GABAERGIC INHIBITION OF STRIATAL SEROTONERGIC TRANSMISSION EXERTED IN THE DORSAL RAPHE REQUIRES THE INTEGRITY OF THE HABENULA

T. Nishikawa, B. Scatton* & A. Serrano, Biochemical Pharmacology Unit, Synthélabo-L.E.R.S., 31 Avenue Paul Vaillant Couturier, 92220 Bagneux, FRANCE.

It has recently been demonstrated that striatal serotonergic neurons are under an inhibitory GABAergic influence which is exerted in the dorsal raphé (Gallager and Aghajanian, 1976 ; Scatton et al, 1982 ; Nishikawa et al, 1983). However, the exact nature of this interaction is as yet unknown. The GABAergic influence might be exerted by afferents arising outside the raphé (e.g. the putative habenulo-dorsal raphé GABAergic pathway) or else be mediated via the GABAergic interneurons which appear to be present in the dorsal raphé. In order to investigate this problem we have studied the effect of an electrolytic lesion of the habenular nuclei on the ability of GABA agonist agents (given systemically or applied directly in the dorsal raphé) to affect striatal serotonergic transmission. Changes in the activity of striatal serotonergic neurons were evaluated by measuring 1) the rate of striatal serotonin (5-HT) synthesis and 2) the release of extracellular 5-hydroxyindoleacetic acid (5-HIAA) from the striatum.

Experiments were performed on male Sprague Dawley rats (170-250g). The synthesis of serotonin was estimated by measuring the accumulation of L-5-hydroxytryptophan (5-HTP) after inhibition of L-aromatic amino acid decarboxylase by NSD 1015 (100 mg/kg ip). 5-HTP was quantitated by high-performance liquid chromatography. The release of extracellular 5-HIAA was measured by differential pulse voltammetry using pyrrolytic carbon fiber electrodes as described previously (Cespuglio et al, 1981; Scatton and Serrano, 1983). Electrolytic lesion of the habenular nuclei was performed bilaterally by means of a monopolar electrode implanted at the following coordinates A 4, L ±0.6, V 5.1 (atlas of König and Klippel) by applying a current of 3.5 mA for 15 sec.

Systemic administration of progabide (50-1200 mg/kg ip) and dipropylacetamide (150 mg/kg ip) to sham-operated animals diminished striatal 5-HTP accumulation by 20 - 30%. Electrolytic lesion of the habenula slightly reduced by itself, and completely antagonized the ability of the GABA agonist agents to diminish, striatal 5-HTP accumulation. In contrast, habenula lesion failed to alter the ability of the serotonin receptor agonist MK212 (10 mg/kg ip) to decrease 5-HT synthesis. Similarly, cessation of impulse flow in the habenulo-raphé pathway (by local injection of tetrodotoxin in the fasciculus retroflexus) blocked the ability of depamide (150 mg/kg ip) to diminish striatal serotonergic transmission. Local injection of GABA (10 and 100 µg), γ-vinyl-GABA (100 µg) or SL 75102 (10 µg), a GABA receptor stimulant, into the dorsal raphé diminished the accumulation of 5-HTP as well as the release of extracellular 5-HIAA in the striatum of sham-operated rats. However, after habenular lesion, intra-dorsal raphé infusion of GABA (100 µg) no longer affected these biochemical parameters.

In conclusion, these results indicate that the GABAergic inhibitory influence on striatal serotonergic neurons which is exerted within the dorsal raphé depends upon the integrity of the habenulo-raphé pathways. GABA receptors may thus be localized on habenular afferents to the dorsal raphé. Alternatively, GABA neuron terminals may impinge on dorsal raphé 5-HT cells, these being inhibited by GABAergic agents only in the presence of a tonic excitatory influence exerted by the habenulo-raphé tract.

Gallager, D. & Aghajanian, G.K. (1976) Eur. J. Pharmacol. 39, 357-364.
Scatton, B. et al (1982) J. Pharmacol. Exp. Ther. 220, 678-688.
Nishikawa, T. & Scatton, B. (1983) Brain Research in press.
Cespuglio, R. et al (1981) Brain Research 223, 299-311.
Scatton, B. & Serrano, A. (1983) Brit. J. Pharmacol. 78, 90P.

FURTHER EVIDENCE THAT CENTRAL BENZODIAZEPINE RECEPTOR LIGANDS MAY EXHIBIT ANXIOGENIC PROPERTIES

W. Kehr and D.N. Stephens*, (introduced by B. Cox) Department of Neuropsychopharmacology, Schering AG, Berlin (West)

Ligands at central benzodiazepine (BZ) receptors have been reported to exhibit pharmacological properties ranging from anticonvulsant (e.g. diazepam) to convulsant activity (e.g. DMCM). In the middle of the range lie substances like Ro 15-1788 which exhibit little intrinsic activity, but which antagonise the effects of both diazepam-like, and convulsant ligands. These three types of ligand have been named agonists, inverse agonists and antagonists, respectively, but the range appears to be continuous. One of the best known properties of BZ agonists is their anxiolytic activity. A limited number of studies suggest that, conversely, inverse agonists may be anxiogenic (Dorow et al 1983; File et al, 1982). In the present study we have sought to provide further evidence for anxiety provoking properties of substances behaving as inverse agonists at BZ receptors.

The ability of 4 BZ-receptor ligands in the antagonist - to - inverse agonist range to antagonise the discriminative stimulus provided by chlordiazepoxide (CDP 5 mg/kg, i.p.) and to substitute for the discriminative stimulus provided by pentylenetetrazol (PTZ, 15 mg/kg, i.p.) was investigated in rats in standard drug discrimination procedures (Stephens et al, 1984). The pentylenetetrazol cue is thought to depend upon the anxiety-provoking properties of this compound (Shearman and Lal, 1979). All substances were administered i.p. in 10 % Cremofor.

The antagonist Ro 15-1788, a benzodiazepine derivative, antagonised the CDP cue with an ED_{50} of 0.6 mg/kg. Ro 15-1788 failed to substitute for PTZ at doses up to 40 mg/kg. CGS 8216, a pyrazoloquinoline, which has been classified as an antagonist, substituted for PTZ (ED_{50}: 5 mg/kg) indicating anxiogenic properties and suggesting that this substance is better regarded as a weak partial inverse agonist.

A ß-carboline, FG 7142, which has been found to be anxiogenic in man (Dorow et al, 1983) also antagonised the CDP cue (ED_{50}: 2.0 mg/kg) and substituted for the PTZ cue (ED_{50}: 10 mg/kg), as did the convulsant ß-carboline, DMCM, at sub-convulsant doses (CDP cue antagonism ED_{50}: 0.2 mg/kg; PTZ cue generalisation ED_{50}: 0.3 mg/kg). Pretreatment with the antagonist Ro 15-1788, 40 mg/kg, antagonised the ability of the two ß-carbolines and of CGS 8216 to substitute for the PTZ cue, without having the ability to antagonise the cue provided by PTZ itself.

These results suggest that DMCM, FG 7142 and CGS 8216 are able to provoke an anxiety-like state in animals through their interaction with benzodiazepine-receptors.

Dorow, R. et al. (1983) Lancet ii 8341
File, S.E. et al. (1982) Neuropharmacol. 21 1033
Shearman, G. and Lal, H. (1979) Psychopharmacol. 64 315
Stephens, D.N. et al. (1984) Psychopharmacol. in press

THE EFFECT OF CIS-2,3-PIPERIDINE DICARBOXYLIC ACID ON THE HIGH PRESSURE NEUROLOGICAL SYNDROME

M.J.Halsey, [1]B.S. Meldrum and Bridget Wardley-Smith*
HPNS Group, Clinical Research Centre, Harrow HA1 3UJ and [1]Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

The exposure of man or animals to increased atmospheric pressure produces a group of symptoms which are known as the high pressure neurological syndrome (HPNS). These include EEG changes, tremors, myoclonus and, in animals, convulsions. The precise mechanism of the HPNS is not fully understood, although several groups of drugs have been shown to be effective in treating the symptoms (Halsey, 1982). 2-amino-7-phosphonoheptanoic acid (2APH) is a selective antagonist of excitation produced by N-methyl-D-aspartate (NMDA), and by the systemic route antagonizes seizures induced by NMDA but not those induced by kainic acid (Czuczwar & Meldrum, 1982). We have previously shown that 2APH, 1 mmol/kg ip provides good protection against the HPNS in rats, with a particularly potent effect against tremor, increasing the onset pressure from 41.5 atmospheres absolute (ATA) to 92 ATA (Meldrum et al, 1983). We have now studied the effect on the HPNS of (±)-cis-2,3-piperidine dicarboxylic acid (PDA), an antagonist of excitation produced by dicarboxylic acids, with some action against NMDA, quisqualate and kainate (Watkins & Evans, 1981).

Male Sprague-Dawley rats (200-250g) were injected ip with PDA (1 mmol/kg adjusted to pH 6.5) or saline 30 min before compression with helium (PO_2 = 0.5 ATA). The detailed protocol has been described elsewhere (Halsey & Wardley-Smith, 1983). The HPNS was assessed by observing the appearance of tremor (measured using a small strain gauge), myoclonus and convulsions. Results are shown in Table 1.

Table 1
Onset pressures for the HPNS in ATA (1 s.e.m.). Results were analysed using Student's t-test after testing for normality with the Shapiro-Wilk test.

	Saline n = 5	PDA n = 5	t-test p
Tremor	37.4 (1.0)	57.4 (1.6)	$<.001$
Myoclonus	80.2 (2.0)	102.2 (1.4)	$<.001$
Convulsion	95.2 (4.3)	118.5 (2.8)	$<.001$

It can be seen that PDA is effective in treating all the motor endpoints associated with the HPNS, with the threshold pressure for tremor being increased by 53%. Myoclonus and convulsion thresholds were also raised, but by only 27% and 24% respectively.

These results parallel those obtained with 2-APH, which was also more effective in treating tremor than myoclonus or convulsions. However, 2-APH had a much greater effect on tremor than PDA, increasing the onset pressure threshold by 122%, compared with only 53% for PDA. This suggests that antagonism of excitation produced by NMDA, and thus presumably antagonism of the action of aspartate, may be more important in protecting against the HPNS than antagonism of excitation produced by the endogenous transmitters acting on the quisqualate or kainate receptors.

Czuczwar, S.J. & Meldrum, B.S. (1982). Eur. J. Pharmacol. 83, 335.
Halsey, M.J. (1982) Physiol. Rev. 62, 1341.
Halsey, M.J. & Wardley-Smith, B. (1983) Neuropharmacology, 22, 103.
Meldrum, B.S. et al (1983) Eur. J. Pharmac. 87, 501.
Watkins, J.C. & Evans, R.H. (1981) Ann. Rev. Pharmacol. Toxicol. 21, 165.

THE ANTAGONISM BY KETAMINE OF N-METHYL D-ASPARTATE IN SLICES OF RAT CEREBRAL CORTEX

N.L. Harrison* and M.A. Simmonds, MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX.

The dissociative anaesthetic, ketamine, has been reported to selectively prevent the excitation of spinal neurones by N-methyl D-aspartate (NMDA), whilst having little effect on excitation by quisqualate and kainate, and to selectively reduce polysynaptic reflexes (Anis et al., 1983; Lodge & Anis, 1982). We have extended these observations in an attempt to study quantitatively the antagonism of NMDA by the anaesthetic.

500 μm thick coronal sections of rat cortex were prepared using a falling razor strip and wedges of tissue were then cut from these sections on either side of the mid-line. The slices were placed in a two-compartment bath (Simmonds, 1978) so that the corpus callosum was in one compartment while the cortical tissue projected through a greased slot into the other. Superfusion of the cortical tissue with glutamate, aspartate, quisqualate or NMDA produced dose-dependent depolarizations of the cortical tissue relative to the corpus callosum.

Because Mg^{2+} has been shown to antagonise NMDA in the spinal cord (Davies & Watkins, 1977), experiments were carried out in a Mg^{2+}-free Krebs solution containing 3 mM K^+ and 2.5 mM Ca^{2+}. Responses to NMDA in this preparation exhibited a similar pharmacological profile to that previously described for NMDA-induced excitation of spinal neurones, in being selectively antagonised by Mg^{2+} and by 2-amino 5-phosphonovalerate (2-APV) (Davies & Watkins, 1982).

Like Mg^{2+} and 2-APV, ketamine selectively reduced responses to NMDA whilst those to quisqualate were unaffected. By constructing dose-response relationships for NMDA, it was established that ketamine caused a parallel shift of the NMDA dose-response curve. Dose-ratios were calculated and a Schild plot constructed for ketamine. This was linear between 3 and 100 μM; least squares regression analysis yielded a "best-fit" value of 1.07 ± 0.06 for the slope of the plot. A pA_2 value of 4.99 was estimated.

These experiments thus confirm that ketamine is a selective antagonist of NMDA and extend these observations to the cerebral cortex.

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Anis, N.A. et al., (1983) Br. J. Pharmac. 79, 565-575.
Lodge, D. & Anis, N.A. (1982) Br. J. Anaesth. 54, 228P.
Simmonds, M.A. (1978) Br. J. Pharmac. 63, 495-502.
Davies, J. & Watkins, J.C. (1977) Brain Res. 130, 364-368.
Davies, J. & Watkins, J.C. (1982) Brain Res. 235, 378-386.

COMPARISON OF THE EFFECTS OF 3-PPP AND ITS ENANTIOMERS ON EMESIS, PLASMA PROLACTIN LEVELS AND CNS DOPAMINE TURNOVER

F. Brown and W. Campbell*, Beecham Pharmaceuticals, Harlow, Essex, CM19 5AD.

The pharmacological profile of the dopamine analogue 3PPP, i.e. 3-(3-hydroxyphenyl) N-n-propylpiperidine, indicates selective presynaptic agonism (Hjorth et al. 1981). The effects of the separate enantiomers on CNS dopamine turnover and motor behaviour indicate that both (+) and (-) 3PPP are presynaptic agonists, whereas at postsynaptic receptors (+)3PPP is an agonist but (-)3PPP an antagonist (Hjorth et al. 1982). We have compared the effects of (±)3PPP and its enantiomers given s.c. in order to see if this agonist/antagonist balance extends to prolactin (prl) and emesis. The concentrations of the dopamine metabolite homovanillic acid (HVA) were also measured to indicate CNS regional dopamine turnover.

Plasma prl (NIADDK radioimmunoassay) and regional HVA (modification of the method of Westerink and Korf, 1976) were measured in samples taken one hour after treatment from male CFY rats weighing 175-200g. Emesis was measured using female beagle dogs weighing approx. 15 kg.

Racemic 3PPP and both enantiomers acted like postsynaptic agonists on emesis and prolactin; potency order (+) > (±) > (-). The effects of (-)3PPP on HVA levels resembled those of antagonists but the effects of racemic and (+)3PPP resembled those of agonists. Results are shown in Table 1.

Table 1 Effects of (+), (-) and (±)3PPP

Response		(+) 3PPP	(-) 3PPP	± (3PPP)
Prl Decrease*			ED_{50} mg/kg	
		1.7	10.5	4.9
Emesis	dose mg/kg	no. of episodes in 3 dogs		
	0.5	0,1,0		
	1.0	1,2,2	0,0,0	0,0,0
	2.0	5,3,3	0,0,0	2,1,0
	4.0		2,0,0	
Striatal HVA Changes † (ED_{50} or ED_{200} mg/kg)		decreased (ED_{50} 9.1)	increased (ED_{200} 9.0)	decreased (ED_{50} >30)

*(±)3PPP and both enantiomers exhibited similar potencies against γ-butyrolactone or α-methyl-p-tyrosine induced hyperprolactinaemia. † effects were similar in nuc. accumbens.

(-)3PPP therefore acts as an agonist on emesis and prolactin although some of its behavioural and biochemical actions apparently involve postsynaptic antagonism. Possible difference between central D2 receptors and lactotrophe- and chemoemetic-receptors are compatible with this dual action of (-) 3PPP.

Hjorth et al. (1981) Life Sci. 28, 1225-1238.
Hjorth et al. (1982) Br. J. Pharmacol. 77, 444P.
Westerink B.A.C. & Korf J. (1976) Eur. J. Pharmacol. 38, 281-289.

SELECTIVE ABILITY OF NEUROTENSIN TO INHIBIT A RAISED MESOLIMBIC DOPAMINE ACTIVITY

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

Intracerebral neurotensin can evoke certain responses characteristic of neuroleptic action (Nemeroff et al, 1982), for example, intra-accumbens neurotensin is reported to decrease the hyperactivity caused by peripherally administered amphetamine (Ervin et al, 1981). However, there is an indication that this 'dopamine antagonist' action of neurotensin may be more selective than that generally attributed to the neuroleptic agents, for whilst the latter are known to also antagonise at extra-pyramidal dopamine mechanisms, neurotensin is reported to be without such effect (Nemeroff et al, 1982). Therefore, in the present studies we compare the abilities of neurotensin and neuroleptic agents to inhibit mesolimbic and striatal dopamine function.

Male Sprague-Dawley rats, 300 ± 25 g, were subject to standard stereotaxic surgery for the implantation of chronically indwelling guides for subsequent drug/vehicle injection (1 µl) at the centre of the nucleus accumbens (Ant. 9.4, Lat. ±1.6, Vert. 0.0) or caudate-putamen (Ant. 7.8, Lat. ±3.0, Vert. +1.5)(Costall et al, 1982, 1983). Locomotor hyperactivity was assessed in photocell cages and asymmetry/circling behaviour was scored on a 0-3 scale (Costall et al, 1983).

Bilateral intra-accumbens amphetamine (0.5-10 µg) caused dose-related hyperactivity. Intra-accumbens pretreatment (30 min) with fluphenazine (0.5-10 ng) or (-)sulpiride (0.1-1.25 ng) dose-dependently antagonised the amphetamine (10 µg) response. Intra-accumbens fluphenazine alone caused an appearance of behavioural depression, and a dose-related decrease in locomotor activity was recorded (0.1-5 ng). In contrast, intra-accumbens (-)sulpiride induced a state of 'alertness' with a stimulation of locomotor activity at doses of 0.5-1.25 ng. Intra-accumbens neurotensin (0.1-1000 ng) similarly caused a state of 'alertness' and behavioural depression was never recorded, indeed, a modest stimulation of locomotor activity was consistently recorded during the 30-60 min period following the administration of 1 ng neurotensin. A 30 min intra-accumbens pretreatment with neurotensin (0.1-1 ng) dose-dependently antagonised the hyperactivity response to a subsequent intra-accumbens injection of 10 µg amphetamine.

Unilateral intrastriatal (-)sulpiride (1-10 ng) or fluphenazine (500-5000 ng) caused ipsilateral asymmetry, associated with circling at higher doses (score 3), on peripheral challenge with apomorphine (0.25 mg/kg s.c.) or amphetamine (2.5 mg/kg i.p.). In contrast, unilateral intrastriatal neurotensin, 100-3000 ng, failed to precipitate asymmetry/circling (or to induce catalepsy/motor depression on bilateral injection).

The present study suggests that, unlike neuroleptic agents, neurotensin has an action to inhibit mesolimbic function in the absence of an inhibitory effect on the striatal system. Furthermore, the inhibitory action in the nucleus accumbens appears to be against a raised rather than a 'normal' dopamine function. When mesolimbic dopamine function is raised by intra-accumbens amphetamine the inhibitory potency of neurotensin is shown to be some 1000 fold greater than previously recorded.

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Costall, B., Domeney, A.M. & Naylor, R.J. (1982) Neuropharmacology 21, 327-335.
Costall, B., Kelly, M.E. & Naylor, R.J. (1983) Eur.J.Pharmac. in press.
Ervin, G.N., Birkeme, L.S., Nemeroff, C.B. & Prange, A.J. (1981) Nature 291, 73-76.
Nemeroff, C.B., Hernandez, D.E., Luttinger, D., Kalivas, P.W. & Prange, A.J. (1982) Ann.N.Y.Acad.Sci. 400, 330-344.

