BEHAVIOURAL ACTIVATION INDUCED BY SPECIFIC AND SELECTIVE α_2-ADRENOCEPTOR ANTAGONISTS IN THE RAT

S.L. Dickinson, B. Gadie and I.F. Tulloch, Department of Pharmacology, Pharmaceutical Division, Reckitt and Colman plc, Kingston upon Hull, HU8 7DS.

Centrally-acting α-agonists and antagonists induce behavioural changes in rodents. α1-Agonists elicit hyperactivity (Clineschmidt et al, 1979), whereas α2-agonists cause sedation and inactivity (Delini-Stula et al, 1979). Although α1-antagonists are reported to decrease activity (Anden et al, 1982), the behavioural effects of specific α2-antagonists remain to be clearly established. Non-specific α2-antagonists such as yohimbine and rauwolscine increase central dopamine (DA) turnover by an action at DA receptors (Scatton et al, 1983) and may thus influence behaviour directly through DA mechanisms. Idazoxan, a selective and specific α2-antagonist which does not affect central DA turnover (Scatton et al, 1983), was previously reported to enhance locomotor and exploratory activity of rats (Dickinson et al, 1986). The present study further investigates the behavioural actions of idazoxan and of the more potent and selective α2-antagonist RX811059 (2-ethoxy analogue of idazoxan, devoid of partial α1-agonist activity, Doxey et al, 1984) in both naive and habituated rats.

Locomotor and exploratory activities of single male Sprague-Dawley rats (250-350 g) were measured using infra-red sensors in an automated arena (60 x 60 cm). Rats were either a) non-habituated and injected in their home cage 10 min before being introduced into the activity arena or b) fully habituated for 120 min in the activity arena before injection.

Non-habituated rats displayed a steady decline (by 90%) of both locomotor and exploratory behaviours over a 60 min recording period. Neither idazoxan (3, 10 and 20 mg/kg,p.o.) nor RX811059 (1, 3 and 10 mg/kg,p.o.) had any effect on this pattern of behaviour (which represents the exploration of a novel environment).

Habituated rats, in contrast, showed consistently low activity levels. Idazoxan (20 mg/kg,p.o.) induced short-lasting behavioural activation (mean 50 min locomotor counts: vehicle, 2744; idazoxan, 7134; n=8, Mann-Whitney U-test, $P<0.05$). Exploratory behaviours were also enhanced. RX811059 (1 mg/kg,p.o.) also significantly enhanced locomotion (mean 50 min locomotor counts: vehicle, 1630; RX811059, 5522, n=8, U-test, $P<0.02$) although the effects on exploratory behaviour were less pronounced.

Selective α2-antagonists therefore induce moderate behavioural excitation but only under conditions of low level baseline activity. A possible mechanism for this behavioural activation may involve stimulation of postsynaptic α1-adrenoceptors subsequent to an α2-antagonist induced enhancement of noradrenaline (NA) release. Alternatively, a functional interaction between NA and DA systems in mesolimbic areas may be involved, although it should be emphasised that behavioural activation induced by α2-antagonists is of a lower magnitude than that observed with DA agonists or DA releasers and falls within the range normally observed in non-habituated rats.

Anden, N-E. et al. (1982). J. Neural. Trans. 55, 111-120.
Clineschmidt, B.V. et al. (1979). Arch. Int. Pharmacodyn. Ther. 242, 59-76.
Delini-Stula, A. et al. (1979). Naunyn-Schmiedeberg's Arch. Pharmac. 307, 115-122.
Dickinson, S.L. et al. (1986). Br.J. Pharmac., 89, 872P.
Doxey, J.C. et al. (1984). Br. J. Pharmac. 81, 181P.
Scatton, B. et al. (1983). Eur. J. Pharmac. 86, 427-433.

THE Ca^{2+} ANTAGONIST PN 200-110 INHIBITS [^{3}H]-DOPAMINE RELEASE FROM NIGRAL BUT NOT STRIATAL SLICES FROM ETHANOL-DEPENDENT RATS

C. Pagonis & J.M. Littleton, Department of Pharmacology, Kings College London, Strand, WC2R 2LS

We have shown that the dihydropyridine (DHP) Ca^{2+} channel "activator" BAY K 8644 potentiates depolarisation-evoked transmitter release to a greater extent in brain preparations from rats made tolerant to ethanol (Dolin et al, 1987a; Dolin et al, 1987b). We have also found DHP binding sites to be present in greater numbers in preparations from ethanol-tolerant rats (Dolin et al, 1987a). In agreement with Middlemiss & Spedding (1985), we found that the DHP Ca^{2+} channel "antagonists" on their own had no influence on depolarisation-induced release of transmitters (Dolin et al, 1987a), thus casting doubt on the physiological relevance of the DHP sensitive channels in this process. Recent evidence (Sanna et al, 1986) has suggested that DHP binding sites are in fact present on nerve cell bodies rather than nerve terminals. If this is the situation, then the DHP Ca^{2+} channel "antagonists" may produce a quantifiable effect if preparations rich in cell bodies are used as the experimental material. We have therefore investigated the effects of a potent DHP "Ca^{2+} antagonist" PN 200-110 on [^{3}H] Dopamine (DA) release from slices of substantia nigra and striatum from the rat brain.

Ethanol tolerance and dependence was induced in male Sprague-Dawley rats (250-300g) by inhalation, as described previously (Lynch & Littleton, 1983). Rats were killed by decapitation while still intoxicated and nigral or striatal slices were taken for estimation of [^{3}H] DA release using a superfusion system with desipramine (5×10^{-7} M) present. The stimulus for release was depolarisation with 25 mM K^+, presented in a low Na^+ medium to minimise the contribution of Na^+/Ca^{2+} exchange and hence expose the DHP-sensitive Ca^{2+} uptake (Turner & Goldin, 1985). Two periods of stimulation were used; S_1 and S_2, each lasting 4 minutes. The efflux of [^{3}H] was converted to a fractional release and the S_2/S_1 ratios compared. Drugs were added 20 minutes before S_2. All experiments were carried out under sodium lighting.

In control preparations the presence of PN 200-110 (5×10^{-7} M) produced a 26% inhibition of [^{3}H] release from nigral slices; S_2/S_1 dropped from 0.719 ± 0.058 to 0.534 ± 0.077 (n=5). This inhibition did not quite reach significance. In nigral preparations from ethanol-dependent rats the 31% inhibition of [^{3}H] release was significant at the $p < 0.02$ level; S_2/S_1 dropped from 0.711 ± 0.045 to 0.493 ± 0.046 (n=5). In striatal slices, however, no inhibitory effects of PN 200-110 were found in preparations from either group.

These experiments provide further evidence that DHP-sensitive Ca^{2+} channels are present on nerve cell bodies in greater number and/or a more active state than in nerve terminals.

Dolin S., et al (1987a) Neuropharmacology (submitted).
Dolin S., et al (1987b) Proc. Brit. Pharmac. Soc. Winter meeting (1986)
Middlemiss D.N. & Spedding M. (1985) Nature 314(7) 94-96.
Sanna E., et al (1986) J. Neurochem. 47(5) 1552-1557.
Lynch M.A. & Littleton J.M. (1983) Nature 303 175-177.
Turner T.J. & Goldin S.M. (1985) J. Neuroscience 5(3) 841-849.

GR38032F, A NOVEL 5-HT_3 RECEPTOR ANTAGONIST, CAN ABOLISH EMESIS INDUCED BY CYCLOPHOSPHAMIDE OR RADIATION IN THE FERRET

P.L.R. Andrews*, H.E. Bailey, J. Hawthorn, R. Stables[1] & M.B. Tyers[1], Department of Physiology, St. George's Hospital Medical School, London, SW17 ORE, and [1]Neuropharmacology Department, Glaxo Group Research Ltd., Ware, Herts., SG12 ODJ.

The most distressing side-effect of radio- and chemo-therapy treatment for cancer is prolonged nausea and vomiting. Currently no totally effective anti-emetic is available, although combinations of drugs have limited success. Advances have been made recently with the discovery that compounds acting on the $5HT_3$ receptors can inhibit vomiting invoked by cytotoxic drugs or radiation. Thus cisplatin-induced emesis is reduced by MDL72222 (Miner & Sanger, 1986) or BRL 24924 (Miner et al., 1986) and cyclophosphamide or radiation-induced vomiting is markedly controlled by BRL 24924 (Andrews et al., 1987). The novel $5HT_3$ receptor antagonist GR38032F (Brittain et al., 1987) was recently reported to inhibit cisplatin-induced emesis (Costall et al., 1987) and in this communication we describe its effects against emesis induced by cyclophosphamide or radiation in the ferret.

Albino or fitch ferrets of either sex (600-1800 g) were used. They were deprived of food overnight and then given 30ml of milk. GR38032F was injected subcutaneously in doses of 0.1-0.5mg/kg and 40 min later the animals were either injected with cyclophosphamide, 200mg/kg i.p. or exposed to a high dose of X-radiation (800 rads, 250 Kv, whole body); the animals were then observed for 4 or 2 h respectively. Results are summarised in Table 1.

Table 1 The inhibitory effects of GR38032F on cyclophosphamide- or radiation-induced emesis in the ferret

Treatment Dose mg/kg s.c.	No.of animals retching/tested	No.of retches (mean ± S.E.M)	No.of vomits (mean ± S.E.M)	Latency to vomit (min) (mean ± S.E.M)
Cyclophosphamide	5/5	52 ± 14	12 ± 2	19.8 ± 3.6
+ GR38032F 0.1	4/4	87 ± 31	12 ± 4	121.1 ± 29.9*
GR38032F 0.5	3/4	2 ± 1*	0	-
Radiation	4/4	62 ± 19	14 ± 3	20.3 ± 1.2
+ GR38032F 0.1	4/4	54 ± 14	11 ± 3	55.3 ± 6.2*
GR38032F 0.5	0/4	0	0	-

* $p < 0.001$

At 0.1mg/kg GR38032F delayed the onset of retching and vomiting but did not reduce their frequency. However, at 0.5mg/kg GR38032F abolished vomiting and markedly reduced retching to cyclophosphamide, and totally abolished both these responses when caused by radiation.

Abdominal vagotomy has a similar effect to GR38032F on the emetic response to radiation and cyclophosphamide (Andrews et al., 1986). It is possible, therefore, that GR38032F acts by blocking $5HT_3$ receptors situated presumably on the vagal afferent input to the emetic co-ordinating areas in the brain stem.

Andrews, P.L.R. et al. (1987) J.Physiol., 382, 47p.
Brittain, R.L. et al. (1987) Br.J.Pharmac., In press.
Costall, B. et al. (1987) Br.J.Pharmac., In press.
Miner, W.D. & Sanger, G.J. (1986) Br.J.Pharmac., 88, 497-499.
Miner, W.D. et al. (1986) Br.J.Pharmac., 88, 374P.

DIFFERENT ANTI-CANCER THERAPIES EVOKE EMESIS BY MECHANISMS THAT CAN BE BLOCKED BY THE 5-HT_3 RECEPTOR ANTAGONIST BRL 43694

E.A. Boyle*, W.D. Miner & G.J. Sanger, Beecham Pharmaceuticals Research Division, Coldharbour Road, The Pinnacles, Harlow, Essex. CM19 5AD

Aggressive anti-cancer cytotoxic therapy, using drugs such as cisplatin, induce severe nausea and vomiting. Single, high, intravenous doses of metoclopramide (Mcp; Maxolon, Beecham Pharmaceuticals) currently provide one of the best means of protection against cisplatin-induced emesis (Gralla et al, 1981).

We have now extended our original proposal that this action of Mcp may be due to 5-HT_3 receptor antagonism (Miner & Sanger, 1986; Miner et al, 1986). For this study, we have used the novel 5-HT_3 receptor antagonist BRL 43694 (Fake et al, 1987), to look for an involvement of 5-HT_3 receptors in the mechanisms of emesis evoked by cisplatin and other cytotoxic drugs or by X-irradiation.

Male ferrets (1-2kg) were used. Routinely, compounds were given via venous cannulae (Miner & Sanger, 1986) and the ferrets were monitored for the onset of vomiting (latency period) and the number of emetic episodes over 120min (post X-irradiation) or 240min (post cytotoxic drugs). BRL 43694 was given by divided dose, 30min before and 45min (cisplatin) or 30min (doxorubicin and cyclophosphamide) after the emetic stimulus. A single injection of BRL 43694 was given 5min before 10min exposure to an X-ray source (approx. 300r min^{-1}; Machlett model OEG-50; 50kV, 20mA, tungsten anode). The results show that BRL 43694 potently inhibits emesis evoked by different cytotoxic drugs or by X-irradiation.

Emetic stimulus	BRL 43694 mg kg^{-1} i.v.	Latency period (min)	Emetic Episodes	N
Cisplatin	0	66.3±5.6	14.2±1.8	4
10mg kg^{-1}	2 x 0.5	***240.0±0	***0	4
	2 x 0.05	***230.5±9.5	***0.5±0.5	4
	2 x 0.005	***183.8±32.6	**1.0±0.6	4
Doxorubicin/ Cyclophosphamide	0	37.1±6.2	24.9±3.5	7
6/80mg kg^{-1}	2 x 0.5	***222.5±8.8	***1.0±0.4	4
X-Radiation	0	19.4±1.1	28.8±1.3	5
	0.5	***120±0	***0	5

means ± SEM Student's 't' test; **P<0.01; ***P<0.001; N=Group size
Note: If the ferret did not vomit, latency period was taken as equal to the observation period (120 or 240 min)

For each emetic stimulus tested, a single dose of BRL 43694 0.5mg kg^{-1} arrested vomiting within 15-60s when given during an emetic episode. In the irradiation studies, an oral dose of BRL 43694 0.5mg kg^{-1} given 60min before exposure, greatly reduced emesis (2.3 ± 2.3 episodes; n=3, P<0.01) compared with controls (26.0 ± 3.5 episodes; n=7), and extended the latency period from 21.0 ± 0.8 to 104.7 ± 15.3 min (P<0.001). These experiments therefore demonstrate the anti-emetic efficacy of BRL 43694 and suggest that 5-HT_3 receptors play a fundamental role in the mechanisms of emesis evoked by different stimuli.

Fake, C.S., King, F.D. & Sanger, G.J. (1987). This meeting
Gralla, R.J. et al (1981). New Eng. J. Med. 305, 905-909.
Miner, W.D. & Sanger, G.J. (1986). Br. J. Pharmac., 88, 497-499.
Miner, W.D., Sanger, G.J. & Turner, D.H. (1986). Br. J. Pharmac., 88, 374P.

EVIDENCE TO SUGGEST THAT REDUCED ANALGESIC EFFICACY OF MORPHINE MAY REFLECT TOLERANCE TO ENDOGENOUS OPIOIDS IN AGED MICE

C.A. Hendrie (introduced by RJ Rodgers), Pharmacoethology Laboratory, School of Studies in Psychology, University of Bradford, BD7 1DP, England.

In view of reported decreases in aged animals of levels of beta-endorphin and concentrations of opiate receptors in regional and whole brain assays (for review see Kramer and Bodnar, 1986) it has been suggested that reduced responsivity to the analgesic effects of morphine may be due to age-dependent alterations in the integrity of endorphinergic mechanisms. However, as many of these age-related changes in endorphinergic function have been reported in rats little is currently known concerning the influence of age on opiate manipulations in mice.

32 10-12 week old and 32 42-44 week old male DBA/2 mice (Bantin and Kingman, Hull) were injected in randomised counterbalanced order with either 0.9% saline, 1mg/kg naloxone, 10mg/kg naloxone or 5mg/kg morphine sulphate (n=8/gp). All injections were performed intraperitoneally in a volume of 10ml/kg. 15 mins after naloxone or 30 mins post-morphine injection tail-flick latencies were recorded with animals then being placed in an observation arena. Behaviour was recorded on videotape for the next 5 mins with the resultant data being analysed in detail by a trained observer who remained blind to drug and age condition throughout. Tail flick data (+/- SEM) are presented below.

