SYNAPTOSOMAL GABA UPTAKE IN MICE SUSCEPTIBLE (DBA/2), AND RESISTANT (C57 B1/6) TO AUDIOGENIC SEIZURES

R.W. Horton, S.A. Prestwich and N.S. Spyrou, Department of Pharmacology, St. George's Hospital Medical School, London SW17 ORE.

DBA/2 mice have a high genetically determined susceptibility to audiogenic seizures (AS). This susceptibility is age related, being maximal at 21-28 days of age and considerably reduced or absent at earlier or later ages. The basis of this age-related susceptibility to AS is not known. Intracerebroventricular injection of GABA uptake inhibitors in AS susceptible DBA/2 mice produce two distinct effects. Nipecotic acid, THPO and cis 4-hydroxynipecotic acid reduce the incidence and severity of AS while diaminobutyric acid and cis-3-aminocyclohexane carboxylic acid induce myoclonus and convulsions in the absence of auditory stimulation (Horton et al 1979; Croucher et al 1983).

The present experiments were undertaken to determine if an abnormality in GABA uptake may be responsible for the age-related changes in AS susceptibility in DBA/2 mice. We have determined the kinetics of [^{3}H] GABA uptake into partially-purified synaptosomes prepared from the cerebral cortex, cerebellum and pons-medulla of DBA/ 2 mice at ages before (13-15 days), during (21-23 days) and after (40-43 days) the period of maximal sensitivity to AS. For comparison, we have studied concurrently the same parameters in age-matched C57 B1/6 mice, a strain resistant to AS at all ages.

Kinetic parameters of ^{3}H GABA uptake in the cerebral cortex are shown in Table 1

Table 1 Synaptosomal [^{3}H] GABA uptake in cerebral cortex

Age (days)	DBA/2		C57	
	Km	Vmax	Km	Vmax
13-15	3.9±0.4	370±60	4.1±0.6	360±40
21-23	5.4±1.1	431±90	4.0±0.8	350±50
40-43	4.4±0.6	270±80	3.3±0.5	180±30

Km = μM, Vmax = p moles/min/mg protein. Means ± S.E.M. for 3-4 determinations. Synaptosomes were preincubated at 37^{O} in the presence of 20μM aminoxyacetic acid, incubated with [^{3}H]GABA (0.25-10μM) for 3 min and uptake terminated by filtration (GF/B). Non-carrier mediate uptake was defined as uptake in the presence of 1mM GABA

Km did not differ with age but Vmax decreased particularly between 21-23 days and 40-43 days. Vmax was greater at every age in the pons-medulla (range 760-360 p moles/min/mg protein) and lower at every age (range 250-90 pmoles/min/mg protein) in the cerebellum than the cerebral cortex. A decrease in Vmax with age was also seen in pons-medulla and cerebellum. There was no difference in Vmax in any region at any age between the two strains. The Km was lower in the cerebellum of DBA/2 mice at 21-23 days (2.0 ± 0.2 V 3.2 ± 0.3) and in the cerebellum at 40-43 days (3.2 ± 0.32 V 4.8 ± 0.18) but did not differ at other ages. Thus we can find no evidence to suggest that the age-related susceptibility of DBA/2 mice to AS are related to an abnormality of neuronal GABA uptake.

We acknowledge the financial support of the Nuffield Foundation.

Croucher, M.J. et al (1983) Euro. J. Pharmacol 89, 217
Horton, R.W. et al (1979) Euro J. Pharmacol 59, 75.

EFFICACY AT THE BENZODIAZEPINE RECEPTOR: IN VIVO STUDIES WITH PYRAZOLOQUINOLINONES

Chloë L. Brown*, B.J. Jones+, I.L. Martin & N.R. Oakley+, MRC Neurochemical Pharmacology Unit, Cambridge CB2 2QH & +Glaxo Group Research Ltd, Greenford, Middlesex UB6 OHE

A novel series of pyrazoloquinolinones were reported to displace potently the benzodiazepines from their CNS binding sites *in vitro*. However, the pharmacological profile suggested that CGS 8216 was an antagonist, CGS 9896 a full agonist and CGS 9895 a partial agonist of benzodiazepine receptors (Yokoyama et al 1982). As we have found CGS 9896 to have only weak anticonvulsant and anxiolytic activity in tests generally predictive for benzodiazepines, we have re-evaluated the potency of CGS 9896 and CGS 9895 in protecting from convulsions induced by pentylenetetrazole, or antagonising the protective effect of diazepam, and compared this with their ability to inhibit benzodiazepine binding *in vivo*.

Male CD1 mice (Charles River) weighing 25-30 g were deprived of food for at least 2 h before administering drugs orally in suspension in acacia. 1 h after drug treatment mice were challenged with 60 mg/kg i.v. pentylenetetrazole. This is an ED100 dose for tonic convulsions in untreated animals. The absence of a full tonic convulsion was counted as protection. Displacement of *in vivo* binding of ^{3}H-flunitrazepam was determined essentially according to the method of Oakley et al (1984). 150 µCi/kg i.v. ^{3}H-flunitrazepam was given 40 min after oral pretreatment and 20 min before death by decapitation. The cortex, cerebellum and hippocampus were rapidly dissected from the brain on ice, weighed and homogenised in 100 vol of 0.1M Tris citrate buffer, pH 7.1 at 0°C using 10 strokes of a teflon-glass homogeniser. Triplicate 1 ml aliquots of the homogenate were filtered through Whatman GF/B filters under vacuum followed by 2 x 5 ml washes with ice cold buffer. Bound radioactivity was determined by conventional scintillation counting. Results are expressed as the % of maximal inhibition determined as that produced by 32 mg/kg diazepam.

Diazepam is a potent antipentylenetetrazole agent and produced 100% protection at doses of 4 mg/kg and above. At 4 mg/kg approximately 63-70% of maximal inhibition is produced in the brain regions studied. CGS 9896 appeared to possess only weak antipentylenetetrazole activity. 3 mg/kg was the MED and even at 100 mg/kg only 60% of animals were protected, although it would appear that virtually all benzodiazepine binding sites are occupied at doses of 30 mg/kg. In contrast to the reported antipentylenetetrazole activity of CGS 9895 we could not detect any protection at doses from 1-100 mg/kg. However, CGS 9895 was able to antagonise the protective effects of 5 mg/kg diazepam at doses closely correlated to those which inhibit the binding of ^{3}H-flunitrazepam *in vivo*.

These data are consistent with CGS 9896 being a partial agonist and CGS 9895 an antagonist of benzodiazepine receptors.

CLB is an SERC CASE student with Glaxo Group Research Ltd.

Oakley, N.R. et al (1984) Neuropharmacology, in press.
Yokoyama, N. et al (1982) J.Med.Chem. 25, 337-339.

MODULATION OF GABA AND BENZODIAZEPINE RECEPTOR ANTAGONIST BINDING BY BARBITURATES IN RAT CORTICAL MEMBRANES

R.W. Olsen & E.H.F. Wong*[1] (introduced by K.J. Watling), Department of Pharmacology UCLA School of Medicine, Los Angeles, California 90024.

[^{3}H] bicuculline methochloride (BMC) has been reported to bind to γ-aminobutyric acid (GABA) receptor sites in the presence of certain anions, such as thiocyanate (SCN^-), which enhance GABA antagonist, but not agonist, binding affinities (Möhler & Okada, 1977; Enna & Snyder, 1977). We have recently described the binding of [^{3}H]-BMC to low affinity GABA agonist sites, assayed in the presence of 0.1M KSCN (plus 10mM $K_2HPO_4KH_2PO_4$, pH7.5; Olsen & Snowman, 1983). Under our assay conditions, a large number of barbiturates lower [^{3}H]-BMC binding in a dose-dependent, stereospecific and picrotoxin sensitive manner (Table I).

Table I EC_{50} (μM) for lowering of [^{3}H]-BMC and [^{3}H]-β CCM binding in rat cortical membranes by barbiturates

	[^{3}H]-BMC (2nM)	[^{3}H]-β CCM (2nM)
Dimethylbutylbarbituric acid	50	40
Secobarbitone	200	110
Pentobarbitone	210	170
(+) Hexobarbitone	260	250
(-) Methylphenylpropylbarbituric acid	280	800
(-) Mephobarbitone	310	220
Phenobarbitone	550	>1000
(+) Mephobarbitone	950	440
(-) Hexobarbitone	>1000	>1000
(+) Methylphenylpropylbarbituric acid	>1000	2000

The benzodiazepine antagonist, β-carboline-3-carboxylic methyl ester (β CCM) has been used to label the benzodiazepine receptor (Braestrup & Nielsen, 1981). Under identical assay conditions, [^{3}H]-β CCM binding showed similar sensitivity to modulation by barbiturates (Table I) with comparable potency and selectivity, but with a smaller maximal effect (40% lowering rather than 100% as for ^{3}H-BMC binding). Scatchard analysis of [^{3}H]-BMC and [^{3}H]-β CCM binding in the presence and absence of a barbiturate indicates that the modulation is due to a lowering in binding affinities with no significant change in the total number of receptor sites. These data demonstrated that barbiturates can allosterically inhibit the binding of [^{3}H]-BMC and [^{3}H]-β CCM. This lends support to the concept of coupling between GABA, benzodiazepine and barbiturate/picrotoxin receptor sites. The similar structure-activity relationship for a series of barbiturates to enhance GABA and benzodiazepine binding, to enhance GABA function and for anaesthetic/hypnotic activity *in vivo* (Olsen, 1982) suggest that this barbiturate receptor site on the GABA receptor complex is likely to play a role in at least some of the pharmacological actions of these drugs.

We thank Dr. Knabe of University of Saarlandes, West Germany for the gift of the isomers of barbiturates used, and acknowledge support from NIH and NSF of U.S.

[1]Present address: Neuroscience Research Centre, Merck Sharp & Dohme, Hoddesdon, Hertfordshire, EN11 9BU.

Braestrup, C. & Nielsen, M. (1981) Nature, 292, 472-474.
Enna, S.J. & Snyder, S.H. (1977) Mol. Pharmac. 13, 442-453.
Möhler, H. & Okada, T. (1977) Nature, 267, 65-67.
Olsen, R.W. (1982) Ann. Rev. Pharmac. Toxicol. 22, 245-277.
Olsen, R.W. & Snowman, A.M. (1983) J. Neurochem. 41, 1653-1663.

EXPERIENCE MIMICS AMPHETAMINE ENHANCEMENT OF CHLORDIAZEPOXIDE STIMULATION IN RATS

Christina Davies* & Hannah Steinberg, Departments of Pharmacology and Psychology, University College London, Gower Street, London WC1 8BT

Variability in responses to benzodiazepines is re-arousing research interest (e.g. File et al, 1983; Bond & Lader, 1983). We here pinpoint past experiences as one source of such variability in rodents: the effective dose of chlordiazepoxide for stimulating spontaneous activities was decreased, and the amount of stimulation increased in rats with experience of an environment different from that in which they were tested. By contrast, rats with previous experience of the test environment itself are known to show little or no stimulation by chlordiazepoxide (e.g. Marriott & Spencer, 1965).

Naive adult female hooded rats were compared with rats given 8 daily 5-minute exposures to an Open Field type arena 35 min after saline injection (Davies et al, 1974). On day 9 all rats were individually tested for 5 min in a Y-maze (Rushton et al, 1973) 35 min after injection with saline or chlordiazepoxide.

Open Field experience, preceded by a saline injection, much enhanced stimulation with the two higher doses; the lowest dose, which had no measurable effect on naive rats, was now stimulant. Drug and experience effects were both statistically significant ($P < .025$ and $< .001$ respectively). Similarly, lack of effect on rearing in naive rats was replaced by stimulation in experienced rats. The high levels of activity obtained in experienced, chlordiazepoxide-treated rats were comparable with those previously obtained by us if similar chlordiazepoxide doses were given to naive rats in combination with a constant small dose of dexamphetamine (Figure 1) (cf Rushton et al, 1973). Behavioural effects of experience plus chlordiazepoxide seem to have been equivalent, both quantitatively and qualitatively, to those of dexamphetamine plus chlordiazepoxide.

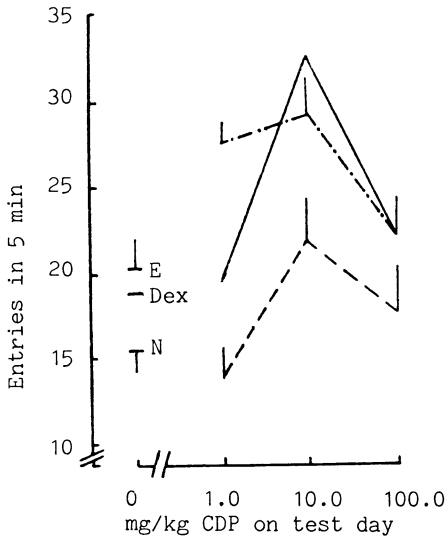

Figure 1 : Number of entries into the arm of a Y-maze in 5 min

Experienced rats (E) (n=7-8)

Naive rats (N) (n=7-8)

Naive rats given 1.18 mg/kg dexamphetamine with their injection of chlordiazepoxide (Dex) (from unpublished data, Steinberg et al).