AN INVESTIGATION OF THE SPECIFICITY OF PRAZOSIN AS AN α_1-ADRENOCEPTOR ANTAGONIST ON SINGLE CORTICAL NEURONES

C.M. Bradshaw, R.D. Sheridan* & E. Szabadi, Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT

There is evidence that the excitatory responses of single cortical neurones to noradrenaline, dopamine and 5-hydroxytryptamine are mediated by pharmacologically distinct receptors: α_1-adrenoceptors, excitatory dopamine receptors and excitatory 5-hydroxytryptamine receptors, respectively (Bradshaw et al, 1983a,b). In the present experiments we examined whether prazosin, a purported specific and potent α_1-adrenoceptor antagonist (Langer, 1980), can discriminate between these responses.

Single spontaneously active neurones were studied in the somatosensory cortex of the halothane-anaesthetised rat. Drugs were applied by microelectrophoresis (Bradshaw et al, 1983a).

The effect of prazosin on responses to phenylephrine and noradrenaline was compared on 8 cells. Acetylcholine was used as a control agonist. Prazosin equally and reversibly antagonised responses to phenylephrine and noradrenaline, while the response to acetylcholine was not affected (percentage change in the size of the response in the presence of prazosin, mean $\pm$ s.e.mean: -91.9 ± 2.5 (phenylephrine); -94.9 ± 2.6 (noradrenaline); -1.2 ± 3.1 (acetylcholine)). The antagonism of the responses to phenylephrine and noradrenaline was statistically significant ($P < 0.001$; Student's t-test).

The effect of prazosin on responses to phenylephrine and dopamine was studied on 16 cells: responses to phenylephrine and dopamine were significantly antagonised (-77.5 ± 5.5 and -83.6 ± 7.9, respectively; $P < 0.001$), while the response to acetylcholine was not diminished ($+20.2 \pm 7.5$).

The effect of prazosin on responses to dopamine and 5-hydroxytryptamine was compared on 11 cells: the response to dopamine was significantly antagonised (-82.3 ± 6.8; $P < 0.001$), while those to 5-hydroxytryptamine and acetylcholine were not reduced ($+32.0 \pm 18.5$ and $+21.1 \pm 9.3$, respectively).

In summary, prazosin proved to be an effective antagonist of excitatory responses of cortical neurones to phenylephrine, noradrenaline and dopamine, while the responses to 5-hydroxytryptamine and acetylcholine were not reduced. The antagonism of the responses to phenylephrine and noradrenaline is consistent with an interaction of prazosin with α_1-adrenoceptors. The antagonism of the excitatory response to dopamine suggests a dopamine receptor blocking property of prazosin, since the excitatory response to dopamine is mediated by receptors that are distinct from α_1-adrenoceptors (Bradshaw et al, 1983b).

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Bradshaw, C.M. et al (1983a) Neuropharmacology, 22, 677-685
Bradshaw, C.M. et al (1983b) Neuropharmacology, In press
Langer, S.Z. (1980) Pharmac. Rev., 32, 337-363

PHARMACOLOGICAL CHARACTERIZATION OF (^{3}H)-COCAINE-BINDING TO RAT STRIATAL MEMBRANES

S.Z. Langer, C. Pimoule, B. Scatton and H. Schoemaker*, Department of Biology, Laboratoire d'Etudes et de Recherches Synthélabo, 58, rue de la Glacière, 75013 Paris, France.

The use of radiolabeled imipramine and desipramine as probes for a regulatory site associated with the neuronal uptake mechanism for serotonin and noradrenaline, respectively, is well established (Langer and Raisman, 1983). However, a molecular probe for a site related to the dopamine transporter has so far not been reported on extensively, although recently ^{3}H-mazindol was advanced as a putative ligand for this purpose (Javitch et al., 1983). The present study examined whether radiolabeled cocaine, a moderately potent dopamine uptake inhibitor, may be used to specifically label the dopamine transporter in the rat striatum.

Male Sprague-Dawley rats (150 - 200 g) were sacrificed and a twice washed crude homogenate of the striatum was prepared by centrifugation at 45000 x g for 10 min. Aliquots of the membrane suspension (25 mg/ml) were incubated with 10 nM ^{3}H-cocaine (spec. act. 35.2 Ci/mmol, New England Nuclear) in 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl buffer (pH 7.4) in a final volume of 560 µl. Following 60 min incubation at 0°C membranes were collected by vacuum filtration over 0.1% bovine serum albumin pretreated Whatman GF/B filters which were washed subsequently with 5 ml buffer. Parallel experiments in which membranes were collected by centrifugation (15 min at 22000 x g) gave identical results, indicating the validity of the filtration method. Specific binding was defined as the difference in membrane-bound radioactivity observed in the absence and presence of 100 µM unlabeled cocaine. Under the conditions employed, specific binding represented 75 - 80% of the total amount of radioactivity bound.

Inhibition of ^{3}H-cocaine binding to rat striatal membranes by unlabeled cocaine was biphasic and indicative of the presence of two saturable binding sites with apparent Kd values of 0.3 and 14 µM. The corresponding binding site densities were 5.2 and 29 pmol/mg protein, respectively. The pharmacological profile of ^{3}H-cocaine binding under these conditions is consistent with its association with a site related to the dopamine uptake mechanism. Binding is inhibited most potently by the dopamine uptake inhibitors nomifensine (IC_{50} = 0.12 µM) and benztropine (IC_{50} = 0.23 µM). IC_{50} values for other biogenic amine uptake inhibitors were (µM): chlorimipramine, 4.4 , fluoxetine, 10.0 ; citalopram, 18.7 ; desipramine, 9.0. Nisoxetine, which inhibits dopamine uptake with an IC_{50} of 0.45 µM, had an IC_{50} value for inhibition of ^{3}H-cocaine binding of 0.3 µM. A moderate stereospecificity of binding could be demonstrated using d- and l-amphetamine, showing IC_{50} values of 11 and 30 µM, respectively. Among the substrates for the dopamine carrier, dopamine inhibited ^{3}H-cocaine binding with an IC_{50} of 11 µM, whereas serotonin and noradrenaline were 3 - 4 times less active.

In conclusion, it may be hypothesized that, in contrast to the rat and mouse cerebral cortex where ^{3}H-cocaine was shown to label a modulatory site associated with the serotonin transporter (Reith et al., 1983), ^{3}H-cocaine binding in the striatum predominantly takes place at the level of the dopamine transporter. As such it may provide a new tool in studying the molecular mechanism of dopamine transport and its alterations following drug treatment or in pathological states.

Javitch, J.A. et al (1983) Eur.J.Pharmacol. 90, 461-462
Langer, S.Z. and Raisman, R. (1983) Neuropharmacol. 22, 407-413
Reith, M.E.A. et al (1983) Mol.Pharmacol. 23, 600-606

LOCUS COEURULEUS LESIONS ABOLISH FEEDBACK OF NORADRENALINE ONTO 'PRESYNAPTIC' α_2-ADRENOCEPTORS IN RAT CEREBRAL CORTEX

S. Baker, I.R. Flockhart[1], I. Marshall*, I.F. Tulloch[1] & D.S. Walter[1], Department of Pharmacology and Therapeutics, The Middlesex Hospital Medical School, London, W1P 7PN and [1]Department of Pharmacology, Pharmaceutical Division, Reckitt and Colman Ltd, Hull, HU8 7DS

The release of noradrenaline in the cortex can be regulated through α_2-adrenoceptors. The location of these receptors remains in doubt as, for example, chemical destruction of the noradrenergic neurons did not alter the binding of ^{3}H-RX 781094 (Langer et al, 1983). The noradrenergic terminals in the cerebral cortex have their cell bodies in the locus coeruleus. The aim of the present work was to study the α_2-adrenoceptor mediated regulation of noradrenaline release in the cortex after lesions of the locus coeruleus.

Sprague-Dawley rats (280-300 g) were given either sham lesions (n=6) or unilateral electrolytic lesions (40 mCoulombs, n=18) in the region of the locus coeruleus (on the right side). When experiments were carried out 8-12 weeks later the noradrenaline concentration of the sham lesioned rats was 223 ± 13 ng/g (mean ± s.e. mean) and 225 ± 11 ng/g in the right and left cortex respectively. In the lesioned group the noradrenaline concentration was reduced (about 70%) to 63 ± 4 ng/g in the right cortex compared with 217 ± 7 ng/g in the contralateral cortex.

Slices of occipito-parietal cortex were incubated in 1.0 μM (^{3}H)-noradrenaline (sp. act. 36 Ci/mmol) for 30 min and were then washed for 120 min. At this time there was less tritium in slices from the lesioned side (54.5 ± 4.6 nCi/mg) compared with the contralateral side (69.4 ± 2.9 nCi/mg; $P<0.01$; paired t-test). The release of tritium from cortical slices was evoked by electrical stimulation (100 pulses, 1.0 Hz, 2 ms, 30 mA). In non-operated rats the fractional release of tritium was reduced by up to 95% by tetrodotoxin 1.0 μM or by use of calcium-free Krebs solution. Clonidine (1.0-100 nM) produced a concentration dependent fall in the tritium fractional release whereas the α_2-adrenoceptor antagonist idazoxan (Doxey et al, 1983; RX 781094; 10 nM-1.0 μM) increased the stimulated overflow of tritium. There were no differences in the control (pre-drug) fractional release of tritium per pulse when comparing slices taken from non-operated rats ($4.5 \pm 0.6 \times 10^{-5}$ and $4.4 \pm 0.6 \times 10^{-5}$, right and left cortex respectively) and the sham lesioned rats ($3.9 \pm 0.8 \times 10^{-5}$ and $4.9 \pm 0.7 \times 10^{-5}$, right and left cortex respectively).

In slices from lesioned rats there was no significant difference between control (pre-drug) fractional release of tritium per pulse on the lesioned and contralateral sides ($3.8 \pm 0.5 \times 10^{-5}$ and $4.7 \pm 0.5 \times 10^{-5}$ respectively). The fractional release of tritium was reduced ($P<0.05$) on both sides by clonidine 30 nM ($2.3 \pm 0.3 \times 10^{-5}$ and $3.9 \pm 0.4 \times 10^{-5}$, lesioned and contralateral sides respectively). There was however a marked difference between sides in the fractional release after idazoxan 1.0 μM. On the contralateral side there was a 172 ± 24% increase above control in the fractional release ($P<0.005$; $11.9 \pm 1.1 \times 10^{-5}$) whereas on the lesioned side there was a non-significant 19 ± 15% increase ($3.9 \pm 0.5 \times 10^{-5}$).

In conclusion, in the cortex some (^{3}H)-noradrenaline may be taken up and released from neurones which possess 'presynaptic' α_2-adrenoceptors but do not originate in the locus coeruleus. The lack of increase of tritium release by idazoxan after lesioning the locus coeruleus suggests that the noradrenergic nerve terminals in the cortex are the source of the amine which, in non-lesioned animals, acts at 'presynaptic' α_2-adrenoceptors to inhibit transmitter release.

Doxey, J.C et al (1983) Br.J.Pharmac. 78, 489
Langer, S.Z. et al (1983) Br.J.Pharmac. 78, Proc. Suppl., 109P

MDL 72145: A POTENT AND SELECTIVE INHIBITOR OF MAO TYPE B

P. Bey, J. Fozard, I. McDonald, M.G. Palfreyman* and M. Zreika, Centre de Recherche Merrell International, 67084 Strasbourg Cedex, France.

A number of inhibitors of monoamine oxidase (MAO) have been described with selectivity for either the A or the B forms of the enzyme. Deprenyl is a selective inhibitor of MAO B which has been evaluated clinically in depression and as an adjunct to the L-DOPA therapy of Parkinson's disease (Mendlewicz and Youdim, 1983). Deprenyl has indirect sympathomimetic activity, probably the result of its metabolism to methamphetamine and amphetamine (Reynolds, et al. 1978), which complicates interpretation of its clinical activity in terms solely of inhibition of MAO B. We describe here the properties of MDL 72145 ((E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine) a selective inhibitor of MAO B which does not have indirect sympathomimetic properties.

MDL 72145 was designed as a potential enzyme-activated, irreversible inhibitor of MAO. In vitro studies using rat brain mitochondrial MAO confirmed the time-dependent pseudo-first order kinetics of irreversible inhibition predicted for the molecule and revealed a marked selectivity for inhibition of the B form of the enzyme (Table 1).

Table 1 Kinetic constants for inhibition of MAO A and B by MDL 72145

MAO A[a]		MAO B[b]	
τ_{50}(min)	K_i (μM)	τ_{50}(min)	K_i (μM)
14.5	130	1.7	40

[a] substrate, 5-HT; [b] substrate, phenylethylamine. Values obtained at 10°C.

Following oral administration to rats, mice and rabbits, MDL 72145 proved a potent and long acting inhibitor of MAO with selectivity for MAO B. For example, the doses required to inhibit rat brain MAO by 50 % following 18 h pretreatment were 0.38 mg/kg for MAO B and 8.0 mg/kg for MAO A. Selectivity was retained following 5 daily treatments with 0.5 or 2.5 mg/kg. Essentially complete inhibition of MAO B in the brain was obtained with the 0.5 mg/kg regime with no associated increase in the cardiovascular responses to either intraduodenally (0.3 to 50 mg/kg) or intravenously (5 to 80 μg/kg) administered tyramine.

The effects of MDL 72145 on the clearance of antipyrine (50 mg/kg given i.v.) were measured in rabbits. MDL 72145 had no effect on antipyrine clearance either at doses selective for inhibition of MAO B (0.5 mg/kg daily for 5 days) or at higher, non-selective doses (10 mg/kg daily for 5 days). Similarly, at MAO B inhibitory doses, MDL 72145 did not prolong pentobarbitone sleeping time in mice nor modify morphine analgesia suggesting minimal effects on drug metabolising enzymes.

Unlike deprenyl, MDL 72145 cannot be metabolised to amphetamine, has no indirect sympathomimetic effects and does not inhibit catecholamine or indoleamine uptake in rat brain synaptosomes at concentrations up to 10^{-4}M.

The selectivity of MDL 72145 for MAO B coupled with its low propensity to potentiate the cardiovascular effects of tyramine and its lack of secondary effects on other systems makes this new inhibitor a useful agent to explore the functional role of MAO B inhibition in man. To this end, preclinical studies are underway. (Alken, et al, 1984).

Alken, R. et al. (1984) Submitted for Presentation, this meeting.
Mendlewicz, J. & Youdim, M.B.H. (1983) Brit. J. Psychiat. 142, 508
Reynolds, G.P. et al (1978) Br. J. Clin. Pharmacol. 6, 542

ATYPICAL DOPAMINERGIC PROFILE OF MINAPRINE, A NEW ANTIDEPRESSANT DRUG

K. Bizière, C. Gueudet, J.P. Kan, J. Souilhac and P. Worms*, Neurobiology, Clin Midy Research Center, Rue Prof. J. Blayac, F.34082, Montpellier Cédex France.

Minaprine (morpholino-2-ethylamino)-3 methyl-4 phenyl-6 pyridazine, dihydrochloride (M) is a new atypical antidepressant drug, for which preliminary pharmacological and clinical data suggested dopamino(DA)-mimetic properties (Bizière et al, 1982 ; Garcia-Maffla, 1981). This prompted us to investigate the effects of M in various models of dopaminergic behaviour.

Female Swiss CD_1 mice (18-22 g) and male Wistar rats (200-230 g)(Charles River France) were used. The interaction with haloperidol (0.25 or 0.75 mg/kg, ip)- induced catalepsy was assessed in rats according to Worms and Lloyd (1980). The induction of stereotyped behaviour by M was followed in rats, every 10 min. for 60 min. after the injection (sc), using the following rating scale (0 : no abnormal behaviour ; 1 : rearing and sniffing ; 2 : gnawing ; 3 : licking and grooming ; 4 : paper and forepaws biting). Turning behaviour was assessed in mice, 7 days after a unilateral 6OH DA lesion of the right striatum (Protais and Costentin, 1976) ; ipsilateral and contralateral turns were noted as + and -, respectively.

At low ip doses, M antagonized haloperidol (0.75)- induced catalepsy in rats (mean ± SEM catalepsy times in sec : controls : 468 ± 29 ; M 1.25 : 280 ± 55** ; M 2.5 : 273 ± 50** ; M 5 : 401 ± 55) ; however, at higher doses, this effect disappeared and M even potentiated the effect of a lower dose of haloperidol (0.25) (controls : 123 ± 50 ; M 10 : 195 ± 60 ; M 15 : 318 ± 68* ; M 20 : 426 ± 68**). In rats, after sc injection, M induced a pattern of stereotyped behaviours mainly characterized by licking, grooming and biting (mean ± SEM stereotypy scores : controls : 0.35 ± 0.18 ; M 0.3 : 1.45 ± 0.22** ; M 1 : 2.4 ± 0.3** ; M 3 : 2.1 ± 0.2** ; M 10 : 1.1 ± 0.1* ; M 30 : 0.3 ± 0.1). This effect was biphasic (bell-shaped dose-response curve) and short-lasting (40 to 60 min.). Under the same conditions, D-amphetamine (5 ip), and apomorphine (1.0 sc), gave respective scores of 1.8 ± 0.2** and 3.2 ± 0.3**. These effects of M (1 sc), D-amphetamine (5 ip) and apomorphine (0.3 sc) were blocked by haloperidol (0.1 mg/kg, ip).

In unilaterally lesioned mice, M (ip) dose-dependently antagonized the spontaneous ipsilateral turning (mean ± SEM number of turns/2 min. : controls : + 11.7 ± 0.5 ; M 0.12 : + 4.6 ± 0.8** ; M 0.5 : + 2.5 ± 0.9** ; M 2 : + 2.1 ± 0.7**). D-amphetamine (5 ip) increased this ipsilateral turning (+ 30 ± 4**) whereas apomorphine (0.5 sc) shifted the vehaviour to contralateral turning (controls : + 10.3 ± 0.4 ; Apo. : - 10.1 ± 0.5). If mice were pretreated (ip) with M, 1 h. before the injection of 6 OH-DA and turning behaviour measured 7 days later, the occurrence of spontaneous turning was dose-dependently diminished (controls : 100 ± 10 % ; M 3 : 69 ± 8 %* ; M 10 : 39 ± 7 %** ; M 30 : 24 ± 7 %**). In this model, nomifensine (20 mg/kg) exhibited the same activity (16 ± 6 %** of controls).

These data suggest that M stimulates central DAergic transmission, at least at low doses. The mechanism whereby M exerts these effects is still unclear, as this drug is completely inactive under in vitro conditions (Kan, personal communication). However, the activity of M in the turning model suggests that it could act through inhibition of DA reuptake. In vivo neurochemical and behavioural experiments are in progress to clarify this mechanism.

* $p < 0.05$ ** $p < 0.01$ vs respective controls (Student's t-test)

Bizière, K. et al (1982) Arzn. Forsch., 32 : 824-831
Garcia-Maffla, A. (1981) Pharmatherapeutica, 2 : 601-606
Protais, P. and Costentin, J. (1976) J. Pharmacol. (Paris), 7 : 251-254
Worms, P. and Lloyd, K.G. (1980) Naunyn-Schmiedebergs' Arch. Pharmacol., 311 : 179.

CHAPS SOLUBILISES D_2 BUT NOT D_1-BINDING SITES FROM RAT STRIATAL PREPARATIONS

P.Jenner, G.J.Kilpatrick*& C.D.Marsden, University Department of Neurology, Institute of Psychiatry & The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

^{3}H-Spiperone selectively labels D-2 binding sites in striatal membranes while ^{3}H-piflutixol identifies both D-1 and D-2 binding sites with equal affinity (Hyttel, 1981). We have compared the binding of these ligands to rat striatal membranes and to a solubilised preparation obtained using the zwitterionic detergent, CHAPS (3-(3-cholamidopropyl)dimethylammonio -1-propane sulphonate).