	Saline (0.9%)	Naloxone (1mg/kg)	Naloxone (10mg/kg)	Morphine (5mg/kg)
10-12 wk	6.1($\pm$0.32)	6.2($\pm$0.36)	6.4($\pm$0.41)	14.5($\pm$0.4)***
42-44 wk	6.3($\pm$0.7)	6.2($\pm$0.6)	4.2($\pm$0.7)*	6.8($\pm$0.5)

*p < 0.05 ***p < 0.001

Analysis of Variance and follow-up Dunnett's 't' tests revealed significant analgesia in 10-12 wk old animals treated with morphine ($t(28)=12.02$, $p<0.001$) but not in 42-44 wk old animals ($t(28)=0.96$,ns). Conversely, 10mg/kg naloxone induced significant HYPERalgesia in 42-44 wk olds ($t(42)+2.8$,$p<0.05$) yet failed to do so in 10-12 wk old mice ($t(42)=0.014$,ns). Importantly behavioural analysis further revealed the highest dose of naloxone to also induce significant increases in body shaking. These data together indicate there to be a significant influence of age on responsivity to morphine and naloxone with (1) 10-12 wk old animals displaying marked morphine analgesia but no influence of naloxone on nociception and (2) morphine failing to induce analgesia in 42-44 wk old mice yet naloxone inducing significant hyperalgesia, a finding which seems to indicate the functional integrity of endorphinergic systems in these animals. Evidence indicates that mice chronically exposed to opioid activating agonistic encounters display tolerance to the analgesic effects of such environmental stimuli (Hendrie, 1985) an effect which is bi-directionally cross-tolerant with morhpine (Rodgers and Randall, 1985). Further, levels of spontaneous intracage fighting have been found to be of sufficient intensity to activate opioid mediated analgesia mechanisms (Hendrie, unpublished observations). Therefore, current data indicating a lack of morphine analgesia yet naloxone induced hyperalgesia and body shaking may be more indicative of tolerance to endogenous opioids in aged mice than a compromised integrity of endorphinergic mechanisms per se.

Hendrie CA in Multidisciplinary approaches to conflict and appeasement in animals and man (ed F. Le Moli) Univ. Parma Press 50 (1985)
Kramer E and RJ Bodnar Neurobiol Aging 7: 185-191 (1986)
Rodgers RJ and JI Randall Pharmacol.Biochem.Behav. 23: 883-887 (1985)

ANXIOGENESIS FOLLOWS ABSTINENCE WITHDRAWAL FROM LONG-TERM TREATMENT WITH DIAZEPAM BUT NOT GR38032F

B. Costall, A.M. Domeney, M.E. Kelly, R.J. Naylor & M.B. Tyers[1], Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP, and [1]Neuropharmacology Department, Glaxo Group Research Ltd., Ware, SG12 0DJ.

In rodent and primate models, the anxiolytic activity of GR38032F (Costall et al, 1987a; Jones et al, 1987) differs from the benzodiazepines (BDZ) in that it does not interact with BDZ binding sites but has highly selective antagonist activity for $5HT_3$ receptors (Brittain et al, 1987). In the present study the effects of GR38032F and diazepam following repeated dosing and subsequent withdrawal in the mouse, rat and marmoset were investigated.

The studies used mice, rats and marmosets placed in situations where anxiety changes could be measured. Mice were placed in a box separated into a black, dimly lit area, and white brightly illuminated area. Naive mice taken from the dark were placed in the white compartment with access to the black section enabled via an opening in the dividing partition. Reduced anxiety was observed as increased behavioural responding (rearings and line crossings) in the white section, whilst anxiogenesis was seen as increased behaviour in the black section (Costall et al, 1987b). In the rat, anxiety changes were assessed in a modified social interaction test (File, 1980), increased interaction being characteristic of reduced anxiety, with reduced interaction during anxiogenesis. A 'human threat' test was employed to assess anxiolytic and anxiogenic action in the common marmoset (Costall et al, 1987a).

Anxiolytic action was sustained on long-term treatment with diazepam, (0.25 to 10mg/kg) and with GR38032F (0.001 to 0.1mg/kg) (7-14 days, twice daily i.p. administrations), with tolerance developing to the sedative effects of 10mg/kg diazepam (3-4 days). Reduced anxiety was seen as increased activity in the white area of the mouse test box (120-180%, $P<0.001$), increased rat social interaction (up to 300%, $P<0.001$) and increased time spent by marmosets in direct confrontation with the experimenter (up to 350%, $P<0.001$), with reduced aggressive postures (reduced by up to 100%, $P<0.001$). On abrupt abstinence withdrawal from diazepam the most dominant behavioural feature observed was anxiogenesis, with particularly marked changes in the rodent (for example, activity in the black section of the mouse test box increased by some 300%); such anxiogenesis was apparent within 8h of the administration of the last dose of diazepam, and persisted for up to 96h. In contrast to these findings, abrupt withdrawal from similar treatments with GR38032F was followed by a slow waning of the anxiolytic action, with a return of anxiety responding to normal control values within 24-48h. Observation of animal behaviour for up to 10 days following abrupt withdrawal from continued treatment with GR38032F failed to reveal any withdrawal anxiogenesis.

It is therefore concluded that GR38032F is not only a potent anxiolytic agent in rodent and primate, lacking sedative potential (Brittain et al, 1987), but that this compound can maintain an anxiolytic profile on long-term use without the withdrawal anxiogenesis on abrupt cessation of treatment observed following treatment with diazepam.

Brittain, R.T., Butler, A., Coates, I.H. et al. (1987) Br.J.Pharmac. 90, 87P.
Costall, B., Domeney, A.M., Hendrie, C.A. et al. (1987a) Br.J.Pharmac. 90, 257P.
Costall, B., Hendrie, C.A., Kelly, M.E. & Naylor, R.J. (1987b) Neuropharmacol. 26, 195-200.
File, S.E. (1980). J. Neurosci. Meth. 2, 215-238.
Jones,B.J., Oakley,N.R. & Tyers,M.B. (1987) Br.J.Pharmac. 90, 88P.

EFFECTS OF BECLAMIDE IN VITRO ON D_2, 5-HT_1 AND 5-HT_2 BINDING SITES

N.A. Darmani, P.J. Nicholls, J. Stolz and R.D.E. Sewell, Welsh School of Pharmacy, UWIST, PO Box 13, Cardiff CF1 3XF

The antiepileptic agent, beclamide, is used in the treatment of behavioural disorders which are a prevalent feature where mental handicap and epilepsy co-exist (Sime and Easby, 1974). One approach to management is treatment with neuroleptics, although their clinical value is restricted by side-effects. In contrast beclamide has fewer side-effects and a wide margin of safety (Hawkes, 1952).

Behavioural disorder patterns of the above type are normally associated with changes in monoamine function in the CNS (Wise et al. 1972). In this context, it is noteworthy that beclamide causes a 3-fold reduction in steady state levels of dopamine (DA) in the striatum of rats (Darmani et al. 1986); together with a 3-fold increase in the concentrations of striatal homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) and in the turnover of DA at this site. In addition, the drug also causes a marked reduction in the levels of striatal 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA). The present study was undertaken to determine whether such changes are due to effects of beclamide on the various receptors for these monoamines.

Male Wistar rats were decapitated and the striatum (^{3}H-spiperone for D_2 binding assays), hippocampus (^{3}H-5-HT for $5HT_1$ binding assays) and frontal cortex (^{3}H-spiperone for $5HT_2$ binding assays) were homogenised according to published methods (O'Boyle and Waddington, 1984; Stolz et al. 1983) with slight modifications. For Scatchard analysis various concentrations of binding ligands were incubated with beclamide (10μM) and, for ligand displacement effects, various concentrations of beclamide (1nM-10μM) were used in the presence of the binding ligands (1nM). Non-specific binding was defined as that remaining in the presence of 1μM butaclamol, 1μM 5HT or 100μM 5HT for D_2, $5HT_1$ and $5HT_2$ assays respectively.

Table 1: Effects of beclamide on radioligand binding parameters at 5HT & D_2 sites.

	$5HT_1$		$5HT_2$		D_2	
	Kd(nM)	B_{max} (fmol/mg Prot.)	Kd(nM)	B_{max} (fmol/mg Prot.)	Kd(nM)	B_{max} (fmol/mg Prot.)
Control	2.64±0.52	187 ± 36	1.2 ±0.19	270 ± 7.8	0.32±0.003	255 ± 6.4
Beclamide (10μM)	2.5 ±0.56	188 ± 42	1.08±0.12	258 ± 10.4	0.3 ±0.015	247 ± 0.33

Values are means ± sem, n=4. P>0.05.

The results presented in Table 1 indicate that in vitro beclamide does not displace the binding ligands and lacks affinity for D_2, 5-HT_1 and 5-HT_2 sites. Since in acute experiments beclamide increases DA turnover and decreases 5-HT and 5-HIAA levels, it is likely that presynaptic release mechanism(s) rather than receptor occupation is involved in the action of this drug. However, it is possible that the drug may also affect adrenoceptor function and this is being currently investigated.

Darmani, N.A. et al. (1986) Br. J. Pharmac. 88, 323P.
Hawkes, C.D. (1952) A.M.A. Archives of Neurology and Psychiatry. 67, 815.
O'Boyle, K.M. and Waddington, J.L. (1984) Eur. J. Pharmac. 98, 433-436.
Sime, D.A. and Easby, P.D. (1974) Br. J. Mental Subnorm. XX, part 2, No. 39.
Stolz, J.F. et al. (1983) Psychopharmacology, 80, 150.
Wise, C.D. et al. (1972) Science 177, 180.

BEHAVIOURAL ANALYSIS OF CHRONIC DOPAMINE D_1 RECEPTOR BLOCKADE IN MICE

K.F. Rooney, R.D.E. Sewell and P.S.J. Spencer, Division of Pharmacology, The Welsh School of Pharmacy, UWIST, PO Box 13, Cardiff CF1 3XF

Chronic administration of the reportedly selective D-1 dopamine antagonist SCH 23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol) has been shown not to produce an up-regulation of D-2 receptors in both rat and mouse striatum over a wide dose range (Creese and Chen, 1985; Rooney et al. 1986). It has however been shown that SCH23390 paradoxically attenuates D-2 induced behaviours in rats (Pugh et al. 1985) which may reflect a functional interaction between the receptor sub-types. The present study examines the effect of chronically administered haloperidol and matched doses of SCH23390 upon D-2 induced behaviours in the mouse.

Male GB1 mice (25-30g commencing weight) received either haloperidol (10mg/kg), SCH23390 (10mg/kg) or saline vehicle, injected once daily for a period of 14 days. Three days after cessation of chronic drug treatment, animals were challenged with either 2mg/kg or 5mg/kg apomorphine injected subcutaneously. Locomotor activity was recorded for 20 minutes post-injection in photocell activity boxes. Stereotyped behaviour was measured using a 6-point scoring system slightly modified from Randall (1985). Animals were observed for 20 second periods at 5 minute intervals for 25 minutes following apomorphine administration.

Table 1

Chronic treatment	Apomorphine-induced locomotor activity count		Apomorphine-induced stereotypy score	
	2mg/kg	5mg/kg	2mg/kg	5mg/kg
SALINE	69.1±4.1	82.8±13.6	6.6±0.4	8.5±0.5
HALOPERIDOL (10mg/kg)	84.9±5.3*	163.1±29.4@	10.5±1.4*	12.0±0.9*
SCH23390 (10mg/kg)	70.3±8.0	72.9±12.0	6.6±0.3	8.2±0.4

n = 10 * = P<0.05 @ = P<0.01

Unlike haloperidol, chronic administration with SCH23390 at the 10mg/kg dose level produces no significant change in apomorphine-induced locomotor activity or stereotyped behaviour. Recently, Hess et al. (1986) reported increases in both stereotypy and locomotor activity in rats challenged with the selective D-2 agonist LY171555 following pretreatment with SCH23390 0.5mg/kg/day for 21 days. Bearing in mind that at the time of testing residual D-1 receptor blockade may persist since the dose of SCH23390 employed was 20 times higher than that used by Hess et al, the difference in these reports may arise from the ability of SCH23390 to functionally diminish D-2 induced behaviours.

This study supports previously reported radioligand binding studies which showed no increase in D-2 receptor density following prolonged D-1 receptor blockade, although caution should be exercised in predicting the beneficial effects of SCH23390 in the clinical situation.

Creese, I. and Chen, A. (1985) Eur. J. Pharmac. 109, 127-128.
Hess, E.J. et al. (1986) J. Pharmac. Exp. Ther. 238, 846-854.
Pugh, M.T. et al. (1985) Psychopharmacology 87, 308-312.
Randall, P.K. (1985) Life Sci. 37, 1419-1423.
Rooney, K.F. et al. (1986) Br. J. Pharmac. 89, 633P.

THE 5-HT_{1A} AGONIST 8-OH-DPAT INCREASES SWEETENED MILK CONSUMPTION IN RATS

J. Coughlan,* C.T. Dourish, F. Gilbert and S.D. Iversen, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, CM20 2QR.

The 5-HT_{1A} agonists 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), buspirone, ipsapirone and gepirone elicit feeding in satiated rats (Dourish et al, 1985, 1986,1987). Feeding induced by 8-OH-DPAT is due to activation of somatodendritic autoreceptors located on 5-HT neurones in the raphe nuclei. (Dourish et al, 1986). Recently, it has been suggested that 8-OH-DPAT-induced feeding may be a non-specific chewing response as 8-OH-DPAT reportedly has no effect on the consumption of palatable sucrose or saccharin solutions (A.J.M. Montgomery, personal communication). The present study examines the effects of 8-OH-DPAT on consumption of another palatable solution, sweetened milk. Male Sprague-Dawley rats (250-350g) were housed individually and habituated over 8 days to drinking a palatable liquid diet (1 part Nestles Sweetened Condensed Milk; 4 parts tap water), which was presented in a 100ml bottle, for 30 min each day. By the end of the habituation period, consumption of the milk was at an asymptotic level. The animals were allocated at random to 6 equal groups which consisted of saline or 8-OH-DPAT at doses of 10, 30, 60, 250 or 500 µg/kg s.c. (n=8/9 rats per group). The animals were allowed to consume some of the liquid diet to achieve partial satiation before 8-OH-DPAT was administered. Thus, the animals were given the diet for 10 min, it was then removed, the drug or vehicle injected (s.c. in the neck) and 10 min later the liquid diet returned. Milk consumption was measured over the next 30 min. ANOVA revealed a significant effect of 8-OH-DPAT on milk consumption [$F(5,44)= 18.75$, $p<0.01$]. The 8-OH-DPAT dose response curve had an inverted U shape, such that 10-30µg/kg increased milk intake whereas 250-500µg/kg of the drug decreased consumption relative to controls. The maximal increase in consumption was produced by 30µg/kg 8-OH-DPAT (Saline, 5.7 ± 0.8; 30µg/kg 8-OH-DPAT 10.32 ± 0.8; $p<0.01$, Dunnett's test). The decrease in milk consumption produced by high doses of 8-OH-DPAT was probably due to the induction of stereotypy, as previously reported (Dourish et al, 1985).

The present data show that a low dose of 8-OH-DPAT can significantly increase consumption of a palatable liquid diet in rats. This observation suggests that 8-OH-DPAT-induced hyperphagia is not simply a reflection of increased chewing behaviour, but may represent an effect of the drug on 5-HT-mediated satiety mechanisms.

Dourish, C.T. et al (1985) Psychopharmacology, 86, 197-204.
Dourish, C.T. et al (1986) Appetite, 7 Suppl., 127-140
Dourish, C.T. et al (1987) This meeting.

EFFECTS OF THE CHOLECYSTOKININ RECEPTOR ANTAGONIST L364,718 ON FOOD INTAKE IN THE RAT

G Hewson, G E Leighton*, R G Hill and J Hughes
Parke-Davis Research Unit, Addenbrooke's Hospital Site, Hills Road, Cambridge. CB2 2QB

Studies showing that intraperitoneal administration of cholecystokinin (CCK) produces satiety in the rat led to the suggestion that endogenous CCK released by ingested food is part of the negative feedback mechanism that terminates eating and elicits postprandial satiety (Gibbs et al, 1973) If this hypothesis is correct, then a CCK receptor antagonist given alone should increase food intake. Proglumide, a weak antagonist at CCK receptors, has been found by some workers to increase food intake (Shillabeer & Davison, 1984) and by others to have no effect (Schneider et al, 1986). We have investigated the effects of the potent CCK receptor antagonist L364,718 (Chang & Lotti, 1986) on the intake of a palatable food in rats allowed free access to their normal diet. Using a different protocol, the ability of L364,718 to antagonise the reduction in food intake produced by cholecystokinin-octapeptide (CCK8) was determined in fasted rats.

Forty individually-housed male Wistar rats (starting weight 175-200 g) were trained to eat palatable food over a 30 min period using a method similar to that of Cooper et al (1985). On experimental days rats were injected with either L364,718 (10-100 ug/kg i.p.) or CCK8 (0.5-5 ug/kg i.p.), 30 min and 5 min before presentation of the palatable food, respectively. Statistical comparisons between the intake of the palatable food (g) of treatment groups with the appropriate controls were made using Kruskal-Wallis one-way ANOVA followed by the Mann-Whitney U-test (two-tailed). The ability of L364,718 (100 ug/kg i.p.) to antagonise the reduction in the intake of powdered food over a 30 min period produced by CCK8 (10 ug/kg i.p.) or bombesin (50 ug/kg i.p.) in 18 h fasted male Wistar rats (175-200 g) was also investigated.

L364,718 significantly increased the intake of the palatable food over the 30 min test period, whereas CCK8 produced a dose-related reduction in the intake of the palatable food (Table 1). In fasted rats, L364,718 antagonised the reduction in food intake produced by CCK8 without affecting the reduction in food intake produced by bombesin. L364,718 itself did not increase food intake in these animals.

