D_1 receptor agonist) there was a decrease (-50 ± 15% n = 3) in DA concentration and no change in AA concentration in the dialysate. There was no detectable change in motor activity. 80-100 mins after the subcutaneous administration of 0.1mg/kg LY 17155 (a specific D_2 receptor agonist) there were small and variable changes in DA and AA concentration in the dialysate and in motor activity.

20-40 min after subcutaneous administration of 0.5mg/kg apomorphine (a non-selective DA receptor agonist) there was a decrease (-70 ± 15% n = 4) in DA concentration and an increase (+240 ± 35% n = 4) in AA concentration in the dialysate. This was accompanied by an increase in motor activity. 20-40 min after local application of 10^{-5}M apomorphine in the striatum there was a decrease (-72 ± 11% n = 3) in DA concentration but no significant change in either AA concentration in the dialysate or in motor activity.

In conclusion, all three DA agonists decreased DA concentration in striatal dialysates but only systemic injection of apomorphine was followed by a significant increase in striatal AA. There was a close parallel between changes in striatal AA and in motor activity following administration of the various DA agonists. In the present study there was no evidence for a relation between DA receptor activity and changes in extracellular AA.

1. Fillenz, M., O'Neill, R.D. & Grünewald, R.A., 1986. In: 'Monitoring neurotransmitter release during behaviour'. Eds. M.H. Joseph et al., Ellis Horwood, pp.144-164.

2. Rebec, G.V., Centore, J.M., White, L.K. & Alloway, K.D., 1985. Science (227), 438-439.

Keywords; dopamine, ascorbic acid, dopamine agonists, microdialysis, motor activity.

DIFFERING EFFECTS OF SUBCHRONIC AND CHRONIC DOSING OF B-HT 920 ON APOMORPHINE-INDUCED STEREOTYPY AND MOTOR ACTIVITY IN RATS

Darmani, N.A., Sewell, R.D.E. and Nicholls, P.J. Department of Pharmacology, Welsh School of Pharmacy, UWIST, Cardiff CF1 3XF

B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo(4,5-d)-azepine) has been shown to be a CNS dopamine agonist with marked selectivity for autoreceptors at low doses (Anden et al. 1982, 1983). At such doses B-HT 920 inhibits locomotion in mice and decreases firing rate in dopaminergic neurons (Anden et al. 1982 and Ericksson et al. 1985). In addition, it produces a dose-dependent retardation of α-methyl-tyrosine-induced reduction of DA content in rat brain and inhibition of δ-butyrolactone-stimulated DA synthesis in rat corpus striatum. These effects are antagonised by spiperone and haloperidol (Mierau 1987 and Brown 1984). There is no effect, however, upon DA-sensitive adenylate cyclase, suggesting B-HT 920 does not act at D_1-receptors. Indeed its effects are characteristic of a dopamine D_2-receptor agonist (Brown et al. 1985). As, on chronic dosing, the above effects may lead to postsynaptic D_2-receptor supersensitivity, this study was undertaken to investigate the chronic effects of B-HT 920 on apomorphine-induced motor activity and stereotypy.

Male Wistar rats were dosed with either B-HT 920 (1mg/kg ip) or saline for 5 and 15 days and treatment was withdrawn for 36h. One hour after habituation to test environment the animals received apomorphine (0.5mg/kg ip), the cumulative locomotor activity was measured every 10 min for a period of 1h using photocell cages and stereotyped behaviour was observed for 2 min in each 10 min period using the scoring system employed by Randall (1985).

In subacute studies (5 days) B-HT 920 caused a significant increase (74.8% $P<0.02$) in motor activity 10 min after apomorphine treatment. However, no significant ($P>0.05$) change in motor activity was observed in chronic studies (15 days). A significant increase of 160% ($P<0.04$) in stereotyped behaviour in subchronically-dosed animals occurred 10 min after apomorphine injection. Such an increase in stereotyped behaviour still persisted in chronically-treated rats (50% $P<0.03$) but the peak difference occurred between 20 and 30 min (87.5% $P<0.03$). The increase in both motor activity and stereotypy seen in subchronically-treated rats may be explained in terms of up-regulation of postsynaptic D_2 receptors due to the aforementioned dopamine autoagonistic activity. The absence of increase in motor activity in chronic studies may be due to either autoreceptor tolerance and/or be attributable to accumulation of B-HT 920 to levels capable of producing agonistic effects on postsynaptic D_2 receptors.

Anden, N.E. et al. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 321, 100.
Anden, N.E. et al. (1983) Acta pharmacol et toxicol. 52, 51.
Brown, F. et al. (1984) Br. J. Pharmacol. 81, 45P.
Brown, F. et al. (1985) Br. J. Pharmacol. 82, 305P.
Eriksson, E. et al. (1985) Life Sciences 36, 1819
Mierau, J. and Schingnitz, G. (1987) 6th International Catecholamine Symposium, 129.

LITHIUM AND HIGH K^+ ALTER AGONIST-STIMULATED INOSITOL POLYPHOSPHATE FORMATION IN RAT CORTEX

P.P. Godfrey, Z. Taghavi, T. Powell and D.G. Grahame-Smith.
Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, OX2 6HE.

Activation of a variety of cell-surface receptors results in a rapid metabolism of phosphatidylinositol 4, 5-bisphosphate (PIP_2) to inositol trisphosphate (IP_3) and diacylglycerol. In rat brain, stimulation with muscarinic cholinergic, α-adrenergic and 5-HT agonists among others, enhances turnover of the phosphoinositides. In most experiments changes in total ^3H-labelled IP levels have been used as a measurement of receptor activation. Recently it has been shown, in carbachol stimulated rat cortical slices, that lithium ions (Batty and Nahorski, 1985) and high concentrations of potassium (Baird and Nahorski, 1986) can specifically alter the formation of inositol tris-and tetrakis phosphates. Here we report changes in inositol polyphosphate levels in stimulated rat cortical slices following treatment with LiCl or high K^+.

Rat cortical slices were prepared as previously described (Godfrey et al, 1985) and were incubated with 40μCi of ^3H-inositol for 60 min. After washing, the slices were incubated for 30 minutes with agonist in the presence of varying Li concentrations (0-10mM) and normal (5mM) or high (20mM) K^+. Incubations were terminated with an equal volume of 10% perchloric acid which was then extracted using the method of Downes et al (1986). Inositol phosphates were then eluted sequentially off Dowex columns using 0.2, 0.4, 0.8 and 1.2M ammonium formate/0.1M formic acid and counted by liquid scintillation spectrometry.

Each agonist tested, carbachol (1mM), noradrenaline (NA; 300 μM) and 5-HT (100 μM) produced time-dependent increases in inositol polyphosphate production. Carbachol addition provoked a very large increase in IP_4 levels (going from 120±20 DPM to 2000 ± 275 DPM). Formation of IP_4 in response to the other agonist was, however, very much smaller. Addition of 10mM LiCl to the medium did not alter the formation of IP_4 in response to NA or 5-HT. The response to carbachol was however markedly affected; the level of IP_4 at 5 min. after stimulation being enhanced and then gradually declining to be significantly below levels in non-lithium treated slices by 30 min. Li^+ also greatly enhanced agonist-stimulated IP_1 and IP_2 formation but had little effect on IP_3 levels.

The production of IP_4 in response to all agonists was potentiated in the presence of 20mM K^+. The levels of IP_1, IP_2 and IP_3 were also significantly increased in the presence of High K^+, though to a smaller extent than IP_4. No significant interactions between Li^+ and high K^+ were observed.

The results suggest that Li^+ and depolarisation can alter, in a specific manner, agonist-stimulated formation of inositol phosphates. The mechanisms underlying these changes are currently unknown.

Godfrey, P.P. et al (1985). Br. J. Pharmac. 84, 112P
Batty, I, and Nahorski, S.R. (1985) J. Neurochem. 45, 1514.
Baird, J.G. and Nahorski, S.R. (1986). Biochem. Biophys. Res. Commun., 141, 1130
Downes, C.P. et al (1986). Biochem. J. 238, 501.

STUDY OF A NOVEL PHOSPHOINOSITIDE-LINKED EXCITATORY AMINO-ACID RECEPTOR SUB-TYPE IN RAT CEREBRAL CORTEX

Wendy Tyler, Stephen P Watson and Philip P Godfrey* Dept. of Pharmacology, South Parks Road and *Dept. of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, OXFORD

In this study, the presence of a novel excitatory amino-acid receptor sub-type in rat cerebral cortex was investigated by observing the effect of various excitatory amino-acids and their antagonists on inositol phosphate formation in intact cell slices.

Rat cortical slices (350 um x 350 um) were incubated in Krebs buffer containing [3H]-inositol for 1 h. Aliquots (40 ul) were then incubated in the presence or absence of agonists and/or antagonists for 30 min at 37 C; the antagonists were added 10 min before the agonists. Total inositol phosphates were separated by Dowex-anion exchange chromatography, and counted in a scintillation counter (Godfrey et al, 1985).

Ibotenate (approx. EC_{50} - 30 uM) and quisqualate (approx. EC_{50} - 10 uM) produced a dose-dependent increase in formation of total inositol phosphates with maximal responses of 165 $\pm$ 5.9% and 154 $\pm$ 5.1% relative to controls, respectively; in contrast, kainic acid, N-methyl-D-aspartate, glutamate and aspartate, at a concentration of 1 mM, produced no significant change in the levels of the inositol phosphates. The response to a maximally effective concentration of ibotenate (100 uM) was not altered significantly in the presence of the general excitatory amino acid antagonist, DL-2-amino-4-phosphonobutyric acid (1 mM), but was increased significantly to 189.0 $\pm$ 7.5 % ($p < 0.05$) in the presence of MK-801 (100 uM) which is a non-competitive antagonist at NMDA receptors.

In the presence of the following ion-channel blockers, tetrodotoxin (300 nM), verapamil (10 uM) and cadmium (0.3 mM) the response to ibotenate (0.3 mM) was 164 $\pm$ 12.5, 156 $\pm$ 5.6 and 164 $\pm$ 12.3 %, respectively. These values are not significantly different from the control response to ibotenate (300 uM) of 161 $\pm$ 6.4 %. This suggests that the effect produced by ibotenate is unlikely to be mediated through the release of other neurotransmitters, or through the entry of Ca^{2+}.

Thus, when we consider that inositol phosphate formation is stimulated by ibotenate (an NMDA-receptor agonist) and by quisqualate (a quisqualate-receptor agonist) only, it seems likely that the effect could be mediated by a novel, excitatory amino-acid receptor, which has been previously defined in rat hippocampus (Nicoletti et al, 1986). This hypothesis is supported by the ability of MK-801 to potentiate the response to ibotenic acid. Ibotenic acid is a potent agonist at NMDA receptors, and the stimulation of these receptors is known to inhibit agonist-induced inositol phosphate formation in cortical slices (Wilkins et al, preceding abstract). The ability of MK-801 to potentiate the response to ibotenic acid, therefore, would appear to result from the block of this inhibitory action.

Godfrey et al (1985) Br. J. Pharmacol. 84 112P
Nicoletti et al (1986) J. Neurochem. 46 40 - 46

MK-801 BLOCKS NMDA BUT NOT KAINATE-MEDIATED INHIBITION OF STIMULATED INOSITIDE TURNOVER IN RAT CEREBRAL CORTEX

C. Jason Wilkins, Philip P. Godfrey and Stephen P. Watson*, Dept. of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road and *Dept. of Pharmacology, South Parks Road, OXFORD.

It has recently been reported that excitatory amino acids inhibit receptor-stimuated phosphoinositide turnover in rat hippocampal slices (Baudry et al, 1986). Here we report that activation of several inhibitory amino acid receptor sub-types has a similar inhibitory effect in rat cerebral cortex.

Rat cortical slices were prepared and incubated as previously described (Godfrey et al, 1985). Slices were labelled for 45 min. with [^{3}H]inositol and then stimulated with agonist in the presence or absence of amino acids for 30 min; amino acids were added 10 min prior to agonist. Incubations were stopped with chloroform/methanol (1:2) and total inositol phosphates (IP) were separated by Dowex anion exchange chromatography.

A maximally effective concentration of carbachol (1 mM) caused between a 300 - 400 % increase of IP relative to controls during a 30 min incubation. This response was inhibited in a dose-dependent manner in the presence of kainic acid (approx. EC_{50} - 20 uM), N-methyl-D-aspartate (NMDA; approx. EC_{50}) and glutamate (approx. EC_{50} 1 mM). The maximal extent of inhibition by all three excitatory amino acids was approximately 80 %. Ibotenate (100 uM) and quisqualate (100 uM) also inhibited this response but, because they also stimulated IP formation (Tyler et al, adjacent poster) they were not used in further experiments. The NMDA antagonists, DL-2-amino-4-phosphovalerate (APV; 1 mM) and MK-801 (100 uM), blocked the inhibitory action of NMDA against carbachol-induced IP formation but had no effect on the action of kainic acid.

Kainic acid also inhibited noradrenaline (300 uM)-, 5-hydroxytryptamine (100 uM)- and K^+ (20 mM)-induced formation of IP. Kainic acid did not significantly affect the [^{3}H]-labelling of the polyphosphoinositides in control or carbachol-stimulated preparations.

The data suggests that inhibition of agonist-stimulated [^{3}H]IP formation in rat cerebral cortex is a general phenomenon of excitatory amino acids and is not mediated by a specific sub-type of receptor. Further, the observation that MK-801, which blocks the opening of the receptor channel associated with the NMDA receptor without affecting the binding of NMDA to its receptor (Iversen et al, 1987), antagonises the response to NMDA, suggests that the inhibitory action of NMDA is related to the passage of ions through the channel rather than the binding of NMDA to its receptor *per se*. The entrance of ions into the cell is thought to be responsible for the neurotoxic action of NMDA and these data therefore suggest that the inhibitory affect of NMDA, and also presumably for the other excitatory amino acids, is a non-specific action caused by the neurotoxic affect of this class of neurotransmitters.

Baudy et al (1986) Nature 319, 329 - 330.
Godfrey et al (1985) Br. J. Pharmacol. 84, 112P.
Iversen et al (1987) Proc. XIth Congress Pharmacol. Sydney, in press.

IDENTIFICATION OF COMPOUNDS SELECTIVE FOR THE HIGH AND LOW AFFINITY STATES OF THE CENTRAL PHENCYCLIDINE (PCP) RECEPTOR

M.A. Sills, P.S. Loo, A.F. Braunwalder, J. Lehmann and M.Williams, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, New Jersey, 07901, U.S.A.

Recent data has shown that PCP and other dissociative anesthetics as well as the novel anticonvulsant, MK 801, non-competitively inhibit N-methyl-D-aspartate (NMDA)-evoked responses (see Kemp et al., 1987). Such findings have focused attention on interactions between NMDA-type receptors and the PCP site. Binding of [^{3}H]TCP, a PCP analog , to well washed rat brain membranes is glutamate-dependent, where L-glutamate (L-GLU) and other NMDA agonists cause a 400-500% increase in TCP binding (Loo et al., 1986). This increase, which is enhanced by Mg^{2+}, occurs via an increase in binding site affinity attributable to the formation of a high affinity (state A) of the PCP receptor (Sills et al., 1987). A lower affinity state (state B) of the receptor comprises 40% of sites in the presence of L-GLU/Mg^{2+}. Under basal conditions where both states of the PCP receptor are present, dexoxadrol and other dissociative anesthetics produce shallow inhibition curves. In the presence of 100 µM-L-GLU and 30 µM-Mg^{2+}, where essentially all binding is shifted to state A ,dissociative anesthetics display steeper inhibition curves that are shifted to the left, indicating selectivity for state A of the PCP receptor (Braunwalder et al., 1987).

In the present study, a series of compounds including MK 801, dexoxadrol, several antidepressants and the antitussive, dextromethorphan, were examined for their relative interactions with states A and B of TCP binding.