Pooled striatal tissue was homogenised in either 10 volumes of 50 mM Tris-HCl 1 mM EDTA buffer (pH 7.4) (solubilised preparations) or 40-50 volumes 50 mM Tris HCl buffer (pH 7.4)(membrane preparation) and centrifuged (48,000 x g for 10 min) and resuspended twice. Membrane preparations were finally resuspended in 270 volumes of 50 mM Tris HCl buffer. For soluble preparations the final pellet was resuspended in 6 volumes of 50 mM Tris HCl 1 mM EDTA buffer containing 5 mM CHAPS and 1 mM dithiothreitol. The preparation was centrifuged at 180,000 x g for 1 h and the supernatant retained.

In membrane preparations specific binding of ^{3}Hspiperone (0.02-1.5 nM) defined using 3 x 10^{-5} M (-)-sulpiride gave a Bmax value of 20.6±1.6 pmoles/g tissue and K_D was 0.040±0.007 nM compared to values for specific ^{3}H-piflutixol (0.1-2.0 nM) binding of Bmax 122 pmoles/g tissue and K_D 0.21±0.02 nM; defined using 10^{-6} M cis-flupenthixol. In soluble preparations Bmax for ^{3}H-spiperone binding (0.1-5.0 nM) was 5.8±0.5 pmoles/g tissue and K_D 0.53±0.02 nM compared to values for ^{3}H-piflutixol (0.3-10 nM) of Bmax 6.4±0.5 pmoles/g tissue and K_D 0.99±0.26 nM. In displacement experiments using membrane preparations, sulpiride (3 x 10^{-5} M) caused approximately 80% of specific ^{3}H-spiperone binding but only displaced approximately 20% of specific ^{3}H-piflutixol binding. Using a solubilised preparation, incorporation of sulpiride (6 x 10^{-5} M) displaced approximately 80% of both specific ^{3}H-piflutixol and ^{3}H-spiperone binding. The specific binding of both ^{3}H-spiperone and ^{3}H-piflutixol to solubilised preparations was displaced stereoselectively by the isomers of butaclamol. Ki values obtained for the displacement of the two ligands by a range of drugs were similar. Most drugs showed a lower affinity to displace specific ^{3}H-spiperone binding from solubilised preparations than from membrane preparations. Sulpiride, however, was more effective in displacing the ligand from solubilised preparations.

Table 1 Ki values for displacement of ^{3}H-spiperone and ^{3}H-piflutixol binding

	Ki (nM)		
	Soluble preparations		Membrane
	^{3}H-spiperone	^{3}H-piflutixol	^{3}H-spiperone
(+)-Butaclamol	11.8	13.8	0.19
(-)-Butaclamol	15,000	79,000	890
Sulpiride	135	180	580
cis-Flupenthixol	9.8	9.4	2.2
Haloperidol	11.6	8.6	0.20
Dopamine	5,200	2,560	220

The similar characteristics of the binding sites for ^{3}H-spiperone and ^{3}H-piflutixol in solubilised rat striatal preparations suggests the presence of D-2, but not D-1, binding sites.

Hyttel,J. (1981) Life Sci. 28, 563

MODULATION OF (^{3}H)-5HT AND (^{3}H)-GABA RELEASE FROM SLICES OF RAT SUBSTANTIA NIGRA BY DOPAMINE AND DOPAMINE AGONISTS

P.Jenner, E.Kelly*& C.D.Marsden, University Department of Neurology, Institute of Psychiatry & The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

Dopamine and dopamine agonists modulate 5-hydroxytryptamine (5HT) (Reubi et al, 1978) and GABA (Reubi et al,1977; Arbilla et al,1981) release from nigral slices. So, presynaptic dopamine receptors may exist on the terminals of nigral 5HT and GABA afferent fibres. We investigate further the ability of dopamine and dopamine agonists to alter ^{3}H-5HT and ^{3}H-GABA release from nigral slices.

Dopamine (100 uM) and apomorphine (100 uM) stimulated the spontaneous release of ^{3}H-5HT from nigral slices (Table 1). The effect of apomorphine, but not dopamine was partially antagonised by haloperidol (1 uM). Dopamine (100 uM) and apomorphine (100 uM) also potentiated the potassium chloride (KCl; 25 mM) evoked release of ^{3}H-5HT, but these effects were not reversed by haloperidol (1 uM). Haloperidol insensitive effects of dopamine and apomorphine on ^{3}H-5HT release may be related to their ability to inhibit ^{3}H-5HT uptake into nigral synaptosomal preparations. Dopamine (100-1000 uM), apomorphine (100-500 uM), amphetamine (100-500 uM) and ADTN (100-500 uM) did not alter the spontaneous release of ^{3}H-GABA from nigral slices. However, apomorphine and ADTN (both 100 uM) inhibited the KCl (25 mM) evoked release of ^{3}H-GABA from nigral slices whereas dopamine (100-1000 uM) and amphetamine (100 uM) did not. The inhibition of ^{3}H-GABA release by apomorphine and ADTN (100 UM) was antagonised by haloperidol (1 uM). The effects of dopamine and apomorphine were also investigated in nigral tissue 21 days following unilateral 6-hydroxydopamine lesions of the medial forebrain bundle. As compared to nigral tissue from the non-lesioned side or unlesioned animals apomorphine's (100 uM) ability to stimulate spontaneous ^{3}H-5HT release was unchanged, whilst dopamine (100 uM) remained unable to modify the KCl (25 mM) evoked release of ^{3}H-GABA.

Table 1 Total % release of ^{3}H-5HT and ^{3}H-GABA from nigral slices for a 6 min period following application of drug/KCl

	Drug treatment	Basal		25 mM KCl stimulated	
		Control	Haloperidol	Control	Haloperidol
^{3}H-5HT	None	10.5±0.3	10.7±0.4	20.4±1.2*	-
	Apomorphine (100 uM)	12.8±0.7*	11.7±0.3**	27.0±1.1	29.2±1.0
	Dopamine (100 uM)	19.2±0.6*	19.7±1.3	42.6±3.3	37.0±2.6
^{3}H-GABA	None	3.1±0.1	3.0±0.1	7.7±0.4*	7.6±0.5
	Apomorphine (100 uM)	3.0±0.1	-	5.7±0.4^x	7.2±0.5$^+$
	Dopamine (1 mM)	3.1±0.1	-	8.1±0.3	-
	ADTN (100 uM)	3.0±0.1	-	6.0±0.3^x	7.0±0.3$^+$
	Amphetamine (100 uM)	2.9±0.1	-	8.2±0.5	-

* significantly different from no drug/KCl treatment; ** significantly different from apomorphine alone; x significantly different from KCl alone; + significantly different from KCl + agonist. $p < 0.05$ for each, Student's t test. n = 4-16.

These data confirm that presynaptic dopamine receptors may modulate ^{3}H-5HT and ^{3}H-GABA release from nigral slices. However, since dopamine was either ineffective or its effects were not reversed by haloperidol the results support the conclusion of Arbilla and colleagues (1981) that dendritic dopamine release may not play a similar role *in vivo*.

Arbilla,S. et al (1981) Br.J.Pharmac. 74, 389
Reubi,J.-C. et al (1977) Nature 268, 652
Reubi,J.-C. et al (1978) Naun.Schmied.Arch.Pharmacol. 304, 271

LESIONS OF THE LOCUS COERULEUS INCREASE THE SENSITIVITY OF CINGULATE CORTICAL NEURONES TO SUBSTANCE P

R.S.G. Jones and H-R. Olpe. Biology Research Laboratories, Ciba-Geigy AG., 4002 Basel, Switzerland.

We recently described the interactions of noradrenaline(NA) and 5hydroxytryptamine(5HT) with substance P(SP) applied iontophoretically to neurones in the anterior cingulate cortex of the rat(Jones and Olpe, I983). 5HT could reduce or potentiate the excitatory responses to SP but these effects were accompanied by respective increases or decreases in baseline firing rate. NA always reduced SP-responses regardless of whether it increased, decreased or had no effect on baseline firing. In the present study we determined the consequences of lesioning the NA- or 5HT-containing afferent pathways to the cingulate cortex on neuronal sensitivity to SP in this brain area.

Electrolytic lesions were placed in the right locus coeruleus(LC) (AP., -I.7 mm: lat., I.I mm: vert., 7.I mm from pia) or in the nucleus raphé medianus(MR)(AP., +0.3 mm: lat., 0.0 mm: vert., 6.5 mm) of male albino rats. Electrophysiological experiments were conducted two weeks later under chloral hydrate anaesthesia using conventional extracellular recording and iontophoretic techniques. In LC experiments the same electrode was used to test responses of 8 cells to SP (80nA, 60s) in the ipsilateral cortex, 8 contralaterally and 8 in an intact rat. Responsiveness to acetylcholine(ACh) was also tested. In MR-experiments 7 cells in a lesioned rat were paired with 7 in an intact rat. SP-responses were quantified by determining the number of spikes above the baseline rate in a 90s epoch starting with the initiation of the SP-application (S_1) and in the subsequent 90s(S_2).

Two weeks after the MR-lesion the 5HT-level in the cingulate cortex was reduced to 35% of that in control rats. However, this was not accompanied by any change in the magnitude of SP-responses(see table) After LC-lesions ipsilateral NA was reduced to 2I% of the contralateral side and to I4% of that in intact rats. Concurrently there was a marked increase in the number of spikes evoked by SP on the ipsilateral side. No alteration in responses to ACh was noted.

	RESPONSE SIZE(mean+s.e.m.)				
	MR-LESION		LC-LESION		
	Intact	Lesion	Intact	Contra	Ipsi
S_1	7I4±93	8I3±I05	80I±84	734±57	I254±II3*
S_2	3I3±72	297±72	32I±49	220±43	500±59*

(p<0.00I compared to contralateral: 't' test)

The present results suggest that the coeruleo-cortical NA projection may exert a functional inhibitory influence on the responsiveness to SP in the anterior cingulate cortex. The lack of effect of MR-lesions probably indicates that the 5HT-system is not similarly involved.

Jones, R.S.G. and Olpe, H-R. (I983) Neuroscience Lett. Supp I4:SI84

ACTIVATION OF THE COERULEO-CORTICAL PATHWAY REDUCES NEURONAL EXCITATION BY SUBSTANCE P IN CINGULATE CORTEX

R.S.G. Jones and H-R. Olpe. Biology Research Laboratories, Ciba-Geigy AG., 4002 Basel, Switzerland.

Noradrenaline(NA) strongly reduces the excitatory response of cells in the anterior cingulate cortex to iontophoretically applied substance P(SP)(Jones and Olpe, I983). Also, lesions of the locus coeruleus(LC) result in an increase in responsiveness of these neurones to SP(Jones and Olpe, this meeting). The present study determined the effect of increasing activity in the coeruleo-cortical pathway on responses of anterior cingulate cortical neurones to SP.

Experiments were performed on I8 male albino rats anaesthetised with chloral hydrate. Conventional techniques were used to record extracellular action potentials and to apply substances by iontophoresis to neurones in layers IV-V (approx) in the anterior cingulate cortex. Lc-activation was achieved by two means. (i) A cannula was stereotaxically placed in the nucleus and small(0.25-2.0ul) injections of l-glutamate($5xI0^{-4}M$) were made. At the end of the experiment a small electrolytic lesion was made at the cannula tip to help verify the injection site. (ii) The antidepressant, Trimipramine(TMI) which is a strong excitant of LC-neurones(Hauser et al., this meeting) was injected parenterally(35mg/kg i.p.).

Injection of glutamate into LC reduced or abolished excitatory responses to SP in the cingulate cortex(20 of 22 cells) with little or no effect on baseline firing. The effect was short lived and showed complete recovery wthin 2-3 mins on most cells. The effect was repeatable on the same cell with subsequent injections of glutamate. Injection of the vehicle alone(4 cells) Or injection of glutamate with the cannula tip Imm dorsal(5 cells) or lateral(7 cells) to LC did not detectably alter responses to SP in the cortex. Also, injection of glutamate into LC did not reduce excitatory responses to iontophoretically applied acetylcholine on 5 cells which did show a reduction of responses to SP. Finally, the occlusive action of glutamate on SP-responses was abolished by administration of propranolol (up to 20 mg/kg i.p.)on 4 of 5 cells tested.

Administration of TMI reduced responses to SP on 5 cells. The time course of this reduction followed that of the activating action of this drug on neurones in LC. In 4 of these studies propranolol, given when the reduction was at a maximum was able to restore SP-responses to pre-TMI levels.

Thus activation of the noradrenergic-pathway to the cingulate cortex, like iontophoretically applied NA(Jones and Olpe, I983) can occlude responses of neurones in this brain area to SP. This, together with the increase in responsiveness to SP seen following depletion of cortical-NA(Jones and Olpe, this meeting) strongly suggests that the amine system may exert a functional inhibitory influence on the peptide in this brain area.

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COMPARISON OF THE EFFECTS OF NALOXONE AND ICI 154,129 ON PLASMA CORTICOSTERONE LEVELS IN NORMAL AND STRESSED MICE

I. Kitchen* and K.M. Rowan, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH.

We have recently shown (Kitchen and McEwan, 1983) that there are differences in the effects of opioid agonists upon plasma corticosterone levels in normal and stressed mice. It is also apparent that effects observed with opioid agonists are not dose-related. In order to study this further we have looked at dose response relationships of two opioid antagonists, naloxone and the putative δ-antagonist ICI 154,129 (Shaw et al., 1982) upon plasma corticosterone levels in normal and ether-stressed mice.

Male albino mice (CD-1 strain) were used in all experiments. Naloxone or ICI 154,129 were dissolved in 0.9% saline and administered intraperitoneally (i.p.) in a volume of 0.2 ml. The procedure for collection of plasma and fluorimetric measurement of corticosterone was as described by Gibson et al. (1979). Corticosterone was measured at 15 minutes after i.p. injection. In addition corticosterone was measured 15 minutes after injection, in mice exposed to ether vapour for 60 seconds at 7½ minutes.

Table 1. Plasma corticosterone levels in mice after i.p. injection of opioid antagonists

PRETREATMENT		PLASMA CORTICOSTERONE (μg/100 ml) 15 min	15 min (ether stressed)
Saline		21.8 ± 1.6	31.8 ± 2.0
Naloxone (mg/kg)	0.05	21.5 ± 2.1	24.8 ± 2.6
	0.1	25.9 ± 3.7	27.5 ± 3.5
	0.5	34.3 ± 4.0 **	38. 8 ± 0.5 **
	1	31.8 ± 3.9 *	37.4 ± 2.7
	5	28.1 ± 2.7	27.0 ± 2.0
	10	13.3 ± 1.6 ***	16.3 ± 1.9 ***
ICI 154,129 (mg/kg)	1	22.8 ± 1.8	27.0 ± 2.2
	10	17.7 ± 1.9	26.4 ± 4.7
	30	25.7 ± 2.5	21.5 ± 4.7

All values are mean ± s.e. mean of between 6 and 12 samples, each from different mice. t-test vs saline * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

In common with the findings of Gibson et al. (1979) naloxone elevated plasma corticosterone, at 0.5 and 1 mg/kg. In contrast, however, a higher dose of naloxone (10 mg/kg) caused a marked inhibition of plasma corticosterone which is similar to the inhibitory effects observed with opioid agonists (Kitchen and McEwan, 1983). It therefore raises the question as to whether naloxone, at this dose is acting as an agonist. Again in common with the findings of Gibson et al. (1979) naloxone at 10 mg/kg inhibited the ether stress-induced rise in corticosterone. However whether this is a true antagonist effect must also be doubted since lower doses of naloxone potentiate the response to ether stress (Table 1). The δ-antagonist ICI 154,129 was without effect at any of the doses tested.

In conclusion the response of corticosterone to naloxone is critical on the dose of antagonist chosen. In addition the lack of effect of the δ-antagonist ICI 154,129 suggests that δ-receptors are unimportant in corticosterone responses to opioids.

Gibson, A., Ginsburg, M., Hall, M. and Hart, S.L. (1979) Br. J. Pharmac., 65, 139-146.
Kitchen, I. and McEwan, A.R. (1983) Br. J. Pharmac., 79, 328P.
Shaw, J.S., Miller, L., Turnbull, M.J., Gormley, J.J. and Morley, J.S. (1982) Life Sci., 31, 1259-1262.

OPIOID RECEPTORS IN THE RABBIT VAS DEFERENS

A.G. Hayes* & A.J. Kelly, Department of Neuropharmacology, Glaxo Group Research Limited, Ware, Herts.

The field-stimulated rabbit vas deferens preparation has been suggested to contain only kappa, not μ, δ or σ, opioid receptors (Oka et al., 1981). The purpose of the present experiments was to confirm this hypothesis and study the profile of a series of kappa agonists in this tissue.

Vasa deferentia from Californian rabbits (2.5 to 3.5kg) were divided into three equal portions and set up in 5ml organ baths under 0.5g tension. They were bathed with Krebs-Henseleit solution, devoid of Mg^{2+} ions, which was bubbled with 5% oxygen/95% carbon dioxide. The vasa deferentia were stimulated via platinum ring electrodes, mounted above and below the preparation, with supramaximal pulses, 0.5ms wide and a frequency of 0.1Hz. The contractions were recorded using an isometric transducer connected to a Bryans recorder. Concentration response curves were constructed from cumulative additions of drug, preliminary experiments having ascertained that cumulative and serial dosing produced similar curves.

Kappa agonists produced concentration related depressions of twitch height in the rabbit vas deferens. The following compounds produced parallel dose-response curves and could achieve maximum depression of twitch: bremazocine (IC_{50} value nM $\pm$ s.e 5.5 $\pm$ 1.1), tifluadom (65 $\pm$ 18), ethylketocyclazocine (EKC 87 $\pm$ 19), ketocyclazocine (318 $\pm$ 129), U-50,488 (370 $\pm$ 75). The effect of EKC was reversed by naloxone with a pA_2 value of 7.54 $\pm$ 0.05, slope = 1.04 $\pm$ 0.02.

Compounds acting at other opioid receptors eg. μ-receptors (morphine, D-Ala^2, $MePhe^4$, Gly-ol^5 enkephalin), δ-agonists (D-ala^2, D-Leu^5 enkephalin) and σ-agonists (cyclazocine, SKF 10,047) were without effect. Absence of μ-receptors was confirmed by the lack of effect on EKC responses of β-funaltrexamine, a selective, irreversible μ-antagonist.

Several compounds which produce kappa agonist activity in the field-stimulated guinea-pig ileum or mouse vas deferens were either inactive in the rabbit vas deferens (ie. nalorphine, butorphanol) or produced very shallow dose-response curves with low maximum effect (ie. Mr 2034, proxorphan). We postulate that these compounds are partial agonists and have insufficient efficacy to produce an effect in the rabbit vas deferens, although they do have sufficient efficacy to produce an effect in the guinea-pig ileum and mouse vas deferens, presumably tissues with a higher spare receptor capacity. If this is the case, then these compounds should antagonise the effect of a full agonist. Consistent with this was the finding that nalorphine, 1-10μM, butorphanol, 0.3-3μM and Mr 2034, 3-10μM, all antagonised the effect of EKC in the rabbit vas deferens, suggesting that they are interacting with the kappa receptor in this tissue.

In conclusion, the rabbit vas deferens seems to contain only kappa opioid receptors and appears to be useful for identifying partial agonists.

Oka, T. et al. (1981). Eur.J.Pharmac. 73, 235-236.

ANTAGONISM OF α-ADRENOCEPTOR-MEDIATED ANTINOCICEPTION IN THE RAT

A.G. Hayes, M. Skingle* & M.B. Tyers, Department of Neuropharmacology, Glaxo Group Research Limited, Ware, Herts.

We have recently reported that both α_1- and α_2-adrenoceptor agonists increase nociceptive paw pressure thresholds in the weanling rat (Hayes et al., 1983). These data are consistent with evidence from Paalzow (1982) who suggested that clonidine, a sympathomimetic agent which has activity at both α_1- and α_2-adrenoceptors, mediates antinociception by two or more receptor systems. The present experiments compare the attenuating effects of WB 4101 and RX 781094, which are selective antagonists for α_1- and α_2-adrenoceptors respectively (Doxey et al., 1983; Drew, 1982), against the antinociceptive effects induced by agonists with differing α_1- and α_2- adrenoceptor selectivity ratios. Agonists employed in these studies included methoxamine and ST 587, selective for α_1-adrenoceptors; UK 14,304, a selective α_2-adrenoceptor agonist; and clonidine.