L364,718	control	10 ug/kg*	30 ug/kg**	100 ug/kg**
g eaten	10.4±1.0	16.0±1.7	20.6±1.7	17.9±1.8
CCK8	control	0.5 ug/kg	1 ug/kg*	5 ug/kg**
g eaten	9.3±2.2	8.5±2.3	5.2±1.2	3.0±1.0

Significantly different from control *p<0.05, **p<0.01
Mann-Whitney U-test. Results are shown as mean±S.E.M. n = 8-10 rats per group.

It is concluded that L364,718 is a potent, selective antagonist of the effects of CCK8 on food intake. The observation that L364,718 increases the intake of palatable food provides some evidence that endogenous CCK is involved in the control of food intake in this model.

Chang R.S.L. & Lotti V.J. (1986) Proc. Natl. Acad. Sci. USA 83 4923-4926.
Cooper S.J. et al (1985) Neuropharmacology 24 877-883.
Gibbs J. et al (1973) J. Comp. Physiol. Psychol. 84 488-495.
Schneider L.H. et al (1986) Peptides 7 135-140
Shillabeer G. & Davison J.S. (1984) Reg. Peptides 8 171-176

INTERACTIONS BETWEEN 5-HT_{1A} AGONISTS ON FOOD INTAKE IN RATS

C.T. DOURISH and F. GILBERT*, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, U.K., CM20 2QR.

8-OH-DPAT, buspirone and ipsapirone (all putative 5-HT_{1A} agonists) dose-dependently increase food intake in rats (Dourish et al., 1986). It has been reported that buspirone and ipsapirone can block the hypothermic and motor effects of 8-OH-DPAT (Goodwin et al, 1986; Smith and Peroutka, 1986). Therefore, we examined interactions between these drugs, as well as the effects of gepirone, another putative 5-HT_{1A} agonist (Eison et al., 1986), on food intake.

Singly housed, normally fed male Sprague Dawley rats (250-300g) were injected s.c. with 1 or 2 drugs or saline between 9.00 and 11.00 a.m., replaced in their home cages and food intake was measured 2,4 and 24 h. after the last treatment. When two drugs were given, 8-OH-DPAT was always injected 30 min. after the other 5-HT_{1A} agonist.

Gepirone dose-dependently increased feeding 2 and 4 h. after injection (Table 1). The magnitude of the feeding response was larger than previously observed with any other 5-HT_{1A} agonist. Buspirone, ipsapirone and gepirone at doses of 2, 4 and 8 mg/kg did not inhibit feeding induced by 8-OH-DPAT (0.4-0.5 mg/kg). Indeed, ipsapirone tended to have an additive effect after 2 h. (3.0±0.3g with 8-OH-DPAT alone, 1.8±0.5g, 1.7±0.4g and 2.0±0.3g with ipsapirone alone at doses of 2, 4 and 8 mg/kg, respectively, and 4.0±0.5g, 4.2±0.5g and 4.0±0.6g when 8-OH-DPAT was injected after ipsapirone at doses of 2, 4 and 8 mg/kg, respectively). None of the drugs or combinations produced any significant alteration of the 24 h. food intake.

Table 1: Effect of gepirone on 2h. and 4h. food intake in free feeding rats

Treatment	n	2h. Food Intake (g)	4h. Food Intake (g)
Saline	18	0.8 ± 0.2	1.5 ± 0.3
Gepirone 0.4 mg/kg	9	1.2 ± 0.4	1.7 ± 0.3
0.8 mg/kg	8	1.2 ± 0.4	1.7 ± 0.3
1 mg/kg	9	2.8 ± 0.5 **	3.0 ± 0.5 *
2 mg/kg	9	3.9 ± 0.7 **	4.2 ± 0.8 **
4 mg/kg	9	4.3 ± 0.3 **	4.6 ± 0.3 **
8 mg/kg	8	5.6 ± 0.4 **	6.1 ± 0.4 **
16 mg/kg	8	5.6 ± 0.7 **	6.8 ± 0.9 **

Values: mean ± S.E.M. Differences from saline: * $p \leq 0.05$, ** $p \leq 0.01$ by Dunnett's test following ANOVA.

We conclude that gepirone is a potent appetite stimulant in rats which probably acts by activation of the raphe somatodendritic 5-HT autoreceptors (Dourish et al., 1986). Further, gepirone, buspirone and ipsapirone do not antagonise 8-OH-DPAT-induced hyperphagia suggesting that these drugs act as 5-HT_{1A} agonists in the feeding model.

Financial support from the "Fonds de la Recherche en Santé du Québec".

Dourish, C.T. et al., (1986) Appetite, 7, Suppl., 127-140
Eison, A.S. et al., (1986) Pharmacol. Biochem. Behav., 24, 701-707
Goodwin, G.M. et al., (1986) Psychopharmacology, 89, 382-387
Smith, L.M. and Peroutka, S.J. (1986) Pharmacol. Biochem. Behav., 24,1513-1519

D_2 DOPAMINE RECEPTORS IN STRIATUM ARE HOMOGENEOUS AS DEFINED BY ANTAGONIST BINDING

M.N. Leonard and P.G. Strange, The Biological Laboratory, The University, Canterbury, Kent, CT2 7NJ.

When studied using the ligand-binding technique with [^{3}H]antagonists, D_2 dopamine receptors generally appear to be a homogeneous population of sites. Sokoloff and colleagues have, however, presented evidence for a subtype of the D_2 dopamine receptor in rat striatum which shows a selectivity for certain substituted benzamide drugs (D_4 site) (Sokoloff et al, 1985). The D_4 sites have also been associated with specific behavioural responses in rodents (Martres et al, 1984). This is of considerable interest both from a theoretical point of view and for the design of new drugs. We have therefore investigated the nature of D_2 dopamine receptors in bovine striatum with particular reference to the interactions of substituted benzamide drugs.

D_2 dopamine receptors in bovine caudate nucleus membranes were labelled with [^{3}H]spiperone (0.25nM approx.) in the presence of 0.3μM mianserin to mask $5HT_2$ serotonin receptors as in Withy et al, 1981. Specific binding of [^{3}H]spiperone was defined by competition with 3μM (+)-butaclamol and bound radioligand was separated by rapid filtration through glass fibre filters. Specific binding was saturable and of high affinity (K_d 168pM, B_{max} 410 fmol/mg protein). Classical D_2 dopamine receptor antagonists competed for [^{3}H]spiperone binding with high affinity and stereoselectivity (K_i(nM), (+)-butaclamol (10) domperidone (7), (-)-butaclamol (>1000)) and their binding could be described as interaction at a single class of binding sites. Substituted benzamide drugs also competed with [^{3}H]spiperone binding in a similar manner (K_i(nM), clebopride (10), DO710 (65), sulpiride (122)) there being no evidence for heterogeneity of the binding sites.

Thus in bovine striatum we can find no evidence for the proposition that D_2 dopamine receptors can be subdivided into pharmacologically distinct subtypes.

Supported by Glaxo Group Research and SERC.

Martres, M.P. et al. (1984) Naunyn Schmiedebergs, Archiv. Pharmacol. 325, 102.
Sokoloff, P. et al. (1985) Naunyn Schmiedeberg's, Archiv. Pharmacol. 329, 236.
Withy, R.M. et al. (1981) J. Neurochem. 37, 1144.

ALTERATIONS IN THE CONTENT OF IRON AND OTHER METAL IONS IN PARKINSONIAN BRAIN

D.T. Dexter, P. Jenner & C.D. Marsden, MRC Movement Disorders Research Group, University Department of Neurology & Parkinson's Disease Society Research Centre, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

The cause of Parkinson's disease is unknown but results in an increase in indices of lipid peroxidation in substantia nigra but not in cerebellum and cortical tissue from Parkinsonian patients, when compared to tissue from age-matched control subjects (Dexter et al, 1986). Iron is a potent catalyst of lipid peroxidation and is contained in high concentrations within the substantia nigra. We now report on the levels of iron, copper, zinc, manganese and lead in substantia nigra, cortex (Brodmann 10) and cerebellum from Parkinsonian and control brains.

Tissue was obtained from patients with Parkinson's disease (mean age 75.7 years ± 1.6) and from control patients (mean age 72.4 years ± 1.4) dying without evidence of neurological disease. Average time between death and removal of brain tissue was 18.3 ± 2.4 h for Parkinsonian patients and 16.2 ± 2.0 h for control patients. Metal ions were solubilized by an adaptation of the method of Smeyers-Verbeke et al (1976) and measured on an ARL inductively coupled plasma (ICP) spectrophotometer.

All five metal ions were detectable in the three areas of control brain examined with iron being in the highest concentration in all regions (Table 1). In the cerebellum no differences in metal ion levels was observed in the Parkinsonian and control patients. Cortical levels of Mn, Pb, Zn and Cu were unchanged in Parkinsonian patients when compared to control patients. However, there was a small increase in iron levels in the Parkinsonian patients. However, while levels of Pb and Mn were not different from controls in the Parkinsonian substantia nigra, there was a marked increase in the levels of iron and Zn. In contrast, Cu levels were reduced in the Parkinsonian substantia nigra when compared to controls.

Table 1 Metal ion levels in the substantia nigra, cerebellum and cortex of Parkinsonian (PD) and control brains

Patient	Number in study	Metal ion level (nmols/g dry weight) Cu	Mn	Fe	Zn	Pb
Cerebellum						
Control	13	560 ± 48	38 ± 2	4676 ± 259	1393 ± 82	87 ± 13
P.D.	11	581 ± 62	41 ± 2	4896 ± 159	1610 ± 95	52 ± 14
Cortex						
Control	13	296 ± 18	32 ± 5	5067 ± 109	3250 ± 124	45 ± 4
P.D.	11	273 ± 18	28 ± 2	5575 ± 219*	3381 ± 179	37 ± 2
Substantia nigra						
Control	9	655 ± 59	32 ± 3	10436 ± 1191	956 ± 71	66 ± 10
P.D.	7	435 ± 51*	33 ± 2	14043 ± 1186*	1436 ± 210*	60 ± 6

Values expressed as mean ± SEM; * $p < 0.05$ compared to controls

We conclude that alterations occur in the levels of Fe, Zn and Cu in the Parkinsonian substantia nigra when compared to control subjects. The increase in iron content of substantia nigra may explain the elevated levels of indices of lipid peroxidation observed in Parkinsonian patients.

Dexter D.T. et al (1986) Lancet, No 8507 vol. II, 639-640
Smeyers-Verbeke J. et al (1976) Clinica Chimica Acta 68, 343-347.

INFLUENCE OF MEDIAL RAPHE NUCLEUS LESIONS ON BEHAVIOURAL RESPONDING TO DOPAMINE INFUSION INTO THE RAT AMYGDALA

Julie C. Barnes, Brenda Costall and R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, West Yorkshire BD7 1DP.

Bradbury et al (1985) showed that the unilateral infusion of dopamine (DA) into the rat amygdala can initiate locomotor hyperactivity, dependent upon cerebral hemispheric dominance as assessed behaviourally in a turn preference test. Barnes et al (1986a) subsequently showed biochemical correlates with inter-hemispheric imbalances in DA and serotonin (5HT) function. To investigate the influence of 5HT on amygdaloid DA function, we have examined the behavioural consequences of unilateral infusions of DA into the amygdala following an asymmetric electrolesion of the medial raphe nucleus (MRN), previously shown to cause disturbances in neurochemical asymmetry within the brain (Barnes et al 1986b). Male Sprague-Dawley rats were preselected in terms of left (LD) or right (RD) hemispheric dominance by measuring spontaneous turn preferences in an open field. Animals were subjected to standard stereotaxic surgery for asymmetric left or right electrolesion of the MRN (stainless steel electrode, 0.65 mm diameter, insulated except at tip, 1mA for 20s; Ant. 0.3, Vert. -2.6, Lat. ±0.2, electrode angled 65° posterior, altas of Konig and Klippel), and concomitant implantation of chronically indwelling cannulae for drug administration into the central area of the amygdala. 14-21 days after surgery, lesioned animals which showed a persistent circling behaviour, the direction dependent on the side of lesion (an indication of a sustained effective asymmetric MRN lesion), and sham control animals (which failed to circle) were subject to a unilateral infusion of DA or vehicle into the amygdala, effected via subcutaneously implanted Alzet osmotic minipumps, infusing at a rate of 0.48µl/h for 13 days. Spontaneous locomotor activity was assessed daily for 1h throughout the infusion.

In sham lesioned animals showing right hemispheric dominance (RD) the unilateral infusion of DA into the amygdala initiated an increased locomotor activity, which was most marked when the infusion was directed into the left (L) amygdala, reaching maximum counts/60 min of 149-198 on days 3, 6, 7 and 12 of infusion ($P<0.05-0.001$, control locomotor activity 74-98 counts/60 min), DA infused into the right (R) amygdala of RD animals initiating only a modest increase in activity on days 4-7 to counts/60 min of 148-154 ($P<0.05-0.01$). The unilateral infusion of DA into the L or R amygdala of LD animals failed to induce changes in locomotor activity throughout the 13 days (counts/60 min of 76-101, $P>0.05$). Asymmetric electrolesion of the MRN in the right hemisphere induced a persistent circling (4-6 revolutions/min) to the left (rendering animals RD) and subsequently inhibited the development of hyperactivity during a unilateral DA infusion into the L or R amygdala (counts/60 min of 65-104, compared with 148-198, $P<0.05-0.001$). Asymmetric electrolesion of the MRN in the left hemisphere initiated a contralateral circling behaviour (5-10 revolutions/min), rendering animals LD and subsequent unilateral infusion of DA into the L or R amygdala initiated an elevation of locomotor activity to counts/60 min of 131-238 (compared with 76-101 counts/60 min of sham control LD animals, $P<0.05-0.001$).

It is concluded that 5HT projections from the MRN exert a marked influence on the laterality of dopaminergic functioning in the amygdala.

This work was supported by the Medical Research Council.

Barnes et al (1986a) Br. J. Pharmac. In press
Barnes et al (1986b) Br. J. Pharmac. In press
Bradbury et al (1985) Neuropharmacol. 24, 1163-1170

EFFECT OF CHRONIC ROLIPRAM TREATMENT ON CLONIDINE-INDUCED BEHAVIOURAL ACTIVITY

D.K. Luscombe, M.R. Mustafa & J.F. Stolz[1], Welsh School of Pharmacy, UWIST, PO Box 13, Cardiff CF1 3XF and [1]School of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY.

Rolipram (ROL), in addition to being a selective inhibitor of phosphodiesterase III, has been shown to possess antidepressant activity in animals (Przegalinski & Bigajska, 1983) and man (Zeller et al, 1984). To examine whether this latter effect is associated with the down-regulation of central α_2-adrenoceptors we have determined the effect of ROL on clonidine - induced hypothermia and locomotor activity in rats (Pilc & Vetulani, 1982; Heal et al, 1983). The effects of both single and chronic doses of ROL have been compared with the phosphodiesterase inhibitors, isobutylmethylxanthine (IBMX) and ICI 63,197, and the tricyclic antidepressant, desmethylimipramine (DMI).

Male Wistar rats weighing 120-160 g at the start of treatment were injected intraperitoneally with either acute (single dose) or chronic (twice daily for 14 days) doses of ROL (5 mg/kg), ICI 63,197 (5 mg/kg), IBMX (5 mg/kg), DMI (10 mg/kg) or vehicle. 24 h after the last treatment, rats received intraperitoneal clonidine (0.1 mg/kg) or saline. Rectal temperature was measured using a thermister probe connected to a digital thermometer before and 60 mins after clonidine administration. Locomotor activity was measured using black photocell activity boxes for a period of 20 mins commencing 40 mins after clonidine dosing. All experiments were performed between 20.00 h and 24.00 h. It was found that none of the drugs administered acutely affected clonidine-induced hypothermia or reduced locomotor activity. Likewise, following chronic dosing with IBMX and ICI 63,197, the behavioural changes induced by clonidine were unaffected (Table 1). In contrast, chronic treatment with ROL and DMI antagonised clonidine-induced hypothermia by 53% and 35% and hypoactivity by 150% and 260%, respectively.

Table 1 Effect of chronic treatment with various drugs on clonidine-induced hypothermia and hypoactivity.

Drug	Change in temp. (°C)	Locomotor activity	n
Saline	1.70 ± 0.18	95 ± 22	9
DMI	0.82 ± 0.16**	329 ± 70**	6
IBMX	1.62 ± 0.21	120 ± 51	6
ROL	1.02 ± 0.22*	225 ± 28**	9
ICI 63,197	1.44 ± 0.30	171 ± 45	5

* $P < 0.05$; ** $P < 0.01$. Student's t-test

These results indicate that the antidepressant effect of ROL, like that of DMI, is associated with down-regulation of central α_2-adrenoceptors. The failure of ICI 63,197 and/or IBMX to antagonise clonidine-induced behavioural activity indicates that the antidepressant effect of ROL is independent of its phosphodiesterase inhibitor property.