Compound	IC_{50} µM (basal)	IC_{50} µM (L-GLU/Mg^{2+})	Ratio
Dexoxadrol	2.0	0.061	32.8
MK 801	2.0	0.009	222
Imipramine	1.0	30.0	0.03
Desipramine	8.0	12.0	0.67
Opipramol	10.0	130	0.08
Dextromethorphan	1.4	2.0	0.7
Dextrorphan	1.6	0.75	2.1

Compounds with reported activity as antidepressants had their displacement curves shifted to the right in the presence of L-GLU/Mg^{2+} (ratios < 1.0). While dextromethorphan caused only a slight rightward shift (ratio = 0.7) , computer analysis (RS-1) indicated that while two binding components were present under basal conditions, the high affinity component (IC_{50} = 35 nM; 25% of total basal binding) is eliminated by L-GLU/Mg^{2+}. Unlike dextromethorphan, the inhibition curve for the narcotic analgesic, dextrorphan, was shifted to the left in the presence of L-GLU/Mg^{2+} (ratio = 2.1).

These data, while somewhat complex in nature, indicate that certain classes of compound have selectivity for one or other of the two states of the PCP binding site labeled by [^{3}H]TCP. The functional significance of these findings and their relation to the molecular mechanisms by which these various classes of compound act, is being further examined.

Braunwalder, A.F. et al., (1987) Fed. Proc. , 46, 1310.
Kemp, J. et al., (1987) Trends Neurosci., 10, 294.
Loo, P et al., (1986) Eur. J. Pharmacol., 123, 467.
Sills, M. A. et al., (1987) Soc. Neurosci. Abstr., 13, in press.

THE SPECIFICITY OF ACTION OF MK-801

H. Callachan, R.F. Halliwell, N.Y. Hather, J.J. Lambert & J.A. Peters (Introduced by I. H. Stevenson). Neuroscience Research Group, Department of Pharmacology and Clinical Pharmacology, Ninewells Hospital, Dundee University, Dundee, DD1 9SY.

MK-801 is a potent anticonvulsant and anxiolytic agent which may be useful in preventing neurodegeneration induced by ischaemia and hypoglycaemia (Woodruff et al, 1987). In rat cortical slices MK-801 selectively blocks NMDA-induced depolarizations in a non-competitive, use-dependent manner (Woodruff et al, 1987) and such an action may explain its central effects. The possibility that MK-801 may also interact with other neuro-transmitter receptors, and ion channels, implicated in anxiolytic and anticonvulsant drug action was investigated electrophysiologically. Additionally, certain pharmacological similarities between MK-801 and phenycyclidine (PCP) prompted us to assess the influence of MK-801 on nicotinic receptors.

Bovine chromaffin cells were isolated, cultured and used 1-7 days after plating (Cottrell et al, 1987). Foetal mouse spinal neurones were isolated and maintained in culture for up to six weeks (Ransom et al, 1978). The NCB-20 neuroblastoma x Chinese hamster brain cell line was cultured according to the methods of MacDermott et al (1979). Voltage-clamp recordings were made at room temperature using the patch electrode technique of Hamill et al (1981). Agonists were applied either iontophoretically, or by local pressure applications from modified patch pipettes.

MK-801 (1-10 μM) had no effect on chloride currents evoked by GABA (100 μM) on bovine chromaffin cells (n = 4). In contrast, the anxiolytic diazepam (1 μM) potentiated GABA-evoked currents to 169.6 ± 9.7% (mean ± S.E.) of control (n = 19). MK-801 (10 μM) had little or no effect on 5HT-induced currents of NCB-20 cells (n=3). In comparison, the anxiolytic and specific $5HT_3$ receptor antagonist GR38032F (10 nM) (Tyers et al, 1987) completely antagonised the 5HT-induced current (n = 3). Voltage-activated sodium currents are blocked by the anticonvulsant carbamazepine (Willow et al, 1986). MK-801 (10 μM) had little or no effect on the amplitude of whole cell sodium currents of bovine chromaffin cells, although a high concentration of MK-801 (100 μM) produced a 23 ± 3.4% block (n = 5).

In preliminary experiments 100 nM MK-801 inhibited NMDA (100 μM coapplied by pressure with 1 μM glycine)-induced whole cell currents (holding potential -60mV) recorded from mouse spinal neurones by approximately 50%. Little or no recovery occurred upon washout out. ACh (100 μM)-induced nicotinic currents of bovine chromaffin cells (holding potential -60mV) were dose-dependently blocked by MK-801 with an IC_{50} of approximately 2 μM (n = 4). The block was reversible on washout (5-10 minutes) and voltage-dependent, the block increasing with hyperpolarization. The antagonism of the ACh-induced current by MK-801 was due to a suppression of the underlying conductance increase rather than a change in its reversal potential.

In conclusion this study confirms that MK-801 is a selective NMDA antagonist although, like PCP, it blocks nicotinic-receptor channels at higher doses. Future studies will investigate the interaction of MK-801 with NMDA receptors in more detail.

We thank the MRC for support, Dr. Iversen and Dr. Woodruff for providing MK-801, and Dr. Oxford for the gift of GR38032F. John Dempster, who developed the computer software, was supported by a Wellcome Research Grant.

Cottrell, G.A. et al (1987) Br. J. Pharmac. 90 491-500.
Hamill, O.P. et al (1981) Pflugers Arch. 391 85-100.
MacDermott et al (1979) Proc. Natl. Acad. Sci. USA 76 1135-1139.
Ransom, B.R. et al (1978) J. Neurophysiol. 40 1132-1187.
Tyers, M.B. et al (1987) Neuroscience Letters, Suppl. 29, 568.
Willow, M. (1986) Trends in Neurosciences 9 147-149.
Woodruff, G.N. et al (1987) Neuropharmacology 26 903-909.

CHARACTERISATION OF THE BINDING OF [^{3}H]CGS 19755, A NEW, HIGH AFFINITY LIGAND FOR N-METHYL-D-ASPARTATE (NMDA) RECEPTORS

D.E. Murphy, A.J. Hutchison, S. Hurt*, M. Williams and M.A. Sills, Research Dept., Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, N.J. 07901, U.S.A. and *DuPont-NEN, Boston, MA 02118, U.S.A.

Radioligands for the NMDA-type excitatory amino acid (EAA) receptor have been typically plagued by low affinities and lack of specificity. Recently, the binding of the NMDA antagonist, [^{3}H]CPP (3-(±)(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) has been characterized (Murphy et al.,1987) Although CPP had higher affinity (Kd = 200 nM) compared to ligands such as L-glutamate (L-GLU) and AP5, separation of bound from free ligand required the use of centrifugation techniques.

CGS 19755 (cis-4-phosphonomethyl-2-piperidine carboxylic acid) has been recently synthesized and identified as an active (IC_{50}[^{3}H]CPP = 50 nM: Lehmann et al., 1987) and selective antagonist of the NMDA-type receptor in a variety of test procedures. The present study reports the characteristics of [^{3}H]CGS 19755 binding in rat brain crude synaptic membranes (CSMs) using both centrifugation (Beckman J 2-21-M, 48,000 x g; 10 min, 4°C) and filtration through glass fibre filters.

Preliminary experiments with [^{3}H]CGS 19755 showed that like CPP, binding was enhanced following detergent (Triton X-100; 0.04%) treatment, reversed by the addition of a large excess (1 mM) of L-GLU and pH-dependent. Equilibrium time course experiments indicated a very rapid association rate of less than 2 min. Binding remained stable for at least 1h with the percent specific binding representing 80-85% of the total counts bound using 1 mM-L-GLU to define non-specific binding.

Saturation experiments generated a curvilinear Scatchard plot using the centrifugation procedure. Based on an approximate specific radioactivity of 30 Ci/mmole, computer analysis (LUNDON-1) of [^{3}H]CGS 19755 binding using 15-20 concentrations of ligand, gave evidence for two distinct binding sites with respective Kd values of 11 and 186 nM. The apparent Bmax values for these two sites were 0.57 and 1.0 pmol/mg protein. In contrast to this finding, filtration assays revealed a single binding component with a Kd value of 30 nM and an apparent Bmax value of 0.65 pmol/mg protein.

Pharmacological characterization of the binding of 10 nM-[^{3}H]CGS 19755 was consistent with the specific labeling of the NMDA-type EAA receptor. L-GLU was nearly 100-fold more active than D-GLU exhibiting a good degree of stereo-selectivity. For both centrifugation and filtration assays, the rank order of activity for a variety of agonists and antagonists was: CGS 19755 = L-GLU > CPP = D-AP5 > DL-AP7 ≥ L- and D-aspartate > NMDA. Quisqualate was only weakly active (IC_{50} = 50 µM). Other EAA agonists such as kainate and AMPA, as well as the non-competitive antagonists, phencyclidine, tiletamine and MK 801 were without significant activity at 100 µM. These results indicate that [^{3}H]CGS 19755 is a selective ligand for the NMDA receptor with an affinity that is superior to CPP and which can be used in a filtration procedure. The significance of the two binding sites obtained using centrifugation conditions is currently being studied.

Murphy, D.E. et al., (1987) J. Pharmacol.Exp. Ther. 240, 778.
Lehmann, J. et al., (1987) Soc. Neurosci. Abstr. 13, in press.

N-METHYL-D-ASPARTATE AND KAINATE-MEDIATED CONTROL OF STRIATAL DOPAMINE RELEASE (DIALYSIS) IN VIVO

C.J. Carter, R. L'Heureux and B. Scatton. Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 31 ave P. Vaillant Couturier, 92220 - Bagneux, France.

Several studies have suggested that glutamate is able to release striatal dopamine in vitro and in vivo (Roberts and Anderson, 1979 ; Chéramy et al, 1986) although the pharmacology of this effect has not been characterized. We have studied the effects of N-methyl-D-aspartate (NMDA) and kainate on striatal dopamine release in vivo, using a trans-striatal dialysis technique (Zetterström and Ungerstedt, 1984).

In halothane-anaesthetised rats implanted with a trans-striatal dialysis cannula perfused with Krebs-Ringer (2 µl/min, 20 min fractions) the intrastriatal infusion of NMDA or kainate (10^{-3}M)for one fraction via the dialysis fiber evokes a large increase in striatal dopamine efflux (1300 and 3400 % basal levels, respectively). The effets of NMDA are totally blocked by the co-infusion of 2-amino-5-phosphono-valerate (5 x 10^{-4} M) which at 10^{-3} M partially blocks the effects of kainate (- 30 %). The effects of NMDA are also blocked by the systemic injection, 30 min before NMDA infusion, of phencyclidine (ID_{50} = 1.5 mg/kg i.p.) or the novel NMDA antagonist ifenprodil (ID_{50} = 1.5 mg/kg i.p.). The effects of NMDA were tetrodotoxin sensitive (1 µM), abolished by prior ibotenate lesions of the striatum (ten days before the NMDA challenge) and totally antagonized by the coinfusion of atropine (10^{-4} M). The effects of kainate were only partially reduced by a higher concentration of atropine (10^{-3}M) and were partially resistant to tetrodotoxin. The striatal infusion of carbachol (10^{-2}M) had a relatively minor effect on striatal dopamine efflux ($\simeq$200 % basal) as compared to NMDA.

These data suggest that the effects of kainate may be mediated partly via liberation of an endogenous NMDA agonist, and partly via a direct action on striatal dopamine terminals. The effects of NMDA appear to be mediated indirectly through the agency of a cholinergic interneurone. However, as carbachol itself has only a weak effect on striatal dopamine release, this neurone would appear to exert a permissive rather than an active role in the effects of NMDA. Kainate and NMDA receptors in the striatum thus control dopamine release via different mechanisms and this distinction should prove useful in characterizing the different striatal circuits involved in the expression of basal ganglion function.

Chéramy, A., Romo, R., Godehen, G., Baruch, P., and Glowinski, J. (1986) Neuroscience 19, 1081-1090.

Roberts, P.J. and Anderson, S.D. (1979) J. Neurochem. 32, 1539-1545.

Zetterström, J. and Ungerstedt, U. (1984) Eur. J. Pharmacol. 97, 29-36.

[^{3}H]-NIPECOTIC ACID BINDING TO GABA UPTAKE SITES IN HUNTINGTONS DISEASE

M.D.C. Simpson, A.J. Cross, P. Slater & G.P. Reynolds[1] Department of Physiology, University of Manchester, Manchester M13 9PT and [1]Department of Pathology, Queens Medical Centre, Nottingham NG7 2UH.

Abnormalities of γ-aminobutyric acid (GABA) containing neurones have been implicated in the pathophysiology of several neurodegenerative disorders. There are however a number of problems involved in studying these neurones in post-mortem brain tissue. Glutamic acid decarboxylase activity is extremely sensitive to ante-mortem hypoxia, and the concentrations of brain GABA rise rapidly after death. It has recently been suggested that ^{3}H-nipecotic acid, a potent inhibitor of neuronal GABA uptake, may label these uptake sites in both rat and human brain (Lloyd & Vargas 1982, Simpson et al 1987). We have examined the usefulness of ^{3}H-nipecotic acid binding as a marker of GABA neurones by comparing changes in ^{3}H-nipecotic acid binding with changes in GABA concentrations in post-mortem brains of patients with Huntingtons disease.

Brains were obtained at autopsy from patients with Huntingtons disease and a group of matched control subjects with no history of neurological or psychiatric disease. The concentration of GABA was determined using HPLC following derivitisation with o-phthaldehyde (Reynolds & Pearson 1987). Sodium-dependent ^{3}H-nipecotic acid binding was determined as described by Simpson et al (1987) using 40nM ligand.

GABA concentrations were markedly reduced in the basal ganglia of Huntingtons disease patients (table).

Table 1. GABA concentrations and ^{3}H-nipecotic acid binding in Huntingtons disease.

BRAIN REGION	GABA CONCENTRATION (μg/g tissue)		^{3}H-NIPECOTIC ACID BINDING (fmol/mg protein)	
	CONTROL	HD	CONTROL	HD
Caudate nucleus	307 ± 79	90 ± 37	292 ± 105	140 ± 59
Putamen	456 ± 66	146 ± 47	110 ± 26	40 ± 10
Temporal cortex	-	-	23 ± 3	28 ± 4

Values are mean ± SD of 9-15 samples.

Similarly, ^{3}H-nipecotic acid binding was markedly reduced in the caudate and putamen. Within the putamen there was a significant correlation between GABA concentrations and ^{3}H-nipecotic acid binding.

The present results suggest that ^{3}H-nipecotic acid binding may be a useful marker of GABA containing neurones in human brain.

[Supported by the North West Regional Health Authority]

Lloyd, K.G. & Vargas, F. (1982) In: Problems in GABA Research, (Okada, Y. & Roberts E. eds) pp. 355-365. Amsterdam: Excerpta Medica.

Reynolds, G.P. & Pearson, S.J. (1987) Neurosci. letts (in press)

Simpson, M.D.C., Cross, A.J., Slater, P. & Deakin, J.F.W. (1987) J. Neural. Transm. (in press).

KYNURENATE DISTINGUISHES IBOTENATE FROM SOME OTHER EXCITATORY AMINO ACIDS

D.A.S. Smith, N.R. Burton & T.W. Stone, Department of Physiology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE

The excitatory amino acids ibotenate, quinolinate, N-methyl-D and DL-aspartate (NMDA and NMDLA) have similar pharmacological profiles (Watkins & Evans 1981: Mayer & Westbrook 1987). However, neurotoxicity data have indicated that the effects of ibotenate, quinolinate and N-methyl-aspartate (NMA) may be distinct (Stone et al., 1987). Neuroexcitation and neurotoxicity are phenomena linked by the excitotoxic hypothesis of Olney (1983). It is possible therefore that there may exist separate receptors for ibotenate, quinolinate and NMA. A direct examination is possible by comparing pA_2 estimates (Waud, 1968) using amino acid antagonists.