Nociceptive pressure thresholds were determined for the hindpaws of weanling rats (AH hooded, male, 50-70g, n = 12 or 18/group) using an 'analgesymeter' (Ugo Basile). Testing, which was performed blind, was carried out at 30min after subcutaneous administration of the drugs. Agonist and antagonist combinations were given as a single injection.

TABLE 1 Effect of selective antagonists on α-adrenoceptor mediated antinociception

Agonist	Antinociceptive ED_{50} (95% confidence limits)mg/kg s.c.		
	Pretreatment with:		
	Vehicle	WB 4101 3mg/kg s.c.	RX 781094 1mg/kg s.c
ST 587	0.53 (0.34-0.78)	> 3.0	1.1 (0.04-32.8)
Methoxamine	3.3 (2.4-4.6)	>27	2.7 (1.6-4.6)
Clonidine	0.07 (0.03-0.13)	0.08 (0.02-0.19)	0.41 (0.16-1.3)
UK 14,304	0.27 (0.03-0.55)	0.12 (0-0.31)	>30

Table 1 shows that the antinociceptive effects of ST 587 and methoxamine were clearly attenuated by WB 4101, 3mg/kg s.c., but not by RX 781094, 1mg/kg s.c.. In contrast, the effects induced by UK 14,304 and clonidine were attenuated by RX 781094, but were not significantly altered by WB 4101. These data suggest that α-adrenoceptor agonists can mediate antinociception by either α_1- or α_2-adrenoceptors.

Doxey, Roach & Smith (1983) Br.J.Pharmac. 78, 489-505
Drew (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 319, 222-5
Hayes, Skingle & Tyers (1983) 80, 572P, July 1983.
Paalzow (1982) J. Pharm. Exp. Ther. 223, 795-800.

THE ANALGESIC MEPTAZINOL DISPLAYS A SELECTIVE INTERACTION AT THE OPIOID μ_1-SITE

Pam Blurton, M.D. Wood & M.G. Wyllie*, Biochemical Pharmacology, Wyeth Institute of Medical Research, Maidenhead, Berks, SL6 OPH.

Pharmacological and biochemical studies have indicated the existence of several distinct opioid receptor/binding sites (μ,δ,κ,etc.). It has been postulated recently (Childers and Pasternak, 1982) that the analgesic action of both opioids and enkephalins is mediated via a sub-type of the μ receptor (μ_1) for which these agents have a high affinity. Some of the other typical actions of opioids are thought to be mediated via sites for which such drugs have a relatively lower affinity (μ_2,δ,κ,σ). The evidence for the existence of the μ_1 site is based largely on results obtained with the putative selective antagonist, naloxonazine. In the present study, the effects of meptazinol, a selective agonist (Blurton et al, 1982; Pasternak et al, 1983), and naloxonazine on high-affinity opioid binding were examined in order to substantiate, or otherwise, the existence of a μ_1 opioid binding site sub-type.

The binding of $|^3H|$-naloxone and $|^3H|$-dihydromorphine was studied in rat whole brain membranes (Childers and Pasternak, 1978). Binding data was analysed by non-linear least squares curve fitting.

Saturation analysis of $|^3H|$-dihydromorphine and $|^3H|$-naloxone binding revealed the presence of two high-affinity components (Table 1). The binding of both agents to the higher affinity site was blocked by pre-incubation of the membranes with naloxonazine. However, this could not be achieved without an appreciable reduction in the binding of the $|^3H|$ opioid ligands to the lower affinity site. Scatchard analysis of $|^3H|$-dihydromorphine binding in the presence of meptazinol (100nM) showed that binding to the higher affinity component was completely suppressed whereas that binding to the lower affinity site was affected only to a negligible degree.

In summary, saturation analysis of $|^3H|$-dihydromorphine and $|^3H|$-naloxone binding is best explained in terms of two separate high-affinity μ binding sites. The selective suppression of one of these sites by meptazinol and a lesser extent by naloxonazine is consistent with this sub-division and the classification of the higher affinity sites as μ_1.

Table 1 Analysis of the two high affinity components of $|^3H|$-opioid binding and the effects of meptazinol (100nM) and naloxonazine (50nM)

Treatment	$|^3H|$-Naloxone				$|^3H|$-Dihydromorphine			
	K_D	Bmax	K_D	Bmax	K_D	Bmax	K_D	Bmax
Control	0.30	22.4	1.6	186	0.50	71	5.0	185
Meptazinol	NA*	NA	NA	NA	ND	ND	6.0	190
Naloxonazine	ND*	ND	1.66	93	0.45	41	4.1	174

* NA = Not analysed
* ND = No binding detected

Blurton, P.A. et al (1982) Br.J.Pharmac. 77, 507p
Childers, S.R. & Snyder, S.H. (1978) Life Sci. 23, 759
Childers, S.R. & Pasternak, G.W. (1978) Cellular & Molecular Neurobiology, 2, 93
Pasternak, G.W. et al (1983) Life Sci. in press

THE EXCITATORY EFFECT OF MORPHINE IN ISOLATED SEGMENTS OF RAT COLON

A.A. Laniyonu*, C.R.McCulloch & D. Pollock, Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, Scotland

Morphine causes isolated segments of rat colon to contract and relax rhythmically. The mechanism of this excitatory effect has been attributed to the displacement by morphine of 5-hydroxytryptamine (5-HT), which acts on smooth muscle to cause it to contract (Burks, 1976). An alternative hypothesis is that morphine causes contractions by inhibiting a tonic neural inhibitory mechanism which normally suppresses myogenic activity (Gillan & Pollock, 1980). This study examined these two hypotheses.

Male Wistar rats (200-250 g) were treated with parachlorophenylalamine (PCPA, 200 mg/kg, i.p. for 4 days) to inhibit 5-HT synthesis. Control rats received saline. The effectiveness of PCPA treatment was investigated by fluorimetric assay of tissue 5-HT levels (Curzon et al, 1981). The excitatory effect of morphine was investigated in the presence of methysergide and in tissues rendered subsensitive to 5-HT by repeated exposure to this amine. The effects of morphine on the motility and responses of the colon to electrical field stimulation (supramaximal voltage, 0.5 ms pulses, 1-20 Hz) were compared with those of apamin, clonidine and tetrodotoxin (TTX). Isolated segments of colon were suspended in organ baths containing Krebs solution (37°C, gassed with 95% O_2 and 5% CO_2). Initially 2 g tension was applied to each tissue and responses were measured isometrically.

Below a threshold dose for each tissue, morphine had no effect. Above this dose the size of the contractions was independent of the dose used. Morphine-induced contractions were unaffected by pretreatment with PCPA, which lowered ($0.01 > P > 0.001$) 5-HT levels in the colon from 3.73 ± 0.83 μg/g (mean ± s.e. mean, n = 5) in controls to 0.41 ± 0.06 μg/g (mean ± s.e. mean, n = 5). Morphine-induced contractions were antagonized by naloxone (2×10^{-5}M) but were unaffected by methysergide (1.2×10^{-5}M).

Rhythmic contractions similar to those produced by morphine (5×10^{-6}-10^{-5}M) were produced by TTX (0.9×10^{-6} g/ml), apamin (5×10^{-7}M) or clonidine (2×10^{-8}M) but these contractions were unaffected by naloxone (2×10^{-5}M). Contractions produced by clonidine were inhibited by yohimbine (10^{-7}M) but not by prazosin (10^{-6}M). Field stimulation at the peak of a contraction produced by morphine, apamin or clonidine caused an inhibition, which was unaffected by atropine (10^{-5}M), phentolamine (10^{-6}M) or propranolol (10^{-6}M) and persisted in colon from rats pretreated with reserpine (2 mg/kg, i.p. for 4 days) or 6-hydroxydopamine (2 x 50 mg/kg, i.p. on day 1 and 2 x 100 mg/kg, i.p. on day 4).

These results suggest that the 5-HT displacement hypothesis (Burks, 1976, Huidobro-Toro & Way, 1981) does not explain the excitatory effects of opiates in the rat colon. It is more likely that morphine, like apamin or clonidine, but acting by a different mechanism, suppresses a tonic inhibitory neural mechanism, which is not the non-adrenergic, non-cholinergic mechanism that persists in the presence of these drugs but could be adrenergic.

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Burks, T.F. (1976) Eur.J.Pharmac. 40, 279-283
Curzon, G. et al (1981) Br.J.Pharmac. 73, 555-561
Gillan, M.G.C. & Pollock, D. (1980) Br.J.Pharmac. 68, 381-392
Huidobro-Toro, J.P. & Way, E.L. (1981) Br.J.Pharmac. 74, 681-694

LOSS OF ACTIVITY OF TACHYKININS IN SMOOTH MUSCLE: RELEVANCE TO POTENCY ESTIMATES IN VITRO

S.J.Bailey* & C.C.Jordan, Department of Pharmacology, University College London, Gower St., London WC1E 6BT.

Differences in the rank orders of potencies of a number of tachykinin (substance P (SP)) analogues on various smooth muscles have suggested the existence of more than one subpopulation of tachykinin receptors (Bailey et al (1982), Gater et al (1982), Lee et al (1982)). Although this possibility has stimulated further experimental work, an alternative explanation for the differing rank orders of potencies seen, that of differential metabolism or uptake of peptide agonists, has received little attention. The importance of recognising this possibility was recently highlighted by Watson (1983). Accordingly, we have compared the rate of loss of biological activity of 8 tachykinin analogues incubated in the presence of segments of guinea-pig ileum or rat colon muscularis mucosae. These preparations were chosen as they exhibit widely differing rank orders of potencies for some tachykinin analogues (Bailey et al (1982)).

Segments of guinea-pig ileum or rat colon muscularis mucosae were prepared as described by Bailey et al (1982). These were thoroughly flushed through with bathing medium (Tyrode solution containing atropine (2μM) and propranolol, phentolamine, indomethacin and mepyramine, all at 1μM) and blotted to remove gut contents. 2 ml aliquots of a 50 nM solution of the peptide under investigation were incubated with pieces of ileum or colon in silanised glass test-tubes at 32 ^{o}C, bubbled gently with 95% O_2 / 5% CO_2. After 2, 5 or 10 minutes the incubation medium was assayed by suitable dilution on pieces of guinea-pig ileum previously calibrated with respect to the peptide under study. Frequent control doses of agonist were given so that corrections could be made for changes in the sensitivity of the ileum with time. Concentrations of agonist remaining after incubation were estimated by extrapolation from the initial calibration of the ileum.

The concentration of peptide agonist remaining at time t was plotted against t. For most analogues, the results were consistent with a simple exponential decay of activity. A half life describing the rate of loss of activity for each analogue was estimated from these plots.

The order of stabilities seen for the various analogues on the 2 tissues was as follows (in decreasing order of stability, half lives in brackets, in minutes):
Guinea-pig ileum : [pGlu^5,MePhe^8,MeGly^9]-SP_{5-11} (DiMeC7) (47) $\approx$ eledoisin (46) > physalaemin (13) $\approx$ kassinin (11) > SP (5) $\approx$ SP_{4-11} (4) > SP-methyl ester (2.4) > eledoisin-related peptide (ERP) (1.6)
Rat colon muscularis mucosae : DiMeC7 (67) $\approx$ physalaemin (35) $\approx$ eledoisin (22) > kassinin (15) > SP_{4-11} (6) $\approx$ SP (5) > ERP (3.2) > SP-methyl ester (1.3)

Thus the relative stabilities of these analogues are similar on 2 tissues displaying widely differing rank orders of potencies. Assuming the apparent relative rates of metabolism we observe reflect those in the biophase, these data suggest such differences cannot by themselves explain the differing rank orders of potency seen on these two tissues. This strengthens the argument in favour of the existence of at least 2 subtypes of tachykinin receptors.

SJB is an MRC scholar.

Bailey et al (1982) Br.J.Pharmac., 75, 114p
Gater et al (1982) Br.J.Pharmac., 75, 341-351
Lee et al (1982) Naunyn Schmiedeberg's Arch.Pharmac., 318, 281-287
Watson (1983) Br.J.Pharmac., 79, 543-552

LACK OF MUSCARINIC BINDING SITES ON CHOLINERGIC NEURONES IN THE HUMAN BASAL FOREBRAIN

J.M. Candy*, R.H. Perry & J.E. Thompson, MRC Neuroendocrinology Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE

One of the best documented neurochemical changes in Alzheimer's disease relates to the cortical cholinergic system and the abnormalities are reflected in a decreased level of choline acetyltransferase (ChAT) activity and acetylcholinesterase (AChE) activity. Interestingly, reductions in ChAT and AChE activities have also been found in the cerebral cortex in cases of Parkinson's disease with cognitive impairment (Perry et al, in press). The importance of the cholinergic input to the cerebral cortex is therefore axiomatic not only for an understanding of its role in cognitive and dementing processes but also for a possible approach to therapeutic intervention.

Current evidence suggests that the nucleus of Meynert supplies the majority of neocortical cholinergic afferents (Johnston et al, 1979). Consistent with the cholinergic nature of the nucleus of Meynert, intense AChE staining is observed and a high level of ChAT activity is present in man (Candy et al, 1981). In senile dementia of Alzheimer type a profound reduction in ChAT activity occurs in the nucleus of Meynert which is not reflected by neuronal loss (Perry et al, 1982). The high level of ChAT activity and intense AChE neuropil staining might suggest the existence of either recurrent axon collaterals or an extrinsic cholinergic input, which is lost in senile dementia. The distribution of muscarinic binding sites in the nucleus of Meynert has therefore been initially investigated using tritium film autoradiography (Palacios et al, 1981; Penney et al, 1981).

Blocks of basal forebrain containing the nucleus of Meynert were obtained from 3 male patients (41, 53 and 64 years with 11, 20 and 8h post-mortem delay respectively), who had no clinical history of neurological or mental illness. The blocks were frozen in melting arcton (Freon 12, ICI) and stored at -70°C. Serial, coronal, 15 m cryostat sections were cut using a modified cryostat (Bright Instruments Limited, Huntingdon), thaw mounted and air dried before storage at -30°C. Sections were preincubated for 10min at 4°C in phosphate buffered saline, pH 7.4 (PBS), followed by incubation for 60min at 4°C either in 1nM (^{3}H) N-methylscopolamine (84.8 Ci/mmol) in PBS or in the presence of the ligand and 1 M atropine to define non-specifically bound ligand. The sections were washed twice for 5min in PBS at 4°C followed by a 10s dip in distilled water at 4°C and rapidly dried under a stream of argon. The sections were exposed to tritium sensitive film (Ultrofilm, LKB) for 8-14 days. Under the conditions used over 95% of the ligand was specifically bound.

High densities of muscarinic binding sites were observed in the caudate nucleus, putamen, anterior hippocampus and in islands of neuropil which surround the anterior commissure. In contrast the internal and external divisions of the globus pallidus and the nucleus of Meynert had only low densities of muscarinic binding sites. These results suggest that the high level of ChAT activity in the nucleus of Meynert is not commensurate with a major cholinergic input at least as reflected by muscarinic receptor binding sites.

Candy, J.M. et al (1981) J. Anat. 133, 123
Johnston, M.V. et al (1979) Proc. Natl. Acad. Sci. 76, 5392
Palacios, J.M. et al (1981) Neurosci. Letters 24, 111
Penney, J.B. et al (1981) Science 214, 1036
Perry, R.H. et al (1982) Neurosci. Letters 33, 311
Perry, R.H. et al. Lancet in press

ACTIONS AND INTERACTIONS OF METHOHEXITONE AT THE SMOOTH MUSCLE-NERVE PREPARATION OF THE RAT ILEUM

F.A.WALI. Anaesthetics Unit,The London Hospital Medical College,Whitechapel, London E1 1BB .

Previous reports (Elliott & Wali,1983 a,b) indicated that methohexitone in low concentrations(0.5-25 $\mu g.ml^{-1}$) may facilitate transmission at the chick neuromuscular junction.Methohexitone also reduced the depolarization and contracture responses produced by acetylcholine(ACh) while it potentiated those produced by tetraethylammonium(TEA) in the isolated chick biventer cervicis nerve-muscle preparation.

In the present study,the effect of methohexitone (1-100 $\mu g.ml^{-1}$) on the spontaneous contractions and on the contractile responses produced by repetitive nerve stimulation at 1-50 Hz, by ACh and by TEA were studied in the isolated rat ileum(autonomic nerve-smooth muscle preparation).

Methohexitone(1-100 $\mu g.ml^{-1}$) produced concentration-dependent contractures in the rat ileum and increased the spontaneous contractions by 30-40% of the control responses(0.5* 0.01 g,n=6). The mean(* SEM) EC50s of the contractures produced by methohexitone was 10* 0.03 $\mu g.ml^{-1}$(n=6) and a mean maximum contracture of 2.1* 0.01 g was obtained by methohexitone (100 $\mu g.ml^{-1}$).

Repetitive nerve stimulation (of the sympathetic and parasympathetic neurones innervating the gut) at 1-10 Hz produced a monophasic response(contraction) in the rat ileum.The mean amplitude of the contractions produced at 10 Hz was 1.5* 0.02 g, n=6. Stimulation at 20-50 Hz produced a biphasic response (i.e relaxation followed by prolonged contraction).The mean amplitude of the relaxations was 0.5* 0.01 g,at 20 Hz,n=6. Higher rates of stimulation (60-100 Hz) caused only relaxations in the rat ileum.Methohexitone (40 $\mu g.ml^{-1}$) reduced the contractions produced at 1-10 Hz, whereas it had little effect on those produced at 20-50 Hz which were only reduced by atropine (500 $\mu g.ml^{-1}$).

ACh (5-100 $ng.ml^{-1}$) and TEA (0.5-5 $mg.ml^{-1}$) produced concentration-dependent contractures in the rat ileum.A mean maximum contracture of 3.5* 0.1 g and 2* 0.1 g was produced by ACh (80 $\mu g.ml^{-1}$) and by TEA (2.4 $mg.ml^{-1}$) respectively(n=6). Methohexitone (40 $\mu g.ml^{-1}$) reduced the contractures produced by ACh and by TEA. The mean EC50s of the contractures produced by ACh in the control Krebs solution and in methohexitone were 20* 1.0 $ng.ml^{-1}$ and 50* 3.0 $ng.ml^{-1}$ respectively(n=6, $P < 0.001$).The corresponding values for TEA were 1.0* 0.03 $mg.ml^{-1}$ and 2.0* 0.01 $mg.ml^{-1}$ (n=6,$P < 0.001$).

The effect of methohexitone,ACh and TEA on the contractile responses produced in the rat ileum (circular and longitudinal smooth muscles) may be more complicated than their effects on the skeletal muscle fibres of the chick biventer cervicis. In the rat ileum,ganglion cells,nerve plexuses and nerve endings from both extrinsic(autonomic nerves) and intrinsic(Auerbach and Meissners plexuses) are in close association with the smooth muscle fibres in the gut.The neurotransmitters released from the autonomic neurones may cause excitation or inhibition of the contractions (Burnstock & Costa,1973; Wood,1975). In conclusion,the actions and interactions of methohexitone with ACh and TEA at the rat ileum may be different from those observed at the chick neuromuscular junction.

Elliott,R.C.& Wali,F.A.(1983a). Neuropharmacology, 22(2),151-155
Elliott,R.C.& Wali,F.A.(1983b). Biochem.Pharmac. 32(4), 671-677
Burnstock,G.& Costa,A.(1973). Gastroenterology, 64 ,141-144
Wood,J.D.(1975). Physiol. Rev., 55 ,307-324

THE EFFECT OF D-PENICILLAMINE AND COPPER ON LYMPHOCYTE CLUSTERING, SECRETION OF IMMUNOGLOBULIN G AND RESPONSES TO INTERLEUKIN 1

J.E. Hawkes & A.J. Kennedy,* Department of Pharmacology, Roche Products Ltd., Welwyn Garden City, Hertfordshire AL7 3AY.