All injections were s.c., at volumes of 2 ml/kg

Bond, A. & Lader, M. (1983) Pharmacol. Biochem. Behav. 18, 294-298P
Davies, C. et al (1974) Psychopharmacologia (Berl.) 36, 263-274P
File, S.E. et al (1983) Pharmacol. Biochem. Behav. 18, 293P.
Marriott, A.S. & Spencer, P.S.J. (1965) Br. J. Pharmacol. 25, 432-441P
Rushton, R. et al (1973) In: The Benzodiazepines, S. Garattini, E. Mussini and L.O Randall (eds). Raven Press, New York, pp. 355-366

DISCRIMINATIVE STIMULUS EFFECTS OF MIDAZOLAM IN RATS

I.C. Rose* and I.P. Stolerman, Departments of Pharmacology and Psychiatry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

Discriminative effects of benzodiazepines can provide behavioural assays useful in studies of their mode of action (Colpaert et al, 1976). We now report preliminary results with midazolam, a recently introduced compound with a short duration of action (Pieri et al, 1981). Rats were trained to discriminate the effects of midazolam maleate (0.4 mg/kg of the base) in a conventional, two-bar operant conditioning procedure (Stolerman and D'Mello, 1981). In 15-min sessions beginning 15 min after administration of midazolam, the animals received food for pressing one of two bars, whereas pressing the other bar produced food in sessions after saline injections. After about 40 training sessions, midazolam and other drugs were tested in groups of 4-8 rats in 5-min sessions during which no responses produced food.

Midazolam (0.05-0.4 mg/kg s.c.) increased the percentage of drug-appropriate responding in a dose-related manner from 11.0 $\pm$ 1.2% after saline (mean$\pm$s.e.m.) to 96.2 $\pm$ 1.2% after midazolam (0.4 mg/kg). Administering midazolam intraperitoneally instead of subcutaneously reduced its potency about ten times. In contrast, administering chlordiazepoxide intraperitoneally instead of subcutaneously increased its potency 10-15 times in rats trained on chlordiazepoxide (5 mg/kg). Midazolam produced detectable effects as early as 2.5 min after injection, they were maximal at 5-15 min, and disappeared after 30-90 min (depending on dose). The optimal time for discrimination training with midazolam appeared to be from 5-15 min after subcutaneous injection, and this was used subsequently.

Chlordiazepoxide HCl (5 mg/kg i.p.), diazepam (0.5-4 mg/kg i.p.) or pentobarbitone sodium (1.25-10 mg/kg s.c.) increased drug-appropriate responding in a manner similar to midazolam. However, quipazine (0.25 mg/kg s.c.), nicotine (0.05-0.4 mg/kg s.c.) or the benzodiazepine antagonist Ro 15-1788 (1-32 mg/kg i.p.) did not increase drug-appropriate responding. Pretreatment with Ro 15-1788 (30 mg/kg) 30 min before testing completely blocked the effect of midazolam (0.4 mg/kg).

These studies suggest that the discriminative effect of midazolam can provide a quantitative, objective index of the drug's central actions. Midazolam-appropriate responding can be produced by other drugs with anxiolytic or sedative-hypnotic effects, but not by all centrally-acting compounds. Comparisons between different benzodiazepines must allow for the finding that relative potencies can differ at least 100-fold depending on route of administration.

We thank the Medical Research Council for financial support and Hoffmann-La Roche for generous gifts of drugs.

Colpaert, F.C. et al (1976) Eur.J.Pharmac. 37, 113-123
Pieri, L. et al (1981) Drug Research 31, 2180-2201
Stolerman, I.P. & D'Mello, G.D. (1981) Psychopharmacology 73, 295-303

FG 7142, A β-CARBOLINE, HAS AN ANXIOGENIC ACTION IN THE SOCIAL INTERACTION TEST

Sandra E. File and Sharon Pellow*. MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy, University of London, UK.

FG 7142 (β-carboline-3-carboxylate methyl amide) inhibits 3H -flunitrazepam binding with an in vivo potency of 37 mg/kg (Jensen et al, 1983). This β-carboline is proconvulsant in rodents (Jensen et al, 1983) and is anxiogenic both in man (Dorow et al, 1983) and in a punished drinking test in rats (Petersen et al, 1983). The social interaction test has proven sensitive to the anxiogenic actions of a variety of substances, including β-CCE (File et al, 1982) and β-CCP (File et al, 1984), and we were therefore interested in whether FG 7142 also had an anxiogenic profile in this test, i.e. a decrease in social interaction without a concomitant decrease in locomotor activity, and without passive body contact.

Pairs of male hooded rats that had previously been singly housed were placed in a wooden test arena, and their social interaction scored by two independent observers for 7.5 min, from a video monitor in an adjacent room. An anxiogenic profile is most clearly seen in this test when animals are tested in low light and have previously been familiarised with the test box. Animals received an i.p. injection of water/Tween-20 vehicle or of FG 7142 (5, 10 or 20 mg/kg) 20 min before testing. Analysis of variance showed that FG 7142 (5, 10 and 20 mg/kg) produced a significant effect on time spent in active social interaction ($F(3, 28) = 8.19$, $p < .0005$), and Dunnett's t-tests showed that all doses significantly reduced social interaction ($p < .01$). There was also a significant effect on locomotor activity ($F(3, 28) = 3.11$, $p < .05$), but Dunnett's tests showed that there was a significant decrease only at the 20 mg/kg dose ($p < .05$).

The anxiogenic action of FG 7142 in man was correlated with high plasma levels of the drug; for this reason, plasma concentrations of FG 7142 were also determined in these rats, and correlated with the behavioural results. For the 5 and 10 mg/kg doses, pairs of rats that had the lowest social interaction scores (i.e. a strong anxiogenic effect) had the highest plasma concentrations of FG 7142, see Table 1. There was no correlation of plasma concentrations with the locomotor activity scores.

Table 1. (a) Mean ± S.E.M. concentrations of FG 7142 (mg/ml plasma) for pairs of rats sampled after social interaction, and (b) Pearson correlation coefficient for the social interaction score of each pair with the plasma concentration.

Dose	(a)	(b)
5 mg/kg	3.92 ± 0.58	-0.60
10 mg/kg	10.23 ± 2.05	-0.74
20 mg/kg	8.92 ± 3.47	-0.20

Dorow R, Horowski R, Paschelke G, Amin M and Braestrup C (1983). The Lancet July 9 : 98-99.
File S E, Lister R G, Nutt D J (1982). Neuropharmacol. 21 : 1033-1037.
File S E, Lister R G, Maninov R, Tucker J C (1984). Neuropharmacol. 23 : 463-66.
Jensen L H, Petersen E N, Braestrup C (1983). Life Sci. 33 : 393-399
Petersen E N, Jensen L H, Honore T and Braestrup C (1983). In Biggio G and Costa E (Eds) : Raven Press, New York.

THE EFFECTS ON SOCIAL INTERACTION OF MICROINJECTIONS OF RO 15-1788 INTO THE NUCLEUS RAPHE DORSALIS OF THE RAT

A. Hobbs ,I.A. Paterson* & M.H.T. Roberts, Department of Physiology, University College, Cardiff, CF1 1XL.

Microinjections of benzodiazepine receptor ligands into the Nucleus Raphe Dorsalis (NRD) of the rat have the same effect as systemic administration in anxiety tests. Thus microinjections of 30 pg of Chlordiazepoxide into the NRD produce anxiolytic effects (Thiebot et al, 1982) and microinjections of 100 pg of Methyl B-Carboline-3-Carboxylate (BCCM) produce anxiogenic effects (Hindley et al, 1984). We have investigated the effects of microinjecting the benzodiazepine receptor antagonist RO 15-1788 into the NRD, using the Social Interaction Test (File, 1980), to determine whether the benzodiazepine receptors in the NRD play an important role in the anxiogenic effects of BCCM.

The experimental procedure has been previously reported (Hindley et al, 1984). Male Wistar rats which had been implanted with steel guide cannulae and had been familiarised with the test box were tested with untreated rats. Five minutes prior to the test the implanted rat was given a microinjection of drug or vehicle (saline). When rats were given an i.p. injection of BCCM this was given either 15 or 5 minutes prior to testing. The social interaction of the treated rat and the locomotor activity of the pair were recorded over a 10 minute period. Four days later the procedure was repeated and the implanted rat was given a microinjection of vehicle or drug so that all implanted animals had received both vehicle and drug. Following the second test the implanted animals were sacrificed for histology.

A total of 42 rats were used in the microinjection studies. Microinjection of 1 ng of RO 15-1788 into the NRD had no effect on the social interaction or the locomotor activity of 6 rats. However, 1 ng of RO 15-1788 reversed the effects on social interaction of 100 pg of BCCM microinjected into the NRD, causing an increase in social interaction of 43±6 seconds (mean±S.E., n=7, $P \overset{3}{4} 0.01$). Intraperitoneal injections of BCCM (2 and 4 mg/kg) decreased social interaction. Microinjections of 1 ng of RO 15-1788 into the NRD reversed the effect on social interaction of BCCM given i.p. (either 4 mg/kg 15 minutes prior to testing or 2 mg/kg 5 minutes before testing) without affecting locomotor activity. The social interaction score of animals given BCCM i.p. was approximately 50% of that of control animals where as the score of animals given BCCM i.p. and RO 15-1788 i.c. was approximately 80% of the score of control animals. Microinjections of RO 15-1788 outside the NRD did not influence the effect of BCCM.
We conclude that the benzodiazepine receptors in the NRD play an important role in the anxiogenic effects of BCCM (as assessed by the social interaction test) because microinjection of the antagonist RO 15-1788 into the NRD blocks the anxiogenic effects of BCCM given either i.p. or directly into the NRD.

File, S.E. (1980) J.Neurosci.Meth. 2, 219-238
Hindley, S.W. et al (1984) Comm. C47 Br.Pharm.Soc. January 1984
Thiebot, M.H. et al (1982) Neuroscience 7, 2287-2294

STEREOTYPY INDUCED BY THE D_2 DOPAMINE AGONIST RU24213 IS BLOCKED BY THE D_2 ANTAGONIST RO22-2586 AND THE D_1 ANTAGONIST SCH 23390

K. M. O'Boyle, M. Pugh and J. L. Waddington*. Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2.

It has recently been possible to investigate the functional role of the D_1 dopamine receptor using the new selective agonist *R*-SK & F 38393 and antagonist SCH 23390 (O'Boyle & Waddington, 1984). While we have identified non-stereotyped behaviours induced by *R*-SK & F 38393 that are selectively blocked by SCH 23390, we have reported that drug actions at the D_1 receptor can also influence classically D_2-mediated behaviours such as stereotypy (Molloy & Waddington, 1984). We have now compared the actions of SCH 23390 and the new D_2 antagonist Ro22-2586 (Davidson et al, 1983) to influence stereotyped behaviour induced by the selective D_2 agonist RU24213 (Euvrard et al, 1980) in male Sprague-Dawley rats.

Relative potencies of drugs to displace the binding of 0.3nM ^{3}H-piflutixol (D_1 receptors) and of 0.1nM ^{3}H-spiperone (D_2 receptors) were determined in rat striatum (O'Boyle & Waddington, 1984). Rats were challenged with 15 mg/kg s.c. RU24213 and stereotypy responses assessed using a rating scale and a behavioural check-list (Molloy & Waddington, 1984). RU24213 and Ro22-2586 selectively displaced ^{3}H-spiperone while SCH 23390 selectively displaced ^{3}H-piflutixol (Table).

	IC_{50} (nM)		$\frac{^3\text{H-PIF } (D_1)}{^3\text{H-SPIP } (D_2)}$
	^{3}H-piflutixol	^{3}H-spiperone	
RU24213	> 50,000	377 ± 84	> 133
Ro22-2586	> 15,000	85 ± 45	> 178
SCH 23390[a]	1.0 ± 0.3	1,565 ± 31	0.0006

Mean ± S.E.mean (n = 3-4). [a]from O'Boyle & Waddington (1984).

Stereotyped behaviour induced by RU24213 was blocked ($P < 0.05$) by Ro22-2586, 40-200 µg/kg 30 min previously. These data are consistent with the conventional view that this behavioural syndrome is D_2-mediated (Seeman, 1980; Laduron, 1982). However, responses to RU24213 were also blocked ($P < 0.05$) by the D_1 antagonist SCH 23390, 40-200 µg/kg 30 min previously. Either the proposed selectivity of one or more of these various agents is mistaken or else there may be functional interactions between D_1 and D_2 receptors. We suggest that these compounds are selective according to current criteria, and therefore that functional interactions between D_1 and D_2 receptors may occur.

This work was supported by the Medical Research Council of Ireland and the Royal College of Surgeons in Ireland. We thank Lundbeck, Roche, Roussel and Schering for gifts of drugs.

Davidson, A.B. et al (1983). Psychopharmacol. 79, 32-37.
Euvrard,C. et al (1980). Neuropharmacol. 19, 379-384.
Laduron, P. (1982). Adv. Biosci. 37, 71-82.
Molloy, A. & Waddington, J.L. (1984). Psychopharmacol., in press.
O'Boyle, K.M. & Waddington, J.L. (1984). Eur. J. Pharmacol., in press.
Seeman, P. (1980). Pharmacol. Rev. 32, 229-313.

LOW LEVEL LEAD EXPOSURE ALTERS THE DEVELOPMENT OF MORPHINE ANTINOCICEPTION IN THE RAT

I. Kitchen, J. McDowell, J.M. Wilson and C. Winder, Department of Biochemistry, University of Surrey, Guildford, Surrey, GU2 5XH

Some neuropsychological disorders of childhood may result from environmental exposure to lead and we have recently shown that low-level exposure to this metal depresses and delays the development of enkephalins in the striatum of neonatal rats (Winder et al. 1983). We have extended these studies by measuring morphine antinociception in the normal and lead-exposed neonate as an indirect measure of opioid receptor system development.