Male TO mice (4-8 weeks) were killed by cervical dislocation, decapitated and brain tissues were quickly removed into ice cold artificial cerebrospinal fluid (ACSF). Tissue wedges (~ 1mm wide), prepared from coronal slices (500μM thick) of neocortex, were placed into two compartment baths at 20-24°C so that the cortical and callosal tissues were separated by a high resistance silicone grease seal. Agonists in ACSF, nominally Mg-free, were applied to the cortical tissue by 2 minute superfusion evoking a DC potential relative to the callosal tissue. A series of log dose response curves were prepared for each agonist in the absence and presence of 50-2000μM kynurenate and dose-ratios calculated. Schild plots were constructed for each agonist. The null hypothesis of conincident regression lines was rejected using analysis of covariance ($F_{3,79}$ =38.68 $p<<0.01$)

TABLE 1. Schild slopes and apparent pA_2 values for kynurenate

	slope ± SEM	apparent pA_2
ibotenate	1.34 ± 0.04	3.89
quinolinate	1.26 ± 0.04	3.65
NMDA	1.42 ± 0.08	3.65
NMDLA	1.41 ± 0.07	3.71
pooled	1.36 ± 0.03	

The only significant contrasts between the corrected means of the regression lines (Armitage, 1985) were between ibotenate and any of quinolinate, NMDA or NMDLA. This implies that the receptor for ibotenate is distinct from that at which quinolinate, NMDLA or NMDA act, at least in the neocortex.

This work was supported by SERC and the Wellcome Trust.

Armitage, P. (1985) Statistical Methods in Medical Research, Blackwell.
Mayer, M.L. & Westbrook, R.L. (1987) Prog Neurobiol 28 197-276.
Olney, J.W. (1983) In Excitotoxins. Fuxe, K., Roberts, P. & Schwarcz, R. (eds.), Macmillan, London.
Stone, T.W. et al. (1987) In CIBA Foundation Symposium 126: Selective Neuronal Death. Wiley, Chichester.
Watkins, J.C. & Evans, R.H. (1981) Ann.Rev.Pharmacol.Toxicol. 21 165-204.

EXCITATORY AMINO ACID EVOKED EFFLUX OF ENDOGENOUS ASPARTATE AND GLUTAMATE IN VIVO

J.H. Connick and T.W. Stone, Department of Physiology, St George's Hospital Medical School, London, SW17 ORE.

Like kainic acid, quinolinic acid has also been shown to require afferent projections in order to manifest its neurotoxic effects (Schwarcz et al., 1984). We have therefore investigated the possible modulation of endogenous amino acid release by quinolinic acid and other related compounds.

Male Wistar rats were anaesthetised with urethane and the cortical surface exposed. A truncated pipette tip (diameter 4mm) was placed on the pial surface and sealed in position with 4% agar in 0.9% saline. A push pull pair of nylon cannulae were inserted into the cup and the cortex superfused with 50mM Tris-HCl pH 7.4 in 0.9% saline at a rate of 100μl per 10 minutes for at least 2 hours before the experiment commenced.

The cup was then drained and the control efflux determined by adding 100μl of oxygenated medium (either Krebs; KH_2PO_4, 2.2mM; $MgSO_4$, 1.2mM; KCl, 2.0mM; Glucose, 10.0mM; $NaHCO_3$, 25.0mM; NaCl, 115mM; $CaCl_2$, 2.5mM, Krebs without magnesium or saline) into in the cup. After 10 minutes, this solution was removed and frozen at -20°C. A second 100μl aliquot of medium containing the compound of interest was similarly introduced into the cup and incubated for 10 minutes before collection and storage. The cortex was then superfused with medium for at least 1 hour between release experiments. The concentration of amino acids within each sample was determined by HPLC analysis as previously described (Connick and Stone, 1986).

Compound	Medium	Aspartate	Glutamate	n
5mM Quinolinic acid	$+Mg^{2+}$	81.6 ± 1.5	97.4 ± 11.1	15
5mM Quinolinic acid	$-Mg^{2+}$	293.7 ± 13.4*	324.2 ± 9.1*	6
1mM NMDLA	$+Mg^{2+}$	142.5 ± 9.6*	151.7 ± 14.3*	5
1mM NMDLA	$-Mg^{2+}$	286.3 ± 12.1***	304.7 ± 14.6***	5
5mM Kainic acid	$+Mg^{2+}$	142.3 ± 8.7**	157.3 ± 11.3***	6
5mM Kainic acid	$-Mg^{2+}$	150.1 ± 9.8***	144.4 ± 9.9***	10

All values are expressed as mean % control efflux ± s.e.mean from (n) experiments. Statistical significance was assessed using a Student's t-test: * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Both NMDLA (1mM) and quinolinic acid (5mM) also induced the release of taurine ($P<0.05$). The quinolinic acid evoked efflux of aspartate and glutamate was significantly attenuated in the presence of 200μM APV ($P<0.05$).

These results add further support to the suggestion that quinolinic acid acts at the NMDLA preferring receptor, and may also explain the requirement for intact afferent projections for the neurotoxic effects of quinolinate to be manifested.

Supported by National Fund for Research into Crippling Diseases.

Connick, J.H. and Stone, T.W. (1986). Biochem Pharmac.35, 3631-3635
Schwarcz, R. et al. (1984). Life Sci. 35, 19-32

EFFECTS OF ACID/BASE CHANGES INDUCED BY BICARBONATE ON NMDA-RECEPTOR MEDIATED DEPOLARISATIONS IN RAT CORTICAL WEDGES

J.A. Aram, S. Zeman and D. Lodge, Department of Physiology, Royal Veterinary College, London NW1 0TU.

Our previous studies show that low pH reduces and high pH enhances epileptiform activity in neocortical slices. Furthermore, increased concentrations of bicarbonate in the superfusing medium to raise extracellular pH above 7.8 induces spontaneous epileptiform discharges (Aram & Lodge, 1987). These *in vitro* findings parallel clinical and *in vivo* observations (Somjen, 1984). Since antagonists of the glutamate receptor subtype N-methyl-D-aspartate (NMDA) block alkalosis-induced and many other types of epileptiform activity *in vivo* and *in vitro* (Aram & Lodge, 1987; Patel et al., 1987), one possible explanation of the enhanced activity in alkalotic medium could be that NMDA-receptor mediated excitation is enhanced. We have attempted to investigate this using the cortical wedge preparation (Harrison and Simmonds, 1985).

In 8 experiments depolarising responses of neocortical neurones to aliquots of the bath applied agonists NMDA (40uM), quisqualate (20uM), kainate (5uM) and potassium (5mM) were examined while changing the bicarbonate concentration in the superfusing medium (equilibrated with 5% CO_2 in O_2). Control respones were first obtained at normal pH 7.3-7.4 (HCO_3 35.5mM). When the pH was lowered to 6.9 (HCO_3 8.55mM) responses to NMDA, quisqualate, kainate and potassium were depressed approximately equally- each by 20-40% from control. On the other hand when the pH was raised to 7.8 (HCO_3 68.38mM), a similar change of pH from control, the responses to all the depolarising agonists were enhanced markedly- each to 75-200% above control. Although NMDA responses appeared to be increased the most, with the variability between preparations this difference did not reach significance. Recovery of agonist responses on replacement of medium of normal pH was readily achieved.

This non-specific action of changes in bicarbonate concentration suggests that changes in membrane excitability rather than direct effects on NMDA receptor-channel complexes underlie the alkalosis-induced enhancement of epileptiform activity. Bicarbonate, however, has actions besides its effect on pH. Therefore the effects of lowering extracellular pH only, by addition of acetic acid, were compared to those produced by reduced bicarbonate concentration on epileptiform activity induced in rat cortical wedges. We found that, at a given pH in the same preparation, acetic acid consistently produced less marked depression of epileptiform activity (n=5). This finding nevertheless, is consistent with the observed non-specific action of bicarbonate on depolarising responses being mediated at least partly through the changes it exerts on extracellular pH.

Aram, J.A. & Lodge, D. (1987) Neurosci. Lett. in press.
Harrison, N.L. & Simmonds, M.A. (1984) Br. J. Pharm. 84, 381-391.
Patel, S., Chapman, A.G., Millan, M.H. & Meldrum, B.S. (1987) In D. Lodge (Ed.) Excitatory Amino Acids in Health and Disease, John Wiley & Sons, in press.
Somjen, G.G. (1984) In P.A. Schwartzkroin and H.V. Wheal (Eds.) Electrophysiology of Epilepsy, Academic Press, London, pp.303-341.

J.A.A. is an M.R.C. scholar.

L-[^{3}H] GLUTAMIC ACID BINDING IN THE VISUAL SYSTEMS OF THE RAT AFTER ORBITAL ENUCLEATION

D.T. Chalmers & J. McCulloch, Wellcome Surgical Institute, University of Glasgow, Garscube Estate, Bearsden Road, Glasgow G61 1QH.

Biochemical and electrophysiological evidence suggests a major role for glutamate in the rat visual system. Using a quantitative autoradiographic technique we have examined the alterations in L-(^{3}H) glutamic acid binding sites which accompany functional disturbances after lesioning of this sensory pathway.

Six male, black-hooded, Long Evans rats (350-450g) were unilaterally enucleated (right eye) under 2% halothane anaesthesia. Twenty four hours post-enucleation local cerebral glucose utilisation (LCGU) was measured with the (^{14}C)-2-deoxyglucose technique (Sokoloff et al. 1977). (^{14}C)-2-Deoxyglucose-6-phosphate was eluted from those sections required for receptor autoradiography, and after a 30 min pre-incubation in 50 mM Tris/HCL (pH 7.4, 4°C), sections were incubated for 45 min at 4°C with 200 nM L-(^{3}H) glutamic acid, non-specific binding being determined in the presence of 1 mM unlabelled glutamate.

The visual system of the hooded rat is 97-98% crossed at the optic chiasma (Jeffery, 1984), thus allowing the ipsilateral (right) hemisphere to act as the reference against which changes in LCGU and L-(^{3}H) glutamic acid binding sites in the visually deprived (left) hemisphere could be contrasted.

Table 1. Changes in local cerebral glucose utilisation and L-(^{3}H) glutamic acid binding 24 hours post-enucleation.

	LCGU (μmoles/100g/min)		Ligand Bound (pmoles/g tissue)	
Visual Structures	R. Hemis.	L. Hemis.	R. Hemis.	L. Hemis.
Visual Cortex (superficial)	97 ± 5	71 ± 6*	131 ± 10	98 ± 8*
Visual Cortex (deep)	94 ± 4	72 ± 5*	106 ± 7	86 ± 6*
Superior Colliculus (superficial layer)	79 ± 7	51 ± 6*	70 ± 6	76 ± 7
Lateral Geniculate Body	95 ± 5	63 ± 3*	81 ± 10	78 ± 9
Non-Visual Structures				
Auditory Cortex	141 ± 9	137 ± 9	122 ± 5	123 ± 9
Hippocampus (mol. layer)	82 ± 3	82 ± 4	140 ± 15	134 ± 16

*$P < 0.05$. Data are mean ± SEM (n=6).

Unilateral eye enucleation produced a significant reduction in LCGU in the contralateral superior colliculus, lateral geniculate nucleus and visual cortex. These functional deficits were accompanied by a significant reduction in L-(^{3}H) glutamic acid binding sites in both superficial and deep layers of contralateral visual cortex. LCGU and glutamate binding were unaltered in non-visual structures.

Both primary (superior colliculus and lateral geniculate body) and secondary (visual cortex) visual structures show a reduction in LCGU. Alterations in L-(^{3}H) glutamic acid binding sites in this polysynaptic pathway are only found in visual cortex - an area receiving no direct retinal input.

We gratefully acknowledge the support of the Wellcome Trust.

Jeffery, G. (1984) Dev. Brain Res., 13, pp 81-96.
Sokoloff, L. et al. (1977) J. Neurochem. 28, pp 896-916.

COMPARATIVE STUDY OF COMPETITIVE AND NON-COMPETITIVE N-METHYL-D-ASPARTATE RECEPTOR ANTAGONISTS AND CEREBRAL GLUCOSE USE

A. Kurumaji, D. Nehls, C. Park and J. McCulloch, Wellcome Surgical Institute, University of Glasgow, Garscube Estate, Bearsden Road, Glasgow G61 1QH, Scotland.

Glutamate is one of the major excitatory neurotransmitters in the CNS and exerts its effects via several distinct receptors named after their preferred agonists, e.g. N-methyl-D-aspartate (NMDA), kainate and quisqualate. NMDA receptors are the best characterised of the subtypes. Competitive, e.g. CPP, as well as non-competitive, e.g. MK-801, NMDA receptor antagonists have provided important insight into the functioning of the receptor at the electrophysiological and molecular level (Murphy et al., 1987; Wong et al., 1986). We investigated the functional consequences (as reflected in local energy generation) in vivo of blockade of NMDA receptors, with peripheral aministration of CPP (30mg/kg,iv) amd MK-801 (0.5mg/kg,iv), both of which cross the blood-brain barrier.

Local cerebral glucose use in the fully conscious male Sprague Dawley rat (320-470g) was measured according to the [^{14}C]2-deoxyglucose technique in exact accordance with the procedural details published previously (Sokoloff et al., 1977). Both CPP and MK-801 were dissolved in saline and were injected intravenously 10 minutes prior to the measurements of cerebral glucose utilisation.

Table 1 summarises the effects of NMDA antagonists on glucose utilisation in the brain areas which exhibit high density of NMDA receptors. Blockade of NMDA receptors with CPP markedly increased glucose use in the hippocampus and the entorhinal cortex, while glucose use was minimally altered in the other limbic system and the cerebral cortices. MK-801 produced heterogeneous patterns of altered cerebral glucose utilisation, with pronounced increases in the limbic system and consistent decreases in the cerebral cortex.

Table 1. Effects of NMDA receptor antagonists on glucose use in the rat brain

	Control (n = 6)	CPP(30mg/kg) (n = 4)	MK-801(0.5mg/kg) (n = 4)
Limbic System			
Hippocampus molecular layer	73 ± 3	102 ± 8 ***	120 ± 4 ***
Hippocampus dentate gyrus	63 ± 2	79 ± 1 **	85 ± 3 ***
Entorhinal cortex	57 ± 3	90 ± 11 **	102 ± 11 **
Posterior cingulate cortex	92 ± 5	97 ± 5	148 ± 11 ***
Anterior thalamic nucleus	109 ± 8	94 ± 6	150 ± 6 **
Cerebral cortex (Layer IV)			
Visual cortex	108 ± 9	105 ± 3	86 ± 6
Auditory cortex	126 ± 7	143 ± 11	94 ± 5 **
Parietal cortex	103 ± 4	112 ± 8	90 ± 6
Sensory-motor cortex	113 ± 7	110 ± 8	73 ± 2 **
Frontal cortex	109 ± 5	109 ± 6	84 ± 3 **

Mean ± SEM (μmol/100g/min) **P<0.01, ***P<0.001 vs control (Student's t-test).

Dynamic alterations in glucose utilisation indicate which polysynaptic neuronal pathways are being activated. The functional consequences in vivo of competitive and non-competitive NMDA receptor blockade appear to be neuroanatomically dissimilar.

The investigations were supported by the Wellcome Trust.

Murphy, D.E. et al (1987) J. Pharmacol.Exp. Ther. 140, 778-786.
Wong, E.H.F. et al (1986) Proc.Natl.Acad.Sci.USA 83, 7104-7108.
Sokoloff, L. et al (1977) J. Neurochem. 29, 13-26.

HOURLY KAINIC ACID HAS BEEN FOUND NOT TO BE AN AGENT OF KINDLING AT THE RAT HIPPOCAMPUS

G. Davies and D.W. Peterson, Physiology and Pharmacology Academic Group,The Hatfield Polytechnic, P.O. Box 109, Hatfield, Herts. AL10 9AB.