D-penicillamine (D-Pen) has modest activity in inhibiting ^{3}[H] thymidine incorporation into human lymphocytes (I50>1mM). However in the presence of copper salts D-Pen markedly inhibits incorporation (Lipsky and Ziff, 1978). In the present studies we have examined the effects of D-Pen on three aspects of lymphocyte and monocyte responses to mitogen. These are the secretion of immunoglobulin G, the cellular clustering responses initiated by mitogen and finally the involvement of interleukin 1.

Immunoglobulin G (IgG) was measured in supernatants from 7 day cultures of human peripheral blood monocytes (HPBM) stimulated with Pokeweed Mitogen (PWM) using a sensitive enzyme linked immunosorbent assay. IgG secretion was inhibited in a dose dependent manner by increasing concentrations of copper sulphate and D-Pen (91% inhibition at 0.15 mM D-Pen and 0.004 mM copper sulphate). D-Pen alone also inhibited IgG secretion in a dose dependent manner (69% inhibition at 0.15 mM). This latter observation has also been reported by Lewins et al, 1982.

Cell clustering of HPBM is stimulated by mitogens and reflects cellular co-operation. Cell clustering initiated by phytohaemagglutinin (PHA) was markedly inhibited by a combination of D-Pen (0.15 mM) and copper sulphate (0.016 mM) and also inhibited by D-Pen alone (0.6 mM). D-Pen and copper also inhibited PWM stimulated cell clustering. We have examined the possibility that these effects may be due to an inhibition of interleukin 1 (IL-1) activity by D-Pen and copper sulphate. A murine thymocyte proliferation test was used to assay a purified IL-1 preparation and to assay IL-1 like activity in a monokine containing supernatant from PHA stimulated HPBM. D-Pen (1 mM) and copper sulphate (0.008 mM) separately or in combination inhibited ^{3}[H] thymidine incorporation into PHA stimulated thymocytes. IL-1 augmentation of PHA stimulated proliferation wasalso inhibited by these concentrations of D-Pen and copper. The threshold concentration for inhibition by D-Pen alone in a series of experiments was 0.1 mM. A similar pattern of inhibitory activities for D-Pen and copper were measured when a crude monokine containing supernatant was used in place of purified IL-1. D-Pen (1 mM) alone inhibited supernatant augmented thymocyte proliferative responses to PHA. These responses were also inhibited by D-Pen (0.1 mM) in the presence of copper (0.008 mM).

Lewins, L.G. et al (1982) J. Rheumatol. 9, 677.
Lipsky, P.E. & Ziff, M. (1978) J. Immunol. 120, 1006.

LEUKOTRIENES C_4 AND D_4 ARE POTENT CONSTRICTORS OF THE PORCINE RENAL VASCULAR BED

L.J.McLeod,† Priscilla J. Piper & A.W.B. Stanton,* Dept. of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons, Lincoln's Inn Fields, London WC2A 3PN, and † Dept. of Physiology, University of Tasmania, Hobart, Tasmania.

Leukotrienes (LT) C_4 and D_4 have been shown to be potent vasoconstrictors in several vascular beds including the coronary circulation of various species in vivo and in vitro (Letts and Piper, 1983; Michelassi et al 1982). We have investigated the actions of LTC_4 and LTD_4 on renal blood flow in anaesthetised pigs.

Nine pigs of either sex were sedated with azaperone (Suicalm, Janssen) and anaesthetised with alphaxalone-alphadolone (Saffan,Glaxo) and thiopentone 5% (Intraval Sodium, May & Baker) given i.v. The animals were intubated through a tracheotomy incision and ventilated with room air. Systemic arterial BP, ECG and heart rate were recorded. The peritoneal cavity was opened and the right or left renal artery cannulated and perfused with heparinised arterial blood from the left carotid artery. The perfusion pressure was regulated to equal BP. Blood flow was measured with an extracorporeal electromagnetic flow probe (Gould) situated in the perfusion line close to the renal artery cannula. LTC_4, LTD_4 or noradrenaline (NA) were given as bolus injections distal to the flow probe. Indomethacin (5 mg kg^{-1}) was given i.v. and doses of LTC_4 or NA repeated.

The renal arteries were removed post mortem and incubated with calcium ionophore A23187 to investigate the release of LT-like material (Piper et al., 1983).

LTC_4, LTD_4 (5×10^{-12} - 5×10^{-9} mol) or NA (10^{-10} - 2×10^{-7}mol) caused dose-related reductions in renal blood flow. LTC_4 was approximately 25 times more active than NA and caused longer-lasting reductions in flow. A slight fall in BP was sometimes recorded after injecting LTC4.

After treatment with indomethacin the dose-response curves for LTC_4 and NA were shifted to the left. The doses of LTC_4 required to cause approximately 50% reduction in flow were 4×10^{-10}mol before and 5×10^{-11}mol after administration of indomethacin. Similar figures for NA were 10^{-8} mol and 10^{-9}mol. Experiments in which LTD_4 was administered indicate that it is more active than LTC_4.

The arteries generated LT-like material in quantities equivalent to 37.2 ± 9.3ng g^{-1} LTD_4 (n=9). This is more than is generated by coronary or pulmonary arteries, furthermore LTs are apparently more active in reducing renal than coronary blood flow.

These experiments show that LTC_4 is a potent constrictor of the porcine renal vascular bed. The indomethacin-induced potentiation of LTC_4 and NA vasoconstriction suggests that as in other species, a vasodilator cyclo- oxygenase product, probably PGE_2 or PGI_2, is generated by the porcine kidney.

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Letts, L.G. & Piper, Priscilla J (1983) Adv. PG, TX, LT Res. 11, 391
Michelassi, F. et al (1982) Science 217, 841
Piper, Priscilla J. et al (1983) Prostaglandins 25, 591

INDOMETHACIN-INDUCED HYPERREACTIVITY OF HUMAN SMALL AIRWAYS TO LTD_4

P.J. Gardiner* & D.M. Stock. Pharmacology Dept., Miles Laboratories Ltd, Stoke Court, Stoke Poges, Slough, SL2 4LY.

Leukotriene D_4 (LTD_4) is a potent contractant of guinea-pig small airways (Drazen et al 1980). This action is partially mediated by de novo synthesis of thromboxane A_2 but is resistant to the leukotriene antagonist FPL55712 (Cuthbert & Gardiner, 1983). In other species such as the cat, sheep and rabbit, however, we have shown that LTD_4 was only a weak contractant of small airways. In a limited study by Hanna et al (1981) LTD_4 was shown to be a weak contractant of human lung strips. We have extended our studies to human small airways and have investigated the action of LTD_4 over a wide dose range alone and in the presence of FPL55712, indomethacin or BW755C.

Human parenchymal lung tissue obtained from surgery for carcinoma of the lung was cut into strips according to the method of Lulich et al (1976) suspended in a bath and superfused with Tyrodes solution (5ml/min) at 37°C gassed with 5% CO_2 in oxygen. Tissue movement was monitored by a Harvard isotonic transducer with a load of 250-500mg.

LTD_4 (10^{-11}- 3 x 10^{-8} Moles) contracted the tissue with a typical slow onset and long duration as expected of a leukotriene. The maximal contractant effect of LTD_4 was comparable to that of $PGF_{2\alpha}$ but exceeded that of histamine or carbachol. FPL55712 (0.1 - 10.0µg/ml) failed to antagonise the leukotriene action but on numerous occasions it directly relaxed the tissue. Indomethacin (1µg/ml) failed to alter the inherent tone of the tissue but potentiated the responses of high doses (10^{-10} - 3 x 10^{-8} moles) of LTD_4. The maximal effect of LTD_4 rose from 80% of the maximal $PGF_{2\alpha}$ contraction to 120%. No such effect was observed for histamine or carbachol on the same preparations. FPL55712 was retested in the presence of indomethacin. The potentiation induced by indomethacin was abolished but no further rightward shift of the LTD_4 dose/response curve occurred. This effect of FPL55712 was not dose related.

BW755C (50µg/ml) was also studied on this tissue both alone and in the presence of indomethacin (1µg/ml). A similar selective potentiation of LTD_4 contractions occurred in both studies.

Taken together, these results demonstrate that LTD_4 potently contracts human small airways in a dose related manner but this action is resistant to FPL55712. The contractions are probably accompanied by an increase in cyclooxygenase products, probably PGE_2 and or PGI_2, which seem to decrease the normal LTD_4 contractions.

Drazen et al (1980) Nat. Acad. Sci. U.S.A., 77; 4354-4358.
Cuthbert, N.J. & Gardiner, P.J. (Poster presentation this meeting).
Lulich et al (1976). Br. J. Pharmac. 58, 71.

PROSTAGLANDIN SYNTHETASE CATALYSED ACTIVATION OF PARACETAMOL BY HUMAN PLATELETS

A. Huggett*, M.A. Orchard & I.A. Blair, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 OHS.

Prostaglandin synthetase (PGS) catalyzes the microsomal oxidation of several compounds including paracetamol (Moldeus et al, 1982). The hydroperoxidase function of PGS is thought to be responsible for the cooxidation of paracetamol. A reactive intermediate is produced which can covalently bind to proteins. This intermediate has the characteristics of a semiquinone radical. In order to further elucidate the mechanism of paracetamol activation by PGS and its relevance in vivo, we have investigated the PGS-mediated covalent binding of paracetamol in human platelet microsomes and also in intact platelets.

Human platelet microsomes were incubated at 25°C with ^{14}C-paracetamol (0.43 mM) in the presence of sodium arachidonate (0.2 mM). Covalent binding to proteins occurred rapidly with maximal binding at 1.5 min. The binding was dependent on sodium arachidonate and protein concentration and could be inhibited by radical scavengers (2,6 xylenol, butylated hydroxytoluene and butylated hydroxyanisole) in a concentration-dependent manner. The activity of PGS was stimulated by the paracetamol as determined by the production of prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2).

The effect of paracetamol on platelet aggregation and arachidonic acid metabolism was investigated in intact platelets (platelet-rich plasma). Paracetamol (0.05 mM) completely inhibited sodium arachidonate (0.34 mM) induced platelet aggregation and substantially reduced PGE_2 and TXB_2 production. The covalent binding of ^{14}C-paracetamol (0.2 mM) to intact washed platelets at 25°C stimulated by sodium arachidonate (0.05 mM) was examined. The time course of binding was slower than that observed in the microsomal preparation with maximal binding at 10 min. Binding was dependent on the presence of sodium arachidonate and increased with increasing paracetamol concentration.

These results show that paracetamol is activated by PGS in human platelets to produce a reactive intermediate which covalently binds to proteins. A radical is involved in this process. The mechanism of activation may be different in the two systems investigated and this raises the question of the relevance of studies of PGS-mediated drug oxidation using purified microsomal systems. The finding that paracetamol covalently binds to platelets may have important consequences for their viability in vivo.

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Moldeus, P. et al (1982) Biochem.Pharm. 31, 1363-1368.

PROSTAGLANDIN RELEASE FROM THE RABBIT ISOLATED PERFUSED KIDNEY AND ITS INHIBITION BY DEXAMETHASONE

C.N. Berry* and J.R.S. Hoult, Department of Pharmacology, King's College, Strand, London WC2.

The rabbit kidney is a major site of prostaglandin synthesis and release, and these compounds may affect the maintenance of renal blood flow and renin secretion from the juxtaglomerular apparatus. We therefore examined prostaglandin release in response to various vasoactive agents known to affect renal vascular resistance, and the effect on this of anti-inflammatory steroids.

Male NZW rabbits (1.5-3.0 kg) were anaesthetised with sodium pentobarbitone (Sagatal, 50 mg/kg, i.v.). After exposing the kidneys, the renal arteries were cannulated with Portex pink Luer-fitting cannulae. The kidneys were then flushed with warmed Krebs' solution and removed to a perfusion chamber and perfused at 12 ml/min with Krebs' solution at 37°C. Potential PG releasing agents were given as short infusions (24 s at 1 ml/min) via a side infusion line. The perfusates were collected for 10 min, acidified to pH 3.0 and loaded onto Waters 'Sep-Pak' C-18 reverse phase columns prewetted with acetonitrile. PGs were eluted with 5 ml ethyl acetate, dried down, resuspended in distilled water and subjected to RIA (double antibody method).

All four vasoactive compounds as well as arachidonic acid caused the release of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (Table 1), without altering thromboxane B_2 output (this was the least abundant product of the 4 assayed, comprising 2.2% of the total). In the case of angiotensin II, release was dose-dependent. We noted that the basal release increased with time, although it was not correlated with perfusion pressure.

TABLE 1. PG RELEASE FROM PERFUSED RABBIT KIDNEY BY VASOACTIVE SUBSTANCES

n	agent (µg)	mean ng/min released ± s.e.m.			
		PGE_2	6-keto-$PGF_{1\alpha}$	$PGF_{2\alpha}$	
11k,33t	basal	6.3 ± 1.0	7.4 ± 0.7	5.3 ± 0.8	
5k,11t	arachidonate,4.	11.3 ± 2.9	17.8 ± 3.4	12.1 ± 2.7	
6k,12t	angiotensin II,4.	30.5 ± 5.2	43.5 ± 11.4	23.0 ± 5.2	
5k,11t	bradykinin,4.	44.5 ± 4.3	34.4 ± 3.4	28.9 ± 6.1	k = kidney
6k,12t	histamine,4.	20.7 ± 3.2	17.1 ± 2.2	15.2 ± 2.7	t = test
6k,12t	noradrenaline,4.	27.7 ± 3.7	28.5 ± 3.0	19.6 ± 2.2	

Dexamethasone, 2 µg/ml perfused for 40 min before challenge, inhibited PG release due to histamine (1 µg)($P < 0.05$, n = 6k,12t), but not that due to bradykinin (1 µg) or arachidonic acid (1 µg). The possibility of this inhibition being mediated by a renal macrocortin-like substance (Cloix et al) should be considered. Our results also imply that there are functionally distinct pools of phospholipase in the rabbit kidney, as demonstrated for the same trigger substances in guinea-pig lung (Robinson & Hoult).

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Cloix, J.F. et al (1983). Br. J. Pharmac. 79, 313-321.
Robinson, C. & Hoult, J.R.S. (1980). Eur. J. Pharmacol. 64, 333-339.

STIMULATORS AND INHIBITORS OF PROSTACYCLIN FORMATION IDENTIFIED BY USING INTACT FRAGMENTS OF RAT CAECUM

C.N. Berry and J.R.S. Hoult*, Department of Pharmacology, King's College, Strand, London WC2R 2LS.

Prostacyclin and other prostanoids have important physiological and pathological actions on the gastrointestinal tract, and may be involved in the therapeutic or side-effects of several classes of drugs which modify gut function. Most methods for examining drug action on the PG system in gastrointestinal tissues utilise homogenates or other subcellular fractions in which cellular integrity is completely lost. We show here that intact fragments of intestinal tissue (in this case, the rat caecum) can be conveniently used to study the actions of drugs which modify the PG system.

Caeca from normal male Wistar rats were cleaned and stored frozen for 1-6 wk; after thawing they were cut into approximately equal fragments (15-60 mg wet weight, mean value 38.8 ± 2.8 mg) and preincubated for 60 min at 4°C in 0.4 ml 50 mM tris buffer pH 7.4 containing the drug of interest (this period allows drug penetration). After transfer to a further 0.4 ml solution, tubes were incubated for 15 min at 37°C, the caecal fragments removed, blotted and weighed. The incubates were assayed for prostacyclin release by radioimmunoassay of the stable hydrolysis product 6-keto-$PGF_{1\alpha}$ using a double antibody method (c.v. inter-assay 8.7%, intra-assay 13.8%, for the last 6 assays; antibody cross-reactivity with other prostanoids <2% except 6-keto-PGE_1, 7.2%, PGE_2, 6.8% and $PGF_{2\alpha}$, 5.8%) and taking direct aliquots of up to 5 µl.

In 15 min, the tissues released substantial quantities of immunoreactive 6-keto-$PGF_{1\alpha}$, e.g. fragments from 63 animals generated 1061 ± 41 pg/mg wet weight tissue, whereas basal "content" (assayed by similar incubation in ethanol) was <300 pg/mg. Release was time-dependent - occurring in exponential fashion over 75 min -, but 15 min incubations were routinely performed for drug studies. Successive transfer of fragments to fresh solutions resulted in step-wise reductions in yield. $PGF_{2\alpha}$ and PGE_2 were released in smaller quantities (18.2% and 5.1%, respectively, of the amount of 6-keto-$PGF_{1\alpha}$).

Known aspirin-like drugs dose-dependently reduced 6-keto-$PGF_{1\alpha}$ release, e.g. flurbiprofen 0.6 µM, mefenamic acid 0.9 µM and indomethacin 13 µM (approx. ID_{50} values shown). Other compounds were characterised as biphasic stimulators of prostacyclin release: 5-aminosalicylic acid (x2.5 stimulation at 500 µM, inhibition at higher concentrations), hydroquinone (x 1.9 at 500 µM, then inhibition) and BW 755c (x 2 stimulation at 50 µM, inhibition above). Further compounds were typified as progressive stimulators : adrenaline (x 2.3 up at 5000 µM, the top concentration tested), phenol (x 3.3 at 5000 µM) and paracetamol (x 3.4 at 2000 µM). The effects of compounds in the latter two classes probably reflect their ability to act as free radical scavengers, thereby preventing self-inactivation of prostacyclin synthetase.

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5-LIPOXYGENASE FROM HUMAN LEUKOCYTES

D.J. Masters and R.M. McMillan* (Introduced by M. Johnson), ICI Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire, U.K.

Arachidonic acid (AA) metabolism via 5-lipoxygenase leads to formation of leukotrienes which are key mediators of allergy and inflammation. Leukocytes from a variety of species, including man, have been shown to release leukotrienes in response to appropriate stimuli but the enzymes responsible for leukotriene biosynthesis have been poorly characterised. Cell-free preparations of 5-lipoxygenase have been reported but to date the only sources of such systems have been leukocyte tumours (e.g. RBL-1 cells) or inflammatory neutrophils from rodents (Jakschik et al, 1980; Ochi et al, 1983). In the present report, a cell-free preparation of 5-lipoxygenase from human leukocytes is described and is compared to the enzyme from RBL-1 cells.

Peripheral human leukocytes were prepared by gelatin sedimentation followed by ammonium chloride lysis of residual erythrocytes. Human leukocytes or RBL-1 cells (5×10^7/ml) were disrupted by Polytron homogenisation and sonication, then the homogenate was centrifuged at 105,000g (60 min; 4°C). The resulting high speed supernatant (HSS) was the predominant source of lipoxygenase activities in both cells. Drugs were pre-incubated with HSS containing 1mM glutathione (15 min; 4°C) before addition of ^{14}C-AA. Incubations were terminated after 20 min. at 37°C by addition of 4 volumes of ethyl acetate. This procedure yielded >85% extraction of ^{14}C-AA and lipoxygenase metabolites, which were separated by thin layer chromatography (petroleum ether:diethyl ether:acetic acid; 50:50:1) localised by autoradiography and quantified by liquid scintillation counting.

Autoradiograms of ^{14}C-AA metabolites from RBL-1 lipoxygenase demonstrated two major radiolabelled products corresponding to 5-HETE and 5,12-di HETEs. In parallel experiments using HSS from human leukocytes, these products were also detected but an additional, less polar metabolite that co-migrated with 12-HETE was formed in this system. In common with the 5-lipoxygenase from RBL-1 cells, lipoxygenase activities from human leukocytes were calcium-dependent. Nordihydroguaiaretic acid, BW755C, phenidone, nafazatrom (Bayer 6575), baicalein, esculetin and quercetin (10^{-8}-10^{-5}M) produced concentration-dependent inhibition of lipoxygenase activities from human leukocytes. In contrast, indomethacin, flurbiprofen, ibuprofen, flufenamic acid and naproxen produced no consistent inhibition at 10^{-4}M. Interestingly the previously reported lipoxygenase inhibitor benoxaprofen (Walker and Dawson, 1978) did not significantly inhibit human leukocyte 5-lipoxygenase under these conditions at concentrations up to 10^{-4}. Comparable effects with each drug were obtained using the RBL-1 enzyme.