Lead was administered at two doses (300 and 1000 ppm) in the maternal drinking water from conception to weaning as previously described (Carmichael et al., 1982). Antinociceptive testing was carried out at 10, 21 and 30 days using the hot water tail immersion test at 50°C. The nociceptive end point was taken as the time of withdrawal of the tip of the tail from the water bath and a maximum cut-off time of 10 seconds was employed. Animals were pretested 15 minutes before injection and 15, 30 and 60 minutes after injection of 0.9% saline or morphine sulphate (at three dose levels) subcutaneously. After experimental procedures were completed trunk blood was collected and blood leads determined by atomic absorption spectrophotometry.

In the 300 ppm lead dosed groups there was a significant impairment of the antinociceptive activity of morphine in 10 day old rats only at 16mg/kg where the peak response latency was reduced from 7.2 $\pm$ 1.1 sec to 4.3 $\pm$ 0.9 sec. In the 1000 ppm lead dose group antinociceptive activity of morphine was observed but this was not dose related. Blood lead measurements at 10 days were 34.7 $\pm$ 1.3 and 47.5 $\pm$ 2.5µg/100 ml for the 300 and 1000 ppm lead dosed groups respectively. The differences in the antinociceptive potency of morphine in normal and lead-exposed animals at 10 days were not observed at later time points (21 and 30 days).

It is suggested that lead may disrupt the ontogeny of opioid receptor systems which mediate the effects of morphine, and that this disruption occurs early in development. It is becoming clear that opioid peptide systems are very sensitive to the toxic effects of lead. Whether this neurochemical toxicity is responsible for the behavioural disorders associated with lead exposure remains to be studied.

Carmichael, N.G., Winder, C. and Lewis, P.D. (1982) Toxicology, 21, 117-128.
Winder, C., Kitchen, I., Clayton, L.B., Gardiner, S.M., Wilson, J.M. and Lewis, P.D. (1983) Toxicol. appl. Pharmac., in press.

MEPTAZINOL AND OPIATE RESPIRATORY DEPRESSION IN THE CONSCIOUS RAT

I.S. Cowlrick* and N.B. Shepperson, Department of Pharmacology, Wyeth Institute of Medical Research, Taplow, Maidenhead.

Unlike most opiate analgesics, meptazinol (Mep) produces analgesia without inducing marked respiratory depression (Goode et al 1979). The interaction between Mep and the opiate receptors inducing respiratory depression has been investigated in the conscious rat.

Right carotid artery cannulae were implanted under halothane anaesthesia 24h before the experiments. The rats (150-200g male) were restrained in perspex tubes and 100µl blood samples were taken from the carotid cannula at 10 minute intervals, for 30 minutes before and 60 minutes after drug administration. Arterial blood carbon dioxide tension (PCO_2, mmHg) was measured as an index of respiratory status.

Mep, 7.5, 15 and 30 mg/kg s.c. (effective analgesic doses, Bill et al, 1983) evoked a weak respiratory depression increasing PCO_2 by 5.7±1.6, 10.3±2.2 and 8.5±4.5mmHg respectively.

Pretreatment with naloxone (0.1mg/kg i.v.) reduced the peak increase in PCO_2 evoked by Mep (15mg/kg s.c.) and morphine (7.5mg/kg s.c.)from 6.2±1.6 and 24.6±7.1mmHg to 0.1±1.6 and 5.7±3.7mmHg respectively.

Animals were given morphine (7.5mg/kg s.c.) in combination with either saline, Mep (15 or 30mg/kg s.c.), or morphine (7.5mg/kg s.c.). The morphine-morphine combination significantly increased the PCO_2 value above that obtained with morphine-saline. In contrast, the Mep-morphine combinations decreased the PCO_2 below that obtained with morphine-saline.

These results demonstrate thatMep has both opioid agonist and antagonist properties.

The analgesic effects of Mep have been shown to be due to both opioid and cholinergic mechanisms in the mouse (Bill et al 1983). Studies on the guinea-pig ileum have demonstrated that only the (-) isomer of Mep possesses cholinergic activity. Pretreatment of rats with atropine (3mg/kg s.c.) caused a significant potentiation of the increases in PCO_2 evoked by Mep (30mg/kg s.c.) Rats were also treated with either the (-) or (+) isomer of Mep (30mg/kg s.c.). The (+) isomer increased the PCO_2 by 9.0±0.9mmHg, but the (-) isomer was inactive. However, when the (-) isomer was given to rats pretreated with atropine (3mg/kg s.c.) it evoked a significant increase in PCO_2 (6.4±1.8mmHg).

These results confirm that in rats meptazinol depresses respiration only weakly. This is attributed to the compound's mixed agonist-antagonist characteristics at opioid receptors and it's ability to facilitate cholinergic transmission.

Bill et al (1983). Br. J. Pharmac. 79, 191-199
Goode et al (1979). J. Pharm. Pharmac. 31, 793-795

CHRONIC ADMINISTRATION OF MORPHINE MOTIVATES LEARNING FOR FOOD REWARDS IN RATS

R. Kumar, E.A. Norris and I.P. Stolerman*, Departments of Pharmacology and Psychiatry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

Rats maintained on large, daily doses of morphine consume substantial amounts of food and water 0-4 hours after being injected (Kumar et al., 1971); the present work tests whether such rats will press bars for food without prior food deprivation. Morphine hydrochloride was administered intraperitoneally in doses which increased progressively to 100 mg/kg/day. After about 6 weeks at this dose the 16 rats were placed in test chambers where animals deprived of food readily acquire the bar-pressing response.

All 16 rats were trained successfully to bar-press and averaged about 150 responses in 20 minute sessions beginning 2 hours after injection of morphine. Initially, the rats were not given access to food between injection and training, but this procedure was found to be unnecessary. Ten animals went on to respond reliably on a fixed-ratio 10 schedule, where food was presented after every tenth bar-press (FR 10). Performance on the FR 10 schedule provided a baseline for further experiments with two subgroups (n = 5 each); the rats were tested twice weekly and training sessions on morphine (100 mg/kg) continued on intervening days.

The mean numbers of responses in 20 min ($\pm$ s.e.m.) under the FR 10 schedule increased in a dose-related manner from 173 $\pm$ 63 when no morphine was injected to 556 $\pm$ 85 after morphine (50 mg/kg) and declined to 318 $\pm$ 63 after morphine (100 mg/kg). For time-course studies, 5-min tests were carried out repeatedly at different times after injection. With morphine (100 mg/kg) response rates were low until 2 h after injection, they reached a maximum at 4 h, and declined again by 8 h. Stereotyped gnawing was evident and this may have been incompatible with bar-pressing. In contrast, response rates were elevated 0.5 h after morphine (25 mg/kg), and reached a maximum of 250 $\pm$ 30 bar-presses in 5 min at 2 h post-injection, as compared with 56 $\pm$ 40 presses 2 h after water. Naltrexone hydrochloride (0.1 - 1.0 mg/kg) injected simultaneously with morphine (100 mg/kg) increased response rates whereas naltrexone (10 mg/kg) drastically suppressed responding.

Chronic administration of morphine in doses associated with dependence can, like food deprivation, motivate bar-pressing for food. FR 10 responding shows the regular pattern typically found under such conditions, it is sensitive to naltrexone, and it is not a consequence of stereotypy or locomotor activity. This novel effect may aid understanding of the strength with which morphine itself can serve as a reward and it is also compatible with evidence reviewed by Sanger (1981) that endogenous opioids may play a role in controlling appetite.

Kumar, R., Mitchell, E. & Stolerman, I.P. (1971) Br.J.Pharmac. 42, 473-484.
Sanger, D.J. (1981) Appetite 2, 193-208.

EFFECT OF ISOMERS ON THE 6-AZA DERIVATIVE OF MIANSERIN ON BEHAVIOUR AND NORADRENALINE METABOLISM IN BULBECTOMIZED RATS

B.E. Leonard & W.T. O'Connor*. Department of Pharmacology, University College, Galway, Republic of Ireland.

Bilateral olfactory bulbectomy in the rat is associated with behavioural changes in exploratory behaviour and passive avoidance conditioning that are largely reversed by chronic, but not acute, antidepressant treatment (Cairncross et al., 1979; Jancsár and Leonard, 1983). The result of such investigations have led to the suggestion that the olfactory bulbectomized rat is a useful model for the selection of potential antidepressant drugs. Detailed studies on the atypical tetracyclic antidepressant mianserin have shown that the hypermotility of the bulbectomized rat in a stressful, novel environment (the 'open field' apparatus) is attenuated by chronic treatment with a dose of mianserin that is equivalent to that used clinically (1 mg/kg). Furthermore, in this animal model only the (+) isomer shows such activity (Leonard, 1982). The aim of the present study was to study the 6-Aza derivative of mianserin (Org. 3770) and its enantiomers on the behaviour of the bulbectomized rat and to determine whether the slight structural modification to the mianserin molecule affects the behaviour of the bulbectomized rat in the 'open field' apparatus. Male Sprague-Dawley rats (250-280g) were used. They were bilaterally bulbectomized as described in detail by Jancsár and Leonard (1981). After recovery from surgery (14 days) groups of 8 rats were injected with Org. 3770 or one of its enantiomers, in a dose of 0.5, 1.0 or 2.0 mg/kg daily for 12 days; their performances in the 'open field' apparatus 24 hr. after the last administration of the drugs were compared with sham operated rats that had also been injected with the drug. Saline injection bulbectomized and sham operated rats acted as controls. One day after the 'open field' exposure the animals were killed and the concentrations of noradrenaline and MHPG determined in the amygdaloid cortex and midbrain by a fluorimetric method (Earley and Leonard, 1978).

The results showed that the racemate attenuated the hypermotility of the bulbectomized rat in the 'open field' apparatus at all doses studied. Only the highest dose of the racemate reduced the activity of the sham operated animals. The behavioural activity would appear to reside in the (+) isomer as the (-) isomer was inactive in this test. Bulbectomy was associated with a decrease in the concentration of noradrenaline and MHPG in the amygdaloid cortex (by 12% and 14% respectively) and midbrain (13% and 18% respectively). Chronic treatment with the racemate was associated with a return of these concentrations to control values. When the enantiomers were tested, it was found that the behaviourally inactive (-) isomer was most effective in normalizing the noradrenaline deficit. It is concluded that there is no correlation between the behavioural activity of the enantiomers of Org. 3770 and changes in the noradrenaline metabolism in the amygdaloid cortex and midbrain.

Acknowledgement. This study was supported by a grant from Organon International B.V., Holland who also supplied Org. 3770 and its enantiomers used.

Cairncross, K.D., Cox, B., Forster, C. & Wren, A.F. (1979). Psychoneuroendocrin. 4, 253-272.

Earley, C.J. & Leonard, B.E. (1978). J. Pharmac. Meth., 1, 67-79.

Jancsár, S. and Leonard, B.E. (1983). In: Frontiers in Neuropsychiatric Research. Ed. Usdin, E., Goldstein, M., Friedhoff, A.J. and Georgotas, A. MacMillan, London. p. 357-372.

Jancsár, S. & Leonard, B.E. (1981). Neuropharmac., 20, 1341-1345.

Leonard, B.E. (1982). Adv. Biochem. Pharmac., 31, 301-319,

EFFECT OF OXYPERTINE VERSUS RESERPINE ON AMPHETAMINE AND METHYLPHENIDATE INDUCED ACTIVITY IN RATS

T. Palomo and I.C. Reid (introduced by C. Geddes), Department of Mental Health, Clinical Research Centre, Royal Cornhill Hospital, Aberdeen.

Amphetamine and methylphenidate are psychostimulant drugs acting on two different neuronal pools of amines, reserpine-resistant and reserpine-sensitive respectively (Scheel-Krüger, 1971). We have previously reported that the monoamine-oxidase inhibitor pargyline prevents the effect of oxypertine on amphetamine induced behaviour (Palomo and Reid, 1983). This suggests that oxypertine is an amine depleter type of drug but, unlike reserpine, it acts on the amphetamine releasable pool of amines. In order to test whether oxypertine acts also on the reserpine-sensitive pool we compared their effects on both amphetamine and methylphenidate induced activity.

68 Albino Sprague-Dawley rats (Charles River U.K. London) were used in these experiments. They all received two i.p. doses of either 4 mg/Kg oxypertine, 5 mg/Kg reserpine or saline. The second dose was administered 18 hours after the first one and then the rats were placed in a hole-board apparatus. 70 Minutes later they received either 8 mg/Kg d-amphetamine i.p. or 50 mg/Kg methylphenidate i.p. The rat motor behaviour was recorded for 4 minutes immediately before the amphetamine (pre-amph) or methylphenidate (pre-meth) and 40 minutes after (post-amph and post-meth respectively). Results in Table 1 show clearly that oxypertine acts on amphetamine but not methylphenidate induced activity whereas reserpine does the opposite. The monoamine-oxidase-inhibitor pargyline (50 mg/Kg i.p.) given four hours before the first dose of reserpine or oxypertine prevents their effect (data not shown).

Table 1: Rat repetitive (stereotyped) motor activity before and after amphetamine (8 mg/Kg) or methylphenidate (50 mg/Kg) x $\pm$ S.E. of mean $n \geq 4$

	AMPHETAMINE		METHYLPHENIDATE	
	Pre-amph	Post-amph	Pre-meth	Post-meth
Saline:	0.0 ± 0.0	163.7 ± 35.2*	2.2 ± 0.9	218.1 ± 4.4**
Oxypertine:	2.0 ± 1.2	10.0 ± 5.9	3.7 ± 2.6	236.7 ± 3.2**
Reserpine:	0.0 ± 0.0	203.0 ± 5.3*	0.0 ± 0.0	6.2 ± 3.6

*, **, $p < 0.01$ compared with pre-amph or pre-meth respectively (Newman-Keuls test).