The kindling phenomenon is a model of epilepsy which is characterised by a long lasting alteration in neuronal function accounting for the increased sensitivity to seizure activity. Kindling promoted by the brief repeated application of a non-polarizing, low intensity electrical stimulus to discrete brain regions has been well documented (Goddard *et al*, 1969) and the stages of the developing epileptiform seizure long been categorised (Racine, 1972). More recently a variety of pharmacologic agents have been found to induce kindling when administered by local brain injection or systemically including a number of cholinergic and dopaminergic agonists (McNamara, *et al*, 1980). Although reports have been made on the anticonvulsant action of amino acid antagonists against electrically kindled hippocampal seizures (Peterson, *et al*, 1984) thus far no results have been reported of an examination of the possibility of excitatory amino acids as agents of kindling. Kainic acid, in activating a specific excitatory amino acid receptor, induces a seizure similar in both electroencephalographic and behavioural effects to hippocampal kindled seizures. The hippocampal formation is rich in glutamatergic neurones and is particularly vulnerable to intraventricular kainate (Nadler *et al*, 1978).

Test doses of kainate (0.2 - 500nmoles) were administered to male Wistar rats (250 - 350g, n=5) over a 5min period via a dialysis probe into the lateral ventricle to produce a dose-response relationship of resulting behavioural and spiking activity, the latter recorded from bipolar electrodes located in the hippocampus, in order to establish a subconvulsant dose to be used as a kindling stimulus. At doses of 0.3 to 0.5nmoles mild signs of seizure (facial clonus, chewing or head nodding) resulted as well as some spiking activity on the E.E.G. Increased spiking was evident at 1.0 to 5.0nmoles and this was accompanied at 50nmoles with 'wet dog shakes' . Full motor convulsion similar to that seen in stage 5 electrically kindled seizure was seen with kainate doses of 250 to 500nmoles.

Subconvulsive doses of kainate (d_1=0.4; d_2=0.5nmoles) were chosen and administered hourly by dialysis into the ventricles of rats (n_1=5; n_2=4), behavioural and E.E.G. changes being monitored continuously. No increase in sensitivity to the kainate doses was observed over the course of 11 stimulations (d_1) or 14 stimulations (d_2).

It must therefore be concluded that at these subconvulsive doses, administered to the lateral ventricle, the excitatory amino acid kainate is not an agent of kindling in the rat hippocampus.

Goddard, G.V., *et al* (1969) Exp. Neurol., 25, 295-330
McNamara, J.O., *et al* (1980) In:'Progress in Neurobiology' Vol.15: Phillis, J.W. and Kerkut, G.A. (eds), pp 139-159. Pergammon Press, Great Britain.
Nadler, J.V., *et al* (1978) In:'Kainic Acid as a Tool in Neurobiology': McGeer, E.G., *et al* (eds), pp 219-237. Raven Press, New York.
Peterson, D.W., *et al* (1984) Brain Research, 311, 176-180.
Racine, R.J. (1972) Electroenceph. clin. Neurophys., 32, 281-294.

ACTIONS OF ECMA ON CHOLINERGIC NEURONES IN THE RABBIT RETINA

Cunningham, J., Neal, M.J., *Pillar, A. & *Atterwill, C.K. St Thomas' Hospital Medical School, London & *Investigative Toxicology, Smith Kline & French Research Ltd, Welwyn U.K.

Ethylcholine mustard aziridinium ion (ECMA) has been proposed as a cholinergic neurotoxin and has been used to produce animal models for Alzheimers Disease (see Fisher & Hanin, 1986, Leventer et al, 1987). In agreement with the more recent in vivo studies we have recently demonstrated in vitro that similar low concentrations of ECMA (12.5 μM) cause a biphasic loss of choline acetyltransferase activity (ChAT) in foetal rat brain reaggregate cultures (Pillar et al, 1987b). Initial direct inhibition is followed by an apparently selective loss of cholinergic neurones. More widespread cytotoxicity occurs at higher concentrations (25-50 μM). The action and selectivity of this neurotoxin have now been further investigated in the rabbit retina. Intravitreal application should also lend itself to controlled conditions of ECMA exposure. The rabbit retina contains different types of intrinsic cholinergic neurones (see Brandon, 1987) and previous studies have already shown that there is a selective and unequivocal destruction of cholinergic cells following intravitreal application in chickens (Morgan & Miller, 1986).

ECMA was freshly prepared from the precursor acetylethylcholine mustard (Salford Ultrafine Chemicals) as previously described (Pillar et al 1987a). 12.5 nmoles or 600 nmoles were injected into the vitreous humour of male albino rabbits under light pentobarbitone anaesthesia, giving final intraocular concentrations of 12.5 & 600 μM respectively. 96 hours later animals were sacrificed and retinae removed, quartered and subjected to neurochemical or histopathological analysis. ChAT, total (^{3}H) choline uptake, glutamate decarboxylase (GAD), GABA - transaminase (GABA-T) and protein were measured. 600 μM ECMA produced a 55% loss of ChAT activity with no significant effects on (^{3}H) choline uptake or GABA-T activity although a small non-statistically significant loss of GAD was seen (35% reduction). In 5 out 7 ECMA treated rabbits tested a loss of the ERG b-wave was seen. At the lower ECMA concentration of 12.5μM a 36% reduction in ChAT was obtained (control = 0.50±0.03 (7), treated = 0.32±0.05 (5) μmols/h/mg protein, $P<0.01$) again with no significant change in total (^{3}H) choline uptake or histopathological appearance.

These data support the findings of Morgan & Miller (1986) who found that low doses of intraocular ECMA (approx 50μM) lesion only two of the three cholinergic cell types found in chicken retina without affecting other cell types. Interestingly, very high concentrations (600μM), which in brain cultures cause more generalized cytotoxicity including astrocytic damage (Pillar et al 1987a), did not lesion other cell types. Furthermore, the residual ChAT activity found after ECMA treatment of rabbit retina in vivo and rat brain cultures in vitro, even at high concentrations, may possibly represent a class of cholinergic cells resistant in some way to ECMA's neurotoxic action (see also Leventer et al 1987).

Fisher, A. & Hanin, I. (1986) Ann. Pharmacology, 26 161-181.
Leventer, S.M. et al (1987) Neuropharmacology, 26 361-365.
Morgan, I.G. & Millar. T.J. (1986) TIPS, July, 264-265.
Pillar, A.M. et al (1987a) Toxicology, (IN PRESS).
Pillar, A.M. et al (1987b) Br. J. Pharmac., This meeting (IN PRESS).
Brandon, C. (1987) Brain Res., 401 385-391.

BINDING OF [^{3}H]SCH 23390 TO POST-MORTEM BRAIN TISSUE IN SCHIZOPHRENIA

C. Czudek & G.P. Reynolds, Department of Pathology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

Of the two types of dopamine receptor in the brain it is the D_2 subtype which is thought to mediate the antipsychotic effect of neuroleptic drugs. The increase of D_2 receptor sites in the brain in schizophrenia is well-established; however the contributions of disease process and effects of drug treatment to this increase have yet to be fully distinguished.

D_1 receptors have been less intensively investigated. The development of a selective D_1 antagonist, SCH 23390, showed this receptor subgroup to be involved in modulating some D_2-related behaviours, and also provided a ligand for the study of D_1 receptors in brain tissue.

While one report identified no difference in binding of SCH 23390 to post-mortem striatal tissue from schizophrenics and control subjects (Pimoule et al., 1985), a recent publication showed a substantial reduction in maximal binding in schizophrenia. We have investigated SCH 23390 binding in such tissue in order to assess how any change in D_1 receptors might relate to the observations of other dopaminergic abnormalities in schizophrenia.

Crude membrane suspesions were prepared and assayed after the method of Billard et al. (1984). Incubation with (3H)SCH 23390 (0.1 - 16 nM) was performed at 37°C for 15 min. Non-specific binding was defined using 10^{-6}M unlabelled SCH 23390. For caudate tissue, Bmax and K_D values were obtained by linear regression analysis of Scatchard plots. The amygdala was investigated using a single 2nM concentration of ligand to conserve tissue.

Table 1 (3H)SCH 23390 binding to human brain tissue

	Caudate Bmax		Amygdala B(2nM)	
	Left	Right	Left	Right
Controls (7)	361(100)	364 (36)	73 (34)	67 (13)
Schizophrenics (14)	362 (69)	374 (84)	69 (16)	64 (15)

Values are mean (s.d.) in fmol/mg protein.

The results are shown in table 1. Contrary to the findings of Hess et al (1987) no reduction in either Bmax or K_D (means 2.2 and 2.5nM for control and schizophrenic groups respectively) values was observed in the schizophrenic group. Nor was any asymmetry found in receptor density, unlike D_2 receptors which are increased in the right striatum, an effect which is accentuated in schizophrenia (Reynolds et al., 1987). Similarly, D_1 receptors do not reflect the indication of presynaptic dopaminergic asymmetry in the amygdala in schizophrenia (Reynolds, 1983). Experiments including 50nM ketanserin to displace binding to $5HT_2$ receptor sites showed this to represent a small (<10%) contribution to SCH 23390 binding in the caudate, and while it displaced more in the amygdala, the proportion (32%) was the same in both controls and schizophrenics. Thus we find no evidence of abnormal D_1 receptor density in schizophrenia.

Billard, W. et al. (1984) Life Sci. 35, 1885-1893.
Hess,E.J. et al. (1987) Life Sci. 40, 1487-1497.
Pimoule, C. et al. (1985) Eur. J. Pharmacol. 114, 235-237.
Reynolds, G.P. (1983) Nature 305, 527-529.
Reynolds, G.P. et al. (1987) Lancet i, 979.

IMMUNOHISTOCHEMICAL DISTRIBUTION OF β-NGF RECEPTORS IN THE FOREBRAIN OF THE RAT

D. Dawbarn*, S.J. Allen, S.J. & Semenenko, F.M.[1], Department of Medicine (Care of the Elderly), Bristol Royal Infirmary, Bristol, BS2 8HW. [1]Department of Oral Medicine and Oral Surgery, Bristol Dental Hospital, Bristol, BS1 2LY.

Nerve growth factor (NGF) is a complex protein consisting of three sub-units α , β and γ . The sub-unit has been sequenced and cloned and appears to be responsible for the observed biological effects of NGF. Recently, β-NGF and the content of β-NGF mRNA have been measured in the central nervous system of the rat and the levels have been found to correlate with distribution of cholinergic innervation. The distribution of β-NGF receptors in the rat brain has been examined (Raivich and Kreutzberg, 1987) by [^{125}I]- β -NGF receptor autoradiography, and in the forebrain, found to coincide with that of cells containing acetylcholinesterase, mainly located in the medial septal nucleus, the vertical and horizontal limbs of the diagonal band of Broca (DBB_{VL} and DBB_{HL}) and the nucleus basalis of Meynert.

In this study we have used the monoclonal antibody 192 IgG (Chandler et al., 1984) to demonstrate the distribution of β-NGF receptors in the forebrain of the rat. Six female Sprague Dawley rats were anaesthetised and perfused with 4% paraformaldehyde. The brains were subsequently removed and frozen sections cut throughout the forebrain. β-NGF receptor immunohistochemistry was performed using the double bridge peroxidase anti-peroxidase technique. Cross-sectional area of immunopositive cells was measured using an image analysis system. β -NGF receptor containing cells were located in the medial septal nucleus, DBB_{VL}, DBB_{HL} and the nucleus basalis of Meynert. Immunopositive cells of the medial septal nucleus first appeared rostrally at the anterior end of the corpus callosum. They were usually bipolar with a mean cross-sectional area of 133um^2. The immunopositive cells of the DBB_{VL} lay ventral and continuous with the medial septal nucleus. These cells usually showed two or three principal dendrites and had a mean cross-sectional area of 199um^2. At the posterior end of DBB_{VL}, labelled cells merged with DBB_{HL}. These cells had a mean cross-sectional area of 173um^2, appeared to be multipolar and were surrounded by a dense plexus of immunoreactive processes. The β-NGF receptor immunoreactive cells of the basal nucleus appeared rostrally, dorsal to the DBB_{HL}, and became more frequent posteriorally where they bordered on the pallidum. These were large multipolar neurones with a mean cross-sectional area of 221um^2. No labelled perikarya were observed in other forebrain regions.

The data reported here demonstrates the direct visualization of β-NGF receptors in neurones of the medial septal nucleus, DBB_{VL}, DBB_{HL}, and nucleus basalis of Meynert. In the rat brain, these are predominantly cholinergic nuclei.

Chandler, C.E., Parsons, L.M., Hosang, M. & Shooter, E.M. (1984) J. Biol. Chem. 259, 6882-6889.
Raivich, G. & Kreutzberg, G.W. (1987) Neuroscience 20, 23-36.

DEVELOPMENT OF A METHOD FOR MEASUREMENT OF FREE MHPG IN BRAIN TISSUES USING HPLC WITH ELECTROCHEMICAL DETECTION

D. J. Heal, M. R. Prow and W. R. Buckett, Research Department, The Boots Company PLC, Nottingham NG2 3AA

3-Methoxy-4-hydroxyphenylglycol (MHPG) is suggested to reflect noradrenaline (NA) which has been released and functionally utilised (Meek and Neff, 1972). In mouse brain it occurs solely in the unconjugated form (Ceasar et al, 1974). We have developed a method for determining MHPG in brain tissue by HPLC with electrochemical detection (HPLC-ELCD). This employs a simple purification step, uses 3-hydroxy-4-methoxyphenylglycol (iso-MHPG) as an internal standard and is based on a method described by Molyneux and Franklin (1985) for measuring MHPG in plasma.

Adult male C57/Bl/601a mice (Olac) weighing 15-35g were used. Brains were homogenised in 5 vols (w/v) 0.1M perchloric acid containing 0.4mM sodium metabisulphite and 0.1μM iso-MHPG (internal standard). This homogenate was centrifuged at 1,100g and 11,600g. 1ml of the resulting supernatant was mixed with 5ml ethyl acetate. After centrifugation at 1,300g, the organic phase was mixed with 1ml 0.08M potassium bicarbonate and recentrifuged at 1,300g. The organic phase was removed and evaporated at 30°C under a stream of nitrogen. Residues were dissolved in 100μl 0.1M sodium acetate/citric acid pH 4.4 buffer containing 8% (v/v) methanol and 4.6mM octanesulphonic acid sodium salt (HPLC mobile phase). Using a WISP 710B autoinjector, 50μl was injected onto the HPLC system which consisted of a Du Pont 870 pump connected to a 25 cm Spherisorb 1 ODS, 5 μm reversed-phase analytical column protected by a 3 cm Brownlee Aquapore RP 300 precolumn. MHPG was detected using a BAS LC-4B amperometric detector with a TL-5A flow cell containing a glassy carbon electrode (potential +0.75V versus Ag/AgCl reference electrode). The flow-rate was 0.85 ml/min. MHPG and iso-MHPG chromatographed with retention times of 7.00 and 9.30 min, respectively. There were no interfering peaks from brain samples, but an unidentified peak occurred after 37 min. Basal MHPG concentration in brain ± s.e. mean was 79.8 ± 1.3 ng/g wet wt which agrees with previously reported values (Ceasar et al, 1974; Buckett and Diggory, 1985). Hence, this is a simple and sensitive HPLC method which does not require the more costly coulometric detector employed by Molyneux and Franklin (1985). Furthermore, it has the advantage over the method of Buckett and Diggory (1985) that an internal standard is incorporated in the procedure.