In summary, human leukocytes contain a calcium-dependent 5-lipoxygenase which should be a useful addition to tumour cell sources of the enzyme.

Jakschik, B.A. et al (1980). Biochem. Biophys. Res. Comm. 95, 103-110.
Ochi, K. et al (1983). J. Biol. Chem. 258, 5754-5758.
Walker, J.R. and Dawson, W. (1978). J. Pharm. Pharmac. 31, 778-780.

THE INFLUENCE OF SEX HORMONES ON AN EXPERIMENTAL MODEL OF THROMBOSIS IN THE RAT

H. Emms* & G.P. Lewis, Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2

Men are more susceptible to cardiovascular disease than premenopausal women whilst the incidence of the disease is similar in postmenopausal women and men of the same age. This difference could be attributable to a protective effect of female hormones. However, when an oestrogen and a progestogen are administered in the form of the oral contraceptive pill they increase the risk of thrombotic disorders. In a model of thrombosis in the rat we have compared males with females and studied the effects of sex hormones in female rats.

Thrombus formation was induced in vivo using the method of Smith and White (1982). Rats were anaesthetized and an extracorporeal shunt, incorporating a length of cotton thread, was inserted between the left carotid artery and the right jugular vein. Blood was allowed to flow over the thread for 15 min. The thread was removed and a second piece of cotton was inserted into the circulation for a further 15 min. The wet weight of thrombus deposited on the threads was determined.

Thrombus deposition was significantly greater in weight-matched males compared with females (31.2 $\pm$ 4.8mg and 12.2 $\pm$ 2.4mg respectively, $p<0.01$) and although not statistically significant, thrombus deposition was also greater in age-matched males (27.7 $\pm$ 6.9 mg). The activated partial thromboplastin time (APTT), prothrombin time (PT) and Russell Viper venom time (RVVT) did not differ between the sexes. Plasma fibrinogen levels in the males were twice those in the females (2.7 $\pm$ 0.1 and 1.2 $\pm$ 0.3 g/l respectively $P<0.001$). The sensitivity of platelets to arachidonic acid (AA), ADP and collagen was similar in both sexes. However, male rat platelets were less sensitive to thrombin than female platelets (EC_{50} 1.3 $\pm$ 0.1 and 1.1 $\pm$ 0.1 u/ml respectively, $P<0.05$) and more sensitive to inhibition by prostacyclin (IC_{50} 2.05 $\pm$ 0.1 and 3.0 $\pm$ 0.1 ng/ml respectively, $P<0.01$).

Female rats were pretreated for 6 weeks with ethynyl oestradiol (EO) or norethindrone acetate (NA) or EO + NA released from silastic capsules implanted under the dorsal skin. The capsules, 9 x 25 mm, contained 30-50 mg crystalline steriod (Henderson, Baker and Fink, 1977). Control rats received empty capsules.

Thrombus deposition was reduced by 78 $\pm$ 12% ($P<0.01$) following treatment with EO. NA had no effect either alone or on the inhibition produced by EO. The APTT, PT and fibrinogen levels were unchanged following treatment whilst the RVVT was shortened in rats treated with EO or EO + NA. Treatment of female rats with 17β-oestradiol (17βO) did not alter platelet sensitivity to ADP, thrombin or PGI_2, however platelet sensitivity to AA was somewhat lower and to collagen it was significantly reduced (EC_{50} control 0.7 $\pm$ 0.07; 17 O 1.4 $\pm$ 0.2 ug/ml, $P<0.01$). The reduction in platelet sensitivity to collagen was accompanied by a reduction in the collagen-induced release of ADP from platelet granules.

In summary, this model of thrombosis in the rat demonstrates the postulated protective effect of oestrogens. Firstly, thrombus deposition was greater in male rats than in females, a difference which could be attributable to higher levels of fibrinogen in males. Secondly, treatment of female rats with EO reduced thrombus deposition, an effect which is unlikely to be due to changes in the coagulation system but which may be associated with changes in platelet reactivity and the platelet release reaction..

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Henderson, S.R., Baker, C. & Fink G. (1977) J. Endocrinol. 73, 441-453.
Smith, J.R. & White, A.M. (1982) Br.J.Pharmac. 77, 29-38.

EFFECT OF THE THROMBOXANE RECEPTOR ANTAGONIST EP 092 ON THE EARLY PHASE OF ENDOTOXIN SHOCK IN THE SHEEP

Roma A. Armstrong, R.L. Jones*, V. Peesapati, S.G. Will & N.H. Wilson, Department of Pharmacology, University of Edinburgh, Edinburgh, EH8 9JZ

Injection of *E. coli* endotoxin into the sheep produces marked changes in lung function: these include vasoconstriction, increased vascular permeability and impaired gas exchange. Thromboxane A_2 has been implicated in the early (0-1.5 h) phase of the shock syndrome (Demling et al, 1981; Smith et al, 1982; Watkins et al, 1982).

In this communication we report the effect of competitive blockade of thromboxane receptors on the early phase of *E. coli* endotoxin shock. EP 092, ± 5-endo-(6'-carboxyhex-2'Z-enyl)-6-exo-{1"-[N-(phenylthiocarbamoyl)-hydrazono]-ethyl}-bicyclo [2,2,1]heptane has properties akin to our previously described compound EP 045 (Jones et al, 1982). Using 11,9-epoxymethano PGH_2 as agonist, affinity constants of 8.7×10^7 on dog saphenous vein, 8.9×10^7 on guinea-pig trachea, 1.8×10^7 on rabbit aorta and 8.2×10^7 M^{-1} on washed human platelets were obtained.

Sheep were anaesthetised with pentobarbitone sodium and allowed to freely breathe 40% O_2/60% N_2. Arterial blood pressure (BP) and pulmonary artery pressure (PAP) were continuously recorded together with intermittent measurements of arterial oxygen tension (PO_2) and cardiac output (CO) (thermodilution technique). After a 1.5 h equilibration period, *E. coli* endotoxin (1 μg kg^{-1}) was injected intravenously and measurements made for a further 3 h. EP 092 was given as a loading dose, 2 mg kg^{-1}, 2 min after the endotoxin, followed by an infusion of 0.1 mg kg^{-1} min^{-1} for 60 min. Control sheep were infused with a matching volume of saline. The results are presented in Table 1. EP 092 prevented the marked rise in PAP, the fall in CO and the respiratory distress due to endotoxin. The fall in arterial PO_2 was reduced and the BP fall was approximately halved. Basal arterial plasma levels of TXB_2 (R.I.A.) were 0.3 ng/ml and rose ∿3 fold at 15 min and >10 fold at 30 min post endotoxin. This was found in both control and EP 092-treated animals.

These results support the role of TXA_2 as the major mediator of the pulmonary vascular changes seen in the early phase of *E. coli* endotoxin in the sheep.

Table 1 Effect of EP 092 on the early phase of endotoxin shock in the sheep

	Control (n=5)		EP 092 (n=5)	
	10' pre-	30' post *E.coli*	10' pre-	30' post *E. coli*
PAP (mm Hg)	16.4 ± 2.0	43.6 ± 3.1	14.0 ± 1.8	16.6 ± 3.0
		P<0.001		NS
BP (mm Hg)	142 ± 3	114 ± 9	139 ± 3	126 ± 5
		P<0.05		P<0.05
CO/kg body wt ($ml\ min^{-1}/kg$)	90 ± 9	78 ± 6	89 ± 7	87 ± 10
		P<0.05		NS
PO_2 (mm Hg)	100 ± 23	42 ± 8	112 ± 22	93 ± 17
		P<0.05		NS

Values are means ± s.e.m. Statistical comparison using paired t test, NS = not significant at P=0.05.

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Demling, R.H. et al (1981) Am.J.Physiol. 240, 348-353
Jones, R.L. et al (1982) Br.J.Pharmac. 76, 423-438
Smith, M.E. et al (1982) J.Surg.Res. 32, 283-288
Watkins, W.D. et al (1982) Prostaglandins 23, 273-285

MODIFICATION OF UTERINE RESPONSES TO OESTROGEN IN THE RAT BY THROMBOXANE RECEPTOR ANTAGONISM

Judith Senior and Kay M. Troughton*, School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

Oestrogens are known to cause an increase in uterine blood flow as well as in uterine weight. Previous work has shown that these parameters can be modified by substances which inhibit the synthesis or action of histamine, kinins and prostaglandins (Phaily & Senior, 1978a). Oestrogen may exert its effect on uterine blood flow by acting directly on vascular smooth muscle or via the oestrogen receptors present in the uterus. In the present study a thromboxane antagonist, EP 092 (±) 5-endo (6'carboxyhex-2'Z-enyl)-6-exo {1"-[N-(phenylthiocarbamoyl)-hydrazono]-ethyl}-bicyclo (2,2,1) heptane, was used to see if there was a relationship between the thromboxanes and the oestrogen response.

Mature rats were used which had previously been bilaterally ovariectomised at least 14 days prior to experimentation. Blood flow was measured in the anaesthetised rat using the microsphere (15μm NEN-Trac) technique (Phaily & Senior, 1978b). Uterine blood flows are expressed using tissue wet weights, both uterine wet and dry weights were recorded.

EP 092 (5mg kg^{-1}) I.V. in a sodium hydroxide (M/400)/sodium chloride (0.9% $^{w}/v$) vehicle) was administered 10 min before and 90 min after 17β-oestradiol (E_2) (0.5 μgkg^{-1} I.V. in propylene glycol (10% v/v)), a dose which gives a maximal uterine blood flow response 180 min later in the spayed rat. 180 min after oestradiol treatment EP 092 had suppressed the oestrogen induced increases in uterine wet and dry weight. However, the oestrogen induced blood flow in the presence of EP 092 was significantly elevated ($P < 0.001$) when compared to that induced by E_2 alone. When EP 092 was administered alone no effect was seen on any of the parameters studied, that is, organ weights or blood flow.

EP 092 has been shown to be a competitive thromboxane receptor antagonist on smooth muscle in vitro (R. L. Jones, personal communication, A. Y. Massele and J. Senior, unpublished observation). This in vivo study shows that EP 092 may be used to modify thromboxane effects in the whole animal. No adverse reactions were noted when EP 092 was administered intravenously in the rat. In the anaesthetised animal pretreated with EP 092 significant changes in blood flow and organ weights were recorded only in the oestrogen stimulated structures. These results suggest that thromboxane receptors are involved in the uterine weight increases following oestrogen treatment. Uterine blood flow responses to E_2 in the rat may be restricted by thromboxane as the thromboxane receptor antagonist increased the flow rate. It is suggested that oestrogen treatment may either stimulate thromboxane formation in the rat uterus or modify the thromboxane receptor population.

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Phaily, S. & Senior, J. (1978a). J.Pharm.Pharmac. 30, 734-735.

Phaily, S. & Senior, J. (1978b). J.Reprod.Fert. 53, 91-97.

A COMPARISON OF THE ACTIONS OF LEUKOTRIENES AND FPL 55712 ON GUINEA-PIG FUNDIC STRIP AND GUINEA-PIG ILEUM

Linda Feniuk, I. Kennedy and C.J. Whelan*, Department of Pharmacology, Glaxo Group Research Limited, Ware, Hertfordshire, U.K., SG12 ODJ.

Although leukotrienes (LT) have actions in many isolated tissue preparations the prototype LT antagonist FPL 55712 (Augstein et al., 1973) does not always act as a competitive antagonist (see Krell et al., 1981). In this paper we describe the actions of leukotrienes and FPL 55712 on guinea-pig fundic strips and compare them with those on guinea-pig ileum.

Experiments with guinea-pig isolated superfused ileum were carried out as described previously (Feniuk et al., 1982). Guinea-pig fundic strips (Kennedy et al., 1982) were mounted in 2 ml organ baths filled with oxygenated Tyrode's solution at 34°C containing atropine (10^{-6} mol/l), mepyramine (3×10^{-6} mol/l) and indomethacin (3×10^{-6} mol/l). Contractions were measured isometrically and concentration-effect curves were constructed cumulatively.

LTC_4 (10^{-10} - 10^{-5} mol/l), LTD_4 (10^{-10} - 10^{-5} mol/l) and LTE_4 (10^{-10} - 10^{-5} mol/l) caused concentration-related contractions of the fundic strip. The rank order of potency was $LTD_4 > LTC_4 \gg LTE_4$, EC_{50} for LTD_4 was 2.8×10^{-9} (95% C.L. 1.9 - 4.7 x 10^{-9}; n = 5) mol/l. LTC_4 (10^{-11} - 3×10^{-4} mol), LTD_4 (10^{-12} - 10^{-9} mol) and LTE_4 (3×10^{-11} mol) also caused dose-related contractions of the ileum. The rank order of potency was the same as that obtained on the fundus, ED_{50} for LTD_4 was 9×10^{-11} (6 - 13 x 10^{-11}; n = 16) mol. On guinea-pig fundus LT concentration-effect curves were parallel to each other and all three compounds were full agonists. In contrast on guinea-pig ileum dose-effect curves for LTC_4 and LTD_4 were parallel to each other but LTE_4 was a partial agonist being equipotent with LTC_4 at threshold concentrations but only achieving about 40% of the maximum produced by the other two leukotrienes.

LT-induced contractions of fundic strips persisted even after repeated washing of the preparation, whereas LT-induced contractions of ileum recovered within 10 min. Therefore, for experiments with fundic strips an experimental design was used in which only one LT concentration-effect curve was obtained in each preparation.

FPL 55712 (10^{-6} - 10^{-5} mol/l) antagonised LTD_4-induced contractions of both preparations. Concentration-effect curves were shifted to the right in a parallel manner by increasing concentrations of antagonist. On ileum a pA_2 value (Arunlakshana & Schild, 1959) of 7.0 (95% C.L. 6.6 - 7.5, n = 4) was obtained whereas on fundus the value was 6.5 (6.0 - 6.9, n = 4). On both preparations the slope of the Schild plot was not significantly different from unity indicating that antagonism of LTD_4 by FPL 55712 was competitive. Furthermore, antagonism of LTD_4 by FPL 55712 was specific since a concentration of 10^{-5} mol/l did not antagonise prostaglandin E_2-induced contractions of either preparation.

Since the rank order of agonist potency for the leukotrienes and the potency of FPL 55712 on guinea-pig ileum and fundic strip are similar, it is likely that the leukotriene receptors in these preparations are the same.

Arunlakshana, O. & Schild, H.O. (1959). Br. J. Pharmac. Chemother., 14, 48.
Augstein, J. et al. (1973). Nature New Biol., 245, 215
Feniuk, L. et al. (1982). J. Pharm. Pharmac., 34, 586.
Kennedy, I. et al. (1982). Prostaglandins, 24, 667
Krell, R.D. et al. (1981). Prostaglandins, 22, 387

THE RELATIONSHIP BETWEEN URINE pH AND URINARY PGE EXCRETION IN THE CONSCIOUS RAT

J.Haylor*,†, C.J.Lote & A.Thewles. †Department of Pharmacology, The University, Sheffield S10 2TN., Department of Physiology, Medical School, Birmingham B15 2TJ.

The excretion of many weak acids or bases can be modified by changes in urine pH due to altered lipid solubility and non-ionic reabsorption in the distal nephron (Milne et al 1958). PGE_2 is a weak acid synthesised in the kidney and its urinary excretion rate has been commonly used as a non-invasive index of its renal synthesis. Experiments have therefore been performed in the conscious rat to study the relationship between urine pH and the renal excretion of PGE.

Female Wistar rats (200-300g) were placed in individual metabolic cages for a 3-hour period following the administration of one of the following oral fluid loads given at 2ml/100g body weight under light ether anaesthesia; sodium chloride 0.9%, sodium bicarbonate 1.0% or ammonium chloride 1.0%. The urine collected was assayed for electrolytes, while the remainder of the sample was immediately stored at-20°C, awaiting assay for PGE. PGE was measured by radioimmunoassay, following acid extraction and silicic acid chromatography as described by Lote et al (1983). Urine flow was not significantly different when a single group of 12 rats were treated with sodium bicarbonate (26.9 $\pm$ 1.9µl/min), sodium chloride (25.6 $\pm$ 1.9 µl/min) or ammonium chloride (29.1 $\pm$ 3.0 µl/min). One week was allowed between each of the treatments given.

In the 3-hour period following treatment with sodium chloride, the urinary PGE excretion rate was 87.9 $\pm$ 20.6 pmole/3h, at a urine pH of 6.03 $\pm$ 0.12. Sodium bicarbonate increased urine pH to 7.72 $\pm$ 0.11 ($P < 0.001$) when compared to sodium chloride, and the PGE excretion rate was some 3-fold higher at 221 $\pm$ 27.7 pmole /3h ($P < 0.001$). Following ammonium chloride administration the urine pH was lower at 5.68 $\pm$ 0.2 ($P < 0.01$) than for sodium chloride, as was the PGE excretion rate at 38.5 $\pm$ 5.3 pmole/3h ($P < 0.05$). The oral fluid loads were designed to give a maximum change in urine pH together with a minimum change in other urinary characteristics. In addition, when rats were treated with sodium chloride, a wide range of urine pH was also found (5.3-6.9) which showed a positive linear correlation ($r = 0.84$ $P < 0.002$) to the urinary excretion rate of PGE.

The results indicate that urine pH may be a determinant of the urinary excretion rate of PGE and question the unqualified use of this parameter as an index of renal PGE synthesis. The experiments may also provide evidence for the passive reabsorption of PGE in the distal nephron.

Lote, C.J. et al (1983) J.Physiol. 336, 39-46.
Milne, M.D. et al (1958) Am. J. Med. 24, 709-729.

INHIBITION AND POTENTIATION OF IMMUNOLOGICAL HISTAMINE RELEASE FROM HUMAN LUNG MAST CELLS BY ADENOSINE

M.K.Church,* S.T.Holgate and P.J.Hughes. Clinical Pharmacology and Medicine 1, Southampton General Hospital, Southampton. SO9 4XY. U.K.

Adenosine (Ado) is a naturally occuring purine nucleoside formed from the cleavage of adenosine 5'-monophosphate by 5'-nucleotidase. Since in asthma Ado is a bronchoconstrictor and is released into the circulation following antigen bronchial provocation in asthma (Mann et al 1983), it may serve as an inflammatory mediator in this disease. In human lung fragments Ado has been shown to both inhibit (Schulman et al, 1983; Vardey et al, 1983) and, at low concentrations, potentiate (Peters et al, 1982) IgE-dependent histamine release (HR). To clarify adenosine's actions on human mast cells we have examined the effects of Ado and its analogues on IgE-mediated HR from mechanically human dispersed lung mast cells (HMC).

Mechanically dispersed HMC were prepared as described by Church et al (1982). Duplicate aliquots containing 10^5 mast cells in HEPES-buffered physiological saline (pH 7.4) were challenged with goat anti-human IgE in the presence or absence of nucleoside. After 15 min incubation at 37^oC the release reactions were terminated by centrifugation at 4^oC and HR measured spectrofluorimetrically.

Preincubation with Ado, 10 μM for 15 min prior to challenge with anti-IgE (1/100) inhibited HR by 28.8 ± 7.8% ($p<0.01$, $n=6$). As the preincubation time with Ado was shortened the inhibitory effect was reduced until, with simultaneous addition of Ado and anti-IgE, HR ws slightly potentiated (3.9 ± 1.3%, $p<0.05$). Addition of Ado at increasing time points after challenge potentiated HR, reaching a maximum of 19.7 ± 3.1% ($p<0.01$) when added 5 min after challenge. Both Ado-induced inhibition and potentiation of HR was dose-related over a range of 0.1 to 100 μM. The degrees of inhibition and potentiation produced by 100 μM Ado were inversely related to the log of the anti-IgE concentration ($r= -0.96$, $p<0.001$ and $r= -0.72$, $p<0.02$ respectively) but were poorly correlated with the amount of HR induced by anti-IgE in the absence of Ado ($r= -0.58$, $p>0.1$ and $r= -0.52$ $p>0.1$ respectively). That both effects of Ado were mediated by stimulation of cell surface Ado receptors was shown by finding that theophylline, 50 μM competitively antagonized, while dipyridamole, 1 μM enhanced Ado-induced inhibition and potentiation of HR.Ado, its analogues L-and D-phenylisopropyladenosine (L- and D-PIA) and 5'-N-ethylcarboxamideadenosine (NECA) all produced concentration-related inhibition and potentiation of HR. The rank order of potency was NECA>Ado>L-PIA>D-PIA suggesting an effect on A_2 receptors. Conversely the "P-site" inhibitor of adenylate cyclase, 2',5'-dideoxyadenosine, supressed HR when added 15 min before or after challenge. Both effects were abolished by dipyridamole, 1 μM, but were unaffected by 50 μM theophylline.