We thank Schering and ICI for the gifts of rolipram and ICI 63,197, respectively.

Heal, D.J. et al (1983) Psychopharmacology, 22, 983-992.
Pilc, A. & Vetulani, J. (1982) Eur. J. Pharmac. 80, 109-113.
Przegalinski, E. & Bigajska, K. (1983) Pol. J. Pharmac. Pharm. 35, 233-240.
Zeller, E. et al (1984) Pharmacopsychiatry, 17, 188-190.

REGIONAL DIFFERENCES IN THE EFFECT OF NICOTINE ON CATECHOLAMINE TURNOVER IN THE RAT CNS

S.N.Mitchell, M.P.Brazell, M.H.Joseph and J.A.Gray.
MRC Brain, Behaviour and Psychiatry Research Group, Department of Psychology, Institute of Psychiatry, London SE5 8AF.

Various studies indicate that nicotine (NIC) stimulates catecholamine (CA) and indoleamine (IA) release in some areas of the rat CNS (Balfour, 1982), based largely on studies using in vitro preparations: slices and synaptosomes. If changes in CA or IA turnover occur in intact animals they may provide a functional index of CNS cholinergic/aminergic interactions. As part of a wider study we have examined the effect of acute NIC on the rates of CA and IA turnover in vivo, in a variety of rat brain regions, estimated by measuring the accumulation of DOPA and 5-HTP, following administration of the decarboxylase inhibitor, NSD-1015.

Male Sprague-Dawley rats (250-300g. Harlan Olac Ltd.) received NIC (0.4 and 0.8mg/kg free base, s.c.) or saline (1ml/kg, s.c.) 15 min. before NSD-1015 (100mg/kg,i.p) Rats killed 30 min. later; brains dissected (Heffner et al. 1980) into: frontal cortex, amygdala, hypothalamus (hyp.), hippocampus (hippo.), nucleus accumbens (n.acc.), globus pallidus, caudate/putamen, septal area and raphe nucleus; sonicated and stored below -40^{o}C until analysed by HPLC-EC.

Both 0.4 and 0.8mg/kg NIC produced a significant increase in DOPA accumulation in the n.acc. in comparison with saline (table 1). In the hyp. and hippo. only the high dose produced a significant effect. No significant change was seen in any other brain region examined, including the caudate/putamen. 5-HTP accumulations were not affected in any areas examined. This effect of NIC was not mediated by its major metabolite (-)-cotinine, as this was ineffective when administered s.c. at the same dose. The effectof NIC on DOPA accumulations was, however, attenuated by administration of an antagonist, mecamylamine, at 5mg/kg i.p.30min. prior to NIC.

DOPA accumulation (ng/g±SE$_{\bar{x}}$)

Dose (mg/kg)	Nucleus Accumbens	Caudate/ Putamen	Hypothalamus	Hippocampus
Saline	1671±146	2134±92	339±12	69±5
0.4	*2352±102	2355±130	**342±6	**82±7
0.8	**2733±236	2437±106	##399±17	**#103±7

(*P<0.05, **P<0.01 -v-saline; #P<0.05, ##P<0.01 -v-0.4mg/kg nicotine; Tukey's test, n = 6)

Nicotine is thus much more effective in stimulating CA turnover in the n.acc. than striatum. This is perhaps unexpected in view of the repeated epidemiological finding of a reduced incidence of Parkinson's disease in cigarette smokers (Baron,1986). Lichtensteiger et al. (1982) have also reported that acute NIC has only a modest effect on dopamine turnover in the rat striatum. In the hyp. and hippo. the increase in DOPA accumulations may reflect an effect on NA turnover. These studies are now being extended using this and complementary extracellular in vivo techniques to examine the effects of acute and chronic NIC administration on CA turnover and release in various brain regions.

Acknowledgements: We thank R.J. Reynolds for financial support.

Balfour, D.J.K. (1982) Pharmacol. Ther., 16: 269-282
Baron, J.A. (1986). Neurology, 36: 1490-1496.
Heffner, T.G. et al. (1980). Pharmacol. Biochem. Behav., 13: 453-456.
Lichtensteiger, W. et al. (1982). Neuropharmacol., 21: 963-968.

COMPARATIVE BINDING CHARACTERISTICS OF μ AND δ SELECTIVE LIGANDS IN MARMOSET AND RAT BRAIN

M. Yeadon and I. Kitchen, Department of Biochemistry, Division of Pharmacology and Toxicology, University of Surrey, Guildford, Surrey, GU2 5XH, U.K.

Opioid agonists produce a dose-related depression of respiration and acute toxicity studies show this to be the cause of death. It is also apparent that primates are more sensitive to this adverse effect than are rodents (Huang et al., 1984). The importance of μ and opioid receptor subtypes in brain areas involved in respiratory control has yet to be unequivocally demonstrated, and the possibility exists that species differences in respiratory depression may be linked with differences in receptor characteristics in pons/medulla regions of the brain. Accordingly, we have studied μ and δ binding in the rat and marmoset brain using the highly selective ligands ^{3}H -[D-Ala^{2}MePhe^4-Glyol^5]enkephalin (DAGO) and ^{3}H [D-Pen^2, D-Pen^5]enkephalin (DPDPE).

Male Wistar albino rats (200-300 g) were killed by cervical dislocation, and adult marmosets (350-450 g) were killed with halothane and CO_2. Binding assays were carried out in Tris HCl, pH 7.4 at 25°C for 60 minutes. K_D and B_{max} values were determined by Scatchard analysis and K_I values against μ and δ binding calculated from Hill plots. K_D values for ^{3}H DAGO in marmoset whole brain and pons/medulla were not significantly different from rat brain in equivalent areas (≅ 1 nM) K_D values for ^{3}H DPDPE although similar in both whole brain and pons/medulla in both species were 5-fold higher in rat brain (≅ 2 nM vs. 10 nM) indicating a lower affinity site (Table 1). Whilst the number of μ-sites were similar in rat whole brain and pons/medulla the latter region in marmoset was enriched by 50% compared with whole brain. The number of δ -sites were 3- and 4-fold lower in pons/medulla regions than whole brain in rat and marmoset respectively.

Table 1. Comparative binding of ^{3}H DAGO and ^{3}H DPDPE in rat and marmoset brain

		^{3}H DAGO		^{3}H DPDPE	
		Whole brain	Pons/medulla	Whole brain	Pons/medulla
RAT	B_{max}	167 ± 11	145 ± 6	149 ± 10	53 ± 7
	K_D	1.1 ± 0.2	1.5 ± 0.07	8.3 ± 0.6	10.3 ± 1.4
MARMOSET	B_{max}	75 ± 2	127 ± 10	74 ± 2	17 ± 1
	K_D	1.1 ± 0.2	0.8 ± 0.1	1.6 ± 0.3	2.4 ± 0.2

Each value is the mean ± s.e. mean of four determinations of B_{max} (fmol mg^{-1} protein) and K_D (nM).

Site selectivity for the opioid drug fentanyl was the same in both species, though affinities were greater in marmoset (rat K_Iμ 3.3 ± 0.5; K_Iδ 246 ± 41nM; marmoset K_Iμ 1.3 ± 0.4; K_I δ 127 ± 17nM). The K_Iμ value for carfentanil was similar in rat (0.42 ± 0.04nM) and marmoset (0.22 ± 0.08nM) but K_Iδ was 10-fold greater in marmoset than in rat (4.4 ± 0.4; 0.42 ± 0.02nM). It is clear that whilst the μ site exhibits similar ligand binding characteristics in rat and marmoset, the δ sites differ in affinity for opioid ligands. To what extent this may contribute to the differential sensitivity of rat and marmoset to opioid-induced respiratory depression remains to be elucidated.

Huang, Z.-M., Zhou, J., Chen, X.-J., Zheng, W.-J., Zhang, H.-P. and Chi, Z.-Q. (1984) Acta Pharmacologica Sinica, 5, 153-158.

A DEVELOPMENTAL STUDY OF κ-OPIOID INDUCED BEHAVIOUR IN THE RAT

Helen C. Jackson and I. Kitchen, Department of Biochemistry, Division of Pharmacology and Toxicology, University of Surrey, Guildford, Surrey, GU2 5XH, U.K.

Several workers have reported behavioural responses to the μ-opioid agonist morphine in infant rats. In contrast, the pharmacological effects of κ-agonists in these animals have received relatively little attention. It was the aim of the current study, therefore, to systematically examine behavioural patterns in 5, 10 and 20 day old rat pups treated with the selective κ-agonist U50,488H (Von Voigtlander et al., 1983).

Behavioural observations were carried out using a time-sampling method (Reinstein & Isaacson, 1977) whereby the behaviours of individually-housed Wistar rat pups (male and female) were recorded for 5 sec every 60 sec over a 60 min testing period. Drugs were administered intraperitoneally in a dose volume of 0.1 ml 20 g^{-1} body weight using a blind protocol. Behavioural scores represented the total number of instances in which each behaviour was observed during the 60 min interval. Mean treatment group scores (n = 8) were statistically compared using non-parametric oneway analysis of variance (Kruskal-Wallis) followed by the Mann-Whitney U test.

U50,488H (1 mg kg^{-1}) induced a 10-fold increase in general activity in 5 and 10 day old rat pups and a 2-fold increase in 20 day old animals. A 10 mg kg^{-1} dose further increased activity in 5 and 10 day old neonates (30-fold) but was without effect in 20 day old rats. The increase in activity represented increases in forward locomotion and wall-climbing activity. It should be noted that locomotion was not significantly enhanced in 20 day old pups by any of the doses of U50,488H tested (0.1 - 10 mg kg^{-1}). The increase in activity induced by U50,488H in 5 and 20 day old rat pups was significantly inhibited by the opioid antagonist naltrexone (0.1 and 1 mg kg^{-1} i.p.) confirming an opioid receptor mediated response. Gnawing, grooming, scratching, rearing, sniffing and stereotyped mouthing behaviours were not fully evident until the animals were 20 days old. At this age U50,488H (1 and 10 mg kg^{-1}) produced a significant reduction in gnawing and grooming behaviour but was without effect on the other activities.

The observed behavioural effects of U50,488H in neonatal rats are in accordance with the report that the κ-agonist ketocyclazocine produces antinociception in young animals (Kitchen & McDowell, 1985) and the results of receptor binding studies which suggest that κ-opioid receptors are present in the rat CNS at birth (Spain et al., 1985). Furthermore, differences between the behavioural profiles of U50,488H (observed in this study) and morphine (Caza & Spear, 1980) in neonatal rats provide further evidence for the existence and differential development of multiple types of opioid receptor from an early age.

This work was supported by the Wellcome Trust.

Caza, P.A. & Spear, L.P. (1980) Pharmacol. Biochem. Behav. 13: 45-50.
Kitchen, I. & McDowell, J. (1985) Toxicol. Lett. 26: 101-105.
Reinstein, D.K. & Isaacson, R.L. (1977) Brain Res. 135: 378-382.
Spain, J.W., Roth, B.L., Coscia, C.J. (1985). J. Neurosci. 5: 584-588.
Von Voigtlander, P.F., Lahti, R.A., Ludens, J.H. (1983) J. Pharmacol. Exp. Ther. 224: 7-12.

THE ANTINOCICEPTIVE EFFECTS OF PD117302, A SELECTIVE KAPPA OPIOID AGONIST

G.E.Leighton, R.G.Hill, D.C.Horwell, J.Hughes, M.A.Johnson, and K.G.Meecham.
Parke-Davis Research Unit, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QB

It is well documented that compounds acting as agonists at the kappa receptor are effective antinociceptive agents in animal models(Tyers,1980,Ward and Takemori,1983).
This report describes the effects of the novel selective kappa agonist PD117302, (±)trans-N-Methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzo[b]-thiophene-4-acetamide (Clark et al. 1987), when tested against thermal, mechanical or chemical nociceptive stimuli.
Male Wistar rats(70-100g)or male CFLP mice(20-25g) (Interfauna,Huntingdon,UK) were used as appropriate for all behavioural testing.The antinociceptive tests used were the rat paw pressure test,mouse acetylcholine writhing test and the mouse hotplate test.Compounds were administered by the intravenous and oral routes in the rat and via the subcutaneous route in the mouse test.For all behavioural tests animals and drug bottles were randomized and results were calculated using custom designed software running on a BBC microcomputer(Cairnduff et al,1985).This program calculates the dose required in each test to produce 50% of the maximum possible effect(MPE50).Results are shown in table 1 in comparison with the kappa agonists ethylketocylazocine(EKC) and U50488 and the mu agonist morphine.

Table 1.

		MPE50(mg/kg)			
	Route of administration	PD117302	EKC	U50488	Morphine
Rat paw pressure	i.v	1.4	0.06	2.0	0.61
	p.o	15.0	70.0	9.6	66.4
Mouse hotplate	s.c	NE	NE	NE	5.4
Mouse ACh writhing	s.c	0.7	0.2	1.3	0.2

NE=no effect at 100 mg/kg

PD117302 displays the antinociceptive profile characteristic of kappa agonists and some mu partial agonists (Tyers,1980) in that it elevates the nociceptive threshold to both a mechanical and a chemical stimulus but does not affect the response to a thermal stimulus.The mu agonist morphine was clearly seen to be active against all three noxious stimuli.
PD117302 was shown to be active in behavioural tests after administration by several routes. Studies of the time course of the effect following oral administration confirm that absorption is rapid with maximum effects being seen within the first 30 minutes.In this property PD117302 resembles U50488 but differs markedly from EKC which exhibits very poor oral bioavailability.

Cairnduff B.et al(1985).Br.J.Pharmacol.86:525P
Clark et al (1987) J. Med Chem (In Press)
Tyers M.B.(1980)Br.J.Pharmacol.69:503-512.
Ward.S &A.E.Takemori(1983)J.Pharmacol.Exp.Ther.224:525-530.

CHRONIC INTRATHECAL TRH ANALOGUE (CG 3509) ALTERS WET-DOG SHAKES, SPINAL CORD CHOLINE ACETYLTRANSFERASE AND INDOLEAMINE LEVELS

K.C.F. Fone* P. Dix, G.W. Bennett & C.A. Marsden, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, U.K.

Thyrotrophin-releasing hormone (TRH), which co-exists with 5-hydroxytryptamine (5HT) in bulbospinal neurones, may regulate spinal motor function, since the peptide facilitates motoneurone excitability in vivo (White, 1985) and induces choline acetyltransferase (ChAT) activity in cultured motoneurones (Schmidt-Achert et al., 1984). In addition, intrathecal injection of a TRH analogue, CG 3509 (orotyl-L-histidyl-L-prolineamide), produces motor behaviours (Fone et al., 1987) including wet-dog shakes (WDS). The aim of the present work was to examine the effect of chronic intrathecal CG 3509 administration on WDS behaviour, 5HT turnover and ChAT activity in vivo.

Male Wistar rats were cannulated intrathecally under sodium methohexitone anaesthesia (60mgkg^{-1} i.p.) so that the cannula tip was at the thoraco-lumbar junction of the cord (Fone et al., 1987). After 7 days recovery, rats received intrathecal injections (10µl washed in with 20µl saline) of saline (n=8) or 2µg CG 3509 (n=8) twice daily (between 08.30-11.30 and 17.30-18.30h) for 5 days. The number of WDS min^{-1} were recorded for 30min after the first, fifth and ninth injections of saline or CG 3509. All rats were decapitated 3-4h after the ninth injection and the thoraco-lumbar cord dissected into dorsal and ventral portions to determine 5HT and 5-hydroxyindole acetic acid (5HIAA) content using high performance liquid chromatography with electrochemical detection and ChAT activity using the radiochemical assay of Fonnum (1975). Values given are mean±s.e.mean and Students' t-test was used for statistical analysis.

Initially CG 3509 produced 157±11 WDS in 30min compared with 1±0 following saline. A significant reduction ($P<0.05$) in the number of WDS occurred with the fifth (67±9) and ninth (68±5) injections of the peptide analogue, although there was no change in WDS behaviour with repeated saline injections. Chronic CG 3509 treatment also significantly increased the ChAT activity ($P<0.01$) in the ventral horn of the thoraco-lumbar spinal cord compared with that in saline treated controls (2.79±0.21 and 2.06±0.10µmol acetylcholine formed h^{-1}g wet weight^{-1}, respectively) without affecting the dorsal horn ChAT activity. The increase in ventral horn ChAT activity was accompanied by a highly significant increase in the ventral horn 5HT level compared with that in saline treated animals (9.34±0.61 and 5.69±0.56ngmg^{-1} protein, respectively) while 5HT levels in the dorsal and 5HIAA levels in both dorsal and ventral horns were unchanged.