Andén et al (1976) proposed that presynaptic α_2-adrenoceptors control NA release in brain. Consistent with this hypothesis, we found that MHPG was dose-dependently reduced 60 min after i.p. injection of the α_2-adrenoceptor agonist, clonidine (1-3000μg/kg). The ED50 was 25 μg/kg and the maximal decrease was 47% ($P<0.01$). The α_2-antagonists, idazoxan (1 mg/kg) and yohimbine (2 mg/kg), induced 21% ($P<0.05$) and 66% ($P<0.01$) increases in MHPG, respectively. However, the α_1-antagonist, prazosin (1 mg/kg), and β-antagonist, pindolol (1 mg/kg), both had no effect on brain MHPG levels. In addition, the 28% ($P<0.01$) decrease in MHPG induced by clonidine (100 μg/kg) was totally abolished by pretreatment with idazoxan (1 mg/kg) or yohimbine (2 mg/kg), but was unaltered by prazosin (1 mg/kg) or pindolol (1 mg/kg).

Andén, N.E., Grabowska, M. & Strömbom, U. (1976). Naunyn-Schmiedeb. Arch. Pharmac. 292, 43.
Buckett, W.R. & Diggory, G.L. (1985) Br. J. Pharmac. 86, Proc. Suppl. 414P.
Ceasar, P.M., Hague, P., Sharman, D.F. & Werdinius, B. (1974) Br. J. Pharmac. 51, 187.
Meek, J.L. & Neff, N.H. (1972) Br. J. Pharmac. 45, 435.
Molyneux, S.G. & Franklin, M. (1985) J. Chromatogr. 341, 160.

CYCLIC AMP ANALOGUES BLOCK CLONIDINE BUT NOT DYNORPHIN A (1-13) INDUCED REDUCTION IN INTRASYNAPTOSOMAL FREE $[Ca^{2+}]_I$

P. Adamson, M. J. Brammer, and I. C. Campbell. Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK

We have previously demonstrated that both the α_2-adrenoceptor agonist clonidine and the preferential Kappa-opiate agonist dynorphin A (1-13) reduce basal intrasynaptosomal free $[Ca^{2+}]$: this can be antagonised by the corresponding antagonists idazoxan and naloxone in rat cerebral cortex (Adamson et al, 1987). Recent evidence suggests a role for adenylate cyclase in the postsynaptic effector mechanisms of α_2-adrenoceptors (Weiner 1979). We have examined the effect of membrane permeant cAMP analogues (dibutyryl cAMP, 8-Bromo-cAMP) on the presynaptic α_2-adrenoceptor and Kappa-opiate receptor mediated reduction in intrasynaptosomal free $[Ca^{2+}]$. For these experiments synaptosomes were prepared according to Gray and Whittaker et al (1962) and Dunkley et al (1986) and were resuspended in Krebs buffer containing 1.0mM $CaCl_2$. $[Ca^{2+}]_i$ was measured using the fluorescent dye Quin 2 (Ashley et al 1984). The results of the studies are shown in Table 1.

TABLE 1

Compound	% change (means ± SEM)	n	Significance (paired t test)
Clonidine (1μM)	-26.5 ± 3.12	6	$P < 0.01$
Dynorphin A (1-13) (1μM)	-19.9 ± 2.50	7	$P < 0.05$
d.b. cAMP (10μM)	+34.41 ± 6.35	16	$P < 0.001$
8-Br-cAMP (10μM)	+46.99 ± 5.48	16	$P < 0.001$
d.b. cAMP (10μM) + clonidine (1μM)	+20.17 ± 3.55	8	$P < 0.01$
d.b. cAMP (10μM) + dynorphin A(1-13)(1μM)	- 0.82 ± 3.54	8	N.S
8-Br-cAMP (10μM) + clonidine (1μM)	+38.83 ± 8.0	6	$P < 0.02$
8-Br-cAMP (10μM) + dynorphin A(1-13)(1μM)	+17.38 ± 5.55	6	$P < 0.001$

We have found that both cyclic nucleotides increase $[Ca^{2+}]_i$ in a dose dependent manner the maximum increases were approximately 50% for 8-Br-cAMP and 35% for the dibutyryl cAMP and occurred at a concentration range of 10-9 μM. IC_{50} values were approximately 4.0μM for both analogues. This effect could be due to an event related to Ca^{2+}-sequestration or a direct effect of the cyclic nucleotide on Ca^{2+}-gating. The effect of clonidine is substantially blocked by the cAMP analogues but the effect of the Kappa-opiate agonist dynorphin (A(1-13) is apparently independent of the cyclic nucleotides. We conclude that these two receptor mediated effects involve different effector mechanisms and it is likely that presynaptic α_2-adrenoceptors are linked to adenylate cyclase.

Ashley, R. H., Brammer, M. J. and Marchbanks, R. M. (1984) Biochem.J. 219, 149.
Adamson, P., Brammer, M. J. and Campbell, I. C. (1987) Eur.J.Pharmacol. 142, 261.
Dunkley, P. R., Jarvie, P. E., Heath, J. W., Kidd, G. I. and Rostas, J. A. P. (1986) Brain Res. 372, 115.
Weiner, N. (1979) Fed.Proc. 28, 2193.

PERFORMANCE OF HOODED LISTER AND SPRAGUE-DAWLEY RATS IN A T-MAZE FOOD REINFORCED ALTERNATION TASK

J.M. Barnes, N.M. Barnes, B. Costall, J. Coughlan & M.E. Kelly, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP

The performance of Hooded Lister (HL) and Sprague-Dawley (SD) rats were compared in a reinforced alternation task using an elevated T-maze (Salamone et al. 1984), and comparisons made of levels of acetylcholine (Ach) and cholineacetyltransferase (ChAT) in the frontal cortex, hippocampus, septum and striatum of the two strains of rats.

For 2 days prior to test, and throughout testing, animals (11 weeks old at the start of experiment) were deprived of food for 23h/day, food being allowed after test, water available 'ad libitum'. Training consisted of paired trials (each pair constituting a run), the first being 'forced' in that one arm was blocked whilst the other was baited, the second being a 'choice' trial in which reward pellets were placed in the arm opposite to that reinforced in the first trial. A correct choice was when the rat entered the arm containing the food. Latency to reward was also recorded for both forced and choice trials. Animals were tested for 9 days or for 5 days (for biochemistry). ChAT was determined using the radioenzymatic technique of Fonnum et al. (1975) and Ach levels by HPLC with electrochemical detection (Barnes et al. 1987).

HL rats made 54% correct choices on day 2, increasing to 62, 78 and 74% on days 3, 4 and 5 respectively. In contrast, SD rats made only 40% correct choices throughout the test period. 'Forced latencies' were significantly higher in the SD than HL rats (e.g. 225 and 137s on day 2, 76 and 41s on day 5 respectively, $P<0.001$) and 'choice latencies' showed similar differences (e.g. 121 and 40s on day 2, 35 and 18s on day 5 respectively , $P<0.001$). ChAT levels of HL rats were significantly higher than in SD rats in the frontal cortex, hippocampus and striatum, but were similar in the septum. Ach levels in HL rats were also higher than in SD rats in the frontal cortex and hippocampus but with no significant differences in the striatum (Table 1).

Table 1. ChAT and Ach levels in Hooded Lister and Sprague-Dawley rats.

	Frontal Cortex	Hippocampus	Septum	Striatum
		ChAT (pmol/min/mg protein)		
Hooded Lister	1095±34**	1594±54**	627±42	2564±96**
Sprague-Dawley	867±39	1092±65	580±30	1948±66
		Ach (pmol/mg wet weight)		
Hooded Lister	28.6±1.7*	30.5±1.6*		82.3± 8.66
Sprague-Dawley	18.6±2.5	20.0±2.6	N.D.	113.5±11.6

n = 8 to 10, S.E.M.s shown. Significant differences between the strains of rats are indicated *$P<0.01$, **$P<0.001$ (Student's t test). N.D. not determined.

In the food reinforced alternation task HL rats show a clear superiority in performance to SD rats. In the HL rats the levels of ChAT and Ach were also higher in the frontal cortex and hippocampus and such measures of increased cholinergic activity may be related to their increased behavioural performance.

Barnes N.M. et al (1987) J. Pharm. Pharmac. 39, 727-731
Fonnum F. (1975) J. Neurochem. 24, 407-409
Salamone J.D. et al. (1984) Behav. Brain Res. 13, 63-70

BLOCKADE OF α_1-ADRENOCEPTORS POTENTIATES THE SUPPRESSION OF LOCOMOTOR ACTIVITY BY κ-OPIOID AGONISTS

G.E.Leighton,R.G.Hill and J.Hughes.
Parke-Davis Research Unit, Addenbrookes Hospital Site,Hills Road,Cambridge,CB2 2QB.

Behavioural sedation is an effect characteristic of agents acting as kappa opioid agonists(Martin et al,1976).The mechanisms underlying this sedative effect are not understood although it has been suggested that the effect could be mediated via an action in the deep layers of the cerebral cortex(Goodman & Snyder,1982) or in brainstem structures particularly the reticular activating system(Kammerling et al,1983).Since monoamines are known to be involved in regulation of the level of consciousness and in the control of locomotor activity it was decided to investigate possible interactions between monoamine systems and the sedative effects of kappa opioid agonists.

The effects of test compounds on locomotor activity were determined in male CFLP mice (30-40grams,Interfauna,Huntingdon) housed individually for the duration of the experiment (2 hours) in cages that formed part of a computer controlled locomotor activity monitoring system. Animals were dosed subcutaneously with saline or a kappa agonist 5 minutes before the start of the data collection period.When antagonists were administered in combination with the kappa agonists these were given 30 minutes beforehand.The kappa agonists used in this study were PD117302 (Leighton et al., 1987) and U50488.The following antagonists were used: ketanserin(5-HT_2 and alpha$_1$ antagonist), ritanserin(5-HT_2 antagonist),methysergide(a nonselective 5-HT antagonist) and prazosin (alpha$_1$ antagonist). Compounds were dissolved in saline and administered in a dose volume of 1ml/100grams body weight.All locomotor activity experiments were started at the beginning of the dark period(18.00hrs) of a twelve hour light twelve hour dark cycle.

PD117302 and U50488 produced clear dose related reductions in locomotor activity(table 1).Because the levels of locomotor activity observed in control animals varied slightly between experimental days all results have been normalized and levels of activity in drug treated animals are expressed as a percentage of the activity seen in the appropriate control group.None of the antagonists studied affected locomotor activity when given alone but ketanserin and prazosin produced a marked potentiation of the suppression of locomotor activity produced by PD117302 and U50488.Methysergide did not affect the response to either of the kappa agonists. Ritanserin, a 5-HT_2 antagonist with a much lower affinity for the alphal adrenoceptor than ketanserin produced only a slight potentiation of the response to PD117302 and was without effect on the response to U50488.

Table 1. Locomotor activity in mice shown as a percentage of the appropriate control group.

	Pretreatment				
	Saline	Ketanserin 1mg/kg	Ritanserin 1mg/kg	Methysergide 3mg/kg	Prazosin 0.5mg/kg
Treatment					
Saline	100±17%	100±18%	100±10%	100±14%	100±14%
PD117302					
3.7mg/kg	73±6	30±7**	55±8	76±8	34±6**
10 mg/kg	34±16	13±4**	-	-	-
30 mg/kg	6±2	2±0.5*	-	-	-
U50488					
3.7mg/kg	85±11	43±9**	77±15	81±6	58±15
10 mg/kg	70±17	13±3**	-	-	13±2**
30 mg/kg	7±3	11±4	-	-	-

*p<0.05,**p<0.01 significantly different from saline pretreated group.Mann-Whitney U test. n=7-9 mice per group.

These results suggest that the interaction seen between ketanserin and kappa agonists on locomotor activity is probably due to antagonism at alpha$_1$ adrenoceptors rather than 5-HT_2 receptor blockade since the effect was mimicked by prazosin but not by the nonselective 5-HT antagonist methysergide.

Goodman,R.R.& Snyder,S.H.(1982)Life Sci.31:1291-1294.
Kammerling,S.G. et al.(1983)Life Sci.33:1893-1843.
Leighton, G.E. et al. (1987). Br. J. Pharmacol. (In Press).

HYPERALGESIA FOLLOWS MORPHINE-INDUCED ANALGESIA: EVIDENCE TO SUGGEST OPIATE RECEPTOR INVOLVEMENT

C.A. Hendrie (introduced by R.J. Rodgers) Pharmacoethology Laboratory, Psychology, University of Bradford BD7 1DP, Yorkshire

Recent evidence suggests that exposure to environmental endogenous opioid activating stimuli induces in addition to analgesia, a hyperalgesic reaction sometime after analgesia has ceased to be detectable (Hendrie, in press). Decreases in sensitivity to noxious stimulation occur 0-45 mins whilst hyperalgesia is observed 70-80 mins post-attack. For several years it has been known that exogenous opiates, such as morphine, exert their influences through interaction with endogenous endorphinergic receptor mechanisms. The following studies were conducted to investigate whether the time course of nociception post-morphine administration would follow a similar pattern to that observed following exposure to opioid activating environmental stimuli.

Naive 20-30g male DBA/2 mice (Bantin & Kingman, Hull, UK) served as subjects. For Experiment 1 mice were injected in randomised, counterbalanced order with saline or morphine (1, 5, 10 or 20 mg/kg). All injections were performed intraperitoneally in a volume of 10ml/kg with tail flick latencies (TFL) being recorded 1 hour prior to and at hourly intervals, up to 6 hours, post-injection. For Experiment 2, saline or morphine injections were given 1 hour after the assessment of baseline TFL, with naloxone (0-10mg/kg) being administered 3 hrs 50 mins later and a second TFL being established 10 mins after that. Data (mean ± SEM) from Experiment 1 are presented below.

morphine (mg/kg)	baseline	Time (hrs post-injection) 1	2	3	4	5	6
0	3.6 ± .3	3.5 ± .5	3.6 ± .3	3.3 ± .4	3.3 ± .4	3.1 ± .4	3.0 ± .5
1	3.7 ± .7	3.5 ± .5	3.2 ± .5	3.3 ± .5	2.7 ± .4	2.6 ± .3	3.0 ± .6
5	3.6 ± .3	5.1 ± .7*	4.1 ± .6	3.6 ± .5	2.4 ± .4	2.4 ± .2	2.6 ± .3
10	4.2 ± .5	7.3 ± 1.3*	5.6 ± .9	3.8 ± .6	2.3 ± .3*	2.9 ± .4	3.1 ± .4
20	3.2 ± .5	12.8 ± .7*	4.8 ± .6	3.5 ± .4	1.5 ± .2*	2.2 ± .3	2.5 ± .4

2-factor Analysis of Variance (ANOVA) revealed significant effects of drug ($F(4, 44) = 7.6$, $p<0.01$), time ($F(6,264)=33.05$, $p<0.01$) and a significant interaction ($F(24,264)=67.91$, $p<0.01$). Follow-up tests revealed there to be significant analgesia in the 5, 10 and 20 mg/kg morphine conditions 1 hour post-injection and significant hyperalgesia in the 10 and 20 mg/kg conditions alone 4 hours post-injection. Experiment 2 revealed these hyperalgesic reactions to be reversed by low doses of naloxone, suggesting its mediation by substrates acting at opiate receptors. In view of this finding it seems unlikely that hyperalgesia following morphine analgesia is an effect of the absence of morphine per se but rather that it is due to the presence ofan opioid inverse agonist, a substrate acting at opiate receptor mechanisms but with effects opposite to those usually ascribed to traditional opiate receptor agonists.

CAN THE MOUSE ABDOMINAL CONSTRICTION TEST BE USED AS A MODEL FOR PERIPHERAL OPIOID ANTINOCICEPTIVE ACTIVITY?

A.G. Hayes & N.J. Hayward, Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DJ.

A modification of the mouse abdominal constriction test has been claimed (Bentley, Newton and Starr, 1981) to detect only peripherally-mediated antinociceptive effects of opioid agonists. In this model, opioid drugs were injected locally (i.e. intraperitoneally), immediately prior to application of the noxious stimulus, at a time when CNS penetration of the drugs had apparently not occurred. The purpose of the present experiments was to further evaluate this model.