The above data suggest that reserpine and oxypertine are amine depleters acting on two different pools of amines. Oxypertine, 4 mg/Kg, seems to deplete the reserpine resistant (amphetamine releasable) pool of amines but appears to be without effect on the reserpine-sensitive one. Thus oxypertine may provide a means to interfere with the pool of newly synthesized amines. However biochemical data is required to confirm the behavioural evidence reported here.

The authors thank Sterling Winthrop and Ciba for gifts of oxypertine and methylphenidate respectively.

Palomo, T. and Reid, I.C. (1983). British Journal of Pharmacology, 80, 455P.
Scheel-Krüger, J. (1971). European Journal of Pharmacology, 14, 47-59.

REGIONAL ACETYLCHOLINESTERASE ACTIVITY IN RAT BRAIN FOLLOWING A CONVULSION

Margaret E. Appleyard*, A.R. Green+ and A.D. Smith, University Department of Pharmacology, South Parks Road, Oxford, and +MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford.

A single electroconvulsive shock (ECS), flurothyl- or bicuculline-induced convulsion produces a rapid rise in seizure threshold when measured by infusion of GABA antagonist drugs, which lasts for somewhat over 3h (Nutt et al, 1981). There is also a rise in brain GABA concentration following an ECS or bicuculline-induced seizure, but this does not seem to be responsible for the rise in threshold since it is elevated for a shorter period than the seizure threshold and flurothyl increases the threshold but not the brain GABA concentration (Bowdler & Green, 1982). In view of the suggested involvement of acetylcholine in seizure disorders (Olney et al, 1983) we have now investigated acetylcholinesterase (AChE) activity in regions of rat brain after an ECS.

Female Wistar rats (150-180g) were given a single ECS (45mA, 1.5s) via earclip electrodes, killed 2, 15, 30, 120 and 180min later and the brain rapidly dissected at 0°C. AChE activity in the total homogenate and 100,000g supernatant (soluble fraction) was measured by the stopped assay version of the Ellman et al (1961) method. Hippocampus, mesencephalon, cortex, striatum and amygdala showed rapid changes in AChE activity in the first minutes following the convulsion whilst brainstem and basal forebrain AChE activity was unchanged. Both hippocampus and midbrain showed a sustained decrease in activity, total AChE activity being decreased by up to 40% within 2min of the convulsion and remaining below control values until 180min (Table). The hippocampal soluble AChE exhibited a similar decrease whilst the soluble AChE in the midbrain showed a variable change (Table). Thirty min after a flurothyl-induced convulsion there was a similar fall in AChE activity in both regions, whilst a subconvulsive shock (5mA, 1.5s) produced no change (Table). This latter treatment does not elevate threshold (Nutt et al, 1981).

The time course of the changes observed in AChE activity in the hippocampus and midbrain correlates well with the time of elevated seizure threshold after a convulsion but further work will be necessary to see whether there is a causal link.

TABLE. Changes in AChE activity following a seizure.

Treatment	Time of measurement	Hippocampus Total	Hippocampus Soluble	Midbrain Total	Midbrain Soluble
Handled	-	39.1 ± 5.3	22.7 ± 5.0	74.8 ± 3.4	54.0 ± 6.2
ECS	2	28.4 ± 2.6*	12.6 ± 1.0*	56.6 ± 3.6*	41.4 ± 6.0
ECS	15	28.9 ± 0.9*	16.7 ± 1.3	55.8 ± 2.5+	67.0 ± 3.7*
ECS	30	31.9 ± 1.9	15.3 ± 1.6	57.2 ± 1.6+	61.2 ± 2.2
ECS	120	27.5 ± 1.8*	12.3 ± 0.8*	47.5 ± 1.5+	47.1 ± 2.9
ECS	180	37.8 ± 1.1	23.6 ± 1.3	77.0 ± 2.2	69.4 ± 3.5*
Flurothyl	30	27.0 ± 2.5*	10.8 ± 0.9*	53.7 ± 4.4+	73.3 ± 4.3*
Subconvulsive	30	53.1 ± 3.7	19.6 ± 0.8	78.1 ± 5.0	56.9 ± 4.0

Different from control *$p < 0.05$; +$p < 0.01$. Results (mean ± s.e. mean of 5 or more observations) expressed in nmol acetylthiocholine hydrolysed $min^{-1}mg$ $protein^{-1}$.

Bowdler, J.M. & Green, A.R. (1982) Br. J. Pharmac. 76, 291-298.
Ellman, G.L. et al (1961) Biochem. Pharmac. 7, 88-95.
Nutt, D.J. et al (1981) Eur. J. Pharmac. 71, 287-295.
Olney, J.W. et al (1983) Nature 301, 520-522.

PRETREATMENT WITH RESERPINE DOES NOT MODIFY THE AMPHETAMINE INDUCED RELEASE OF ENDOGENOUS DOPAMINE IN STRIATAL SLICES

Sonia Arbilla, T. Dennis, S.Z. Langer, Ruth Niddam* and B. Scatton. Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo, 58 rue de la Glacière, 75013 Paris, France.

The central stimulant actions of amphetamine (AMPH) are not altered in animals in which the brain stores of catecholamines are depleted with reserpine (RES) suggesting that amphetamine causes these behavioural effects by releasing dopamine (DA) from a reserpine-resistant pool (see Moore, 1977). However, no direct biochemical evidence for this proposal has been provided. In order to test this possibility we have presently investigated the effect of AMPH on the release of endogenous DA from striatal slices after RES-pretreatment in the rat.

Striatal slices were perfused with Krebs medium at a rate of 0.5 ml/min and 2 ml samples were collected. The release of endogenous DA was elicited by electrical stimulation (3 Hz, 2 msec, 24 mA, 2 min) in the presence or absence of nomifensine 10 µM or by 2 min exposure to AMPH 10 µM in the presence or absence of pargyline 10 µM. Endogenous DA was measured, in the perfused medium by radioenzymatic assay (Da Prada M. and Zürcher G. 1976) and in the tissue by high performance liquid chromatography with electrochemical detection (Semerdjian-Rouquier et al., 1981).
In untreated animals, in the absence of nomifensine, the spontaneous outflow of DA (Sp) and the electrically evoked release of DA above Sp (S) were 17 ± 2 pg/sample (n=7) and 28 ± 6 pg (n=7) respectively. In the presence of nomifensine Sp was 286 ± 39 pg/sample (n=9) and S : 2021 ± 200 pg (n=9).

Table 1 : Effects of RES treatment on the AMPH-induced release of endogenous DA.

DA	UNTREATED RATS	RESERPINE TREATED RATS
AMPH stimulation (pg) (a)	362 ± 5.3 (3)	374 ± 15.4 (4)
Tissue content (ng/g)	5515 ± 874.0 (3)	134 ± 17.2 (4)*

Pargyline 10 µM was present in the Krebs. (a) . DA released above Sp during 20 min following a 2 min exposure to 10 µM AMPH. ( ) : number of experiments.
* $p < 0.001$ when compared with the untreated group.

RES (5 mg/kg, s.c., 24 hr) treatment resulted in 97% reduction of DA content in the striatum (Table 1). In the presence of pargyline, in untreated and RES treated rats, AMPH released similar amounts of endogenous DA (Table 1). In the absence of pargyline, similar qualitative results were obtained.

The present results show that, although the endogenous stores of DA were almost totally depleted by RES treatment, the amount of DA released by AMPH remained unaffected. In addition, they support the view that the stimulant effects of AMPH in RES treated animals are mediated through the release of DA from a RES resistant pool (Cantrill et al., 1983) and not by a direct effect of AMPH on an AMPH recognition site (Paul et al., 1982). Consequently, the present "in vitro" results are consistent with the inability of RES to modify the behavioural effects of AMPH.

Cantrill R. et al. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322 : 322-324
Da Prada M. and Zürcher G. (1976) Life Sciences 19 : 1161-1174
Moore K.E. (1977) Biological Psychiatry 12 : 451-462
Paul S.M. et al. (1982) Science 218 : 487-490
Semerdjian-Rouquier L. et al. (1981) Chromatography 218 : 663-670

AN IMMUNOHISTOCHEMICAL STUDY TO LOCALISE HISTAMINERGIC NEURONES IN INVERTEBRATE AND VERTEBRATE NERVOUS SYSTEMS

R. Leslie, N.N. Osborne*, S. Patel and A. Peard, Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW. U.K.

It has been proposed that histamine has a transmitter role in vertebrate and invertebrate nervous systems (Weinreich, 1978; Schwartz *et al*, 1980). There is, however, a lack of proof that histamine is restricted to specific neurones in the vertebrate CNS, although biochemical results from hand-dissected neurones in invertebrate nervous systems such as the snail brain have provided persuasive evidence (Weinreich, 1978). It would seem essential that we have histochemical proof of the restriction of histamine to specific neurones.

Recently, Wilcox and Seybold (1982) described a method for producing an anti-serum to a histamine/methylated BSA complex and showed the occurrence of histamine-like neurones in the rat brain; however, proof of the specificity of the anti-serum for localising histamine was questionable.

In our studies (Osborne and Patel, 1984) we raised an anti-serum to immunogen as described by Wilcox and Seybold (1982) and subjected nervous tissues from the rat and snails (*Helix Aspersa* and *Lymnea stagnalis*) to immunofluorescent histochemical analysis. A high density of histamine-immunoreactive varicose fibres was observed in the rat median eminence. Scattered fibres and weakly stained cell bodies could be seen in other parts of the diencephalon. Some large cell bodies in the ventral horn of the spinal cord also exhibited immunoreactivity. Sections of the snail brain exhibited more dramatic activity with intense fluorescence associated with specific neurones and some fibres in the neuropile regions.

Our anti-serum was clearly 'staining' specific molecules but it was still necessary to prove that it was revealing histamine. An anti-serum to a conjugate of carnosine produced in the same way as the histamine one gave negative results. The most persuasive evidence was the finding that a defined neurone in *Lymnea stagnalis* which had previously been shown to contain histamine by microchemical analysis (Turner & Cottrell, 1977) reacted positively to the antibody. We interpret these data as showing that our anti-serum recognises histamine, and conclude that histamine is therefore restricted to specific neurones in vertebrate and invertebrate nervous systems.

R.L. is on leave from the Department of Anatomy, Dalhousie University, Halifax, Nova Scotia, Canada.

Osborne, N.N. & Patel, S. (1984) in press.
Schwartz, J.C., Pollard, H. & Quach, T.T. (1980) J.Neurochem. *35*, 26-38.
Turner, J.D. & Cottrell, G.A. (1977) Nature *267*, 447-448.
Weinreich, D. (1978) in Biochemistry of Characterised Neurones (ed. Osborne, N. N.) Pergamon Press, pp. 153-175.
Wilcox, B.J. & Seybold, V.S. (1982) Neuroscience Letters *29*, 105-110.

DL-(^{3}H)-2-AMINO-4-PHOSPHONOBUTYRATE LABELS POSTSYNAPTIC EXCITATORY AMINO ACID RECEPTORS IN RAT STRIATUM

S.P. Butcher, J.F. Collins[1] & P.J. Roberts, Department of Physiology & Pharmacology, University of Southampton, Southampton, SO9 3TU and [1]Department of Chemistry, City of London Polytechnic, London, EC3N 2EY.

L-2-amino-4-phosphonobutyrate (L-APB) is a potent synaptic depressant, although it has not been resolved whether its effects are pre- or post-synaptic (Koerner & Cotman, 1981; Davies & Watkins, 1982). The binding of DL-^{3}H-APB to glutamate-sensitive sites on striatal synaptic plasma membranes (SPM's) is preferentially inhibited by L-APB (Butcher et al., 1983). We have recently reported that L-APB-sensitive sites in striatum are not localised on cortico-striatal glutamatergic terminals (Butcher et al., 1984). In this study, we have combined binding and transmitter release experiments to investigate the likely postsynaptic localisation of L-APB-sensitive receptors in the rat striatum.

Male Wistar rats (190 g) were injected unilaterally into the striatum with 5 nmol kainate (pH 7.4) (coordinates: AP +7.9, Ml +2.2, DV -5.3; Konig & Klippel, 1967), or with 6-hydroxydopamine (6-OHDA) into the substantia nigra (coordinates: AP +2.4, ML +1.8, DV -8.3), in order to destroy respectively, striatal interneurones and the nigro-striatal dopaminergic terminals. At various times following lesioning, DL-^{3}H-APB binding to striatal SPM's was determined (Butcher et al., 1983). In addition, striatal slices (0.25 x 0.25 mm) were allowed to accumulate ^{3}H-GABA or ^{3}H-dopamine (both 10^{-7} M), and the release characteristics of these substances investigated.

It was found that L-glutamate and L-APB did not influence the spontaneous, or the Ca^{2+}-dependent release (15 mM K^+) of ^{3}H-GABA. However, ^{3}H-dopamine release was increased by both drugs, suggesting that L-APB-sensitive receptors may be present on dopaminergic terminals. The binding studies demonstrated that kainate lesions produced a profound (at least 50%) decrease in specific DL-^{3}H-APB binding. A smaller decrease was observed in the 6-OHDA-treated animals.