Chronic CG 3509 administration produced a tolerance to WDS behaviour without affecting the time course of the response, as would be expected from receptor down-regulation rather than an alteration in the metabolism of CG 3509. The selective elevation in 5HT without any alteration in 5HIAA levels in the ventral horn produced by CG 3509 treatment, could be due to an increase in synthesis or reuptake of 5HT and may represent a functional consequence of the co-existence of TRH with 5HT in this region. The finding that chronic CG 3509 administration elevated ventral horn ChAT activity implies that TRH may exert a trophic influence on mature motoneurones in vivo and is particularly relevant to the suggested therapeutic use of TRH to treat motor neurone disease.

We thank the MRC and the Wellcome Trust for financial support.

Fone, K.C.F., Bennett, G.W. & Marsden, C.A. (1987) Neuropharmacol. In Press.
Fonnum, F. (1975) J.Neurochem. 24, 407-409.
Schmidt-Achert,K.M., Askanas, U. & Engel, W.K. (1984) J.Neurochem. 43, 586-589.
White, S.R. (1985) Brain Res. 335, 63-70.

ACTIVATION OF EXCITATORY AMINO ACID RECEPTORS INCREASES CALCIUM MOBILISATION IN SYNAPTOSOMES FROM RAT CORTEX

Celestine T. O'Shaughnessy & P. Slater, Department of Physiological Sciences, University of Manchester Medical School, Manchester M13 9PT

Activation of excitatory amino acid receptors, particularly those subserved by N-methyl-D-aspartate (NMDA), have previously been shown to enhance the uptake of $^{45}Ca^{++}$ into rat cortical slices (Lodge et al., 1986).

In this study we have used a crude synaptosomal preparation from adult rat cortex and have monitored free calcium levels during incubation with NMDA using the fluorescent probe, quin-2 (Ashley et al., 1984).

Synaptosomes were prepared by differential centrifugation in 0.32M sucrose (Gray & Whittaker, 1962). For loading with quin-2, the P2 pellet was resuspended in well-oxygenated Krebs-HEPES buffer containing 1mM calcium (no magnesium) at 0.8 g eq./ml and incubated for 20 min at 37°C under oxygen in the presence of 50 µM quin 2 acetoxymethylester. The suspension was then diluted ten-fold and the incubation allowed to proceed for a further 40 min. The tissue and medium were then separated by centrifugation, the pellet gently resuspended in an equal volume of ice-cold Krebs-HEPES and kept oxygenated on ice for up to 2 hours. 2 ml of this suspension was centrifuged for 20 s in an Eppendorf desk centrifuge, the pellet resuspended in 2 ml warm buffer and after 5 min equilibration at 37°C, fluorescence was measured in a Perkin-Elmer LS-3 spectrofluorimeter (excitation wavelength 339 nm; emission wavelength 490 nm).

Basal calcium levels were 213 ± 16 nm (mean ± SEM, n = 11). Both KCl (15 - 60 mM) and veratridine (10 - 100 µM) elevated $[Ca^{++}]$ in a dose-dependent manner with maximal increases of about 300 nM. L-Glutamate, quisqualate and NMDA all elevated $[Ca^{++}]$. Responses to 100 µM NMDA (200 nM increase in Ca^{++}) were completely blocked by physiological concentrations of magnesium, 10 µM 2-amino-5-phosphonovalerate and 40 µM phencyclidine.

These results suggest that activation of NMDA-preferring receptors can increase intrasynaptosomal calcium levels and that such effects can be inhibited by specific NMDA antagonists.

Ashley, R.H., Brammer, M.J. & Marchbanks, R.M. (1984) Biochem. J. 219, 149.
Gray, E.G. & Whittaker, V.P. (1962) J. Anat. 96, 79.
Lodge, D., Aram, J.A., Church, J., Davies, S.N., Martin, D., O'Shaughnessy, C.T. & Zeman, S. (1986) IUPS, 16, 371 (Proc.)

CHLORIDE IONS ARE INVOLVED IN 'DESENSITISATION' TO N-METHYL-D-ASPARTATE IN RAT CORTICAL SLICES

A.A. Miller, K.T. Sparrowhawk and P.L. Wheatley, Dept of Pharmacology I, Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS.

The application of maximally-depolarising concentrations of excitatory amino acids, e.g. N-methyl-D-aspartate (NMDA), to slices of rat cortex induces an irreversible 'desensitisation' to subsquent applications of the agonists which may reflect their neurotoxicity. Excessive influx of CA^{2+} and/or Cl^- has been implicated in this neurotoxic action and we have therefore investigated the roles of these ions in 'desensitisation' using rat cortical slices prepared to record NMDA-evoked DC depolarisations as previously described (Wheatley, 1986).

Experiments in a control medium were performed on preparations continuously superfused with a Mg^{2+}-free Kreb's solution containing 200nM tetrodotoxin of the following composition (all mM): NaCl, 124; KCl, 5; KH_2PO_4, 1.2; $NaHCO_3$, 25.9; glucose, 9.9: $CaCl_2$, 2.0. In preliminary experiments, a series (n=4) of single concentration-response curves were obtained to the application of NMDA in increasing concentration for 30s at 10 min intervals to determine NMDA concentrations producing 'desensitisation'. Typically, 'desensitisation' was observed following the application of NMDA at 50 or 100μM. The requirement for maximal depolarisation to evoke 'desensitisation' was investigated in a series of experiments (n=4) by generating an initial partial concentration-response curve up to an NMDA concentration of 25μM and then repeating the concentration- response curve until a maximal depolarisation was observed. In this situation concentration-response curves were superimposable, i.e. no 'desensitisation' was observed following submaximal responses to NMDA. The roles of Ca^{2+} and Cl^- in 'desensitisation' were investigated by obtaining two successive full concentration-response curves to NMDA in slices (n=4-8 per group) superfused with one of the following: a) normal medium; b) medium from which $CaCl_2$ was omitted ('Ca^{2+}-free'); c) medium in which NaCl and KCl were replaced with 62mM Na_2SO_4 and 2.5 mM K_2SO_4 and 65mM sucrose was included ('Cl-free'); d) medium containing one of a range of concentrations, 1-10μM, of the Cl^- transport inhibitor ethacrynic acid. The degree of 'desensitisation' was determined by comparing the maximal response obtained in the second of each pair of concentration-response curves with that obtained in the first curve.

The second maximum response was reduced to a mean of 59% of the first curve maxima in control medium and to 55% in 'CA^{2+}-free' medium. In 'Ca^{2+}-free' medium the mean EC_{50} value for NMDA was significantly reduced ($p < 0.0001$) to 5.7μM compared with a control value of 12.2μM, as was the mean slope, as determined from the first curves. This may reflect the removal of divalent cationic block of the NMDA-receptor gated channel as previously reported (Ault et al., 1980). In 'Cl^--free' medium or media containing ethacrynic acid, 5 or 10μM, the mean second concentration-response curve maxima were reduced to 87-95% of the first curve maxima. These reductions were significantly ($p < 0.05$) less than those observed in control medium.

Comparison with the results of studies of the neurotoxic action of excitatory amino acids (Rothman, 1985), leads us to conclude that 'desensitisation' to NMDA is a functional manifestation of the neurotoxic action of NMDA. This arises as a consequence of excessive cation-coupled Cl^- entry and leads to irreversible changes in the internal neuronal environment.

Ault et al., (1980). J. Physiol. (Lond)., 307, 413.
Rothman, S.M. (1985). J. Neurosci., 5, 1483.
Wheatley, P.L. (1986). Br. J. Pharmac. 87, 159P.

ROLE OF THE SUBSTANTIA NIGRA IN THE EXPRESSION OF DOPAMINE D_1 AND D_2 BEHAVIOURS

G.H. Fletcher and M.S. Starr, Department of Pharmacology, The School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX.

Dopamine D_1 and D_2 receptors are differentially localised in the striatum and are conceivably associated with different striatal efferent pathways. In this study we sought to establish whether the behavioural deficits caused by interrupting the striatonigral outflow specifically disrupt D_1 rather than D_2-mediated motor behaviours, as was recently suggested by Herrera-Marschitz and Ungerstedt (1984).

Wistar albino rats were first pretreated with 6-hydroxydopamine (8 µg base in 4 µl) in one medial forebrain bundle, in order to render one hemisphere supersensitive to the effects of D_1 and D_2 stimulants. One week later, these rats were observed to posture and to rotate contralaterally, and to exhibit characteristic stereotypies (on a rating scale of 0-3) when injected s.c. with apomorphine (D_1/D_2 agonist), SKF 38393 (D_1), lisuride (D_2), RU 24213 (D_2) or LY 171555 (D_2). All responses were dose-dependent.

A secondary kainate lesion (1 µg in 1 µl) of the ipsilateral substantia nigra pars reticulata modified the components of D_1 and D_2 motor responding differentially. Apomorphine-induced circling was greatly attenuated (0.05 mg/kg) or even reversed (0.5 mg/kg), while posture and grooming were significantly reduced. On the other hand, sniffing and head movements were facilitated. These changes were often evident as soon as one day after kainate administration and showed no signs of recovery three months later. Intranigral kainate similarly altered the patterns of responding to SKF 38393 (2.5 & 10 mg/kg), lisuride and RU 24213 (0.1 & 0.5 mg/kg) and LY 171555 (0.05 & 0.25 mg/kg). No such deficits in behaviour occurred in rats injected intranigrally with 1 µl saline. Although rotation and posture were greatly diminished in doubly-lesioned rats, all four selective dopaminomimetics (not apomorphine) nevertheless promoted forward locomotion, especially at the higher doses. Post-mortem histological examination revealed extensive loss of compacta dopamine cells and reticulata neurones, consistent with the accurate placement and effectiveness of the two neurotoxins.

These results suggest the integrity of the striatonigral connection is crucial for the full expression of both D_1 and D_2-mediated circling, posture and grooming, but not for locomotion, sniffing and head movements. It seems likely, however, that these last three resistant motor components do not ordinarily emanate from the striatum, but from other structures (possibly nucleus accumbens). Thus when the whole spectrum of motor events was elicited by injecting the D_1 and D_2 agonists (0.5-5 µg in 1 µl) directly into the supersensitive caudate nucleus, each one of these motor activities was abolished completely by additionally lesioning the corresponding nigra with kainate.

In contrast to the findings of Herrera-Marschitz and Ungerstedt (1984), our present data show the striatonigral connection is just as important for registering behaviours mediated via striatal D_2 receptors as it is for conveying D_1-dependent information.

Herrera-Marschitz, M. & Ungerstedt, U. (1984) Brain Res. 323, 269-278.

CHARACTERISTICS OF [^{125}I]-BOLTON-HUNTER LABELLED CHOLECYSTOKININ BINDING IN HUMAN BRAIN

A.J. Cross[1], P. Slater & W. Skan, Department of Physiology, University of Manchester, Manchester M13 9PT and [1]Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ.

Cholecystokinin (CCK)-like peptides have been identified in human brain, where they are present in neurones (Emson, Rehfield & Rossor, 1982). Ligand binding studies have identified binding sites for CCK-8 and CCK-33 in several tissues, and these binding sites probably reflect functional CCK receptors. Recently it has become apparent that CCK receptors may be heterogeneous, and in particular the receptors in brain may differ from peripheral CCK receptors (Innis & Snyder, 1980). In the present study we have characterised ^{125}I-Bolton-Hunter labelled CCK-8 (^{125}I-BHCCK) binding in human brain.

Brains were obtained at autopsy, cut into 1cm thick coronal sections and snap-frozen in isopentane at -40°C. 20μ thick cryostat sections were used for binding studies. Sections were incubated for 1h with 50pM ^{125}I-BHCCK in 50 mM TRIS HCl pM 7.7 containing 5mM $MgCl_2$, 1mM DTT, 0.02% bacitracin and 0.2% BSA. The sections were then washed in buffer and water and dried. Bound radioactivity was either determined by scintillation counting or visualised by autoradiography.

^{125}I-BHCCK binding was saturable and displaced by several CCK/gastrin peptides (Table). ^{125}I-BHCCK binding sites were heterogeneously distributed in brain.

Inhibition of ^{125}I-BHCCK binding in human cerebellum

Neuropeptide	IC_{50}(nM, mean ± SEM)
Caerulein	0.41 ± 0.10
CCK-8	1.9 ± 0.45
CCK-33	7.1 ± 0.70
Gastrin 1-17	35 ± 3.0
CCK-8 (non-sulphated)	52 ± 17
CCK-4	> 1000

Highest binding was observed in the deep layers of cerebral cortex and in the cerebellum. Within the basal ganglia ^{125}I-BHCCK binding was greatest in caudate nucleus and putamen with lower levels in globus pallidus.

The results suggest that ^{125}I-BHCCK binding sites in human brain in general resemble those of guinea-pig brain in terms of both their regional distribution and pharmacological properties (Mantyh & Hunt, 1985; Van Dijk et al, 1984).

We are grateful to the SERC for financial support.

Emson, P.C., Rehfield, J.F. & Rossor, M.N. (1982) J.Neurochem. 38. 1177
Innis, R.B. & Snyder, S.M. (1980) Proc. Natl.Acad.Sci. 11,6917.
Mantyh, C.R. & Hunt, S.P. (1985) Eur.J.Pharmacol. 113.137.
Van Dijk, A., Richards, J.G., Trzeciak, A., Gillessen, D. & Mohler, H. (1984) J.Neurosci. 4. 1021.

FAILURE OF ICI 169,369, PROPRANOLOL AND CYANOPINDOLOL TO ANTAGONISE 8-OHDPAT-INDUCED ROTATION IN THE RAT

T.P. Blackburn, B. Cox and D.A. Martin. ICI Pharmaceuticals Division, Bioscience Dept. II, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

The rotational behaviour induced by 5-HT agonists has been investigated in rats with unilateral lesions of the dorsal raphe nucleus (DRN). We have previously reported (Blackburn, et al., 1984) that the agonist; 5-methyoxy-N, N-dimethyl-tryptamine (5-MeODMT), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) and 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)(1H) indole (RU24969) induce a characteristic contralateral rotational behaviour in this model and these agonists have all been reported to have a greater affinity for the $5HT_1$ binding site than the $5HT_2$ site (Tricklebank, 1985).

The selective $5\text{-}HT_2$ antagonists ketanserin and pirenperone injected s.c. at non-sedative doses failed to inhibit this rotational behaviour (Table 1), unlike the classical 5-HT antagonist methysergide which we have previously shown to be an effective antagonist in this model (Blackburn et al., 1987). The chemically novel $5\text{-}HT_2$ antagonist, ICI 169,369 (2-(2-dimethylamino-ethylthio)-3-phenyl-quinoline hydrochloride) also failed to antagonise 8-OHDPAT- induced rotational behaviour (Table 1) at doses reported to inhibit the $5\text{-}HT_2$ mediated behaviours of 5-HTP-induced head twitch in mice and fenfluramine-induced hyperthermia in the rat (Blackburn, et al., 1987).

The purported $5\text{-}HT_1$ antagonists, (-)-propranolol and (±)-cyanopindolol, (Table 1) have also been evaluated in this model at doses claimed to be effective in-vivo against some aspect of the 8-OHDPAT-induced behavioural syndrome in the rat (Tricklebank, 1985) and found to be inactive. 8-OHDPAT is currently widely used as an agonist at the $5\text{-}HT_{1A}$ receptor subtype. The results are consistent with other findings with 8-OHDPAT (Tricklebank M.D., 1985) that not all components of 8-OHDPAT-induced behaviours are mediated by a propranolol sensitive $5\text{-}HT_1$-like recognition site.

Table 1 EFFECT OF VARIOUS ANTAGONISTS ON 8-OHDPAT INDUCED ROTATION IN 5,7-DHT DRN LESIONED RATS.

ANTAGONIST & Dose		Mean no. of turns in 2 hrs 8-OHDPAT 2mg/kg:s.c.		STATISTICAL SIGNIFICANCE (Students) (t-test
		Alone	+ Antag.	
(+/-)	CYANOPINDOLOL 1 mg/kg	401 +/- 48 (n=7)	327 +/- 49 (n=7)	N.S
(-)	PROPRANOLOL 1 mg/kg	364 +/- 89 (n=4)	452 +/- 70 (n=4)	N.S.
	KETANSERIN 0.5 mg/kg	365 +/- 65 (n=6)	358 +/- 93 (n=6)	N.S
	PIRENPERONE 0.05 mg/kg	404 +/- 42 (n=7)	340 +/- 40 (n=7)	N.S
	ICI 169369 10 mg/kg	401 +/- 37 (n=12)	337 +/- 59 (n=12)	N.S

Blackburn, T.P., Kemp, J.D., Martin, D.A. and Cox, B. (1984) Psychopharmacol. 83, 163-165

Blackburn, T.P. , Thornber, C.W., Pearce, R.J. and Cox B.,(1987) Br. J. Pharmac. (in press).

Tricklebank, M.D., (1985) Trends Pharmacol. Sci. 61, 403-407.