Mice (male, CRH, 17-23g) were dosed with opioid agonists, either s.c. or i.p., concurrently with a dose of 3mg/kg i.p. acetylcholine. The number of abdominal constrictions were counted for a period of 2 min, starting 1 min after administration of drug and acetylcholine.

The opioid agonists all caused a dose-related inhibition of abdominal constrictions, but their potency was similar whether administered locally (i.p.) or remotely (s.c.) as shown in Table 1.

TABLE 1 Antinociceptive Activity of Opioid Agonists in the Mouse Abdominal Constriction Test when Administered 1 min Before Testing

DRUG	ED_{50} $mgkg^{-1}$ (confidence limits) (n=12) s.c.	i.p.
U50488H	0.57(0.27-1.35)	0.12(0.05-1.28)
Tifluadom	0.03(0.01-0.06)	0.10(0.03-0.38)
Ethylketazocine	0.01(0.007-0.038)	0.02(0.013-0.064)
Fentanyl	0.02(0.007-0.043)	0.04(0.014-0.159)

One explanation for these findings is that the opioid agonists penetrate into the CNS very rapidly. This suggestion is lent further credence by the fact that tifluadom produced potent activity in two central antinociceptive models when administered s.c. 1min before testing (ED_{50} in tailflick test = 0.08mg/kg; hot plate test = 0.28mg/kg). Also, naloxone (5mg/kg i.p. given 1 min before testing) antagonised the effect of tifluadom (given 30 min before testing) in the abdominal constriction test (dose ratio = 9.1, 95% confidence limits = 4.03-30.11). This suggests that naloxone is capable of antagonising centrally-mediated effects within 1 min of administration.

This study suggests that the modified abdominal constriction test is not a reliable model of peripheral antinociceptive activity, since centrally mediated opioid effects are observed after just 1 min. Thus, an antinociceptive action at peripheral opioid receptors can only be reliably demonstrated with a compound that does not enter the CNS.

We are grateful for the technical assistance of Mrs. R. Sargent and Miss S. Curson.

Bentley, G.A., Newton, S.H. & Starr, J. (1981) Br. J. Pharmac. 73, 325-332.

CLASSIFICATION OF MUSCARINIC RECEPTOR SUBTYPES IN THE CONSCIOUS MOUSE

K.A.Bevan, J.C.Patterson, W.W.Templeton, Department of Development Biology, Pfizer Central Research, Sandwich, Kent, CT13 9NJ.

The classification of muscarinic receptors into M_1 (neuronal), M_2 (atrial) and M_2 (smooth muscle) is largely based on the receptor affinities of 3 compounds, pirenzepine, AF-DX-116 and 4-DAMP, respectively. An M_3 receptor has also been proposed, though no selective antagonists are available for this receptor. Although the central nervous system (CNS) is likely to contain more than one of these receptor subtypes, their underlying physiological functions are unknown. To characterise further the receptors present in the CNS, salivary gland, and eye, the effects of a number of muscarinic antagonists ((Table 1) on mydriasis and on oxotremorine-induced tremors and salivation have been assessed in mice. Antagonists were administered i.p. or i.c.v. 5 mins before oxotremorine (200 ug/kg i.v.). The mice were scored for tremors, and salivation, 5 min after oxotremorine injection and ED_{50} values calculated. Mydriasis was assessed 5 min after antagonist administration and an ED_{200} (doubling of pupil size) obtained. Potency of each compound, relative to atropine sulphate, is shown in Table 1.

Table 1: Relative potencies of muscarinic antagonists to cause mydriasis and to inhibit tremor and salivation (less than 1 = less potent).

		i.p.		i.c.v.
Antagonist	Mydriasis	Tremor	Salivation	Tremor
Atropine SO_4	1	1	1	1
Atropine $MeNO_3$		<0.015	0.35	1.15
Pirenzepine	0.007	0.004	0.03	0.31
4-DAMP	0.18	0.04	0.49	0.56
AF-DX-116	<0.0006	0.008	<0.017	not active

The rank orders of potencies of the compounds tested with respect to tremor, salivation and mydriasis are consistent with the involvement of different receptor subtypes, however interpretation of the data is complicated by the fact that the compounds have different absolute receptor potencies and different degrees of CNS penetration. 4-DAMP, AF-DX-116 and pirenzepine administration i.p. are all much less active than atropine sulphate versus oxotremorine-induced tremor which could reflect their relatively greater polarity and reduced CNS penetration. When the blood brain barrier is circumvented (by i.c.v. drug administration) pirenzepine, 4-DAMP and atropine are approximately equiactive while AF-DX-116 is inactive at the maximum dose injectable. On the basis that AF-DX-116 is at least 10 times more potent on the M_2 atrial type receptor than pirenzepine or 4-DAMP, it is unlikely that this receptor subtype is involved in the induction of tremor. Similarly, as 4-DAMP is more than 100 times more potent than pirenzepine at the M_2 ileal type receptor, this receptor can be largely discounted in the tremor response. However, in vitro (radioligand binding) affinities of pirenzepine and 4-DAMP for the M_1 receptor are comparable which could implicate this receptor.

The rank order of compound potency in mydriasis production and antagonism of salivation favours a common receptor subtype. Relative potencies of 4-DAMP and pirenzepine suggest a receptor resembling the M_2 (smooth muscle) subtype although pirenzepine is more active than would be predicted from in vitro studies. Definite classification of receptor subtypes awaits more selective, potent and lipophilic compounds.

We thank Dr. R.B. Barlow for 4-DAMP and Boots Company plc for pirenzepine.

BEHAVIOURAL AND BIOCHEMICAL ACTIONS OF THE CHOLINESTERASE INHIBITOR TETRAHYDROAMINOACRIDINE

A.J. Hunter, T.K. Murray, A.J. Cross, J.A. Osborne and A.R. Green, Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ.

Tetrahydroaminoacridine (THA) is a centrally active cholinesterase inhibitor which has been reported to be beneficial in the treatment of Alzheimer's Disease (AD) (Summers et al 1986). In addition to its anticholinesterase activity, its therapeutic benefit may be due in part to direct actions at muscarinic receptors or to pharmacokinetic factors. We have compared the behavioural effects and duration of action of THA in the rat and the mouse, and examined the effects of THA on muscarinic receptors in vitro.

Behavioural observations were carried out on male Lister Hooded rats or C57Bl mice (Olac, Bicester, U.K.). In the mouse THA (5-20 mg^{-1}/kg s.c.) and the rat 5-20 mg^{-1}/kg s.c.) produced a dose-dependent decrease in temperature and an increase in salivation, lacrimation and tremor. In the rat it also decreased rearing in an open field and increased chewing. These changes were similar to those seen with physostigmine (0.3-0.6 mg/kg, s.c.). In further experiments carried out in rats and mice, 6 groups of animals (n = 6-8) were injected with vehicle, and 6 groups with 20 mg/kg of THA.

	RAT			MOUSE		
TIME (hr) After THA injection	DECREASE IN TEMP (°C) mean±SEM	SALIVATION (mg) mean±SEM	MEDIAN TREMOR SCORE	DECREASE IN TEMP (°C) mean±SEM	MEDIAN SALIVATION SCORE	MEDIAN TREMOR SCORE
0.5	1.1 ± 0.1**	95 ± 10	3	5.3 ± 0.2**	2	3
1.0	2.5 ± 0.2**	66 ± 8	3	9.1 ± 0.5**	1	2
2.0	2.6 ± 0.3**	81 ± 5	3	10.3 ± 0.8**	1	1
4.0	3.8 ± 0.2*	66 ± 11	2	3.2 ± 0.7**	0	0
8.0	1.6 ± 0.2**	0	1	2.1 ± 1.1	0	0
24.0	0.7 ± 0.5	3 ± 1	0	1.35 ± 1.8	0	0

Significantly different from control group **$p < 0.001$; *$p<0.01$.

Data shown for THA treated animals only. Control animals showed no tremor or salivation at any time point.

As shown in the Table, the duration of action of THA in the rat was almost twice the duration in the mouse, with some behavioural effects still present at 8 hours. This contrasts with the behavioural effects of physostigmine in the rat and the mouse, which disappear by 1 hour post-injection.

THA was found to be a non-competitive inhibitor of both [^{3}H]-pirenzepine (1nM) binding to rat cortical M_1 receptors (IC_{50} 0.9 ± 0.2 μM) and [^{3}H]-NMS (1nM) binding to rat heart M_2 receptors (IC_{50} 7 ± 1μM). The present results suggest that any clinical advantage of THA over physostigmine in AD may relate in part to its duration of action.

Summers, W.K. et al (1986) New Eng. J. Med. 315: 1241-1245.

THE POTENCY OF (+)-MUSCARINE ON TWO MUSCARINIC RESPONSES ON THE ISOLATED SUPERIOR CERVICAL GANGLION OF THE RAT

M.J. Gilbert & N.R. Newberry, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR.

Two responses can be evoked by (±) muscarine on the rat superior cervical ganglion: a pirenzepine-sensitive depolarisation and a hyperpolarisation which is less sensitive to pirenzepine but antagonised by gallamine (Newberry et al, 1985). Classical studies, now textbook facts (Bowman and Rand, 1980; Waser, 1961), demonstrated that the naturally occurring (+) muscarine is over 100 times more potent than (-) muscarine and twice as potent as the racemate. We sought to re-examine this stereoselectivity by comparing the potency of (+) muscarine with that of the racemate on the two muscarinic responses described above.

D.C. potential changes induced by 1 minute agonist applications, were recorded using a grease-gap technique from the internal carotid nerve of rat desheathed, isolated superior cervical ganglia (Newberry et al, 1985). Concentration-depolarisation relationships were determined in a Krebs-bicarbonate medium containing 2.5mM $CaCl_2$, 3mM K^+ and 0.1μM TTX. To facilitate observation of the hyperpolarisation, the medium was altered so as to contain 0.1mM $CaCl_2$ and 0.3μM pirenzepine. (±) muscarine chloride was obtained from Sigma, (+) muscarine iodide (≃ 95% pure) was kindly supplied by Dr. D.B. Taylor (U.C. Santa Barbara) and pirenzepine dihydrochloride was a gift from Boots p.l.c.

The pEC_{50} (-log (EC_{50})) values (mean ± S.D, n) of (+) and (±) muscarine at depolarising the ganglion in (2.5mM Ca^{2+}) were 7.1 ± 0.1, 8 and 7.0 ± 0.1, 10, respectively. At an approximate EC_{50} concentration of 1μM, the (+) isomer was 1.3 ± 0.5 (n = 9) times more potent than the racemate at evoking the hyperpolarisation (in 0.1mM Ca^{2+}, 0.3μM pirenzepine).

Given that it is difficult to detect a two fold potency difference on this preparation, these results confirm the previous findings that (±) muscarine is approximately as potent as (+) muscarine and extend them to include two neuronal muscarinic responses in the rat.

Bowman, W.C. and Rand, M.J. (1980) "Textbook of Pharmacology" (2nd Edn.) Ch.10 p.28. Oxford, Blackwell.
Newberry, N.R., Priestley, T. and Woodruff, G.N. (1985) Eur. J. Pharmac., 116, 191-192.
Waser, P.L. (1961) Pharmac. Rev., 13, 465-515.

PHARMACOLOGICAL DIFFERENCES BETWEEN CHOLINESTERASES IN THE RAT SUPERIOR CERVICAL GANGLION (SCG) AND CEREBRAL CORTEX

K.E. Binns, S.B. Freedman, N.R. Newberry, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR

Cholinesterase (ChE) inhibitors potentiate the nicotinic response to acetylcholine (ACh) on the rat SCG (Brown 1966). We have compared the pharmacology of the ChE responsible for this effect with that of the ChE in cortical homogenates.

DC potential changes, to 1 minute applications of ACh, were recorded (at 25°C) from the postganglionic trunk of rat, isolated, desheathed SCG (Newberry et al, 1985). 1μM N-methylatropine and 0.1μM tetrodotoxin were routinely included in the superfusing medium. The concentration of ACh (x) equieffective to the control 3mM ACh response was determined after 90 min. in the presence of an enzyme inhibitor. Possible antinicotinic activity was monitored by a change in the response to 200μM carbachol. Cholinesterase activity in rat cerebral cortical homogenates was determined spectrophotometrically (Ellman et al, 1961).

Table 1 Pharmacological Activity of Cholinesterase Inhibitors

Compound	Cholinesterase in Cortex [A]		Nicotinic responses on Superior Cervical Ganglion[B]		
	IC_{40} (μM)	Max. Inhibition (%)	Concn. (μM)	Shift in ACh Potency (3mM/x)[C]	Carbachol Response[D] (%)
Neostigmine	0.1	100	10	96 (76,120)	100 ± 12
THA[F]	0.04	100	10	25 (21,30)	97 ± 15
BW284C51[G]	0.002	80	1	0.95 (0.71,1.2)	107 ± 13
Ethopropazine	-	24[E]	1	0.52 (0.41,0.66)	88 ± 2[H]
No drug	-	-	-	1.0 (0.82,1.5)	112 ± 4[H]

A: n = 3; B: n = 4-12; C: geometric mean (-s.e.m., +s.e.m.); D: mean ± s.e.m., pre-drug control = 100%; E: at 100μM; F: 9-amino 1,2,3,4 tetrahydroamino-acridine G: 1,5-bis-(allyldimethylammoniumphenyl)-pentan-3-one; H: significantly different (P = 0.05 level, ANOVA).

THA was more potent than neostigmine in inhibiting cortical cholinesterase activity, but neostigmine was a more effective potentiator of ACh on the SCG. Despite its high potency in the cortex, BW284C51 did not potentiate ACh in the intact SCG. Ethopropazine also failed to potentiate the response to ACh in the SCG, but it possessed antinicotinic activity. The last two results were interesting since, at 1μM, BW284C51 and ethopropazine were effective and selective inhibitors of acetylChE and butyrylChE, respectively, in homogenates of rat SCG (Vigny et al, 1978). Our results suggest that the enzyme(s) responsible for exogenous ACh breakdown in the intact SCG differ from those in homogenized preparations from SCG or cortex.

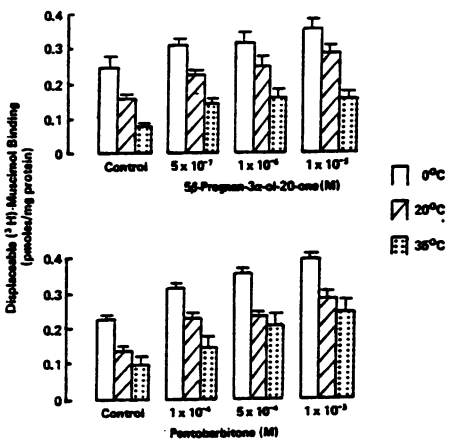
Brown, D.A., (1966) Brit. J. Pharmac., 26, 511-520.
Ellman, G.L., et al (1961) Biochem. Pharmac., 7, 88.
Newberry, N.R., et al (1985) Eur. J. Pharmac., 116, 191-192.
Vigny, M., et al (1978) Proc. Natl. Acad. Sci., (USA) 75, 2588-2592.

PENTOBARBITONE AND 5β-PREGNAN-3α-OL-20-ONE: MODULATORS OF THE $GABA_A$ RECEPTOR COMPLEX

J.L.W. Yau, D.J.K. Balfour & I.H. Stevenson, Department of Pharmacology & Clinical Pharmacology, University of Dundee Medical School, DUNDEE. DD1 9SY.

The binding of GABA to the $GABA_A$ receptor complex has been shown to be enhanced by both barbiturates and some endogenous steroids. (Whittle and Turner, 1982; Majewska et al, 1986). However, the mechanisms involved in these effects are not fully understood. The purpose of the present study was to examine the effects of temperature on the modulation of the $GABA_A$ receptor by steroids and barbiturates.