These results favour the idea that the majority of L-APB-sensitive sites are localised postsynaptically in the rat striatum, with a smaller population occurring on the afferent dopaminergic terminals. While there is no direct evidence available from electrophysiological studies, it seems likely that the action of L-APB in the striatum is that of an agonist, mimicking the effects of L-glutamate.

Acknowledgement: This work was supported by an S.E.R.C. project grant to P.J.R. We thank Dr Dick Young (NEN) for his continued interest in this work.

Butcher, S.P., Roberts, P.J. & Collins, J.F. (1983) Br. J. Pharmac. 80, 355-364.
Butcher, S.P., Collins, J.F., Roberts, P.J. & De Souza, R.J. (1984) J. Physiol. (Proc.) In Press.
Davies, J. & Watkins, J.C. (1982) Brain Res. 235, 378-386.
Koerner, J.F. & Cotman, C.W. (1981) Brain Res. 216, 192-198.
Konig, J.F.R. & Klippel, R.A. (1967) The Rat Brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Robert E. Kreiger Publishing Co. Inc. New York.

ENDOGENOUS PHENOLAMINES : DO THEY HAVE A DIRECT POSTSYNAPTIC ACTION ON DOPAMINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM?

Sonia Arbilla, P. Baud*, R. Cantrill and S.Z. Langer. Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo, 58, rue de la Glacière, 75013 Paris, France.

Phenolamines originated from β-phenylethylamine (PEA), a naturally occuring analog of amphetamine (AMPH), are detectable in trace amounts in brains of several species. We have compared the effect of PEA, tyramine (TYR) and octopamine (OCT) with AMPH on the electrically evoked release of ^{3}H-acetylcholine (^{3}H-ACh) from rat striatal slices. Exposure to AMPH produces an inhibitory effect on neurotransmission from the cholinergic striatal interneuron through the activation of inhibitory dopamine receptors via the release of newly synthetized dopamine (Cantrill et al., 1983).

Rat striatal slices were labelled with ^{3}H-methylcholine and perfused in Krebs medium in the presence or absence of pargyline (10 μM). In the presence of pargyline, 4 experimental groups were studied : untreated, reserpinized (RES, 5 mg/kg s.c., 24hr), RES + α-methyl-tyrosine (αMpT 300 mg/kg i.p., 2hr) and RES + αMpT+sulpiride (SULP 0.1 μM during the superfusion). The release of ^{3}H-ACh was elicited by two periods of electrical stimulation (S_1 and S_2) at 1 Hz, 16 mA for 2 min. Drugs were added 20 min before S_2.

Table 1 : Effects of PEA, AMPH, TYR and OCT on the electrically evoked release of ^{3}H-ACh from rat striatal slices.

	μM	S_2/S_1			
		UNTREATED	RESERPINE	RES + αMpT(a)	RES+αMpT+SULP(a)
Control	-	0.77 ± 0.03	0.89 ± 0.02	0.87 ± 0.03	0.81 ± 0.06
AMPH	1	0.42 ± 0.04*	0.22 ± 0.03*	0.51 ± 0.01*	0.88 ± 0.04**
PEA	10	0.20 ± 0.02*	0.18 ± 0.01*	0.35 ± 0.02*	0.86 ± 0.03**
TYR	10	0.21 ± 0.04*	0.43 ± 0.02*	0.73 ± 0.02*	N.T.
OCT	10	0.44 ± 0.06*	0.88 ± 0.01	0.90 ± 0.02	

Pargyline (10 μM) was present in the medium throughout the experiment. (a) : Krebs with αMpT 100 μM. * $p < 0.005$ vs the corresponding control. ** $p < 0.001$ vs RES + αMpT alone. N.T. . not tested. Values are mean ± SEM from 3 to 9 experiments per group.

In the absence of pargyline, and in contrast with AMPH, the three trace amines tested (OCT, TYR, PEA) were inactive at inhibiting the electrically-evoked release of ^{3}H-ACh. In the presence of pargyline, PEA, TYR and OCT inhibited the electrically-evoked release of ^{3}H-ACh from the striatal cholinergic interneuron (see ratios S_2/S_1 in table 1). Pretreatment with RES abolished the inhibitory effects of OCT on cholinergic neurotransmission but PEA, AMPH and TYR still retained their activity. After RES pretreatment combined with αMpT, PEA, AMPH and TYR were still able to inhibit the electrically-evoked release of ^{3}H-ACh (Table 1). These inhibitory effects were antagonized by SULP 0.1 μM (Table 1). Our data suggests that trace amines inhibit transmitter release from the striatal cholinergic interneuron when monoamine oxidase is inhibited. PEA appears to be the most active. The inhibitory effects of PEA and AMPH on ^{3}H-ACh release seem to be mediated by a special RES resistant pool of dopamine since they are antagonized by SULP. However, a direct effect of PEA on the post-synaptic D_2 receptor can not be excluded. AMPH significantly increases the content of PEA in rat brain (Chuang et al. 1982) and therefore it is possible that PEA may contribute to the effects of AMPH.

Cantrill R. et al. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322 . 322-324
Chuang L.W. et al. (1982) Eur J Pharmac 81 : 385-392

SIMULTANEOUS MEASUREMENT OF ENDOGENOUS DOPAMINE AND CHOLECYSTOKININ (CCK) RELEASE FROM RAT STRIATAL SLICES

J.B. Hutchison*, S.R. Nahorski & J. Strupish. Department of Pharmacology and Therapeutics, University of Leicester, University Road, Leicester. LE1 7RH. U.K.

Dopamine and the 'COOH' terminal octapeptide of cholecystokinin (CCK-8) are present in high concentration in rat striatum (Hokfelt et al. 1980), and recent evidence suggests that dopamine may modulate CCK release (Meyer & Krauss, 1983). In the present experiments we have examined depolarisation-evoked release and possible interaction of dopamine and CCK in striatal slices. Pooled slices (0.3 x 0.3 mm approx. 30 mg w.w.) from two animals were divided into two chambers (vol: 0.5 ml) and superfused (0.5 ml/min) with Krebs bicarbonate solution containing 30 μM bacitracin. Dopamine concentrations in the superfusate were measured by high performance liquid chromatography with electrochemical detection (Nahorski & Strupish, 1981), whilst CCK was measured by radioimmunoassay using a COOH terminal specific antiserum (Dockray, 1980).

Basal dopamine release (approx. 0.4 pmol/mg protein) was elevated to 26.2 ± 2.7 pmol/mg protein/min (n = 4) by a 4 min pulse of 45 mM K^+ (replacing Na^+). Under these conditions, there was a five-fold increase in CCK in the perfusate (from 0.6 to 2.4 ± 0.6 fmol/mg protein/min). The time course of CCK and dopamine release was similar, peak responses occurring within 3-4 min of the start of the stimulus, returning to basal levels in a further 6-8 min.

In three experiments, preparations were exposed to a gradient of calcium (0-2.1 mM over 30 min) in the presence of 40 mM K^+. The K^+ stimulated release of both CCK and dopamine were found to be entirely Ca^{2+} dependent, the threshold concentration of calcium being approximately 0.5 mM with maximal release apparent between 1-1.25 mM Ca^{2+}. Pulses of the alkaloid veratrine (4 min at 10^{-5} M) also stimulated dopamine (0.3 to 8.9 ± 2.3 pmol/mg protein/min) and CCK (0.5 to 3.2 ± 0.3 fmol/mg protein/min) release by a calcium-dependent mechanism.

Amphetamine (10^{-5} M) increased basal dopamine release between 4-5 times, and doubled the release of the amine in response to veratrine (10^{-5} M). Although basal CCK release was not influenced by amphetamine alone, veratrine-stimulated release (10^{-5} and 5 x 10^{-6} M) was reduced by 60 ± 6% and 68 ± 10% respectively.

In conclusion, the characteristics of dopamine and CCK release in response to high K^+ and veratrine have been studied in a superfusion system. Both substances are released by a calcium-dependent mechanism, and evidence is provided that a markedly reduced veratrine-stimulated CCK release accompanies amphetamine-stimulated dopamine release.

The authors thank Professor G.J. Dockray for the gift of the CCK antiserum.

This work was supported by a Nuffield Scholarship and the M.R.C. JBH is a Foulkes Fellow.

Dockray, G.J. (1980) Brain Res. 188, 155-165
Hokfelt, T. et al (1980) Neurosci. 12, 2093-2124
Meyer, D.K. and Krauss, J. (1983) Nature 301, 338-340
Nahorski, S.R. and Strupish, J. (1981) J.Physiol. 316P

AMINO ACID LEVELS IN MICE RESISTANT AND SUSCEPTIBLE TO AUDIOGENIC SEIZURES (AGS)

W.E. Davies & C.D. Owen (introduced by P.B. Bradley), Department of Pharmacology, Medical School, Birmingham, B15 2TJ

Alterations in the concentrations of free amino acids in affected parts of the brain have been reported in many experimentally induced epilepsies, the most common being decreases in GABA and glutamate content, along with a rise in glycine levels (Emson, 1978). In addition, van Gelder (1982) has proposed that imbalances in taurine, glutamate, and glutamine are involved in many epilepsies.

DBA/2J mice have a high genetically determined susceptibility to audiogenic seizures. This susceptibility is age-related, being maximal at about 29 days after birth, the seizure incidence showing a reduction or absence at earlier and later ages. The basis of this age-related seizure susceptibility is unknown, although several factors have been implicated, amongst them defects in amino acid neurotransmission. Drugs that increase brain GABA levels reduce susceptibility to and severity of audiogenic seizures, whilst both GABA and taurine, when injected intracerebroventricularly into audiogenic-seizure susceptible rats, exert a dose-dependent anti-convulsant effect, with taurine the most potent (Jobe, 1981).

We have measured the levels of five amino acids in the Organ of Corti and in three areas of the brain concerned with the auditory pathway - the cochlear nuclei, the inferior colliculi, and the primary auditory cortex. Levels were also measured in the cerebellum. The determinations were done at various ages during and after the period of maximum audiogenic seizure susceptibility and compared with the levels in age-matched controls (BALB/c).

Amino acid levels were measured by an HPLC method similar to that of Lindroth & Mopper (1979).

The results of this study show that the levels of glutamate, aspartate, glycine, taurine and GABA in all areas examined in the DBA/2J mouse were not significantly different to those in AGS-resistant mice of a comparable age. Further analysis revealed that there were no significant differences in the ratios of the concentrations of these amino acids between the two strains of mice. However both strains exhibited a variation in amino acid levels with age in all brain areas examined, the levels of glutamate, aspartate, glycine and GABA increasing and that of taurine decreasing as the animal matured. No significant changes were noted in the Organ of Corti. Of particular interest were the marked increases in glutamate and aspartate in the cochlear nuclei with age, a change characteristic of neurotransmitters during development. This finding supports Wenthold and Gulley's (1977) theory that glutamate or aspartate may be the secondary auditory transmitter (i.e. the transmitter released by VIIIth nerve endings in the cochlear nucleus).

These results indicate that variations in levels of these amino acids are not important in the audiogenic susceptibility of DBA/2J mice. However this does not preclude defects in binding, release, or uptake of these compounds as being involved in the aetiology of this form of epilepsy.

Emson, P.C. (1978) in Taurine and Neurological Disorders (Barbeau A. & Huxtable R.J., eds) Raven Press pp 201-216
van Gelder N.M. (1982) Adv. Exp. Biol. 193 pp 239-256
Jobe, P.C. (1981) in Pharmacology of Hearing (R. Don Brown & E.A. Daigeault, eds) John Wiley pp 271-304
Lindroth P. & Mopper K.V. Anal. Chem. 51 pp 1667-1674
Wenthold, R.J. & Gulley, R.L. (1977) Brain Res. 138 pp 111-123

EVIDENCE FOR NEUROTENSIN AS AN INHIBITORY NEUROTRANSMITTER IN THE GUINEA-PIG ILEUM

M. Goedert, J.C. Hunter* and M. Ninkovic. (Introduced by I.L. Martin)
MRC Neurochemical Pharmacology Unit, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH, U.K.

Neurotensin is a thirteen amino acid peptide that is widely distributed in both central and peripheral tissues. One of the highest concentrations of neurotensin-like immunoreactivity is found in the small intestine, where the immunoreactive material is present in both mucosal endocrine-like cells and in nerve fibres (Nemeroff and Prange, 1982; Reinecke et al, 1983).

We have investigated the distribution of specific ^{3}H-neurotensin binding sites in the guinea-pig ileum by autoradiography and we have characterised these binding sites by using a test-tube binding assay performed on ileal homogenates (Goedert et al, 1984). ^{3}H-neurotensin binding sites were present as two separate bands located over the circular and longitudinal muscle layers. Saturation analysis indicated the presence of one binding site with a maximum number of binding sites of 12 fmol/mg protein and an equilibrium dissociation constant of 2.4 nM. The potencies of neurotensin and of various neurotensin fragments in competing with ^{3}H-neurotensin for its specific binding site were similar to the structure-activity relationships obtained in biological assays (Nemeroff and Prange, 1982).