AUGMENTATION OF MORPHINE-INDUCED CHANGES IN CEREBRAL DOPAMINE METABOLISM IN RATS TREATED CHRONICALLY WITH NALTREXONE

Liisa Ahtee, L. Martti J. Attila, Heimo Haikala, Kristin R. Carlson and Auli Vuorela, Division of Pharmacology, Department of Pharmacy, University of Helsinki, Kirkkokatu 20, SF-00170 Helsinki, Finland

Chronic naltrexone treatment has been shown to enhance the antinociceptive effect of morphine as well as the binding of opioid ligands to μ- and δ-sites (Tempel et al, 1985). To further investigate the role of opioid receptors in the regulation of cerebral dopaminergic neurones we studied the effects of 3, 10 and 30 mg/kg of morphine (2 h, s.c.) on the concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3MT) in the striatum, limbic forebrain, frontal cortex and hypothalamus of rats one day after withdrawal from 14 day-treatment with naltrexone. Naltrexone was administered by Alzet mini-osmotic pumps model 2002. DA, DOPAC, HVA and 3MT were estimated by using HPLC and electrochemical detection (Haikala, 1986).

Morphine elevated the DOPAC and HVA concentrations dose-dependently in the striatum, limbic forebrain and frontal cortex. It also clearly elevated the hypothalamic HVA concentration but only tended to elevate the hypothalamic DOPAC concentration. In naltrexone-treated rats morphine elevated the DOPAC and HVA concentrations more or in smaller doses than in control rats. In control rats 30 mg/kg of morphine diminished the striatal 3MT concentration by 34% but not the limbic one. In naltrexone-treated rats 10 mg/kg of morphine significantly diminished both striatal and limbic 3MT concentrations (Table 1).

Table 1 Percentage changes of cerebral dopamine metabolites induced by 10 mg/kg of morphine in control (C) and naltrexone-treated (NTX) rats

	DOPAC		HVA		3MT	
	C	NTX	C	NTX	C	NTX
Striatum	173± 8	221±16***	227± 8	273±23***	98±4	82±3**
Limbic forebrain	206± 7	186±12	268±13	295±36	105±6	65±3***
Frontal cortex	230±10	318±37*	279±18	447±60***	not determined	
Hypothalamus	121± 6	139±11	162± 8	228±17**	not determined	

*P<0.05, **P<0.01, ***P<0.001 compared with corresponding control

Our results suggest that the opioid receptors which mediate the opioid regulation of dopaminergic neurones become supersensitive during chronic naltrexone treatment. Furthermore, our results demonstrate that morphine acts on cerebral dopaminergic neurones by at least two mechanisms both of which are potentiated by chronic naltrexone treatment. In small doses morphine enhances the formation of DOPAC and HVA, whereas in larger doses it especially in the striatum causes a decrease of 3MT concentration.

Supported by the Research Council for Medicine of the Academy of Finland. The Alzet pumps were purchased by USPHS Grant DA 02226 (KRC).

Haikala, H. (1986) Naunyn-Schmiedeberg's Arch.Pharmac. 334,373
Tempel, A. et al (1985) J.Pharmac.exp.Ther. 232,439

NORADRENALINE STIMULATES PI METABOLISM IN RAT RENAL CORTEX SLICES

R.J. Plevin, Department of Physiology and Pharmacology, University of Southampton, Southampton, SO3 5NH. (Introduced by J.A.Poat)

The renal proximal tubule has been identified as one site of action of the neural control of renal solute and water transport. Current evidence suggests that this mechanism operates via tubular α-adrenoceptors. Further, renal cortical membranes possess binding sites for α1-adrenoceptors as demonstrated by the use of specific ligands such as prazosin (McPherson and Summers, 1981). In vitro preparations respond to noradrenaline with an increase in pump activity (Brunton, Parsons and Poat, 1978). The present study examines the involvement of the secondary messenger phosphatidylinositol pathway in this response.

Kidney cortex slices were cut on a McIlwain tissue-slicer (3 x 150 μm). The slices were washed for 30 minutes at 37°C with three changes of Krebs bicarbonate buffer and then pre-labelled by incubation with (^{3}H)-myo-inositol (10 μCi/ml) for 3 hours in Krebs' bicarbonate buffer containing 10 mM LiCl. Residual isotope was removed by washing and 25 μl aliquots (2.01-2.40 mg/protein) were dispensed into vials containing buffer and antagonist where appropriate. After 10 min incubation the reaction was started with the addition of agonist and terminated after 45 min by the addition of chloroform/methanol (2/1 v/v). Labelled inositol phosphates were extracted by the addition of chloroform (0.31 mls) and water (0.31 mls) and separated by Dowex formate ion exchange chromatography (Berridge, Downes and Hanley, 1982).

Both adrenaline and noradrenaline stimulated the accumulation of tritium from (^{3}H)-myo-inositol into (^{3}H)-inositol-1-phosphate in the presence of LiCl with EC_{50} values of 0.86 ± 0.31 and 1.12 ± 0.22 μM (n = 4) respectively. The α1-adrenoceptor agonist phenylephrine was active with an EC_{50} 4.16 ± 1.21 μM (n = 3). Clonidine, isoprenaline and dopamine were without effect at concentrations up to 100 μM. The ability of α-adrenoceptor antagonists to block the noradrenaline-stimulated response was tested. The order of potency of antagonists was prazosin IC_{50} 4.43 ± 0.32 nM > indoramine IC_{50} 126.5 ± 26 > phentolamine IC_{50} 218.0 ± 3.2 nM >> yohimbine IC_{50} 6980 ± 1252 nM (n = 3).

These results indicate that in renal tissue noradrenaline will stimulate the phosphatidylinositol pathway by interacting with an α1-adrenoceptor and stimulation of this secondary messenger system may lead to a change in transport function.

Berridge MJ, Downes CP and Hanley MR (1982) Biochem. J. 206 587-595.
Brunton J, Parsons BJ and Poat JA (1978) J. Physiol. 284 73-74p.
McPherson GA and Summers RJ (1981) J. Pharm. Pharmacol. 33 189-191.

R.J. Plevin is an S.E.R.C. scholar.

EFFECT OF ROLIPRAM ON HISTAMINE- AND ADENOSINE-INDUCED [^{3}H]-CYCLIC AMP ACCUMULATION IN GUINEA-PIG BRAIN SLICES

A.M. Brown, J. Donaldson & S.J. Hill, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, U.K.

Histamine can increase cyclic AMP levels in brain slices by stimulating H_2-receptors directly linked to adenylate cyclase or by stimulating H_1-receptors which act indirectly to augment the cyclic AMP responses to H_2- or adenosine A_2-receptor stimulation (Hill et al., 1981; Al-Gadi & Hill, 1985). Rolipram has been reported to be a centrally acting phosphodiesterase (PDE) inhibitor which does not block adenosine receptors (Schwabe et al., 1976). The present study was undertaken to investigate the effects of this PDE inhibitor on cyclic AMP responses to histamine and adenosine in slices of guinea-pig cerebral cortex.

Accumulation of ^{3}H-cyclic AMP was determined using a modification of the ^{3}H-adenine prelabelling technique (Schimuzu et al., 1969). Prelabelled slices were incubated in the presence or absence of rolipram (0.1 mM) for 20 min at 37°C prior to the addition of agonist for a further 10 min. ^{3}H-cyclic AMP was isolated by sequential Dowex-alumina chromatography (Minneman et al., 1979).

Histamine (1 mM) and adenosine (0.1 mM) stimulated ^{3}H-cyclic AMP accumulation by 8.0±0.6 fold (n=23) and 51.4±5.3 fold (n=10) (over basal levels) respectively. The response to histamine was abolished by the selective H_2-antagonist tiotidine (3×10^{-5} M) and reduced by 50% by the H_1-antagonist mepyramine (1 μM) (n=5 in each case). In addition, histamine (1 mM) was able to produce a 2.7±0.1 fold augmentation (n=10) of the response to adenosine (0.1 mM). This augmentation was antagonised by mepyramine but not by the H_2-antagonist cimetidine (0.1 mM).

Rolipram produced a large potentiation of the response to histamine alone (5.3±0.5 fold, n=13) and studies with H_1- and H_2-antagonists suggested that the pharmacological profile of the response to histamine was not affected by the PDE inhibitor. Rolipram had a much smaller effect on the larger responses produced by adenosine alone and by a combination of adenosine (0.1 mM) and histamine (1 mM), producing a 1.7±0.3 and 1.2±0.1 fold potentiation respectively (n=7 in each case). Furthermore, studies in which the levels of ^{3}H-cyclic AMP were followed after removal of the adenosine (with adenosine deaminase 1.2 U/ml) or histamine (with tiotidine 3×10^{-5} M) stimulus indicated that there was still substantial PDE activity in the presence of rolipram. Under these conditions, levels of cyclic AMP fell by 50% within 5 min following removal of the agonist stimulus.

These results suggest that rolipram is not an effective inhibitor of cyclic AMP breakdown at high levels of cyclic AMP. This may be due to competition between cyclic AMP and rolipram for the active site of the enzyme. Alternatively, since rolipram preferentially impairs Ca^{2+}/calmodulin-independent PDE activity (Davis, 1984), it is possible that cyclic AMP metabolism at high levels of the substrate is more dependent on the calcium-sensitive enzyme which has a higher Km for cyclic AMP.

We thank the Wellcome Trust for financial support and Schering AG for the gift of rolipram (ZK 62711).

Al-Gadi, M. & Hill, S.J. (1985). Br. J. Pharmac., 85, 877-888.
Davis, C.W. (1984). Biochim. Biophys. Acta, 797, 354-362.
Hill, S.J. et al. (1981). J. Neurochem., 37, 1357-1360.
Minneman, K.P. et al. (1979). Molec. Pharmac., 16, 21-33.
Schimuzu, H. et al. (1969). J. Neurochem., 16, 1609-1619.
Schwabe, U. et al. (1976). Molec. Pharmac., 12, 900-910.

PROLONGED REVERSAL OF PARKINSONISM IN MPTP-TREATED PRIMATES FOLLOWING SUSTAINED-RELEASE 4-PROPYL-9-HYDROXYNAPHTHOXAZINE

C.E. Clarke, S. Boyce, S.M. Stahl* and A.R. Crossman
Experimental Neurology Group, Department of Cell and Structural Biology, The Medical School, University of Manchester, Manchester, M13 9PT and *Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR

4-Propyl-9-hydroxynaphthoxazine (PHNO) is a highly potent D-2 dopamine receptor agonist which has been shown to be active both in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated marmosets (Jenner et al., 1986) and idiopathic Parkinson's disease in man (Stoessl et al., 1985). A possible limitation to its potential clinical usage is its relatively short duration of action. We now report the results of experiments with a novel sustained-release formulation of the drug in the MPTP-treated macaque monkey.

Three Macaca fascicularis received total cumulative doses of 3.2-4.4 mg/kg MPTP i.v. over 10-12 weeks at 1-2 week intervals. MPTP was discontinued when the severity of an animal's parkinsonism reached stage IV on the Hoehn and Yahr scale (1967). Three to 9 months post-MPTP, trials were commenced with a novel formulation of PHNO designed to be released over a 12 h period following oral administration. Animals were sedated with low doses of ketamine (1.4-1.7 mg/kg), placed in a primate chair, and either PHNO (4, 8, 12 mg) or a placebo pill administered. The primates were then transfered to an observation cage fitted with 3 photoelectric activity counters and monitored for 12-15 h with the aid of a video camera.

MPTP produced a classical parkinsonian syndrome (Mitchell et al., 1985) which progressed in a step-wise predictable manner. At the time of PHNO administration, parkinsonism was stable in all animals. Placebo trials demonstrated complete recovery from sedation within 2 h. Sustained-release PHNO reversed the parkinsonian syndrome in a dose-dependent manner in all monkeys (Table 1). This commenced 3.5-6.0 h after administration and lasted 4-9 h, although preliminary observations suggest that a minor residual effect was still present 24 h after the largest dose. The therapeutic dose (i.e. that required to reverse parkinsonism without causing excessive hyperactivity or dyskinesia) ranged from 1.4-1.7 mg/kg. The onset of action may be hastened by providing a priming oral dose of unformulated PHNO with the slow-release preparation.

Table 1 Cumulative 12 hour activity counts in 3 MPTP-treated primates following sustained-release PHNO (means of 2 trials)

PHNO dose (mg)	0	4	8	12
CYN 112	984	1754	4115	-
CYN 114	761	645	1623	3063
CYN 116	781	1401	3566	4534

These results confirm that PHNO is an excellent anti-parkinsonian agent, the action of which can be prolonged by utilising a sustained-release oral formulation. The therapeutic potential for such a preparation in the management of idiopathic Parkinson's disease is considerable.

Hoehn, M.M. et al. (1967) Neurology 17, 427.
Mitchell, I.J. et al. (1985) Neurosci. Lett. 61, 195.
Jenner, P. et al. (1986) Brit. J. Pharmacol. 89, 626P.
Stoessl, A.J. et al. (1985) Lancet ii, 1330.

CHARACTERISATION OF A SEMICARBAZIDE-SENSITIVE AMINE OXIDASE IN THE CAT UTERINE ARTERY

J. Elliott and B.A. Callingham, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD.

There have been many reports of amine oxidase activities, resistant to irreversible acetylenic inhibitors of monoamine oxidase (MAO), but inhibited by semicarbazide, since the first report by Coquil et al (1973; see Callingham & Barrand, 1987). These semicarbazide-sensitive enzyme activities (SSAO) have been found in many tissues in a variety of species including vascular smooth muscle (Wibo et al, 1980) and brown adipose tissue (Barrand & Callingham, 1984) where they appear to be located mainly in the plasmalemma. This enzyme group differs from MAO in several respects following from the absence of FAD as a cofactor as well as in the high affinity shown by SSAO towards benzylamine as substrate. However, there is no known physiological role for SSAO.

In an attempt to determine whether or not SSAO could be detected in vascular tissue of the cat, uterine arteries were obtained from anaesthetised cats undergoing routine ovarohysterectomy. Arteries from groups of cats (5-7, total of 29 animals) were pooled, homogenised in 1mM K phosphate buffer (1:30 w/v) and assayed for activity against ^{14}C-benzylamine (2.5-20μM) as substrate. The time course of the metabolism of benzylamine was examined and the period over which it was linear established. In addition the sensitivity of the deamination to a range of potential enzyme inhibitors was also determined. Kinetic parameters were calculated by the method of Wilkinson (1961).

Incubation in the presence of 10^{-6}M selegiline (deprenyl; a selective inhibitor of MAO-B at this concentration) caused between 10 and 20% inhibition of the deamination of benzylamine, indicating a small contribution from MAO-B over this range of substrate concentrations. In consequence, all kinetic determinations were carried out in the presence of 10^{-6}M selegiline. The Km for benzylamine in cat uterine artery homogenates was 8.46± 0.42μM with a Vmax of 7.57± 0.55 nmol h^{-1}(mg protein)$^{-1}$ (n=5 groups). Clorgyline (10^{-6}M), β-aminoproprionitrile (10^{-4}M) and diethyl- dithiocarbamate (10^{-4}M) were without significant effect, indicating the absence of MAO-A, lysyl oxidase and of copper in the enzyme, respectively. The semicarbazide was an effective inhibitor of the deamination of benzylamine while kinetic analysis suggested that the inhibition was mixed and was consistent with the inhibitor binding to a prosthetic group. The activity was also reduced when tyramine, isoamylamine and 5-hydroxytryptamine (all at 2.5 x 10^{-4}M) were present in the medium. This enzyme activity in cat uterine arteries, capable of deaminating benzylamine in the presence of inhibitors of MAO, appears to possess the properties of SSAO seen in other tissues and species. However, the specific activity in cat uterine artery (5.0 nmol h^{-1}[mg protein]$^{-1}$), compares with a value of 70 nmol h^{-1} (mg protein)$^{-1}$ for rat aorta examined under the same conditions. Tyramine, isoamylamine and 5-hydroxytryptamine interact with the enzyme and may even be substrates for it. The presence of SSAO activity in yet another vascular bed may indicate that it has some role to play in the control of blood flow.

J.E. is in receipt of a Wellcome Veterinary Research Training Scholarship

Barrand, M.A. & Callingham, B.A. (1984) Biochem. J. 222, 467-475
Callingham, B.A. & Barrand, M.A. (1987) J. Neural. Trans. in press
Coquil, J.F. et al (1973) Br. J. Pharmac. 48, 590-599
Wibo, M. et al (1980) Eur. J. Biochem. 112, 87-94
Wilkinson, G.N. (1961) Biochem. J. 80, 324-332

EFFECTS OF GAMMA-CARBOXYGLUTAMIC ACID AT ACIDIC AMINO ACID RECEPTORS AND RECOGNITION SITES

William F. Hood, Thomas H. Lanthorn, Jacquelyn Michel, Joseph B. Monahan, Linda M. Pullan and Randall K. Rader. CNS Diseases Research, G.D. Searle & Co., St. Louis, MO 63198, U.S.A.