Crude synaptosomal membranes were prepared from the cerebral cortex of male Sprague-Dawley rats using the procedure of Olsen et al (1981). The effects of pentobarbitone and 5β-pregnan-3α-ol-20-one on (^{3}H)-muscimol binding to the $GABA_A$ receptors was measured at three temperatures (0°C, 20°C, 35°C) using the protocol described by Marangos and Crawley (1982). The (^{3}H)-muscimol concentration in each incubation was 5nM. Non-displaceable binding was measured in the presence of 100μM GABA. The results of the study, presented below, show that increasing the assay temperature decreased (^{3}H)-muscimol binding (F temperature (2,21) = 39; P<0.001). Both the steroid and the barbiturate increased displaceable ^{3}H-muscimol binding (F drug (3,27) = 113; P<0.001 and 291; P<0.001 respectively). The effect of the steroid was unaffected by the assay temperature whereas the interaction between the effect of pentobarbitone and the assay temperature was significant (F drug by temperature (6,27) = 4.3; P<0.01). Scatchard plots, using (^{3}H)-muscimol concentrations between 1 and 100nM indicated that the increased binding evoked by both compounds reflected an increase in the density of the binding sites rather than a change in their affinity for muscimol.



The results are means ± SEM of 4 experiments performed in triplicate.

It is concluded that both 5β-pregnan-3α-ol-20-one and pentobarbitone increase the apparent density of $GABA_A$ recognition sites in brain membranes but that the differences in temperature dependence suggest that the two compounds may exert their effects via different mechanisms.

Majewska, M.D. et al (1986). Science, 232, 1004-1007.
Marangos, P.J. and Crawley, J.N. (1982). Neuropharmacol. 21, 81-84.
Olsen, R.W. et al (1981). Fedn. Proc. 40, 309.
Whittle, S.R. & Turner, A.J. (1982). Biochem. Pharmacol., 31, 2891-2895.

SOCIAL ISOLATION IN THE RAT DOES NOT AFFECT BENZODIAZEPINE RECEPTOR BINDING

Delyth A. Rich, Vivienne Parker[1], P. Cariuk, A. Morinan[1] and R.W. Horton. Department of Pharmacology, St. George's Hospital Medical School, London SW17 ORE and [1]Department of Biological Sciences, North East Surrey College of Technology, Reigate Road, Ewell, Epsom KT17 3DS.

The socially isolated rat has been proposed as an animal model of anxiety. These animals exhibit a variety of behavioural deficits (reduced exploratory activity in a novel environment, increased hyponeophagia and longer escape time from an open cage) compared to rats housed in social groups (Parker & Morinan,1986). These behavioural deficits are attenuated by acute administration of various anxiolytic drugs, including benzodiazepines and ethanol (Morinan & Parker,1986). Since many anxiolytic drugs interact with the GABA receptor complex we have compared benzodiazepine (BZ) binding sites and the ability of GABA to stimulate BZ binding (a $GABA_A$-mediated effect) in several brain regions of isolated and social rats.

Male Sprague Dawley rats were weaned at 21 days of age and housed singly (isolated) or in groups of 4 to a cage (social) for a 21 day post-weaning period. On the following day, rats were killed by decapitation and the frontal cortex, hippocampus, amygdala and cerebellum dissected and frozen. Crude membrane fractions were prepared by repeated homogenisation and centrifugation and stored at -20°C. On the day of assay the membranes were washed 5 times with 50 mM Tris-citrate pH 7.1. The number and affinity of BZ binding sites was determined by saturation binding of [^{3}H]flunitrazepam (6 concentrations, 0.5-10 nM). GABA stimulation (9 concentrations, 0.1-100 μM) of [^{3}H]flunitrazepam (1 nM) binding was determined in the presence of 200 mM NaCl. Samples were incubated for 60 min at 0°C and specific binding was defined with 2 μM clonazepam.

The Bmax (Table 1) and affinity of BZ binding sites did not differ significantly between social and isolated rats in any of the 4 brain regions studied.

Table 1 The Bmax of BZ binding sites in the brains of social and isolated rats.

	social rats	isolated rats
frontal cortex	1.86 ± 0.07	1.80 ± 0.13
hippocampus	1.65 ± 0.08	1.71 ± 0.10
amygdala	2.06 ± 0.12	2.21 ± 0.12
cerebellum	0.93 ± 0.12	0.85 ± 0.05

Values are means ± s.e.m (pmole/mg protein) n = 6 cortex or n = 8 other regions.

The maximal % stimulation of BZ binding by GABA and the GABA concentration required for 50 % maximal stimulation of BZ binding did not differ significantly between social and isolated rats in any of the brain regions studied.

These results suggest that the behavioural deficits in isolated rats do not result from differences in the number or affinity of BZ binding sites or from differences in coupling of BZ and $GABA_A$ binding sites. These results do not preclude the possibility that functional differences in BZ binding sites may occur between social and isolated rats,perhaps mediated by differing concentrations of endogenous modulators for these sites.

Morinan, A. & Parker, V. (1986) Br. J. Pharmac. 89, 868P
Parker, V. & Morinan, A. (1986) Neuropharmacology 25, 663

FLUMAZENIL (RO 15-1788) REDUCES SINGLE-DOSE NITRAZEPAM TOLERANCE IN MICE

J.C. Garratt, J.P. Gent, J.R.M. Haigh & M. Feely, Departments of Pharmacology and Medicine, University of Leeds, Leeds LS2 9JT

Tolerance to the anti-pentylenetetrazole (PTZ) effects of benzodiazepines (BZ) is now well established, but both the degree of tolerance and its speed of onset vary considerably with different compounds. We have recently found that nitrazepam (NZ) produces marked tolerance which develops rapidly and maximally within 12 h after a single dose (Garratt et al., 1987). This provided a suitable tool to study the effects of the BZ antagonist flumazenil (RO 15-1788; FL) on the development of tolerance.

Convulsive threshold was assessed by measuring the minimum convulsant dose (MCD) of i.v. PTZ required to elicit a clonic convulsion in unrestrained mice (see Gent et al., 1986). NZ and FL were dissolved in vehicle and given s.c. 30 min (NZ) and 35 min (FL) before testing with PTZ, since the peak effect of NZ occurred 30 min after administration. Groups of 5 mice (adult male Tuck No. 1, 22-30 g in weight) were used, and results expressed as mean ± s.e. mean MCD of PTZ. Significance of differences between groups was assessed with Student's t-test.

Preliminary experiments had shown that a dose of 0.6 mg/kg NZ was submaximal, and that 4 mg/kg FL was the minimum dose required to block the effect of NZ completely; these doses were used throughout the experiments described here. The results are shown in Table 1.

Table 1. Effects of combinations of NZ and FL on MCD of PTZ

Group No.	Drug treatment at stated times before PTZ test				MCD of PTZ	Compared with group	Significance (P)
	36h	24h	12h	0.5h			
1	-	-	-	vehicle	38.1 ± 0.8	-	-
2	-	-	-	FL	38.6 ± 0.6	1	>0.1
3	-	-	-	NZ	109.3 ± 2.0	1	<0.001
4	-	-	-	FL+NZ	40.7 ± 2.5	1	>0.1
5	-	-	NZ	NZ	67.5 ± 2.2	3	<0.001
6	-	-	FL+NZ	NZ	100.3 ± 0.9	5	<0.001
7	-	FL	FL+NZ	NZ	107.1 ± 1.7	3	>0.1
8	FL	FL+NZ	NZ	NZ	69.6 ± 3.8	5	>0.1

NZ produced a marked anticonvulsant effect (group 3), which was completely blocked by FL (group 4). FL alone had no effect (group 2). Clear single-dose tolerance developed to NZ (group 5), but when the first dose of NZ was accompanied by FL, the subsequent tolerance to NZ was much reduced (group 6), although still significant (comparing groups 6 and 3, $P<0.01$). There appeared to be no single dose tolerance to FL since when this last sequence was preceded by FL alone (group 7) no significant tolerance to NZ was observed. Finally, the effect of FL seemed transient, since administration of NZ evoked single-dose tolerance even after prior administration of FL and FL+NZ (group 8).

Garratt, J.C., Gent, J.P., Feely, M. & Haigh, J.R.M. (1987) Eur. J. Pharmac., (in press).
Gent, J.P., Bentley, M., Feely, M. & Haigh, J.R.M. (1986) Eur. J. Pharmac., 128, 9-15.

2,6-DIISOPROPYLPHENOL POTENTIATES GABA-MEDIATED TRANSMISSION IN THE OLFACTORY CORTEX

G.G.S. Collins & J. Anson, University Department of Pharmacology and Therapeutics, Royal Hallamshire Hospital, Sheffield S10 2JF.

2,6-Diisopropylphenol (DIP) is an intravenous general anaesthetic recently introduced into clinical use. Little has been reported concerning its actions at a cellular level and so we decided to study its effects on synaptic transmission and the actions of transmitter candidates in the olfactory cortex.

Rat olfactory cortex slices (500μm thick) were preincubated and perfused at room temperature (Pickles & Simmonds, 1976) with salt solution containing 0.2% v/v dimethyl sulphoxide and the surface field potentials evoked on supramaximal stimulation of the lateral olfactory tract (50μs pulse width, 0.033Hz) recorded using a chlorided silver wire electrode. DIP (Aldrich Chemical Company) was dissolved in dimethylsulphoxide and diluted with the perfusion solution.

Perfusion of slices (n = 6) with DIP (10 to 200μM) caused a concentration-dependent, partially reversible increase in the area of the potential known as the late N-/I-wave complex, implying a potentiation of GABA-mediated transmission (Pickles & Simmonds, 1978). This possibility was explored in two further series of experiments:-

1. Using a twin-stimulus paradigm (Pickles & Simmonds, 1978), DIP (50μM) caused a small but consistent increase in the latency of the population spike evoked by the second of two stimuli (n = 3), indicating potentiation of GABA-mediated postsynaptic inhibition. Moreover, DIP (50μM) markedly potentiated the reduction in the amplitude of the potential known as the N-wave during the period of the late N-wave evoked by a preceding stimulus (n = 4) in a partially reversible manner, suggesting increased GABA-mediated presynaptic inhibition. This was confirmed by the finding that the percentage reduction in excitability of the lateral olfactory tract terminals (Cain & Simmonds, 1982) caused by GABA (0.33mM) of 17.8 ± 1.8 was significantly potentiated ($P < 0.01$) in the presence of DIP (50μM) to 51.8 ± 8.4 (mean percentage reductions in antidromic compound action potential amplitude ± s.e.mean, n = 6).

2. In slices perfused in such a manner that surface depolarizations evoked by exogenous agonists could be recorded (Brown & Galvan, 1979), DIP (50μM) shifted the log dose-response curve to GABA to the left in a parallel manner (n = 5). As similar shifts were observed in the dose-response curves to 3-aminopropanesulphonic acid and muscimol (n = 3), it is unlikely that DIP potentiates the actions of GABA by inhibiting its uptake. DIP alone (200μM) did not depolarize slices (n = 4).

These results show that at low concentrations, DIP potentiates GABA-mediated transmission in the olfactory cortex, probably by interacting with the GABA receptor complex. Although several other general anaesthetics also possess this property (Scholfield, 1980), it is unclear whether enhancement of GABA-mediated transmission is responsible for the depressant actions of these drugs in the central nervous system.

Brown, D.A. & Galvan, M. (1979) Br. J. Pharmac. 65, 347-353.
Cain, C. R. & Simmonds, M.A. (1982) J. Physiol. 332, 487-499.
Pickles, H.G. & Simmonds, M.A. (1976) J. Physiol. 260, 475-486.
Pickles, H.G. & Simmonds, M.A. (1978) J. Physiol. 275, 135-148.
Scholfield, C.N. (1980) Pflüg. Arch. 383, 249-255.

DEFEAT ANALGESIA IS POTENTLY INHIBITED BY COMPOUNDS 'SELECTIVE' FOR EITHER NEURONAL OR NON-NEURONAL BENZODIAZEPINE BINDING SITES

J.I. Randall* and R.J. Rodgers, Pharmacoethology Laboratory, School of Studies in Psychology, University of Bradford, West Yorkshire BD7 1DP, U.K.

The involvement of benzodiazepine (BZP) recognition sites in the mediation of non-opioid defeat analgesia in male mice has been suggested by the observation that the reaction is dose-dependently blocked by Ro15-1788 and diazepam (Rodgers & Randall, 1987). Follow-up studies have, however, failed to provide support for this hypothesis in that defeat analgesia is unaffected by even high doses of other BZP recognition site ligands (Randall & Rodgers, 1987). Ineffective compounds include chlordiazepoxide, midazolam and ZK93423 (full agonists) as well as CGS9896 and ZK91296 (partial agonists). Since diazepam has affinity for both neuronal and non-neuronal BZP sites, we have examined the effects of several compounds with preferential affinity profiles on the analgesic consequences of defeat experience. In this context, clonazepam has preferential affinity for neuronal sites whilst Ro05-4864 (4'-chlordiazepam) and PK11195 (1-(2-chlorphenyl)-N-methyl-N-(1-methyl-propyl)-3-isoquinoline carboxamide) display preferential affinity for non-neuronal sites. Furthermore, PK14067 and PK14068 (1- and d-enantiomers of N,N-diethyl-α-methyl-2-phenyl-4-quinolinepropanamide) have recently been shown to stereospecifically bind to non-neuronal sites, with only the former binding with high affinity (Dubroeucq et al, 1986).

10-18 week old male DBA/2 mice (Bantin & Kingman, Hull) were housed 10/cage in a temperature-controlled room (24±1°C) under a reversed 12hr light cycle. All testing was conducted under dim red light during the dark phase of the cycle. Nociceptive latencies were assessed by traditional tail-flick assay using a cut-off of 8 seconds. For defeat experience, intruder mice were individually placed into the home cage of an aggressive resident conspecific and removed immediately upon display of the species-typical upright submissive posture. All drugs were ultrasonically dispersed in distilled water to which Tween 80 had been added, and were administered intraperitoneally (i.p.; 10ml/kg) thirty minutes before testing. All compounds were tested over wide dose ranges on both basal nociception and defeat analgesia.

Statistical analysis (ANOVA) indicated that, with the exception of PK14068 which per se induced analgesia (2.5-10mg/kg, $p<0.001$), none of the compounds tested significantly altered basal tail-flick latencies. In defeat studies, all control groups showed pronounced analgesia ($p<0.001$), a reaction that was potently blocked by clonazepam (≤0.06mg/kg), PK11195 (≤5mg/kg, and by PK14067 (≤10mg/kg) but not PK14068 (1.25-20mg/kg). Although Ro05-4864 totally blocked the reaction at 2.5 and 20mg/kg, only partial inhibition was evident at intermediate doses. Present data clearly show that defeat analgesia in male mice is potently inhibited by ligands selective for either neuronal or non-neuronal BZP sites. However, they fail to explain why a number of other BZP ligands are unable to alter the reaction. It is suggested that a BZP site-independent action, common to all active compounds, is the most parsimonious interpretation of findings to date.

The work was supported by M.R.C. We thank Professor W Haefely and Dr G Richards (Hoffmann-La Roche, Basel) and Dr R Michaud (Rhone Poulenc Sante, Gennvilliers) for the kind gifts of compounds used.

Dubroeucq M-C et al (1986) Eur. J. Pharmacol., 128, 269-272.
Randall J.I. & Rodgers R.J. (1987) Br. J. Pharmac., 91, 346P.
Rodgers R.J. & Randall J.I. (1987) Psychopharmacology 91, 305-315.

EVIDENCE FOR DEFICITS IN CARDIAC FUNCTION IN VITRO AFTER KINDLING IN VIVO WITH THE ANXIOGENIC β-CARBOLINE, FG7142

S.C. Stanford*, D. Gettins & H.J. Little[1]. Department of Pharmacology, Middlesex Hospital Medical School, Cleveland Street, London WC1 and [1]Department of Pharmacology, University Walk, Bristol.