A possible physiological function of ileal neurotensin receptors was studied in the guinea-pig ileum circular smooth muscle which is known to be innervated by a large proportion of the ileal non-adrenergic inhibitory nerves (Hirst and McKirdy, 1974). The preparation was set up as previously described (Brownlee and Harry, 1963). Contraction of the muscle tone was induced by the tachykinin kassinin (10^{-7} M) and all experiments were performed in the presence of atropine (5×10^{-6} M) and guanethidine (2×10^{-5} M). Neurotensin produced a tetrodotoxin-resistant, dose-dependent relaxation (IC_{50} 7×10^{-9} M), whereas the relaxation consequent to field stimulation of the inhibitory nerves was frequency-dependent and tetrodotoxin-sensitive. In addition, the bee venom apamin (3×10^{-8} M) inhibited both the neurotensin-mediated relaxation and the relaxation produced by nerve stimulation. A direct relationship between the effects of neurotensin and of nerve stimulation was investigated by incubating the smooth muscle preparation in the presence of a carboxy-terminus directed neurotensin antiserum (Goedert et al, 1984) at a dilution of 1 in 20 for 3 h prior to nerve stimulation. Incubations performed in the presence of the same dilution of preimmune serum served as controls. The nerve stimulation-induced smooth muscle relaxation was consistently inhibited at all frequencies tested (mean inhibition at 2 Hz 75±4%, n=4) in the presence of neurotensin antiserum, whereas the preimmune serum did not affect the relaxation consequent to nerve stimulation.

In conclusion, the present results indicate that neurotensin may function as an inhibitory non-adrenergic, non-cholinergic neurotransmitter in the guinea-pig ileum

Brownlee, G. and Harry, J. (1963) Br. J. Pharmac. 21, 544-554.
Goedert, M. et al (1984) Brain Res., in press.
Hirst, G.D.S. and McKirdy, H.C. (1974) J. Physiol. 238, 129-143.
Nemeroff, C.B. and Prange, A.J. (Eds) (1982) Ann. N.Y. Acad. Sci. 400, 1-444.
Reinecke, M. et al (1983) Neurosc. Lett. 37, 37-42.

MODULATION BY CONVULSANTS OF THE RELEASE OF 5-HYDROXYTRYPTAMINE INDUCED BY LOW CHLORIDE CONCENTRATIONS

R.J. Boakes[1], J.D. Turner* and M.A. Virmani. [1]MRC Neuroendocrinology Unit, Newcastle General Hospital, and Department of Psychiatry, Newcastle University.

We have recently shown that superfusion of medium containing low $[Cl^-]$ over rat cortical slices preincubated in the presence of 5-hydroxytryptamine elicits release of this transmitter candidate (Boakes et al, 1984). In order to establish whether this release can be ascribed to movement of Cl ions through membranes or to actions at receptors which are dependent on the presence of Cl^-, we have measured this release in the presence of picrotoxin and strychnine, convulsant agents which are thought to act at receptors associated with membrane Cl^- channels.

In each experiment 8 slices were prepared from the cerebral cortex of 1 rat and preincubated in Krebs bicarbonate medium containing 10nM $[^3H]$-5-hydroxytryptamine at 37°C. The slices were then transferred to individual water-jacketed chambers and superfused at 0.5ml min^{-1} with Krebs medium containing 0.1mM pargyline. After equilibration the superfusate was collected as fractions and the fractional rate coefficient (FRC; Bowery et al, 1976) calculated from the radioactivity. In all experiments low $[Cl^-]$ values were obtained by substituting propionate for Cl^- in the superfusing medium. The presence of picrotoxin in the superfusing medium delayed the appearance of labelled 5-hydroxytryptamine in the superfusate and decreased the maximum release attained. In a typical experiment the FRC of radiolabelled 5-hydroxytryptamine reached 5.4±0.25 (±s.e. mean n=4), 10 min after reducing medium $[Cl^-]$ to 3mM in control conditions, and 4.2±0.40 (n=4, difference from control $P < 0.05$) in the presence of 0.1mM picrotoxin. This concentration of picrotoxin did not affect the basal release of $[^3H]$-5-hydroxytryptamine in normal (129mM Cl^-) medium or the release induced by superfusion of medium containing 30mM K^+, made isotonic by reducing Na^+. Strychnine similarly delayed the appearance of labelled 5-hydroxytryptamine in the superfusate and reduced the FRC attained 10 min after reducing $[Cl^-]$ to 3mM. In the presence of 0.1mM strychnine the FRC was reduced from 3.75±0.4 to 1.90±0.25 (results from a representative experiment, n=4 in both conditions, probability of difference $P < 0.01$).

Experiments to determine the actions of compounds which are thought to increase Cl^- permeability have so far proved inconclusive. While the presence of 5mM glycine, taurine or proline in the superfusion medium potentiated the release of labelled 5-hydroxytryptamine induced by low $[Cl^-]$, basal release was also increased under these conditions. GABA (0.1mM) had no effect on low-$[Cl^-]$ release whereas 5mM GABA inhibited release, and muscimol in concentrations up to 1mM had no significant effect on basal release or on the release induced by low $[Cl^-]$.

M.A.V. is supported by Newcastle H.A.

Boakes, R.J. et al (1984) J. Physiol. 346,38P
Bowery, N.G. et al (1976) Nature 264, 281-284

CHRONIC ENHANCEMENT OF DOPAMINE AGONIST ACTION AFTER INTRA-ACCUMBENS DOPAMINE INFUSION

Brenda Costall, Annette M. Domeney* & R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

Dopamine (DA) infused into the nucleus accumbens of rat brain for 13 days can alter locomotor responding to the DA agonist (-)N-n-propylnorapomorphine [(-)NPA] for many weeks post-infusion. The most striking change is in those animals pre-selected as low-responders to (-)NPA: post-infusion they show markedly exaggerated hyperactivity to (-)NPA (Costall et al, 1982). The present study investigates whether this enhanced hyperactivity can occur to other dopamine agonists and, in particular, to l-dopa.

Sprague-Dawley rats, selected as low hyperactivity responders to (-)NPA, measured using 'photocell cages' (Costall et al, 1982), were subject to standard stereotaxic surgery for the implantation of chronically indwelling guide cannulae for subsequent bilateral infusion of dopamine (25 µg/24 h, 0.48 µl/h) into the nucleus accumbens. 14-16 weeks post-infusion they were challenged with (-)NPA, pergolide, LY141865 and l-dopa plus benserazide (50 mg/kg i.p., 30 min pretreatment). Comparisons were made with the responses of normal non-treated rats and rats receiving intra-accumbens vehicle infusion 14-16 weeks previously.

In control animals, non-treated or vehicle infused, (-)NPA caused the typical profile of hyperactivity responding (at 0.05 mg/kg s.c. onset was within 120 min, intensity 10-25 counts/5 min, duration 200-220 min) but pergolide (0.025-0.5 mg/kg s.c.), LY141865 (0.0125-0.5 mg/kg s.c.) and l-dopa (50-200 mg/kg i.p. after benserazide) each failed to cause any locomotor hyperactivity responding in the control animals. In contrast, 14-16 weeks after infusing DA into the nucleus accumbens all DA agonists and l-dopa were shown to initiate marked hyperactivity. The profile of responding was similar for each agent with onset and the attainment of maximum occurring within the 60-120 min period following injection and the total duration being 3.5-4.5 h. Similarly, for each agent (with the exception of l-dopa) hyperactivity was either very marked or absent with a reduction in numbers of animals responding with decreasing dose rather than a reduction in intensity. Thus, 0.5 mg/kg s.c. pergolide caused a hyperactivity of maximum intensity 95±8 counts/5 min; pergolide at 0.05 mg/kg s.c. caused a similar response but was inactive at 0.025 mg/kg. Doses of LY141865 in the range 0.5-0.0125 all caused marked hyperactivity of intensity 88±9-84±8 counts/5 min, with lower doses inactive. (-)NPA caused hyperactivity of intensity 86±9 counts/5 min at 0.05 mg/kg s.c.; similar intensity was recorded at lower doses but in only 75% (0.0125-0.025 mg/kg s.c.) or 40% (0.003 mg/kg s.c.) of animals. Lower doses were ineffective. Of all agents tested l-dopa (after benserazide) caused the most marked hyperactivity in those animals previously subject to intra-accumbens DA infusion (100±4 counts/5 min at 200 mg/kg i.p.) with dose-related reductions in intensity (62±7 counts/5 min at 100 mg/kg, 22±2.2 counts/5 min at 50 mg/kg). Haloperidol (0.5 mg/kg i. p., 30 min) was shown to antagonise the hyperactivity induced by all agents.

Thus, the long-term consequence of intra-accumbens DA infusion to change the basal responsiveness to (-)NPA such that initially low responders become high responders can be detected using other DA agonists and l-dopa. The change for these other agents is particularly striking in that they normally fail to cause hyperactivity. The marked hyperactivity obtained to l-dopa challenge would question whether, at a pathophysiological level, a temporarily raised limbic DA function may allow the development of an enhanced response to a subsequent increase in DA activity.

The work was supported by the Medical Research Council.

Costall, B. et al (1982) Neuropharmacology 21, 327-335.

DOPAMINE D_2 RECEPTOR ANTAGONISM ENHANCES SALT PREFERENCE IN THE RAT

S.J. Cooper* & D.B. Gilbert, Department of Psychology, University of Birmingham, Birmingham B15 2TT.

Mogenson & Wu (1982) reported that administration of spiroperidol to the central nucleus of the amygdala in the rat significantly enhance the intake of a mildly aversive 1.5% NaCl solution without change in water consumption in a two-bottle preference test. Our study investigated the effects of systemic administration of 3 compounds with dopamine receptor-blocking activity (sulpiride, clozapine, pimozide) on salt preference in water-deprived male and female rats.

Subjects were 24 female (150-200 g) and 24 male (250-380 g) General strain hooded rats from our colony. They were housed in same-sex pairs with free access to standard food, and kept on a 12 h light: 12 h dark cycle. The animals were well-adapted to a daily 22 h water-deprivation schedule. In the saline preference tests, rats were placed individually in wooden boxes, each with two drinking tubes. Animals were allocated to 3 choice conditions: 0.125% NaCl v water; 0.6% NaCl v water; 1.7% NaCl v water (the first two NaCl concentrations are preferred to water, the last concentration is less preferred). Eight males and 8 females were in each group. Fluid consumptions (ml) from both tubes were measured in a 15 min test. Each animal served as its own control, and received each injection according to a balanced design.

Sulpiride (1.0, 3.0, 10 and 30 mg/kg, i.p. 1 h before the test) had no effect on total fluid consumption in male and female animals. In contrast both clozapine (0.3, 1.0, 3 and 10 mg/kg, i.p. 1 h before testing) and pimozide (0.1, 0.3, 1 and 3 mg/kg, i.p. 3 h before testing) dose-dependently produced marked reductions in total fluid consumption in both sexes. There was no evidence for an attenuation of salt preference following injection of the 3 drugs. Instead, sulpiride consistently enhanced salt preference across the 3 salt concentrations examined. Clozapine and pimozide, too, despite their overall antidipsogenic action elevated salt preferences in the rats.

If sulpiride can be accepted as a specific dopamine D2 antagonist (Kebabian et al 1982), then three conclusions follow. First, antidipsogenic effects of some neuroleptics (e.g. Rolls et al. 1974) are not mediated by D2 blockade. Second, enhanced preference for salt in a choice test can be obtained by D2 receptor blockade. Third, the theory that dopamine receptor blockade attenuates rewarding properties of stimuli (Wise et al. 1978), does not apply to salt preference.

We thank Chemitechna, Sandoz and Janssen for gifts of sulpiride, clozapine and pimozide, respectively.

Kebabian, J.W. et al. (1982) In, The Benzamides (J. Rotrosen and M. Stanley, eds), pp.17-49. Raven: New York.
Mogenson, G.J. & Wu, M. (1982) Brain Res. Bull. 8, 685-691
Rolls, E.T. et al. (1974) Psychopharmacol. 38, 219-230
Wise, R.A. et al. (1978) Science, 201, 262-264

^{45}Ca UPTAKE IN THE AORTA AND MESENTERIC ARTERY IN RABBITS WITH PERINEPHRITIS HYPERTENSION

M.T. Hall, C.A. Hamilton[1] K.A. Kane, J.L. Reid[1] I.W. Rodger & R.M. Wadsworth, Department of Physiology & Pharmacology, University of Strathclyde, Glasgow G1 1XW and [1]Department of Materia Medica, University of Glasgow, Glasgow G12 8QQ.

There is evidence that hypertension is associated with functional changes in arterial smooth muscle as suggested by the hyper-reactivity to noradrenaline found in perinephritis hypertension (Hall et al, 1983). In an attempt to look further at this question we have studied ^{45}Ca uptake in the aorta and mesenteric artery of both normo- and hypertensive rabbits. Rabbits were either sham operated or one kidney was wrapped in cellophane to induce perinephritis hypertension. Animals were used 4-6 weeks after operation by which time the hypertensive group had a mean blood pressure of 116 ±7 mmHg compared to 79 ±3 mmHg in the sham group. ^{45}Ca uptake was measured in segments of the thoracic aorta and in the main ileal, duodenal and colonic branches of the mesenteric artery using the method of Hay & Wadsworth (1984). The results are summarised in Fig. 1.

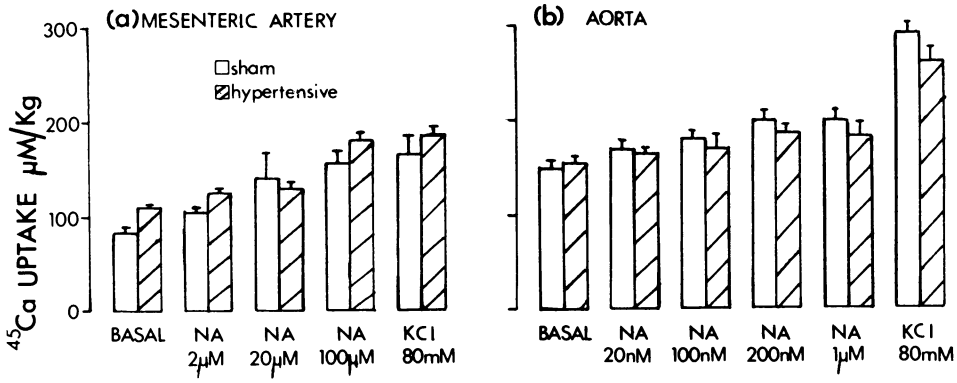

Figure 1 Basal and noradrenaline (NA) and potassium chloride (KCl) stimulated ^{45}Ca uptake in the (a) mesenteric artery and (b) aorta of sham operated and hypertensive rabbits.