These results suggest, that in common with human basophils (Church et al, 1983) inhibition and potentiation of HR from HMC by Ado was mediated by an interaction with A_2 receptors. The direction and magnitude of the response is dependent on the time of Ado addition relative to immunological challenge, and upon the strength of immunological stimulation respectively.

Church, M.K. et al (1982) J. Immunol. 129, 2116.
Church, M.K. et al (1983) Br. J. Pharmac. In press.
Mann, J.S. et al (1983) Clin. Sci. 65, 22P.
Peters, S.P. et al (1982) J. Allergy Clin. Immunol. 69, 150.
Schulman, E.S. et al (1983) J. Immunol. 129, 2662.
Vardey, C.J. et al (1983) Eur. J. Pharm. in press.

INHIBITION OF HISTAMINE RELEASE FROM HUMAN BASOPHILS BY "P-SITE" INHIBITORS OF ADENYLATE CYCLASE

M.K.Church,S.T.Holgate and P.J.Hughes.* Clinical Pharmacology and Medicine 1, Southampton General Hospital, Southampton. SO9 4XY. UK

Immunological activation of human basophils and mast cells is associated with a transient activation of adenylate cyclase and subsequent increase in cellular levels of cyclic AMP which precede the onset of mediator secretion (Hughes et al, 1983; Ishizaka et al, 1983). This increase in cyclic AMP activates protein kinases which are intimately involved in the coupling of immunological activation to the secretory process (Holgate et al, 1980). In contrast, mediator release induced by the calcium ionophore A23187 (which bypasses the IgE-Fc receptor) is associated with a fall in cyclic AMP levels due to activation of Ca^{++}-dependent cyclic AMP phosphodiesterases (PDE) (Chabot et al, 1981). To clarify the role of cyclic AMP in basophil histamine secretion, we have investigated the effects of ribose-modified adenosine analogues which inhibit adenylate cyclase at its P-site, on anti-IgE- and ionophore A23187-induced histamine release (HR).

Human leucocytes, separated from venous blood (Lichtenstein and Osler, 1964), were suspended in HEPES buffered physiological saline (pH 7.4) containing 0.03% human serum albumin. Duplicate aliquots containing 10^5 basophils were challenged with either goat anti-human IgE or A23187 in the presence or absence of nucleoside. After 45 min at 37^oC the release reactions were stopped by centrifugation at 4^oC and HR measured spectrofluorimetrically.

2',5'-dideoxyadenosine (DDA) added 15 min before or after challenge with anti-IgE caused a dose-dependent inhibition of HR, the concentrations required to inhibit HR by 25% (IC) being 45 and 1455 μM respectively. The effect of DDA on A23187-induced HR differed markedly. When added 15 min before or after challenge, DDA had no effect on HR. However, simultaneous addition of DDA and A23187 produced a dose-dependent inhibition of HR, the IC_{25} being 300 μM (n=15). The inhibitory effects of DDA on anti-IgE- and A23187-induced HR were unaffected by theophylline, 50 μM, (a concentration that does not inhibit PDE but does antagonize the effects of adenosine at cell surface receptors) or homocysteine, 100 μM (a concentration which potentiates adenosine's inhibitory effects on transmethylation reactions). The purine transport inhibitor dipyridamole, 1 μM, significantly reduced the effects of DDA ($p<0.01$) suggesting that DDA was acting at an intracellular site. Furthermore 2'-deoxyadenosine (DA) and 9-β-D- arabinofuranosyl-adenosine (ARA-A) also caused dose-dependent inhibition of HR. The rank order of potency DDA>DA>ARA-A suggested that inhibition of HR by these compounds was mediated via an interaction with intracellular P-sites which inhibit adenylate cyclase activity.

These studies indicate a role for cyclic AMP in activation-secretion coupling of basophils at two levels, one associated with membrane events which occur following perturbation of IgE-Fc receptors and the other being independent of receptor activation.

Chabot, J.A. et al (1981) Life. Sci. 28, 1155.
Holgate, S.T. et al (1980) J. Immunol. 124, 2095.
Hughes, P.J. et al (1983) Biochem. Pharmacol. In press.
Ishizaka, T. et al (1983) J. Immunol. 130, 2357.
Lichtenstein, L.M. & Osler, A.G. (1964) J. Exp. Med. 120, 507.

GASTRIC DAMAGE INDUCED BY TOPICAL SALICYLATE CAN BE POTENTIATED BY ASPIRIN OR INDOMETHACIN

G. Steel & B.J.R. Whittle*, Dept. of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

Oral administration of sodium salicylate (SS) induces only a low degree of gastric erosions compared to that induced by comparable oral doses of aspirin in the rat. This has been interpreted to reflect the inability of SS to inhibit the biosynthesis of gastric mucosal prostaglandins, unlike aspirin or other non-steroid anti-inflammatory agents (Whittle et al., 1980). In addition, pretreatment with SS can prevent subsequent induction of gastric erosions by aspirin or indomethacin (Ezer et al., 1976) presumably by preventing the gastric cyclo-oxygenase inhibition by these latter agents (Ligumsky et al., 1982). In the present study, the effect of prior inhibition of prostaglandin biosynthesis on the gastric damage induced by SS has been investigated in the rat.

Sodium salicylate (100-200 mg kg^{-1}, p.o.) dissolved in distilled water was administered into the gastric lumen of fasted (18h) unanaesthetised male rats (180-220 g) in a volume of 0.1 ml/100 g body weight. To inhibit gastric cyclo-oxygenase, rats were pretreated with aspirin (100 mg kg^{-1}) or indomethacin (5 mg kg^{-1}), administered subcutaneously 1 h prior to challenge with SS. These doses have previously been demonstrated to inhibit gastric mucosal prostacyclin generation ex vivo by 75-90% (Lidbury et al., 1982). The degree of macroscopic gastric damage after 3 h was assessed in a coded fashion and expressed in terms of an erosion index.

Subcutaneous pre-treatment with aspirin (100 mg kg^{-1}), in a dose itself giving only a low erosion index after 3 h, (0.3 ± 0.1, n=12), significantly potentiated the erosion score tenfold following oral administration of SS (100 mg kg^{-1}) from 0.8 ± 0.3 (n=35) to 10 ± 2 (n=19; $P < 0.001$). This enhanced effect was not simply due to the administration of a higher total dose of salicylate, since SS (200 mg kg^{-1}, p.o.) administered alone gave only a low erosion index (2 ± 1, n=5). Furthermore, the enhanced erosion score observed with oral SS following prior cyclo-oxygenase inhibition with parenteral aspirin was comparable to that observed with aspirin (100 mg kg^{-1}) administered orally (14 ± 2, n=24). Likewise, prior subcutaneous administration of indomethacin (5 mg kg^{-1}) in a dose itself giving an erosion index of 3.6 ± 1.5 (n=12) significantly potentiated the irritancy of orally-administered SS (100 mg kg^{-1}) giving an erosion index of 10 ± 2 (n=13; $P < 0.01$).

The present findings contrast with previous studies which showed that simultaneous or prior administration of SS can protect against aspirin- or indomethacin-induced gastric lesions (Ezer et al., 1976). In the current studies following prior cyclo-oxygenase inhibition with parenteral aspirin or indomethacin, SS induced substantial gastric damage. Since SS can act locally as a topical gastric irritant and 'barrier breaker' (Davenport, 1965) these findings are comparable to the observations that the gastric damage by another topical irritant, taurocholate, is significantly potentiated by prior inhibition of gastric mucosal prostaglandin production (Lidbury et al., 1982). The present results thus support the concept of synergistic interactions between topical irritation and the inhibition of gastric cyclo-oxygenase in the pathogenesis of gastric erosions following oral administration of anti-inflammatory drugs. Furthermore, it is apparent that the full gastric damaging potential of orally-administered aspirin (which can act as both a topical irritant and cyclo-oxygenase inhibitor) can be conferred on sodium salicylate by the prior inhibition of gastric prostaglandin biosynthesis.

Davenport, H.W. (1965). Gastroenterology 49, 189-196.
Ezer, E. Palosi, E., Hajos, Gy. & Szporny, L. (1976) J. Pharm. Pharmac. 28, 655-656.
Lidbury, P., Steel, G. & Whittle, B.J.R. (1982). Br. J. Pharmac., 75, 10P.
Ligumsky, M., Hansen, D.G. & Kauffman, G.L. (1982). Gastroenterology, 83, 1043-1046.
Whittle, B.J.R., Higgs, G.A., Eakins, K.E., et al. (1980). Nature, 284, 271-273.

THE CELLULAR ELECTROPHYSIOLOGICAL EFFECTS OF NICAINOPROL ON DOG ISOLATED CARDIAC TISSUES. COMPARISON WITH REFERENCE DRUGS

G. Hamon*, N.A.T. Lusamvuku & M. Worcel, Centre de Recherches Roussel-Uclaf, BP.9, F93230 Romainville, France

Nicainoprol (RU 42 924) [8-(3-isopropylamino-2-hydroxypropoxy)-1-nicotinoyl-1,2, 3,4 tetrahydroquinoline] is a new orally active antiarrhythmic drug, very efficient against experimental arrhythmias, devoid of negative inotropic effects and possessing someβblocking properties. We have investigated its cellular electrophysiological effects on isolated dog Purkinje fibres and ventricular muscle under normal conditions. The preparations were superfused with a physiological salt solution containing 5.4 mM KCl and were stimulated at a cycle length of 800 msec. The results were analyzed after a 30 min perfusion period in the presence of either drug.

Nicainoprol exerted concentration-dependent effects on Purkinje action potentials (AP) parameters : at $3x10^{-6}$M, AP amplitude was decreased by 3.8±0.9%, Vmax by 16.5±3.4%, AP duration (measured at 90% repolarization) by 12.4±1.5%, plateau amplitude was reduced by 11.9±1.5% and effective refractory period (ERP) by 9.4±1.0% (n=10). At 10^{-5}M,AP amplitude was decreased by 10.3±2.9%, Vmax by 27.2±2.1%, AP duration by 26.3±1.5%, plateau amplitude by 27.0±2.4%, and ERP by 20.1±2.3% (n=12). Conduction time at the Purkinje fibre-ventricular muscle junction (P-V junction) was increased by 14.6±3.4% at $3x10^{-6}$M (n=6) and by 34.3±5.2% at 10^{-5}M (n=8) nicainoprol. The only parameter of ventricular AP modified was AP duration which was increased by 5.7±1.7% at $3x10^{-6}$M (n=6). All modifications were statistically significant ($p < 0.02$).

Under the same experimental conditions, lignocaine showed qualitatively similar effects, although it was much less efficient than nicainoprol in depressing Purkinje AP parameters (at 10^{-5}M, Vmax was decreased by 3.5±1.3%, AP duration by 13.3±1.7%, ERP by 10.9±2.1%, n=7) and in delaying conduction time at P-V junction (+7.1±0.4% at 10^{-5}M, n=7). On the other hand, the effects of disopyramide and propafenone on Vmax of both Purkinje and ventricular cells were similar and these effects as well as the effect on conduction time at P-V junction were comparable to those of nicainoprol. However, the ventricular AP duration and ERP were always prolonged by both disopyramide and propafenone while AP duration and ERP of Purkinje cells were increased by disopyramide but only slightly prolonged by lower concentrations of propafenone.

Thus, given its cellular electrophysiological effects, nicainoprol appears to belong to the I_B class of antiarrhythmic drugs (lignocaine-like drugs) according to Singh and Vaughan-Williams (1972), although it does not affect phase 0 of ventricular AP. On the other hand, the cellular electrophysiological effects of propafenone on dog preparations are close to those of disopyramide (I_A class type).

Singh BN and Vaughan-Williams, M (1972) Cardiovas. Res. 6, 109-119.

INHIBITION OF VASOCONSTRICTION TO CIRAZOLINE IN PITHED RATS BY NIFEDIPINE AFTER PHENOXYBENZAMINE

A. de Jonge, M.J. Mathy, M.J.M.C. Thoolen, P.B.M.W.M. Timmermans*, B. Wilffert & P.A. van Zwieten, Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands.

The imidazoline derivative cirazoline, 2-cyclopropylphenyloxymethyl-Δ2-imidazoline, has been characterized as a selective agonist of postjunctional vascular α_1-adrenoceptors in the pithed rat (Van Meel et al, 1981; Ruffolo et al, 1983). Accordingly, its log dose-vasopressor effect curve is vitually unaffected by previous treatment with yohimbine or rauwolscine (1 mg/kg), whereas prazosin (0.1 mg/kg) induces an appreciable (parallel) rightward displacement. Unlike the α_1-adrenoceptor selective imidazolidine derivatives St 587, 2-(2-chloro-5-trifluoromethylphenylimino)imidazolidine (De Jonge et al, 1981) and Sgd 101/75, 2-(2-methylindazol-4-imino)imidazolidine (Mathy et al, 1983), cirazoline causes pressor effects in pithed rats which are relatively uninfluenced by the calcium entry blocker nifedipine (up to 3 mg/kg) (Timmermans et al, 1983). This finding indicates that cirazoline is able to contract vascular smooth muscle without a major involvement of an entry of extracellular calcium. In the present communication we report on the identification of a calcium influx dependent mechanism of vasoconstriction to cirazoline after treatment with phenoxybenzamine.

All experiments were performed on male normotensive Wistar rats (200 - 250 g), anaesthetized with hexobarbitone (150 mg/kg, i.p.) which were subsequently pithed and artificially ventilated with room air. Dose response curves to cirazoline were constructed 15 min after i.a. treatment with nifedipine (0.1 - 3 mg/kg) and/or phenoxybenzamine (3 - 1000 µg/kg, i.v., -1 h). Maximal increases in diastolic pressure (mm Hg) were measured.

Phenoxybenzamine (3 - 100 µg/kg) produced virtually parallel rightward displacements of the log dose-pressor effect curve of cirazoline. These pretreatments depressed the maximum by approximately 25 mm Hg only. In pithed rats treated with 100 µg/kg of phenoxybenzamine, the pressor responses to cirazoline were now markedly sensitive to depression by nifedipine (0.1 - 1 mg/kg). Following 1 mg/kg of this calcium entry inhibitor the vasocontrictor potency of cirazoline (up to 1 mg/kg) was completely abolished. On the other hand, the previous treatment of pithed rats with nifedipine (1 mg/kg) caused a profound sensitivity of cirazoline's vasoconstriction to inhibition by phenoxybenzamine (3-100 µg/kg). Under these circumstances, 100 µg/kg of phenoxybenzamine was sufficient to completely eliminate the pressor effects to this agonist.

The findings show that after phenoxybenzamine treatment the vasoconstriction to cirazoline in pithed rats can be modified by calcium entry blockade. Furthermore, pretreatment with nifedipine induces an enhanced effectiveness of phenoxybenzamine with respect to its inhibition of cirazoline's pressor effects. These data suggest that phenoxybenzamine differentially affects calcium influx dependent and independent mechanisms of vasoconstriction thereby exhibiting a preference for the latter process. In addition, cirazoline is able to initiate both mechanisms of vasoconstriction.

De Jonge, A. et al (1981) Life Sci. 28, 2009
Mathy, M.J. et al (1983) Br.J.Pharmac. in press
Van Meel , J.C.A. et al (1981) J.Pharmac.Exp.Ther. 219, 760
Ruffolo, R.R., Jr. et al (1983) Naunyn-Schmiedeberg's Arch.Pharmac. 322, 93
Timmermans, P.B.M.W.M. et al (1983) Naunyn-Schmiedeberg's Arch.Pharmac. in press

COMPARISON OF CIS AND TRANS DICLOFURIME WITH VERAPAMIL AS CALCIUM-ANTAGONISTS

M. Gittos and M. Spedding*, Centre de Recherche Merrell International 16, rue d'Ankara, 67084 Strasbourg-Cedex, France.

Diclofurime (ANP-4364) is a vasodilator which has calcium-antagonist properties (Bessin & Thuillier, 1975; Moore et al., 1981), although these have not been fully defined. We have compared the effects of the isomers of diclofurime with verapamil in order to ascertain the nature of the calcium antagonist activity (Spedding, 1982 a,b).

In K^+-depolarized taenia preparations from the guinea-pig caecum set up as previously described (Spedding, 1982a) diclofurime displaced cumulative concentration-response curves to Ca^{++} to the right. The apparent pA_2 for the trans isomer was 8.3±0.2 (slope of Schild plot, -0.909±0.123, n=7) whereas the cis isomer was less active (6.6±0.1, slope -0.972±0.118, n=4). Verapamil has a pA_2 of 7.8±0.1 (Spedding, 1982a). Trans diclofurime (0.1 µM) relaxed established Ca^{++} (100 µM)-induced contractions rapidly (< 10 min), indicating rapid onset of the inhibitory effects, which also occurs with verapamil (Spedding, 1982a).

Guinea-pig left atria preparations were set up in Tyrode solution at 31°C and stimulated electrically (for 3 msec at 0.2-2 Hz with threshold voltage). Trans-diclofurime and verapamil (0.1-10 µM) were equipotent inhibitors of high frequency (2Hz) stimulation but did not inhibit responses at 0.2 Hz indicating use dependent inhibitory effects. The cis isomer was 10-fold less effective. The inhibitory effects of all the drugs were reversed by increasing the Ca^{++} in the bathing fluid to 6 mM. Both trans-diclofurime (1-10 µM) and quinidine (1-10 µM) reduced maximum following frequency (MFF) of the atria preparations and this effect was not reversed by Ca^{++} (6 mM). Verapamil reduced MFF by < 20% at 10 µM. Cis-diclofurime was 2-3-fold less potent than the trans isomer but was more potent than verapamil.

In pithed rat preparations infused with angiotensin II (Spedding, 1982b) trans diclofurime (0.1-10 µmol/kg i.v.) and verapamil had equivalent hypotensive activity but diclofurime caused a greater increase of PP intervals. Trans-diclofurime (10 µmol/kg, i.v.) caused a remarkable, long-lasting and atropine-resistant bradycardia due to a combination of factors. The PP and PR intervals were prolonged (as were the QRS and RT intervals) and in some rats the P wave was inhibited and/or 2°AV block developed. The hypotensive effects of cis-diclofurime in this model occurred at 10-fold higher doses (1-30 µmol/kg) but the cis isomer was only 2-3 fold less potent than the trans isomer in prolonging PP intervals.

Thus diclofurime resembles verapamil in its actions in smooth muscle and in the heart but may have some additional membrane stabilizing properties, the predominance of which vary with the isomer.

Bessin, P. & Thuillier, J. (1975) C.R. Hebd. Seances Acad. Sci. Ser. D.281, 463-466.
Moore, N. et al (1981) J. Pharmac. (Paris), 12, 331.
Spedding, M. (1982a) Naunyn-Schmiedeberg's Arch. Pharmac., 318, 234-240.
Spedding, M. (1982b) J. Cardiovasc. Pharmac. 4, 973-979.

LOSS OF RESPONSE TO POSITIVE INOTROPIC DRUGS DURING HIBERNATION IN GROUND SQUIRRELS

W.F. Dryden, Department of Pharmacology, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada

During hibernation, the normally sensitive myocardium of Richardson's ground squirrel, responds poorly or not at all to cardiac glycosides or noradrenaline (Charnock, Dryden, Skoog & Lauzon, 1980a; Charnock, Dryden & Marshall, 1983).