FG7142 is a partial inverse agonist at benzodiazepine receptors. A single injection of this drug has anxiogenic actions (Dorow et al., 1983; File et al., 1985) and lowers seizure threshold (but does not normally cause convulsions). Repeated administration of FG7142 causes "chemical kindling" and full convulsions are seen (Little et al., 1984), a procedure which also increases β-adrenoceptor number in mouse cerebral cortex (Stanford et al., 1986). In view of this effect on β-adrenoceptors and the association between chronic stress and cardiac dysfunction (Corley et al., 1977), we have investigated cardiac function ex vivo after kindling with FG7142.

Male Sprague-Dawley rats (initially 150-170g) were given FG7142, 20 mg kg^{-1} i.p. three times weekly for five weeks; animals showing at least one full seizure were classed as 'kindled'. Controls received vehicle injections only (Tween 80, 0.5%). Animals were killed, one week after their last injection, by cervical dislocation. The hearts were removed and cardiac function assessed using the Langendorff method: force of contraction, coronary perfusion pressure and heart rate were measured after administration of pulse doses of noradrenaline (NA, dose range: $1x10^{-11}$ - $5x10^{-8}$Moles).

Peak increase in force of contraction after NA (g):

Dose NA (Moles)	$1x10^{-11}$	$5x10^{-11}$	$2x10^{-10}$	$1x10^{-9}$	$2x10^{-9}$	$1x10^{-8}$	$5x10^{-8}$
Control (n=7)	0.6±0.3	1.4±0.2	2.9±0.4	3.2±0.7	3.0±0.4	3.3±0.3	3.4±0.4
Kindled (n=7)	0.4±0.3	0.9±0.1*	1.9±0.3*	2.2±0.3	2.2±0.4	2.5±0.5	2.1±0.4*

* $P < 0.05$, Student's t-test, (cf. controls). Values show mean ± s.e.m.

The hearts from animals kindled with FG7142 showed consistently lower inotropic responses to noradrenaline. There was also a difference in baseline force measurements: those of hearts from FG7142 treated animals were lower at all times (approximately 65% of controls). Both basal heart rate and peak chronotropic responses to noradrenaline were consistently lower in hearts from kindled animals, but the differences were small. Neither basal nor peak changes in coronary perfusion pressures after noradrenaline showed any consistent differences between the two groups. The deficit in myocardial function caused by FG7142 may be due to: the anxiogenic actions of FG7142, the convulsions produced by kindling, or peripheral actions of FG7142. Experiments are in progress to distinguish between these possibilities.

We thank Beecham's Pharmaceutical Division for FG7142.

Corley, K.C., Shiel, F., Mauck H., Clark L.S. & Barber J.H (1977) Psychophysiol 14, 322-328.
Dorow,R., Horowski, R., Paschelke,G., Amin,M. & Braestrup,C. (1983) Lancet 11, 98.
File, S.E., Pellow, S. & Braestrup, C. (1985) Pharmac. Biochem. Behav. 22, 941-944.
Little, H.J., Nutt, D.J. & Taylor, S.C. (1984) Brit. J. Pharmac. 83, 951-958
Stanford, S.C., Little, H.J., Nutt, D.J. & Taylor, S.C. (1986) Euro. J. Pharmac. 129, 181-184.

LOCAL CEREBRAL GLUCOSE UTILIZATION DURING ANXIETY AND SEIZURES INDUCED BY FG 7142

J.A. Pratt & D.J. Laurie, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW

We have previously demonstrated that the benzodiazepine receptor ligand FG 7142 increases local cerebral glucose utilization in specific components of the limbic system (File, McCulloch & Pratt, 1986). We now contrast alterations in function related glucose use during anxiety and convulsive states after FG 7142 administration using the quantitative ^{14}C-2- deoxyglucose technique (Sokoloff et al., 1977) in conscious male hooded rats.

Measurements were initiated 10 min after FG 7142 (1-10 mg/kg i.v.) or vehicle (1% Tween 20 in saline) and the rate of glucose utilization was determined in 65 brain regions. FG 7142 (3 & 10 mg/kg) effected increases in glucose utilization which were mainly confined to areas of the limbic system (Table 1). In some structures, such as the hippocampal molecular layer and the basolateral amygdala significant increases in glucose use were observed at doses of 3 mg/kg but not at 10 mg/kg. In 32 of the structures examined glucose use was unchanged.

Following the administration of FG 7142 (10 mg/kg), 30% of animals developed myoclonus and motor seizures. In these animals, increases in glucose use occurred in several structures of the limbic system, when compared to an equivalent non-seizure group. In addition, structures associated with motor control such as the sensory motor cortex, subthalamic nucleus and globus pallidus displayed significant increases in glucose use during seizures.

These data indicate that there is overlap between limbic structures important in anxiety, and seizure generation.

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

Structure	Vehicle (n=6)	FG 7142 (mg/kg) 3.0 (n=4)	10.0 (n=7)	10.0 (n=3) Seizure group
Anterior thalamus	108±5	143±11**	133±4*	144±8
Mammillary body	97±4	141±6**	130±4**	147±3^{+}
Dentate gyrus	70±2	88±6*	84±4*	94±3
Hippocampus:-				
CA 2 oriens	62±3	108±8**	77±5	98±8
CA 2 molecular layer	86±3	113±6**	90±3	96±5
CA 2 radiatum	60±3	76±3	67±2	71±3
Amygdala (basolateral)	81±3	101±8*	86±3	99±2
Amygdala (cortical)	63±5	76±9	69±2	87±6^{+}
Septum (medial)	76±3	103±7**	92±3*	117±10^{+}

Data are presented as mean glucose use (μmol/100 g/min) ± SEM
*P<0.05, **P<0.02, compared to vehicle, Bonferroni test.
$^{+}$ P<0.05, compared to non-seizure group (10mg/kg), Student's t-test.

We thank Ferrosan for FG 7142, the Wellcome Trust for financial support and Dr J McCulloch, University of Glasgow for the use of densitometric facilities.

File, S.E., McCulloch, J. & Pratt, J.A. (1986) Br.J. Pharmac 89, 871P
Sokoloff, L. et al. (1977) J. Neurochem 28, 897-916.

A RESPONSE TO CHOLECYSTOKININ ON SUPRAHYOID MUSCLE TWITCHING PARTLY MEDIATED VIA OPIATE RECEPTORS

C. R. Gardner, Roussel Laboratories, Kingfisher Drive, Covingham, Swindon, Wiltshire

In a pharmacological investigation of suprahyoid muscle twitching (SMT) in urethane-anaesthetised, nialamide-pretreated (50mg/kg i.p., 18h prior), male, Wistar rats it was observed that sulphated cholecystokinin octapeptide (CCK8, 5-100ug/kg i.v.) induced a biphasic response. Initially large amplitude twitches were induced but were replaced within 30 sec by a more consistent inhibition of twitching (with respect to pre-dose spontaneous activity). This inhibition was less marked on higher twitch-frequency baselines and slowly reduced over 8 - 22 min when control level was regained in most but not all preparations. Caerulein was more potent, with a minimal effective dose of lug/kg i.v. Responses were qualitatively similar on spontaneous twitching or on twitching induced by amphetamine, fenfluramine or picrotoxin.

Responses to either CCK8 or caerulein were inconsistently antagonised by proglumide at doses which could be administered intravenously (up to 10mg/kg i.v.), however, the inhibitory component was blocked by naloxone (0.5-2mg/kg i.v. n=8) (Fig. 1).

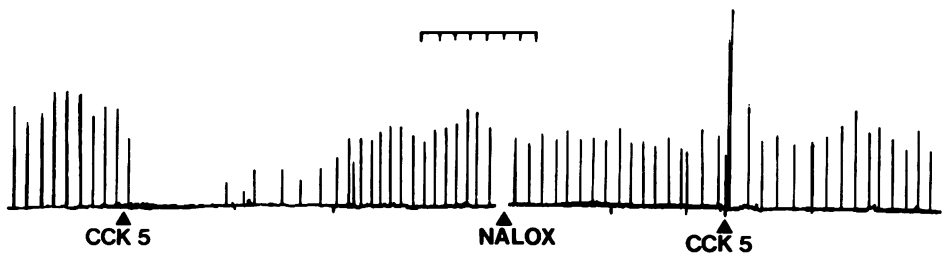


Figure 1. Effect of naloxone 2mg/kg (NALOX) on responses to CCK8 (ug/kg i.v) on SMT. There was a 12 min gap between traces. Time bars - minutes.

Benzodiazepines have been shown to antagonise central and peripheral effects of CCK8 (Bradwejn & de Montigny, 1984; Kubota et al., 1985b). In one study the benzodiazepine antagonist Ro 15-1788 antagonised CCK-induced analgesia (Kubota et al., 1985a). However, doses of the benzodiazepine agonist RU 32007 (N-ethyl loprazolam, 0.2-1mg/kg i.v. n=6) and the inverse agonist DMCM (0.5mg/kg i.v. n=4) which respectively induce sub-maximal decreases or increases in twitching, did not substantially alter the response to CCK8 or caerulein when control responses at similar rates of twitching were compared. Ro 15-1788 (2-4mg/kg i.v.) had little effect on twitching alone but fully antagonised RU 32007 and DMCM. These doses of Ro 15-1788 (n=4) also did not affect responses to CCK8 or caerulein.

Bradwejn, J. & de Montigny, C. (1984) Nature 312, 363-364.
Kubota, K. et al., (1985a) Jap. J. Pharmacol. 37, 101-105.
Kubota, K. et al., (1985b) Eur. J. Pharmacol. 110, 225-231.

AGENT ENHANCING GABA COUPLED CHLORIDE IONOPHORE. FUNCTION: EFFECTS IN A SOCIAL INTERACTION MODEL OF ANXIETY

A.P. Guy and C.R. Gardner, Roussel Laboratories, Wiltshire

It is currently considered that there is a supramolecular complex consisting of recognition sites for GABA ($GABA_A$ receptors), benzodiazepines and an allosteric site labelled by picrotoxin. This receptor complex gates a chloride ion channel (Olsen, 1981).

Recently a number of agents have been found to enhance chloride ionophore function by interaction with the picrotoxin recognition site as labelled by [^{35}S]TBPS (Ramanjaneyulu and Ticku, 1984). Six agents having such activity were tested in a social interaction (S.I.) model of anxiety in the rat (Guy and Gardner, 1985). The difference in median values from Control values (ranges of control data: S.I. = 20.5 - 31 secs; Locomotion [LOCO; counts on Varimex activity meter] = 410-560 counts during 5 min observation) were recorded for drug treated groups (n=8 pairs in all cases) Diazepam was tested as a reference anxiolytic.

DRUG	DOSE (mg/kg p.o. 60 mins before test)	CHANGE FROM CONTROL S.I. (secs)	LOCO (counts)
Diazepam	6	+29.5*	-25.0
Tracazolate	100	+26.0*	-89.0*
Tofisopam	50	+11.5	+5.0
Methaqualone	20	+29.0*	-80.0*
Etomidate	20	+23.0*	-120.0*
LY 81067	20	+10.0*	-35.0
Pentobarbitone	20	+19.5	-90.0*

All compounds tested increased S.I. and these changes were significant (* = $p < 0.05$ by Mann-Whitney "U" test) with the exception of tofisopam, which is in agreement with Pellow and File (1985). Locomotion was reduced by compounds displaying overt CNS depressant properties (methaqualone, etomidate and pentobarbitone) but also by the non-sedative tracazolate. In a relatively non-anxious condition in which animals were tested with their cagemates, none of these agents increased S.I. However, LOCO was still reduced by methaqualone and etomidate but not by tracazolate. This suggests that factors other than CNS depression may contribute to the reduction in LOCO observed in the anxious condition.

Guy A.P. and Gardner C.R. (1985) Neuropsychobiology 13(4): 194-200.
Olsen R.W. (1981) J.Neurochem 37(1): 1-13.
Pellow S. and File S.E. (1985). Drug Dev. Res. 7: 61-73.
Ramanjaneyulu R. and Ticku M.K. (1984). J. Neurochem 42(1): 221-229.

Gifts of etomidate (Janssen), LY 81067 (Lilly), tofisopam (Pierre Fabre) and tracazolate (ICI) are gratefully acknowledged.

USE OF A COMPUTER ASSISTED METHOD TO COMPARE THE PATTERNS OF BEHAVIOUR PRODUCED BY THE ANORECTIC DRUGS AMPHETAMINE AND FENFLURAMINE

G.E.Leighton,R.W.Marshall*,G.Hewson,R.G.Hill and J.Hughes.
Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge, CB2 2QB and *Department of Pharmacology and Therapeutics,University of Wales College of Medicine, Heath Park, Cardiff.

Drugs that reduce food intake can do so as a consequence of altering feeding behaviour in a variety of different ways(Blundell et al,1976),for example a drug can decrease food intake as a consequence of reducing the duration of each meal or by reducing the frequency of meals i.e. by prolonging postprandial satiety.A reduction in food intake can also occur as a result of nonspecific suppression of behaviour which is manifested as a reduction in locomotor activity and water intake in addition to alterations in feeding behaviour.A computer assisted method has been developed to measure and analyse feeding and drinking behaviour in rodents concurrently with measurements of locomotor activity.

The system consists of a rack of twelve polycarbonate cages each fiited with a detachable food hopper and water bottle holder. Infrared emitters and detectors are fitted to each of the food hoppers and water bottles so that each time the animal starts feeding or drinking the appropriate infrared beam is broken and remains broken until feeding or drinking ceases.The system is capable of monitoring both the number of visits the animal makes to the food hopper and/or water bottle(i.e the number of beam breaks) and the amount of time spent eating and/or drinking(i.e.the length of time for which the beam remains broken).Each cage is also bisected by a single infrared beam which is broken every time the animal moves around the cage and thus gives a gross measure of locomotor activity. Data from this system are collected and analysed using a B.B.C. microcomputer.The system is extremely flexible with regard to experimental design and data can be collected and analysed according to any one or more of the following components; locomotor activity,feeding frequency, feeding duration,drinking frequency,and drinking duration.

Results are shown (table 1) from an experiment in which the effects of fenfluramine and amphetamine (both given at doses known to suppress food intake)on different components of behaviour are compared. Amphetamine(1 mg/kg) or fenfluramine (3 mg/kg) were given subcutaneously to individually housed male rats (Lister-Hooded, 325-350g,Olac). In each experiment 6 saline treated animals were compared with 6 drug treated animals, values shown are means + SEM for activity measured over two hours.

Table 1.

	Saline	Fenfluramine	Saline	Amphetamine
Locomotor activity.	162.2+46.9	193.3+24.6	154.3+30.7	619.5+101.5*
No of visits to food hopper.	21.0+3.4	22.6+4.2	17.3+3.6	55.3+15.3*
Time spent feeding(mins).	23.6+4.1	9.1+4.7*	20.2+2.4	18.8+5.6
No of visits to water bottle.	15.6+3.9	26.5+3.4	16.8+4.4	47.7+12.5
Time spent drinking(mins).	1.7+0.1	0.3+0.1*	2.2+0.3	2.1+0.6

*$p<0.05$ compared to saline controls(Kruskall Wallis one way ANOVA).

The data shown here demonstrate the different patterns of behaviour produced by the anorectic drugs fenfluramine and amphetamine. Amphetamine stimulates locomotor activity and increases the number of visits the animals make to the food hopper and water bottle without affecting the amount of time spent either feeding or drinking whilst fenfluramine does not affect locomotor activity or the number of visits to the food hopper but does increase the number of visits to the water bottle and decreases the time spent feeding and drinking.

Blundell et al(1976)J.Pharm.Pharmacol:28:471-477.