Four distinct physiological receptors for acidic amino acids have been postulated. One of these, the L-2-amino-4 phosphonobutanoic acid (L-AP4) receptor, is defined by the actions of L-AP4 at low micromolar concentrations. At these concentrations, L-AP4 blocks synaptic transmission at some glutamatergic synapses in the central nervous system, including the lateral perforant path-dentate gyrus (LPP-DG) synaptic response in the rat hippocampus (Koerner & Cotman, 1981). A selective recognition site has been described for L-AP4, but this does not appear to correspond to the physiological receptor (Fagg and Lanthorn, 1985).

Only a few agonists of L-AP4 have been described and the structural requirements for activity at this receptor are unclear. Nevertheless it has been noted that all potent agonists contain an omega phosphonic acid group which can have two negative charges (Fagg, Foster, Harris, Lanthorn and Cotman, 1982). Gamma-carboxyglutamic acid (Gla) contains two carboxyl groups at the omega terminal and can also have two negative charges. In order to examine the importance of a double negative charge on activity at the L-AP4 receptor, we have examined the effects of Gla on the LPP-DG synaptic response in the in vitro rat hippocampal slice (Fagg and Lanthorn, 1985) and on radioligand binding to acidic amino acid receptors. Perforant path synaptic responses were stimulated in, and recorded from, the outer molecular layer in the hippocampal slice. The amount of LPP-DG response present in the perforant path response was estimated from the effect of 20μM D,L-AP4 (Koerner, Johnson, Freund, Robinson and Crooks, 1983). L-Gla reduced the LPP-DG response by 34.8 ± 2.3% (n=6) at 100μM, 48.5 ± 2.9% at 300μM (n=2) and 63.2 ± 4.3% at 1,000μM (n=5). No extracellular signs of depolarizing activity were seen. L-Gla had no effect on the Schaffer collateral/commissural-CA1 response (less than 10% reduction at 100μM, n=4). D-Gla had minimal effects on the LPP-DG response (less than 10% reduction at 1,000μM, n=4). D,L-Gla exhibited low affinity for the three acidic amino acid recognition sites (Monahan and Michel, 1987) with K_i values for the AAA1, AAA2, and AAA3 sites of 97, 43 and >300μM, respectively. The results indicate that L-Gla acts like L-AP4, selectively blocking the LPP-DG response without apparent depolarization. The results are consistent with the hypothesis that two negative charges at the omega terminal confer L-AP4-like activity. Previous studies have shown that the L-isomers of AP4 and serine-O-phosphate are more active than the D isomers.(Koerner and Cotman, 1981). The present study extends the observation of a stereoselective interaction with L-AP4 receptors to the isomers of Gla as L-Gla was more potent than D-Gla.

Fagg, G.E. et al (1982) Neuroscience Lett. 31, 59-64.
Fagg, G.E. and Lanthorn, T.H. (1985) Br. J. Pharmac. 86, 743-751.
Koerner, J.F. and Cotman, C.W. (1981) Brain Res. 216, 192-198.
Koerner, J.F. et al (1983) Brain Res. 272, 299-309.
Monahan, J.B. and Michel, J. (1987) J. Neurochem. in press.

QUANTITATIVE ANALYSIS OF GAMMA-AMINOBUTYRIC ACID (GABA) AGONISTS AND ANTAGONISTS ON CELL ACTIVITY IN RAT CEREBELLAR SLICES

J. Bagust, C.R. Gardner*, S. Hussain & R.J. Walker, School of Biochemical and Physiological Sciences, Southampton University, Southampton SO9 3TU and Roussel Laboratories Ltd.*, Kingfisher Drive, Covingham, Swindon, Wilts. SN3 5BZ.

The GABA antagonists bicuculline, its salts and picrotoxin have selective sites of action in a number of preparations (Simmonds 1982; Kemp et al 1986). Bicuculline is believed to act as a competitive antagonist at the $GABA_A$ receptor while picrotoxin probably interacts with a site associated with the chloride ionophore. This difference in the site of action is supported by evidence from binding studies where bicuculline but not picrotoxin reduces binding of GABA and muscimol to cell membrane preparations (Lloyd & Dreksler 1979). In a previous study muscimol, δ-aminolaevulinic acid (DALA), taurine and β-alanine were shown to inhibit spontaneous activity recorded from cells in the Purkinje cell layer in rat cerebellar slices (Bagust et al 1986). In the present study these agonists were quantitatively evaluated against selected antagonists using the same preparation as previously described.

Table: Slopes of Schild plots and derived pA_2 values for antagonists of muscimol, taurine, β-alanine and DALA; $n > 4$.

Agonist	Antagonist	pA_2	Slope	Correlation Coefficient
Muscimol	Bicuculline methoiodide (BMI)	5.92	1.28	0.99
Muscimol	Pitrazepin	5.97	1.02	0.98
Muscimol	Picrotoxin	5.71	0.94	1.0
Taurine	BMI	5.64	0.97	0.99
Taurine	Picrotoxin	6.14	0.70	1.0
β-Alanine	BMI	6.32	0.56	0.98
β-Alanine	Picrotoxin	5.75	0.71	0.98
DALA	BMI	5.60	1.22	0.99

Slopes of Schild plots, correlation coefficients and derived pA_2 values for antagonists to muscimol, taurine, β-alanine and DALA are shown in the table. Against muscimol, pA_2 values for BMI, pitrazepin and picrotoxin were 5.92, 5.97 and 5.71 respectively. These values are in reasonable agreement with those obtained by other workers (Kemp et al 1986). The slopes obtained were close to one. Taurine and DALA gave pA_2 values of 5.64 and 5.60 respectively against BMI, with slopes approaching unity. Although β-alanine was antagonised by BMI with a pA_2 of 6.32 the slope was 0.56. Picrotoxin antagonised taurine and β-alanine with pA_2 values of 6.14 and 5.75 respectively and slopes well below one. All Schild plots were computer fitted using a least squares linear regression method.

The results indicate that the inhibitory effects of muscimol, taurine and DALA are probably competitively antagonised by BMI. Muscimol is also competitively antagonised by pitrazepin. Quantitative analysis indicates these agonists act at the $GABA_A$ receptor complex. It is possible that β-alanine may act at a different site. The slope values for picrotoxin against taurine and β-alanine would suggest that picrotoxin in acting noncompetitively. Strychnine showed only weak antagonism against muscimol and taurine and no quantitative data was obtained.

Acknowledgements: We are grateful to Sandoz for a supply of pitrazepin and SH is a CASE-SERC student in conjunction with Roussel.

Bagust, J. et al (1986). Br. J. Pharmac. 89, 864P.
Kemp, J.A. et al (1986). Br. J. Pharmac. 87, 677-684.
Lloyd, K.G. & Dreksler, S. (1979). Brain Res. 163, 77-87.
Simmonds, M.A. (1982). Eur. J. Pharmac. 80, 347-358.

DRUG DISCRIMINATION STUDIES WITH BUPRENORPHINE: FURTHER EVIDENCE FOR A MORPHINE-LIKE OPIATE RECEPTOR INTERACTION

Blackman, D.E., McCarthy, P.S.* and Naylor, V.J.H., Department of Psychology, University College, P.O. Box 78, Cardiff, CF1 1XL, and *Department of Pharmacology, Reckitt and Colman, Dansom Lane, Hull, HU8 7DS.

Buprenorphine is an opioid partial agonist with a high affinity for both mu and kappa opioid receptor subtypes (Richards et al 1985a). Investigations into the agonist effects of buprenorphine at the mu and kappa receptor have generally concluded that buprenorphine has low mu intrinsic activity and undetectable kappa intrinsic activity (Hayes et al 1986; Takemori et al 1986; Richards et al 1985b). The present studies were conducted to investigate the discriminative stimulus properties of buprenorphine with respect to other opioids considered to act at mu, kappa and sigma receptor subtypes.

Discrimination training was conducted using rats (male, Sprague Dawley, Cardiff University strain, approximately 300 g) in standard two lever operant chambers, according to a fixed-ratio 10 schedule for a food pellet reward. Using this procedure rats (n=12) were trained to discriminate buprenorphine (0.03 mg/kg s.c.) from saline (1 ml/kg s.c.). Incorrect responses were not rewarded. An animal was considered to have acquired the discrimination of buprenorphine from saline when it had attained a mean of 85% correct responding for at least ten consecutive days. Novel opioids were then tested for their ability to produce responding of a similar pattern to that of the training drug ('generalisation').

The results of the drug discrimination trials demonstrated a clear generalisation to both morphine (0.3-5 mg/kg s.c.) and buprenorphine (0.003-0.3 mg/kg s.c.). Drugs with various degrees of agonist effects at kappa receptors (Takemori et al 1986) such as ethylketocyclazocine (0.01-1 mg/kg s.c.), cyclazocine (0.02-2 mg/kg s.c.) and nalorphine (0.3-3 mg/kg s.c.) completely failed to generalise to buprenorphine. Phencyclidine (0.02-2 mg/kg s.c.) totally failed to generalise as did (±)N-allylnormetazocine (0.3-3 mg/kg s.c.). (+)N-Allylnormetazocine ((+)NANM) and (-)N-allylnormetazocine ((-)NANM) also failed to be discriminated as buprenorphine-like (0.3-3 mg/kg, s.c., both drugs). The failure of (-)NANM was surprising as this compound produces generalisation in the opposite direction (Blackman et al 1986). Although (-)NANM has a pharmacological profile consistent with a mu receptor partial agonist it also interacts with phencyclidine binding sites (Blackman et al 1986). This additional characteristic of (-)NANM may be a causative factor in the failure in this instance to generalise to buprenorphine.

The failure of compounds having selective agonist actions at any but the mu opioid receptor to generalise to buprenorphine is consistent with the proposal that buprenorphine exerts its agonist effects at the mu receptor.

Blackman, D.E. et al (1986). Br. J. Pharmac. (In press).
Hayes, A.G. et al (1986). Br. J. Pharmac., 88, 867-872.
Richards, M.L. and Sadee, W. (1985a). Eur. J. Pharmac., 114, 343-353.
Richards, M.L. and Sadee, W. (1985b). Pharmaceutical Res., 4, 178-181.
Takemori, A.E. et al (1986). Eur. J. Pharmac., 123, 357-361.

AF64A INDUCES IMPAIRMENT OF MORRIS WATER MAZE AND HYPERACTIVITY IN THE RAT AT DOSES WHICH DO NOT CAUSE WIDESPREAD NECROSIS

J.G. Gobert, A.J. Gower, I. Hanin[1], P. Jamsin, D. Rousseau and E. Wülfert, UCB, Secteur Pharmaceutique, Braine l'Alleud, Belgium and [1] Loyala University Stritch School of Medicine, Maywood, Illinois, USA.

Ethylcholine mustard aziridinium ion (AF64A) is considered as a relatively selective neurotoxin for cholinergic neurones (Fisher et al., 1982). However, there is some controversy about its use, particularly in view of the variability in effective doses used and the reports of non-specific necrosis (Colhoun and Rylett, 1986). In this study we determined the effects in rats of low concentrations of AF64A injected intracerebroventricularly (icv) on activity and acquisition of the Morris Water Maze and carried out an histological examination at the end of the study.

Male, Sprague Dawley rats (280-310 g) were used. AF64A was prepared from AF64 as described by Fisher et al., 1982 and the solution controlled using NMR analysis. The rats were anaesthetized with tribromoethanol, 250 mg/kg ip and AF64A (0.3, 1.0 and 3.0 nmol) injected in a dose-volume of 3 µl into each lateral ventricle over 4 min. AF64A was kept on ice during infusion. Control animals received an equivalent dose-volume of vehicle. Three days post-operatively the activity of the rats was measured using a 1 m^2 Open Field. The distance per min, number of rearings and time spent in the corners and centre were recorded over 10 min. From 15 to 19 days post-operatively, acquisition of spatial learning was tested using a Morris Swimming Maze (Morris, 1981). In this test, using external cues, the rat learns to swim to a hidden platform in a circular pool (diameter 1.4 m) filled with opaque water. The rat was allowed 120 sec to locate the platform; in the event of failing to do so, the rat was placed on the platform. Each rat received 4 trials per day, each one at a different point of departure in the pool, for 5 consecutive days. The time to reach the platform and the distance swum were recorded. Histological examination of the brains was carried out 1 month after operation. The rats were perfusion-fixed and the brains removed. 5 µ sections were stained with luxol fast blue and cresyl violet.

AF64A caused hyperactivity which was maximal at 1.0 nmol. In the Morris test, AF64A caused dose-dependent impairment of acquisition, observed as an increase in the time to reach the platform and the distance swum. There was no effect on the speed of swimming. Histologically, 0.3 nmol bilaterally produced gliosis along the cannula tract and some necrosis of the dorsal fornix. At 1.0 nmol similar effects occurred but there was also slight ventricular dilatation and slight necrosis of the hippocampus bordering the site of injection. At 3.0 nmol, more widespread damage was observed : marked ventricular dilatation and necrosis of structures around the site of injection, in particular parts of hippocampus, fornix and septum.

It is therefore apparent from these experiments that it is possible to obtain behavioural changes, namely hyperactivity and impaired spatial learning in the rat following icv injection of low doses of AF64A which do not cause widespread necrosis.

Colhoun, E.H. and Rylett, R.J. (1986) Trends Pharmacol.Sci. 7 : 55-58.
Fisher, A. et al. (1982) J. Pharmacol. Exp. Ther. 222 : 140-145.
Morris, R.G.M. (1981) Learning Motivation 12 : 239-260.

MONOAMINE RECEPTOR BINDING PROFILE OF THE $^{5}HT_{1A}$ ANTAGONIST SPIROXATRINE

B.S. Alexander & M.D.Wood, Wyeth Research (UK) Ltd, Huntercombe Lane South, Taplow, Berks. SL6 0PH

Spiroxatrine (1-oxo-4-phenyl-8-(1,4-benzodioxan-2-ylmethyl)-2,4,8-triazospiro [4,5] decane) is a 5-HT1A antagonist that, it has been suggested, may be useful in the characterisation of 5-HT1A receptors (Nelson and Taylor, 1986). Although spiroxatrine has been shown to be selective for the 5-HT1A receptor amongst 5-HT receptor types, its selectivity amongst other receptor types is not known. We have therefore studied the interaction of spiroxatrine with monoaminergic receptor binding sites in the rat C.N.S. and with the recently identified 5-HT1C binding site (Blurton & Wood, 1986).

Binding assay mixtures comprised of radioligand and membranes incubated with varying concentrations of spiroxatrine in Tris-HCl buffer (50mM, pH 7.4) for 20-30 min at 37°C. 5-HT1A binding was studied using 1nM 3H-8-OH-DPAT using hippocampal membranes, whereas 3H-5-HT (2nM) was used to label the 5-HT1B and 5-HT1C sites in rat striatum in the presence of 250nM mianserin or 500nM (-)-propranolol to occlude 1C and 1B sites respectively. 3H-Ketanserin (1nM) was used to label 5-HT2 sites in the frontal cortex and 3H-spiperone (0.5nM) labelled D2 sites in the striatum. Cortical membranes were used to study the binding of tritiated prazosin (0.2nM), rauwolscine (1nM, in the presence of 300 nM spiperone to occlude 5-HT1A binding according to Broadhurst et al, 1986) and dihydroalprenolol (0.7nM) to α1-, α2- and β-adrenoceptors respectively.

Spiroxatrine was a potent blocker of 5-HT1A binding with an IC50 of 8.56nM ± 2.62 (n=3) and was only weakly active at other 5-HT sites (30% inhibition by 10µM spiroxatrine at the 5-HT1B site and IC50's of 1400 ± 250nM and 1413 ± 363 nM at the 5-HT1C and 5-HT2 sites respectively. Spiroxatrine, however, bound at nanomolar concentrations to D2, α1 and α2 sites with IC50 values of 10.8nM ±1.0, 177nM ± 7.2 and 32.0nM ± 8.2 respectively. Spiroxatrine displayed a low affinity for the 3H-DHA β-binding site (IC50 = 8230nM ± 890, n=5).

In agreement with the studies of Nelson and Taylor, spiroxatrine showed selectivity for the 5-HT1A binding site compared to the 5-HT1B, 5-HT1C and 5-HT2 binding sites. However, care should be taken in the interpretation of in vitro and in vivo studies using spiroxatrine as it also binds with high affinity to dopamine D2 receptors and α1- and α2-adrenoceptors.

Blurton, P.A and Wood, M.D. (1986) J. Neurochem. 46, 1392-1398
Broadhurst A.M., Wood M.D. & Wyllie M.G. (1986) BPS London,C80
Nelson, D.L and Taylor, E,W. (1986) Eur. J. Pharmacol. 124, 207-208

THE EFFECT OF ARGININE VASOPRESSIN (AVP) ON THE ELECTROENCEPHALOGRAM (EEG) OF CONSCIOUS RATS

Ebenezer, I.S. (Introduced by J.W. Thompson). AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, CB2 4AT.