In the mesenteric arterial preparations from both normo- and hypertensive rabbits, increasing concentrations of noradrenaline (2-100 μM) produced a graded increase in ^{45}Ca uptake above the basal level (Fig. 1a). Concentrations of noradrenaline (100 μM) and KCl (80 mM), which produce maximal contractions in this tissue, produced similar increases in ^{45}Ca uptake (Fig. 1a). In the aortic preparations from both normo- and hypertensive animals noradrenaline (20 nM-1 μM) caused a less graded increase in ^{45}Ca uptake than seen in the mesenteric arterial preparations, and the maximum uptake obtained with noradrenaline (1 μM) was significantly less ($P<0.001$) than seen with KCl (80 mM). No significant difference was found in the noradrenaline or KCl stimulated ^{45}Ca uptake between the normo- and hypertensive tissues (Figs. 1a and 1b). However, there was a significant difference ($P<0.005$) in the basal uptake between the normo- and hypertensive mesenteric but not the aortic preparations.

The observed increase in the basal ^{45}Ca uptake in the mesenteric arteries, with or without alterations in Ca efflux or intracellular sequestration, could explain their hyper-reactivity in hypertension.

This work was supported by the University of Strathclyde R & D Fund.

Hall, M.T. et al (1983) Br. J. Pharmac. 80, 702P
Hay, D.W.P. & Wadsworth, R.M. (1984) Br. J. Pharmac. In press

INTERACTION OF MAITOTOXIN WITH VOLTAGE SENSITIVE CALCIUM CHANNELS IN CULTURED NEURONAL CELLS

S.B. Freedman*[1], D.M. Miller, R.J. Miller and D.R. Tindall, Depts. of Pharmacology and Physiol. Sci., University of Chicago and Depts. of Physiology and Pharmacology and Botany, Southern Illinois University.

Maitotoxin is a water soluble toxin extracted from the toxic dinoflagellate Gambierdiscus Toxicus (Dickey et al., 1984). It has been shown that this toxin contracts smooth muscle and causes transmitter release from cultured PC12h pheochromocytoma cells (Ohizumi and Yasumoto, 1983; Takahashi et al., 1982). It has been suggested that the toxin activates voltage sensitive calcium channels. We investigated this question by utilizing cultured NG108-15 (neuroblastoma x glioma) cells under growth conditions where they express voltage sensitive calcium channels. The pharmacological characteristics of channels in these cells have been extensively described (Freedman et al., 1984). $^{45}Ca^{2+}$ uptake was measured as described previously (Freedman et al., 1984).

In NG108-15 cells, depolarizing stimuli such as 50 mM K^+ or the alkaloid batrachotoxin cause an immediate increase in net $^{45}Ca^{2+}$ uptake. Maitotoxin (> 10 ng/ml) also produced an increase in net $^{45}Ca^{2+}$ uptake. However, the effect of the toxin was slower only becoming apparent at incubation times > 2 minutes. Following this lag period, maitotoxin induced $^{45}Ca^{2+}$ uptake increased over the time course of the assay period reaching levels at least as great as those produced by 50mM K^+ after 15 minutes. Neither depolarizing agents nor maitotoxin stimulated $^{45}Ca^{2+}$ uptake into cultured 3T3 fibroblasts. In NG108-15 cells, the effects of depolarization or of maitotoxin could be blocked by organic calcium antagonists e.g. nitrendipine D-600 and diltiazem and by inorganic calcium antagonists e.g. Cd^{2+}. The inhibition curves for nitrendipine against maitotoxin and 50mM K^+ stimulated $^{45}Ca^{2+}$ uptake differed in shape.

Normally, the novel dihydropyridine BAY K8644 enhances $^{45}Ca^{2+}$ uptake into NG108-15 cells in the presence of a depolarizing stimulus (e.g. 50 mM K^+ or batrachotoxin) but has no effect at resting membrane potentials (5 mM K^+). However, in the presence of 100 ng/ml maitotoxin, BAY K8644 (10nM-1μM) greatly enhanced $^{45}Ca^{2+}$ uptake even if no other depolarizing stimulus was present.

The effects of maitotoxin were not blocked by tetrodotoxin (10^{-6} M). However, if Na^+ in the assay buffer was replaced by choline maitotoxin induced $^{45}Ca^{2+}$ uptake was reduced and the lag period lengthened. Na^+ replacement did not alter the effect of 50 mM K^+. Maitotoxin did not stimulate Na^+ uptake into NG108-15 cells.

In preliminary experiments maitotoxin did not alter the kinetics of ^{3}H-nitrendipine binding to its receptor in isolated rat brain synaptosomes.

It is suggested that MTX alters the voltage dependency of voltage sensitive calcium channels so that they now open at resting membrane potentials. This would be analogous to the effect of batrachotoxin on voltage sensitive sodium channels. In addition, the effect of maitotoxin is partly dependent on the presence of Na^+. It is possible that Na^+ facilitates the binding or entry of the toxin into the target cell.

Dickey, R.W., Miller, D.M. and Tindall, D.R. (1984). J. Am. Chem. Soc. (in press).
Freedman, S.B., Dawson, G., Villereal, M. and Miller, R.J. (1984). J. Neurosci. (in press).
Ohizumi, Y. and Yasumoto, T. (1983). J. Physiol. 337, 711-721.
Takahashi, M., Ohizumi, Y. and Yasumoto, T. (1982). J. Biol. Chem. 257, 7287-7289.

[1]Present address: Neuroscience Research Centre, Merck Sharp & Dohme, Hertford Road, Hoddesdon, Hertfordshire, EN11 9BU.

THE INFLUENCE OF DILTIAZEM AND NIFEDIPINE ON THE RESPONSE OF THE RAT KIDNEY TO MODEST RENAL NERVE STIMULATION

E.J. Johns. Department of Physiology, The Medical School, Birmingham B15 2TJ.

It is becoming accepted that calcium entry blocking drugs act as α-adrenoceptor antagonists on vascular smooth muscle causing vasodilation (van Zwieten et al., 1983). Within the kidney the renal sympathetic nerves also act directly on the proximal tubular cells to increase sodium reabsorption which is mediated via α_1-adrenoceptors (Hesse & Johns, 1983, 1984). The aim of this study was to determine whether diltiazem or nifedipine could in any way affect nerve mediated tubular sodium reabsorption.

Anaesthesia of male Sprague-Dawley rats (375-425g) was induced with 240 umol/kg sodium pentobarbitone i.p. Cannulation of a carotid artery and a jugular vein allowed measurement of blood pressure and infusion of saline (6.6 ml/h), inulin and drugs, respectively. The left kidney was exposed using a midline abdominal incision, the ureter cannulated and a flow probe placed on the renal artery. The renal nerves were sectioned and cleared for stimulation (15v, 0.2 msec), using frequencies to give a 12-18% reduction in renal blood flow (RBF). Experiments were begun 2h after completion of surgery and consisted of 20 min clearance periods, two before and two following one period of nerve stimulation. A mean of the non-stimulation clearances was compared to the stimulation period. Measurements began 30 min after the start of drug infusion when blood pressure had stabilised.

In six rats infused with saline mean blood pressure was 127±4 mmHg throughout the experiment. Renal nerve stimulation reduced RBF (21.09±1.99 ml/min/kg) by 12% ($p<0.01$), did not change glomerular filtration rate (GFR, at 3.49±0.15 ml/min/kg) while urine flow rate (UV, at 47.2±5.0 ul/min/kg), absolute sodium excretion ($U_{Na}V$, at 10.74±1.09 µmol/min/kg) and fractional sodium excretion (FE_{Na}, at 2.59%) decreased by 32% ($p<0.01$) 34% ($p<0.001$) and 33% ($p<0.01$), respectively. Diltiazem was infused at 10 µg/min/kg into a group of 5 rats and blood pressure was 104±5 mmHg. Stimulation of the renal nerve significantly reduced RBF by 18% ($p<0.01$), GFR by 9% ($p<0.05$), UV by 38% ($p<0.001$), $U_{Na}V$ by 44% ($p<0.02$) and FE_{Na} by 37% ($p<0.01$). A group of 6 rats infused with 20 µg/min/kg diltiazem had a mean blood pressure of 94±5 mmHg and renal nerve stimulation caused reductions of 17% ($p<0.01$) in RBF and 23% ($p<0.02$) in GFR. UV, $U_{Na}V$ and FE_{Na} fell by 46%, 62% and 54% (all, $p<0.01$) respectively when the nerves were activated. Four rats were infused with nifedipine at 1.0 µg/min/kg and had a blood pressure of 102±6 mmHg and renal nerve stimulation reduced RBF by 17% ($p<0.001$), did not change GFR but decreased UV by 41%., $U_{Na}V$ by 47% and FE_{Na} by 36% (all, $p<0.05$). The group of 5 rats infused with 2 µg/min/kg nifedipine had a blood pressure of 90±5 mmHg and renal nerve stimulation reduced RBF by 14% ($p<0.01$), GFR by 24% ($p<0.05$), UV by 53% ($p<0.01$), $U_{Na}V$ by 62% ($p<0.01$) and FE_{Na} by 43% ($p<0.05$). Basal values of renal function variables were similar in all groups of animals.

These results show that during renal nerve stimulation, causing a modest reduction in RBF, vasodepressor doses of diltiazem and nifedipine impair the ability of the kidney to regulate GFR, however, the neurally induced increase in tubular sodium reabsorption is not affected. It would seem that these calcium entry blockers do not interfere with the action of the renal nerves at the level of the nephron.

Hesse, I.F.A. & Johns, E.J. (1983) J. Physiol. 339, 32P.
Hesse, I.F.A. & Johns, E.J. (1984) Br. J. Pharmac. London meeting.
van Zwieten et al. Hypertension 5, suppl. 11, 11-18.

RELEASE OF ADENOSINE AND ITS METABOLITES FROM HUMAN LEUKOCYTES ACTIVATED WITH THE CALCIUM IONOPHORE A23187

S.T. Holgate & J.S. Mann*. Medicine 1, Southampton General Hospital, Southampton SO9 4XY.

The naturally occuring purine nucleoside adenosine is a pharmacological regulator of mast cell mediator release, lymphocyte mitogenesis, neutrophil superoxide generation and excitation contraction-coupling of smooth muscle. These effects occur through an interaction of adenosine with specific cell surface receptors which either stimulate (A_2) or inhibit (A_1) adenylate cyclase. Adenosine produces bronchoconstriction in asthma which is antagonised by low concentrations of theophylline, suggesting a receptor-mediated effect (Cushley et al, 1983). Since bronchoconstriction in asthma is associated with infiltration of the airways with inflammatory cells we have investigated the potential of human leukocyctes to release adenosine and related products upon cell activation with the calcium ionophore A23187.

Human leukocytes were separated from whole blood by dextran sedimentation (Lichtenstein & Osler 1964). The intracellular nucleotide pools were labelled by incubating 10^8 leukocytes in HEPES buffered salt solution (pH 7.4) containing 30 µCi [^{3}H] adenine/ml for 5 mins at 37°C. Duplicate aliquots of 1.5×10^6 labelled leukocytes in HEPES buffer were challenged with A23187 (0.1-3.0 µM). The cells and supernatants were separated by centrifugation and the labelled products quantified by scintillation spectrometry. Individual nucleotides and nucleosides were identified both by thin layer chromatography (Norman et al, 1973) and reverse phase high performance liquid chromatography (Gehrke et al, 1978). In parallel experiments unlabelled leukocytes were challenged with A23187 and basophil histamine release measured spectrofluorometrically.

[^{3}H] adenine was rapidly taken up by leukocytes 75% being incorporated into the purine nucleotide pool (ATP:ADP:AMP 2:1:1). Both unstimulated leukocytes and cells challenged with A23187 released [^{3}H] products identified as hypoxanthine, inosine and adenosine in the ratio of 65:8:16. Spontaneous release of [^{3}H] adenosine and its metabolites occurred for the first 10 mins of incubation after which it plateaued at 17.2±1.5% (mean± SEM) of the total label incorporated. A23187, 1.0 µM stimulated release of purine nucleosides and histamine from leukocytes which reached maximum at 45 mins of 28.2±4.8% and 46.4±9.3% respectively. A23187 (0.1-3.0 µM) produced parallel concentration-related release of labelled purine nucleosides and histamine. Further studies showed that both lymphocytes (n=6) and granulocytes (n=4) of 86-96% purity had the capacity to release adenosine and its metabolites upon stimulation with A23187.

Thus this study demonstrates that activation of inflammatory cells with influx of calcium ions stimulates the release of adenosine and its related metabolites in parallel with other indices of cell activation. These observations suggest that activated inflammatory cells are capable of releasing purine nucleosides which may interact with other mediators to cause bronchoconstriction in asthma.

Lichtenstein L.M. & Osler A.G. (1964) J. Exp. Med. 120, 507-529
Norman G.A., Follett M.J. & Hector D.A. (1974) J. Chromotogr. 90, 105-111.
Gehrke C.W., Kuo K.C., Davis G.E., Suits R.D., Waalkes T.P. & Borek E. (1978) J. Chromotogr. 150, 455-76.
Cushley M.J., Tattersfield A.E.T. & Holgate S.T. (1983) Agents Actions. 13 (Suppl) 109-113.

HISTAMINE INHIBITION OF ACETYLCHOLINE-INDUCED BRADYCARDIA IN THE GUINEA-PIG ISOLATED RIGHT ATRIUM

I. Marshall and P.M. Roberts*, Department of Pharmacology and Therapeutics, The Middlesex Hospital Medical School, London W1P 6DB.

Concentrations of histamine which increased atrial rate also attenuated bradycardia of the guinea-pig isolated right atrium caused by acetylcholine. Both effects were mimicked by the selective histamine H_2-receptor agonist dimaprit and were competitively inhibited by the histamine H_2-receptor antagonist cimetidine, 10μM (Marshall and Roberts, 1984). Subsequently, however, cimetidine 100μM, which also competitively inhibited the effects of histamine on atrial rate, was shown to have no greater effect on the attenuation by histamine of acetylcholine-induced bradycardia than did cimetidine 10μM. In the present study the difference between the effects of histamine on atrial rate and on acetylcholine-induced bradycardia has been examined.

Right atria were removed from male Dunkin-Hartley guinea-pigs. Decreases in atrial rate caused by 1μM acetylcholine were measured either alone or in the presence of histamine receptor agonists and antagonists. Changes in atrial rate were measured in beats per minute.

The increase in atrial rate caused by dimaprit was competitively inhibited by cimetidine, 10 and 100μM. In contrast to the effects of histamine, attenuation by dimaprit of acetylcholine-induced bradycardia was also competitively inhibited by cimetidine, 10 and 100μM. In the presence of cimetidine 100μM, mepyramine 0.3μM had no effect on the increase in atrial rate caused by histamine, but caused a six-fold shift to the right of the concentration related reduction by histamine of acetylcholine-induced bradycardia. To examine the role of histamine H_1-receptors, the effects of the selective histamine H_1-receptor agonist 2-pyridylethylamine (2-PEA) were examined. 2-PEA 10μM-1mM, in the presence of cimetidine 100μM, caused a concentration related increase in atrial rate. 2-PEA 0.3 and 1mM also significantly inhibited bradycardia caused by acetylcholine. The β-adrenoceptor antagonist propranolol, 0.3μM, abolished the increase in atrial rate caused by 2-PEA, but only partially inhibited the attenuation by 2-PEA of acetylcholine-induced bradycardia. In the presence of cimetidine 100μM, propranolol 0.3μM had no effect on the increase in atrial rate caused by histamine, but caused an approximately two-fold shift to the right of the concentration related reduction by histamine of acetylcholine-induced bradycardia.

In conclusion, histamine and dimaprit increased atrial rate by an action at histamine H_2-receptors. 2-PEA increased atrial rate by an action involving β-adrenoceptors. Dimaprit attenuated acetylcholine-induced bradycardia by an action at histamine H_2-receptors. The reduction by 2-PEA of acetylcholine-induced bradycardia was only partially inhibited by propranolol, suggesting an effect involving both β-adrenoceptors and a second site of action. The attenuation by histamine of acetylcholine-induced bradycardia was partially inhibited by cimetidine. In the presence of cimetidine 100μM, the effects of histamine on acetylcholine-induced bradycardia were also reduced by mepyramine, and to a lesser extent by propranolol. These results suggest that histamine attenuated bradycardia caused by acetylcholine through histamine H_1- and H_2-receptors, and possibly also β-adrenoceptors.

P.M.R. holds an SERC CASE award in collaboration with S.K. and F. Research Limited.

Marshall, I. and Roberts, P.M. (1984) Br. J. Pharmac. Proc. Suppl., in press.

INHIBITION OF HISTAMINE RELEASE FROM HUMAN MAST CELLS BY NIFEDIPINE AND NICARDIPINE

M.K. Church, S.T. Holgate* and Y-Y. Kim. Clinical Pharmacology and Medicine 1, Southampton General Hospital, SO9 4XY, U.K.

Calcium entry blockers such as nifedipine inhibit bronchoconstriction induced by antigen inhalation in allergic asthma (Henderson et al, 1983). While calcium influx is involved both in mast cell mediator secretion and excitation-contracton coupling of airway smooth muscle, which of these events is involved in the protective effect of calcium entry blockers in antigen-induced asthma has not been defined (Barnes, 1983). To investigate this we have observed the effects of two dihydropyridine calcium entry blockers, nifedipine (nif) and nicardipine (nic) on histamine release from human mast cells induced immunologically by anti-IgE and non-specifically by the calcium ionophore A23187.

Enzymatically dispersed human mast cells were prepared from fresh lung and tonsil (Church et al, 1983). Duplicate aliquots containing 10^5 mast cells in HEPES-buffered salt solution (pH 7.4) with 0.9mM calcium and 0.2 mg/ml human serum albumin were challenged with goat anti-human IgE (1/300 dilution) or A23187 (0.3 μM) in the presence and absence of nif or nic (0.01-100μM). All incubations were carried out in the dark. After 15 min at 37° the release reactions were terminated and histamine released by the secretogogues measured spectrofluorimetrically. This assay for histamine was not affected by either nif or nic.

In the absence of drugs, net release of histamine induced by anti-IgE from lung mast cells (HLMC) and tonsillar mast cells (HTMC) was 10±2% and 15±3% respectively. Corresponding histamine release induced by A23187 was 36±5% and 35±5%. Both nif and nic caused a concentration-related inhibition of histamine release induced by anti-IgE from HLMC, geometric mean IC_{30} values being 10uM and 4.4μM which were not significantly different. Similar inhibition of IgE-dependent histamine release was observed with HTMC (nif IC_{30} 47μM, nic IC_{30} 21μM). Nif and nic also caused a concentration-related inhibition of histamine release from HLMC induced by A23187 (nif IC_{30} 67μM, nic IC_{30} 14 μM). In contrast to anti-IgE, inhibition of A23187- induced release by the highest concentration of the drugs (100μM) was significantly greater for nic (94±2%) than for nif (36±11%) $p<0.005$. Almost identical results were obtained with HTMC.

Thus both nifedipine and nicardipine inhibited histamine release from human mast cells *in vitro*. However the concentrations required for this effect are in excess of those quoted for blockade of calcium entry and are far in excess of plasma levels achieved with therapeutic doses. Taken with the findings that nifedipine inhibits bronchoconstriction induced by histamine, methacholine, exercise and inhalation of cold air, inhibition of IgE-dependent mediator release is unlikely to contribute significantly to the protective effect of calcium entry blockers in asthma.

Henderson, A.F. Dunlop, L.S. & Costello,J.F. (1983), Agents Actions, 13 (Suppl.); 209-210.
Barnes, P.J. (1983), Thorax, 38; 481-485.
Church, M.K., Mageed, R.A.K. & Holgate, S.T. (1983), Int. Archs Allergy appl Immun., 72; 188-190.

PREVENTION OF AXONAL TRANSPORT DEFECTS, IN VAGUS AND SCIATIC NERVES OF DIABETIC RATS, BY ALDOSE REDUCTASE INHIBITION

P. Fretten, D.R. Tomlinson & J. Townsend*, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK.

Short-term streptozotocin (STZ)-induced diabetes in rats impairs orthograde axonal transport of choline acetyltransferase (ChAT) in the sciatic nerve. This defect is associated with accumulation of sorbitol, formed from glucose by aldose reductase, and depletion of *myo*-inositol in the nerve. All three defects are prevented by treatment with one aldose reductase inhibitor (Mayer & Tomlinson, 1983). The present study was designed to examine the effect of a different aldose reductase inhibitor (ICI 128436, 3-(4-bromo-2-fluorobenzyl)-4-oxo-3H-phthalazin-1-ylacetic acid) on these phenomena in sciatic and vagus nerves. Male Wistar rats (350-360 g) were made diabetic with STZ (50 mg/kg i.p.) and half of the animals were given ICI 128436 (25 mg/kg p.o. daily) for the following 3 weeks. An untreated control group was run in parallel. On day 20 the left sciatic and vagus nerves were constricted under halothane anaesthesia and the animals killed 24 h later. Accumulation of ChAT activity proximal to the constrictions was measured in both nerves as an index of axonal transport of the enzyme. The contents of sorbitol and *myo*-inositol were measured in the right sciatic nerve. All methods have been described by Mayer & Tomlinson (1983). The results in Table 1 show that treatment of the diabetic rats with ICI 128436 prevented the accumulation of sorbitol in the nerve. The inhibitor also maintained normal *myo*-inositol levels. The untreated diabetic rats showed deficits in orthograde transport of ChAT in both vagus and sciatic nerves, defects which were prevented by ICI 128436. These findings implicate either sorbitol accumulation or *myo*-inositol depletion in the axonal transport defects and suggest that aldose reductase inhibition may be of value in acute neurological defects in diabetes.

Table 1. Mean values (±SEM) after 3 weeks' diabetes (number of rats in brackets)

	ChAT accumulation (nmol ACh/h/nerve)		Sciatic nerve content of (nmol/mg)		Blood glucose
	Vagus	Sciatic	Sorbitol	*myo*-Inositol	(mmol/L)
Controls-untreated (n=13)	2.29±0.14 *	4.58±0.46 ***	0.17±0.05	3.54±0.22 ***	5.1±0.7
Diabetics-untreated (n=14)	1.76±0.14 ***	2.96±0.33 **	2.03±0.28	2.66±0.18 ***	18.3±0.7
Diabetics-ICI 128436 (n=10)	2.58±0.20	4.16±0.30	0.19±0.04	3.41±0.18	18.6±0.7

*p<0.05, **p<0.02, ***p<0.01 by unpaired t tests (masssive differences untested).

This work was supported by the British Diabetic Association and ICI Pharmaceuticals Division.

Mayer J.H. & Tomlinson D.R. (1983) Diabetologia 25, 433-348.

LY1411865 INDUCED HYPOTHERMIA: A POSSIBLE D_2 DOPAMINE RECEPTOR MEDIATED EFFECT

N.A. Moore*, I.A. Pullar, F.C. Risius, N.C. Tye, (introduced by W. Dawson)
Lilly Research Centre Ltd., Eli Lilly and Company, Erl Wood Manor,
Windlesham, Surrey GU20 6PH

A number of workers have proposed that dopamine receptors maybe subclassified as linked (D_1) or non-linked (D_2) to adenylate cyclase (Kebabian and Calne, 1979). Characterisation of the type of dopamine receptors involved in a number of behaviours and physiological functions has proved difficult because of the lack of specificity of the various dopamine agonists and antagonists. However, a number of selective D_1 and D_2 agonists and antagonists are now available. Since dopamine agonists have been shown to lower body temperature (Cox, Kerwin and Lee 1978, Doggett & Moore, 1979) we have looked at the effect of SKF38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl 1H 3-benzazepine) and LY141865 (trans-(±)-4,4a,5,6,7,8,8a,9 octahydro-6-propyl-2H-pyrazalo (3,4-g) quinoline di HCl), selective D_1 and D_2 agonists respectively (Lehmann et al., 1983) on body temperature of mice.

Female albino CFI mice (20-25 g) were used in all the experiments. Rectal temperature was measured immediately prior to and every 30 mins for up to 5 hrs following treatment with LY141865 or SKF38393. In some experiments animals were pretreated with various antagonists or their respective vehicles 1 hr prior to the start of the experiment. All the experiments were carried out in a room maintained at 21±2°C.

LY141865 (1.25 mg/kg - 10 mg/kg s.c.) produced a dose related fall in body temperature which had a rapid onset and reached a maximum 1 hr after administration. SKF 38393 (2.5 - 50 mg/kg s.c.), however failed to lower body temperature at any dose tested. The dopamine antagonists haloperidol (0.25 - 0.5 mg/kg i.p.) and sulpiride (20 mg/kg i.p.) significantly ($P<0.05$ and $P<0.01$ respectively) antagonised the hypothermic response produced by LY141865 (2.5 mg/kg s.c.). They also caused a flattening and shift to the right in the dose response curve produced by LY141865 (1.25-5.0 mg/kg s.c.). The cis isomer of flupenthixol (0.25 mg/kg i.p.) also reduced the hypothermic response produced by LY141865 ($P<0.02$), while the trans isomer had no effect. SCH 23390 [(R)-(+)-8-chloro 2,3,4,5,-tetrahydro-3 methyl-5-phenyl-1H-3 benzazepin-7-ol] a selective D_1 antagonist (Iorio et al, 1983), at doses which antagonised apomorphine induced climbing (unpublished observation) (6.25 - 12.5 mg/kg i.p.) had no effect on the hypothermic response produced by LY141865. These results therefore suggest that the hypothermia produced by dopamine receptor agonists is mediated by the D_2 receptor.

We wish to thank M.S. Davis and G. Hampton for expert technical assistance and Janssen, Delagrange, Lundbeck, Schering Plough and S.K.F. for their generous supply of compounds.

Cox, B., et al. (1978). J. Physiol. 282, 471-483.
Doggett, N.S., Moore, N.A. (1979). Br. J. Pharmac. 67, 434P.
Iorio, L.C., et al. (1983). J. Pharm. Exp. Ther. 226, 462-468.
Kebabian, J.W., Calne, D.B., (1979). Nature 277, 93-96.
Lahmann, J., et al. (1983). Eur. J. Pharmac. 88, 11-26.