Electrophysiological data which has been previously presented indicate that s.c. administration of vasopressin increases the level of arousal in conscious animals (Burns et al, 1984; Ebenezer, 1984). The present study was undertaken (i) to see if s.c. vasopressin altered the EEG in a manner consistent with an increase in arousal, and (ii) to find out more about the possible mechanisms involved.

Female Wistar rats (n=8) were chronically implanted under Equithesin anaesthesia with stainless steel screw electrodes for recording of EEGs between frontal and parietal cortical sites. EEG signals were amplified by high gain a.c. amplifiers (time constant 0.1s) and recorded on magnetic tape for subsequent off-line spectral analysis. The EEG was digitized at a sample rate of 128Hz and power density spectra were calculated for blocks of 20 min in epochs of 2s by fast Fourier transformation using an Apricot Xen computer. The power spectrum was divided into 5 frequency bands i.e. 0 - 4Hz, 4 - 8Hz, 8 - 16Hz, 16 - 22Hz and 22 - 35Hz. On test days the rats were allowed to adapt to the recording chamber for 1h before a 20 min sample of EEG was recorded. The rats were then injected with either AVP (1, 5 or 10μg/kg s.c.) or desglycinamide AVP (DGAVP) (10μg/kg s.c.) and the EEG was recorded for a further 60 min. In control experiments the rats received physiological saline. Statistical analysis was carried out using analysis of variance and the paired t-test.

AVP produced dose-related changes in the spectral power of the EEG as compared with saline. AVP (1μg/kg) caused no significant change in the EEG. However, the higher doses caused significant ($P<0.05$) decreases in power, which are consistent with increases in the level of arousal. AVP (5μg/kg) (n=5) produced significant changes 20-60 min after administration, and mainly affected the 0 - 4Hz, 4 - 8Hz and 16 - 22Hz frequency bands. AVP (10μg/kg) (n=5) produced significant decreases in power throughout the recording period. During the first 20 min, changes were observed in the 16 - 22Hz and 22 - 35Hz frequency bands, but during the next 40 min, decreases in power were noted in all 5 bands. In contrast, DGAVP (10 μg/kg) (n=4) produced no significant changes in spectral power compared with saline. It is of interest to note that rats given saline, DGAVP or AVP (1μg/kg) spent most of the time either sitting quietly or sleeping, while rats given AVP (5 and 10μg/kg) displayed behavioural changes similar to those described previously (Ebenezer, 1984).

The present results confirm and extend previous findings (Burns et al, 1984; Ebenezer, 1984), and show that AVP (5 and 10μg/kg) produces changes in the EEG that are consistent with a moderately aroused state. The mechanism involved seems to be related to the pressor actions of the peptide because (i) only doses that increase systemic blood pressure (see Koob et al, 1986) maintain an activated EEG, and (ii) a high dose of DGAVP, which is virtually devoid of vasopressor activity, did not effect the EEG. It is unlikely that the drug could have had a direct central effect as it is now well established that vasopressin does not enter the brain from the systemic circulation (see Koob et al, 1986).

Burns, B.D. et al. (1984) J. Physiol., 349, 26P.
Ebenezer, I.S. (1984) Br. J. Pharmac., 81, 108P.
Koob, G. et al. (1986) Peptides, 7, 213-218.

MUSCARINIC RESPONSES ON ISOLATED SUPERIOR CERVICAL GANGLIA FROM RATS OF DIFFERENT AGES

G.P. Connolly and N.R. Newberry, Neuroscience Research Centre, Merck Sharp and Dohme Research Laboratories Ltd., Terlings Park, Harlow, Essex, CM20 2QR

Age-related reductions in the responsiveness of central and peripheral tissues to muscarinic agonists have been reported (Lippa, Loullis, Rotrosen, Cordasco, Critchett and Joseph, 1985; Elfellah, Johns and Shepherd, 1986). Muscarinic agonists evoke two potentials on rat superior cervical ganglia: a depolarising response which is more sensitive to the competitive antagonist pirenzepine than is a preceeding smaller hyperpolarising response (Newberry, Priestley and Woodruff, 1985). We have tested for age-related changes in those responses using our previously described techniques.

The excised ganglia, one from each rat, were desheathed and placed in 3 compartment baths, with pre- and postganglionic trunks protruding through greased gaps in the barriers between the compartments, and continually superfused with medium at 25°C. Each ganglionic potential was recorded differentially between the ganglion body and postganglionic trunk. The potency of muscarine to depolarise the ganglion was determined using a semicumulative concentration-response regimen with 10 minute intervals between the 1 minute application periods. The pA_2 for pirenzepine was calculated from dose-ratios produced by 0.3μM pirenzepine, applied for 30 minutes, using $pA_2 = -\log[B]+\log(DR-1)$. The presence of the smaller hyperpolarising response was assessed under conditions optimal for its detection: 0.3μM pirenzepine, 0.1mM $CaCl_2$ in medium (cf normal 2.5mM) with 1μM carbachol as agonist. A summary of the results from this series of experiments is shown below.

Age (Weight)	4-5 days (9 - 12 g)	6-7 weeks (150 - 200g)	Over 2 years (360 - 800g)
pEC_{50} muscarine[A]	6.42 ± 0.14 (10)[C]	6.93 ± 0.08 (6)	6.81 ± 0.08 (7)
pA_2 pirenzepine[A]	8.48 ± 0.41 (9)[D]	8.52 ± 0.19 (5)	8.64 ± 0.18 (5)
hyperpolarisation [B]	9/9	5/5	4/4

A : Means ± SD(n), B: frequency of detection, C : significantly different and D : not significantly different when compared to the other age groups (at P = 0.05, Kruskal-Wallis ANOVA).

It appears that both the depolarising and hyperpolarising muscarinic responses on the rat superior cervical ganglion are present at a very early age and remain over the age range tested. The only significant age-related change that we could detect was that the potency of muscarine on the neonatal ganglion was less than on ganglia from older rats. This difference may be associated with steps subsequent to receptor activation since the pA_2 values for pirenzepine were not significantly different.

Elfellah M.S., Johns A. and Shepherd, A.M.M., (1986) J. Card. Pharmac. 8, 873-877.
Lippa A.S., Loullis C.C., Rotrosen J., Cordasco D.M., Critchett D.J. and Joseph J.A. (1985) Neurobiol. Aging. 6, 317-323.
Newberry N.R., Priestley T. and Woodruff G.N., (1985) Eur. J. Pharmac. 116, 191-192.

REGULATION OF α_2-ADRENOCEPTORS AFTER MANIPULATION OF NORADRENERGIC TRANSMISSION IN THE RAT BRAIN

J.A. García-Sevilla & M.T. Giralt, Department of Pharmacology, Faculty of Medicine, University of the Basque Country, Leioa, Spain.

Neurotransmitter receptors appear to be modulated by drugs modifying neurotransmission processes through indirect mechanisms which involve the respective transmitter (e.g. Lee et al. 1983). Thus, presynaptic alpha-2 adrenoceptors can be activated or desensitized by antidepressant drugs through endogenous noradrenaline (NA) as the responsible agonist (García-Sevilla & Zubieta 1986). This study was designed to directly evaluate brain alpha-2 adrenoceptor changes after manipulation of synaptic NA pools by drugs known to affect noradrenergic transmission. The specific binding of ^{3}H-clonidine (K_D, dissociation constant and Bmax, maximum number of binding sites) to brain membranes (pooled from 6 rats) was used as a biochemical index to quantitate alpha-2 adrenoceptors.

In the hypothalamus (control values: K_D=5.5±0.5 nM; Bmax=144±11 fmol/mgP; n=17) prolonged inhibition of NA synthesis with α-methyl-p-tyrosine (α-MPT, 150 mg/kg; 7 days) or treatment with reserpine (0.25 mg/kg; 14 days) reduced the NA content by 40-85% ($P<0.001$), respectively, which also resulted in marked reductions of K_D (48±4 - 58±6%; n=9; $P<0.001$). In cortex, brainstem and striatum K_D values were also decreased (36±2 - 51±4%; n=21; $P<0.001$). Bmax values were not alter except in the hypothalamus and cortex (30±6% decrease;n=6; $P<0.05$) after α-MPT. In contrast, chronic administration of the MAO inhibitors clorgyline (1 mg/kg) and tranylcypromine (5 mg/kg; 7 or 14 days) increased hypothalamic NA content by 100% ($P<0.001$) which led to marked reductions of Bmax (32±7 - 38±7%; n=12; $P<0.001$) whithout altering K_D values. Similarly, prolonged inhibition of NA neuronal uptake with cocaine or protriptyline (10 mg/kg; 21 days) also resulted in significant decreases in Bmax (20±4 - 24±3%; n=7; $P<0.05$) with no alterations in K_D. Similar results were obtained in cortex after treatment with MAO inhibitors.

Acute treatments (2 h) with α-MPT, reserpine and MAO inhibitors induced similar but less marked effects. However, acute treatments with uptake inhibitors did not change ^{3}H-clonidine binding parameters. In vitro the inhibition of the radioligand by the various drugs was weak and competitive (K_i: 1-5000 μM) indicating that the changes observed after the acute or long-term treatments were not due to direct interactions with the receptor.

It is suggested that drugs which deplete endogenous NA up-regulate alpha-2 adrenoceptors (increased affinity of ^{3}H-clonidine binding sites) while drugs which increase the intraneuronal and/or synaptic NA pools down-regulate the receptors (decreased number of ^{3}H-clonidine binding sites). These adaptive receptor changes appear to be dependent on NA availability.

Supported by CAICYT Grant No. 1018/81.

García-Sevilla & Zubieta (1986) Br. J. Pharmac. 89, 673.
Lee et al. (1983) Science 220, 626.

NON COMPETITIVE ANTAGONISM BY CALCIUM OF THE INHIBITORY EFFECT OF ADENOSINE ON NEUROMUSCULAR TRANSMISSION

J.A. Ribeiro & A.M. Sebastião. Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781 Oeiras, Portugal

At the rat diaphragm neuromuscular junction low calcium bathing solutions enhance the depressing effect of adenosine on neuromuscular transmission (Ribeiro, 1982). However, in this preparation the effect of adenosine is only evident when the safety margin of transmission is previously inhibited. To further investigate the role of calcium on the action of adenosine on neuromuscular transmission we used the frog sartorius nerve-muscle preparation, where adenosine is effective in the absence of previous neuromuscular depression.

The experiments were performed at room temperature (22 - 25° C) on the innervated sartorius muscle of the frog. The nerve was stimulated at a constant rate (0.2 Hz) with supramaximal rectangular pulses and the muscle twitches in response to nerve stimulation were recorded isometrically at a resting tension of 50 mN. The normal bathing solution (pH 7.0) contained (mM): NaCl 117, KCl 2.5, Na_2HPO_4 1, NaH_2PO_4 1, $MgCl_2$ 1.2, $CaCl_2$ 1.8.

In the presence of normal calcium (1.8 mM) adenosine (2.5 - 100 μM) caused a concentration-dependent decrease in neuromuscular transmission. High calcium concentrations (2.5 - 3.6 mM) shifted to the right, and low calcium concentration (1.3 mM) shifted to the left the concentration-response curve for the inhibitory effect of adenosine on neuromuscular transmission. Both the maximal effect of adenosine and its apparent affinity were affected by calcium (Figure 1), which suggests that calcium antagonizes the effect of adenosine in a non competitive manner.

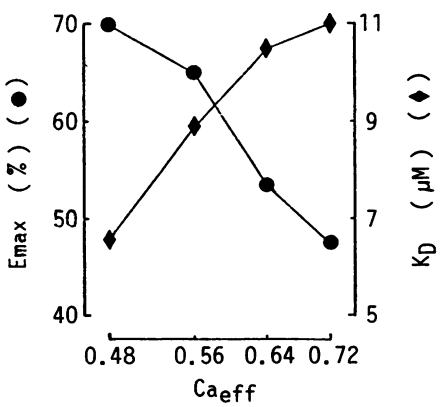

Figure 1 Influence of extracellular calcium on the maximal effect (Emax) and apparent affinity of adenosine on transmission at the frog neuromuscular junction. The ordinates represent Emax (left, ●) or K_D (right, ◆) values for adenosine calculated from the regression lines (method of the least squares, correlation coefficients 0.999) of the double-reciprocal plot of the concentration-response curves for the inhibitory effect of adenosine in the presence of the different calcium concentrations. In the abscissae are represented (log scale) the effective calcium (Ca_{eff}) in the bath calculated as (Dodge & Rahamimoff, 1967): $Ca_{eff}=Ca^{2+}/1+Ca^{2+}+Mg^{2+}x1/3$). Each point is the average of 7 to 10 experiments.

The results support the idea that the recently proposed A_3 adenosine receptor (Ribeiro & Sebastião, 1986) that mediates the inhibitory action of this nucleoside on neuromuscular transmission is negatively linked to calcium.

Dodge, J.A., Jr. & Rahamimoff, R. (1967) J.Physiol.Lond. 193, 419-432
Ribeiro, J.A. (1982) Arch int. Pharmacodyn. 255, 59-67
Ribeiro, J.A. & Sebastião, A.M. (1986) Prog.Neurobiol. 26, 179-209

DHP-SENSITIVE Ca^{2+} CHANNELS, INOSITOL LIPID BREAKDOWN AND FUNCTIONAL RESPONSES IN VASA DEFERENTIA FROM ETHANOL-DEPENDENT RATS

C H BRENNAN, S J CHARLES and J M LITTLETON
Department of Pharmacology, King's College, Strand, London WC2R 2LS

We have suggested that an increase in the number of dihydropyridine (DHP)-sensitive Ca^{2+} channels on central neuronal bodies is associated with ethanol physical dependence in the rat (Pagonis and Littleton, 1987). The function of these channels is obscure but the DHP Ca^{2+} channel activator BAYK8644 is known to potentiate inositol phospholipid (IPL) breakdown in brain preparations (Kendall & Nahorski, 1985), an effect increased in ethanol dependence (Dolin et al., 1987). As the role of both DHP-sensitive Ca^{2+} channels and IPL breakdown is more clearly characterised in smooth muscle we have investigated whether similar changes occur in these tissues. The rat vas deferens was chosen because after chronic ethanol administration it is known (DeMoreas & Capaz, 1984) to become more sensitive to stimuli which act through voltage-dependent Ca^{2+} channels (eg K^+-depolarisation) and IPL breakdown (eg α_1-adrenoceptor agonists).

Vasa deferentia were dissected from male Sprague Dawley rats made tolerant to, and dependent on ethanol (Lynch & Littleton, 1983). For measurement of contractile responses the sustained response of the epidydimal third was used routinely. Tissue from the whole organ was used when measuring IPL breakdown as no significant difference between the response of the epidydimal and prostatic tissue was detected in previous experiments. Inositol phospholipid breakdown was assessed using a modified version of Berridge (1983) (Hudspith et al., 1987).

As described by DeMoraes and Capaz (1984) vasa from ethanol-treated rats were hyper-reactive to all the stimuli tested including depolarisation, noradrenaline and carbachol. All these responses were inhibited by DHP Ca^{2+} antagonists (nifedipine or nitrendipine 1 μM). Both K^+- and electrical depolarisation produced an accumulation of ^{3}[H]-inositol phosphates which was significantly greater ($p \leq 0.05$) in preparations from ethanol-dependent rats. The K^+-depolarisation-induced IPL response was not sensitive to prazosin (10 μM) or atropine (10 μM), concentrations which completely blocked receptor-mediated responses, nor was it mimicked by $\beta\gamma$ -methylene ATP, suggesting that the cause was not released transmitter. The ^{3}[H]-inositol phosphate accumulation caused by exogenous noradrenaline (10 μM) was also greater in preparations from ethanol treated rats. Lastly, BAYK8644 (10^{-8} M) produced marked accumulation of ^{3}H-inositol phosphates in preparations from ethanol-treated rats but no effect on control preparations.

The results suggest that during chronic administration of ethanol an up-regulation in DHP-sensitive Ca^{2+} channels occurs in peripheral as well as central tissues. The change seems to be associated with an increased sensitivity of the inositol lipid signalling system and increased functional responses.

BERRIDGE, M J (1983) Biochem J, 212, 849-858.
DeMORAES, S and CAPAZ, F R (1984) J Pharm Pharmacol, 36, 70-72.
DOLIN, S, HUDSPITH, M, PAGONIS, C, LITTLE, M, LITTLETON, J (1987) Neuropharmacology. In press.
HUDSPITH, M, BRENNAN, C H, CHARLES, S and LITTLETON, J M (1987) N Y Acad Sci. In press.
KENDALL, D A and NAHORSKI, S R (1985) Eur J Pharmacol, 115, 31-36.
LYNCH, M J and LITTLETON, J M (1983) Nature, 303, 175-177.
PAGONIS, C and LITTLETON, J M (1987) This meeting.