This loss of response has been associated with a fall in ^{3}H-ouabain binding sites and myocardial ($Na^{+}+K^{+}$) ATPase activity (Charnock, Simonson, and Dryden, 1980b) and with changes in the staircase phenomenon and calcium sensitivity. It was not known whether the loss of positive inotropic response was associated only with the two membrane receptors, or if it was a general phenomenon based on a common mechanism of inotropy. The present study extends the observation to a group of inotropic agents thought to act by different mechanisms from cardiac glycosides.

Wild adult ground squirrels were trapped in May and kept singly in cages until used. Summer animals were sacrificed in the month of June, after 1 year in captivity. Hibernating animals were used between December and February, seven to nine months after capture. Left atria and papillary muscles were suspended in Krebs Henseleit solution (3mM Ca^{++}) at 37°C and stimulated at 1Hz with a supra-maximal pulse 5 ms in duration. Tension was measured isometrically using Dynamometer UFI force transducers and recorded on a Grass model 7 polygraph. Tissue resting tension was set to produce 70% maximum twitch tension. Drugs were added to the bath cumulatively and the response measured once the twitch height reached a new equilibrium. The responses to calcium were as previously reported. Tissues from summer animals responded well to dobutamine ($ED_{50}=5x10^{-6}M$) Anthopleurin-A. ($ED_{50}=5x10^{-9}M$) and theophylline ($ED_{50}=1.6x10^{-5}M$). In each case the response of the atria was greater in magnitude than the response of the papillary muscles. Tissues from animals in hibernation, however manifested no increase in twitch tension over the same range of drug concentrations.

The concentration-effect curves obtained for the negative inotropic drugs, quinidine, verapamil and nifedipine were the same from both summer and hibernating animals.

The results indicate that loss of positive inotropic response is not unique to either the adrenoceptor or cardiac glycoside "receptor" in hearts from hibernating animals, and that the physiological change most probably occurs at a more fundamental level than membrane receptors. The equi-effectiveness at different seasons of the cardiac suppressant drugs would suggest dependence on particular calcium movements for the basal muscle twitch at all times, but the loss of a positive inotropic response may indicate the loss of a separate source of calcium during the hibernating period.

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Charnock et al (1980a) Comp. Biochem. Physiol. 65B, 675-680.
Charnock et al (1980b) Comp. Biochem. Physiol. 65B, 681-686.
Charnock et al (1983) Br. J. Pharmac 78, 151-158.

BINDING OF (^{125}I)IBE2254 TO α_1-ADRENOCEPTORS (α_1R) AND α_1R ACTIVATED PHOSPHATIDYLINOSITOL TURNOVER IN INTACT DDT$_1$ CELLS

D. Hoyer*[a], E.E. Reynolds and B.B. Wolfe (Introduced by B.P. Richardson). Department of Pharmacology, University of Pennsylvania Medical School, Philadelphia/PA 19104. [a]Present address: Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland.

DDT$_1$ is a cell line derived from vas deferens of a syrian hamster that possesses androgen, glucocorticoid (Norris and Kohler, 1977) α_1-adrenergic (α_1R)(Cornett and Norris, 1982) and β-adrenoceptors (βR)(Garner et al., 1982). The βR are linked to adenylate cyclase. Interactions of agonists with α_1R have been correlated with calcium fluxes and/or phosphatidylinositol (PI) turnover in many systems. We have investigated the breakdown of PI into Inositol-1-phosphate (IlP) associated with the activation of α_1R in intact DDT$_1$ cells. The cells were preloaded with [^{3}H]inositol which is incorporated into PI and the accumulation of [^{3}H] IlP was measured in the presence of LiCl which blocks the IlP phosphatase. DDT$_1$ cells were incubated in the presence of noradrenaline, EC_{50}=0.6 µM, or phenylephrine, EC_{50}=6.0 µM. The increase in IlP accumulation was blocked by prazosin, IC_{50}=0.3nM, but not by yohimbine below 0.1 µM. The increase in IlP accumulation was linear for up to 72 minutes, with no sign of desensitization, and could represent up to 235 times the basal level. We have also investigated the binding of the antagonist [^{125}I]IBE2254 (IBE)(Engel and Hoyer, 1981) to α_1R in intact cells. The binding of IBE was rapid, reversible and saturable. K_D=110±13 pM, Bmax=113000±13000 receptors/cell (R/C) n=25. Both saturation and competition experiments indicated a single class of binding sites with the following pK_i-values, characteristic for α_1R: prazosin 9.9, phentolamine 7.5, yohimbine 6.4 noradrenaline 5.7, phenylephrine 4.7. These values are in good agreement with IC_{50} or EC_{50}-values for PI breakdown. The quality of specific binding and the density of α_1R/cell were inversely related to the density of cells in the growth medium. Under low cell density conditions < 40'000 cells/ml, the number of α_1R could be as high as 220'000 R/C, whereas under high cell density conditions, > 300'000 cells/ml, the number of α_1R could be as low as 30'000 R/C. The accumulation of IlP was also dependent on the cell density, and was 2.5 to 3 times higher in low density as compared to high density conditions. βR on intact DDT1 cells were studied using the antagonist [^{125}I]Iodopindolol (IPIN). The binding of IPIN was saturable with a K_D of 64±7 pM and a Bmax of 8160±1270 R/C, n=12. As for α_1R, the density of βR was inversely correlated with the density of cells. The Bmax varied between 4'000 and 16'000 R/C in high and low cell density conditions. In conclusion, binding to βR and α_1R in intact DDT$_1$ cells is easy to perform with IPIN and IBE, however, the quality of the binding and the density of both receptor types are inversely related to the density of cells in the growth medium. Furthermore, stimulation of α_1R leads to a large increase in PI breakdown which varies according to the density of α_1R/cell and to the growth conditions. DDT$_1$ cells provide an elegant system for studying the simultaneous regulation of both α_1R and βR and related biochemical events (PI turnover, calcium movements and adenylate cyclase) on the same intact smooth muscle cells, as well as the regulation of these receptors by other hormones (androgens or corticoids).

Cornett, L.E. and Norris, J.S. (1982) J.Biol.Chem. 257, 694-697
Engel, G. and Hoyer, D. (1981) Eur.J.Pharmacol. 73, 221-224
Garner, J. et al. (1982) Fed.Proc. 41, 1511
Norris, J.S. and Kohler P.O. (1977) Endocrinology 100, 613-618
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FACTORS INVOLVED IN BP RECOVERY FOLLOWING α-ADRENOCEPTOR ANTAGONISM: STUDIES IN LONG EVANS AND BRATTLEBORO RATS

T. Bennett, S.M. Gardiner & M.J. Winn*, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH

There is current interest in the role of angiotensin II and vasopressin in limiting the hypotensive effect of α-adrenoceptor antagonists (Waeber et al, 1983). In the present study we measured BP responses to the α_1-antagonist prazosin (1 mg/kg i.v.) followed by the α_2-antagonist RX781094 (Doxey et al, 1983) (0.75 mg/kg i.v.) in the presence and absence of the converting enzyme inhibitor captopril (2 mg/kg; 1 mg/kg/h), in rats congenitally unable to synthesize vasopressin (Brattleboro rats), and in the parent strain (Long Evans; LE). Rats were anaesthetized (sodium methohexitone i.p.) and the jugular vein and abdominal aorta were cannulated. Systolic and diastolic BP were recorded 4.5 - 5 h later when the rats were fully conscious. Atropine (2.5 mg/kg; 1 mg/kg/h) and propranolol (1 mg/kg; 0.5 mg/kg/h) were administered to prevent changes in heart rate. The results of the experiment are shown in the table.

Table The effects of prazosin (1 mg/kg) and RX781094 (0.75 mg/kg) on BP (mean ± s.e. mean) in Long Evans and Brattleboro rats

		Long Evans		Brattleboro	
		Control n=9	Captopril n=6	Control n=6	Captopril n=6
Basal		138±2/88±2	143±5/91±5	138±2/89±2	135±3/86±4
+Prazosin	1 min	96±4/59±3	95±4/55±2	82±3/50±3	76±6/46±5
	45 min	122±2/82±1	123±7/79±2	127±2/84±2	101±8/65±5
+RX781094	1 min	103±3/64±4	92±4/55±2	99±3/60±3	79±4/46±3
	45 min	120±2/80±1	121±2/76±3	120±3/79±2	90±5/52±5

In the absence of captopril, prazosin caused a greater fall in BP in the Brattleboro rats than in the LE rats, but during the following 40 min BP returned to similar levels in the two groups. Following administration of RX781094 in the presence of prazosin, there was no difference between the two strains in the initial hypotension or recovery of BP. In the LE rats, the presence of captopril did not affect the BP change following administration of prazosin. Addition of RX781094 caused a greater fall in BP in the presence of captopril, but 40 min later there was no difference between the BP of the control and captopril-treated LE rats. In the Brattleboro rats the initial fall in BP in response to prazosin was unaffected by captopril, but BP recovery was attenuated. Subsequent administration of RX781094 caused BP to fall to a lower level in the captopril-treated Brattleboro rats than in the controls, and in these conditions there was little recovery of BP in the former group.

These results suggest that following prazosin, postjunctional α_2-adrenoceptors may contribute to the maintenance of BP. It is likely that in vasopressin-deficient rats, BP recovery following α_1- and α_2-adrenoceptor antagonism largely depends upon activation of the renin-angiotensin system since it is markedly attenuated by captopril. In LE rats, when angiotensin II production is inhibited, there is some recovery of BP following α_1- and α_2-adrenoceptor antagonism which may be due to vasopressin.

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Waeber, et al (1983) J.Pharmacol.Exp.Ther. 225(2), pp.442-446
Doxey et al (1983) Br.J.Pharmac. 78(3), pp.489-505

FORSKOLIN AND INOTROPIC ACTIVITY IN THE RAT AND GUINEA-PIG ISOLATED HEART: MODIFICATION BY THE β-ADRENOCEPTOR?

Elisabeth Buschmans*, D.J. Hearse and A.S. Manning, The Heart Research Unit, The Rayne Institute, St. Thomas' Hospital, London, SE1.

Forskolin has been reported to induce dramatic increases in the cyclic AMP content of a number of tissues by a mechanism which is independent of the beta adrenoceptor, probably involving direct stimulation of adenyl cyclase. In the heart the forskolin-induced increase in cyclic AMP has been associated with a simultaneous increase in force and rate of contraction. In order to characterize the activity of this novel inotropic agent in the rat and guinea-pig and also confirm a receptor-independent mechanism, isolated hearts from both species were exposed to various concentrations of the drug. Hearts were aerobically perfused in the Langendorff mode with Krebs Henseleit buffer containing 11 mM glucose at 37°C and pH 7.4. Intraventricular pressure was measured via an in-dwelling catheter attached to a pressure transducer, heart rate was electronically derived from the pressure trace. Cyclic AMP was measured by a protein binding assay (Brown et al. 1971). The minimum concentration of forskolin needed to induce a change in contractile performance was 2 nM in the rat and 20 nM in the guinea-pig heart. Maximal effects were obtained with 2 uM forskolin in both species. Perfusion of the guinea-pig heart with a submaximal concentration of forskolin (20 nM) increased heart rate by 7%, left ventricular pressure by 2% and cyclic AMP was increased 3-fold from 3.96 ± 0.3 to 12.4 ± 3.0 nM/g dry wt. In the rat heart the corresponding figures were 20% and 25% increase in heart rate and left ventricular pressure and a 7-fold increase in cyclic AMP (from 4.25 ± 0.2 to 30.7 ± 1.5 nM/g dry wt). In the rat heart inclusion of high concentrations of the beta-blockers propranolol (1.6 uM) or timolol (2.6 uM) caused a significant reduction (but not a complete abolition) of the forskolin-induced increase in heart rate and left ventricular pressure. Cyclic AMP content was reduced from 30.7 ± 1.5 to 5.7 ± 0.6 nM/g dry wt in the timolol series and to 4.1 ± 0.5 in the propranolol series, values which approached the forskolin-free control (4.3 ± 0.2 nM/g dry wt).

Before interpreting these surprising findings as evidence for a receptor involvement in the actions of forskolin we investigated whether the results could be explained by endogenous catecholamines - an effect which might reasonably be expected to be sensitive to beta-blockers. Rats were subjected to pre-treatment with reserpine (2.5 mg/kg i.v. 48 h before and 5.0 mg/kg i.v. 24 h before perfusion in order to deplete endogenous catecholamine stores. The results showed that when these hearts were perfused with forskolin there was again an increase in tissue cyclic AMP content and a corresponding increase in contractile function. However, inclusion of timolol now failed to reduce this contractile response or attenuate the forskolin-induced increase in cyclic AMP.

In conclusion forskolin possesses a potent ability to increase tissue cyclic AMP in the heart, however, this property appears to be influenced by endogenous catecholamines which appear capable of some form of potentiation. This latter effect is sensitive to receptor mediation. Thus any studies of receptor independent, forskolin mediated events may require depletion of endogenous catecholamines.

Brown, B.L., Albano, J.D., Ekins, R.P., Scherzi, A.M. (1971) Biochem J, 121: 561

ANTAGONIST EFFECTS OF IDAZOXAN AND YOHIMBINE AGAINST SEVERAL α_2-ADRENOCEPTOR AGONISTS IN PITHED RATS

T.L. Berridge, J.C. Doxey, A.G. Roach, Diane A. Strachan and Narinder K. Virdee. Pharmacology Department, Reckitt and Colman, Hull, HU8 7DS.

We have previously shown that yohimbine was equally effective against the α2-agonist effects of UK-14,304 in the vas deferens (pre α2), heart (pre α2) and diastolic blood pressure (DBP - post α2) in pithed rats (Doxey et al., 1982). Idazoxan (RX 781094), however, was significantly more potent than yohimbine against UK-14,304 in the vas deferens; the two antagonists being equipotent on the UK-14,304 DBP responses. We have now compared the effects of idazoxan and yohimbine against UK-14,304, guanabenz, guanoxabenz and B-HT 920, four selective α2-adrenoceptor agonists (Cambridge, 1981; Doxey et al., 1981; Van Meel et al., 1981) in three α2-adrenoceptor systems (see above).
Male Sprague-Dawley rats (300 - 350g) were pithed and BP measured from a cannulated carotid artery. Drugs were injected into a femoral vein. Cumulative i.v. doses of the agonists required to reduce a sympathetic tachycardia (90-110 beats/min; 0.1 - 0.3 Hz, 0.5 ms, 60v continuous stimulation of thoracic cord) or contractions of the vas deferens (6Hz, 50 μs, 40V for 2 s every 30 s stimulation of lumbar cord) by 50% or agonist doses increasing DBP by 50 mm Hg in non-stimulated animals (ED50 values; μg/kg) were calculated from dose-response curves obtained in groups of rats (n=5-11/group) pretreated 10 min beforehand with either i.v. saline, idazoxan or yohimbine. All rats were given atropine (1 mg/kg) and stimulated rats also received d-tubocurarine (3 mg/kg). Non-stimulated rats received propranolol (1 mg/kg).
The agonist ED50 values at the three α2-receptor sites after the various pretreatments are listed in Table 1 below.

Table 1. Agonist ED50 values after treatments. Values in small print refer to agonist dose-ratio shifts. * indicates that antagonism produced was significantly greater ($P<0.05$ anova) than that produced by the other antagonist.

AGONIST	α2-SYSTEM	AGONIST ED50 (μg/kg iv) SALINE 1ml/kg iv	IDAZOXAN 1mg/kg iv		YOHIMBINE 1mg/kg iv	
UK-14,304	POST α2- D.B.P.	4·6 ± 0·9	57·3 ± 10·7	12·4	49·7 ± 6·4	10·8
	PRE α2 - VAS DEF.	2·5 ± 0·4	235 ± 35·9	93·9*	35·0 ± 9·1	14·0
	PRE α2 - CARDIAC	1·9 ± 0·4	65·5 ± 13·3	34·5*	29·3 ± 4·0	15·4
B-HT 920	POST α2- D.B.P.	16·3 ± 1·6	133 ± 37·5	8·2	342 ± 59·6	21·0*
	PRE α2 - VAS DEF.	9·1 ± 2·2	423 ± 75·0	46·5	209 ± 26·7	23·0
	PRE α2 - CARDIAC	2·4 ± 0·4	128 ± 51·5	53·3	234 ± 80·5	97·5
GUANABENZ	POST α2- D.B.P.	13·4 ± 1·3	173 ± 52·0	12·9	94·1 ± 26·6	7·0
	PRE α2 - VAS DEF.	17·6 ± 2·3	941 ± 151	53·5*	108 ± 15·2	6·1
	PRE α2 - CARDIAC	3·3 ± 0·5	203 ± 62·5	61·5	122 ± 32·9	37·0
GUANOXABENZ	POST α2- D.B.P.	101 ± 12·3	2520 ± 855	25·0	948 ± 99·2	9·4
	PRE α2 - VAS DEF.	47·2 ± 5·5	2168 ± 401	45·9*	265 ± 41·7	5·6
	PRE α2 - CARDIAC	8·5 ± 0·6	343 ± 70·3	40·3	260 ± 44·3	30·6

All agonists were significantly weaker on DBP than the two prejunctional systems except guanabenz which exhibited its weakest activity in the vas deferens. The smallest ED50 values for each agonist were found at pre α2-adrenoceptors in the rat heart. Idazoxan produced significantly greater antagonism of the agonists (except B-HT 920) than yohimbine in the vas deferens. In contrast, yohimbine was more potent than idazoxan against the DBP effects (and to a lesser extent the pre α2 cardiac effects) of B-HT 920. These results show that in vivo antagonist potencies at α2-adrenoceptors vary greatly depending upon the agonist and test system chosen. The variations in α2-agonist and antagonist potencies in these three systems may reflect differences in α2-receptors as well as differences in tissue distribution of the compounds. Tissue distribution of both agonists and antagonists should be borne in mind when determining selectivity values in vivo.

Cambridge, D. (1981). Eur. J. Pharmac., 72, 413-415.
Doxey J.C. et al. (1981). J. Auton. Pharmac., 1, 157-169.
Doxey J.C. et al. (1982). Br. J. Pharmac., 77, Proc. Suppl. 532P.
Van Meel et al. (1981). J. Pharmac. exp. Ther., 219, 760 - 767.

BORDETELLA PERTUSSIS VACCINE CONSTITUENTS CAUSE DISTURBANCE OF AUTONOMIC RECEPTOR FUNCTIONING WITHIN THE CARDIOVASCULAR SYSTEM OF THE RAT

D.J.de Wildt* and F.P.Nijkamp+. Laboratory for Pharmacology, National Institute of Public Health, P.O.Box 1, 3720 BA Bilthoven, The Netherlands; + Rudolf Magnus Institute for Pharmacology, University of Utrecht, Utrecht, The Netherlands

Whether the cardiovascular system is primarily involved in the adverse reactions e.g. shock sometimes occuring after B.pertussis vaccination has never been thoroughly investigated. Recent studies (De Wildt et al., 1983a; De Wildt et al, 1983b) demonstrate a severe disturbance of beta 2-adrenergic and cholinergic responsiveness of the cardiovascular system in rats 4 days after vaccination with either plain or combined whole cell pertussis vaccines. Based upon indirect evidence it was postulated that a heat-labile component of the vaccine which is assumed to be lymphocytosis promoting factor (LPF) rather than the heat-stable endotoxin (LPS) is responsible for the observed autonomic hyporesponsiveness.

In the present study autonomic receptor function was studied in rats 5 h and 4 days following i.v. administration of different doses of isolated and purified LPF and LPS. Blood pressure was recorded in urethane anaesthetized rats from a cannulated carotid artery. Heart rate was derived from the blood pressure trace by a cardiotachometer.

Purified LPF caused a dose dependent reduction of the fall in blood pressure elicited by the beta 2-adrenoceptor stimulant salbutamol (up to 90-100% inhibition). The negative chronotropic action induced by the muscarinic agent arecoline was also strongly reduced (up to 90% inhibition). These inhibitory effects of LPF paralleled a reduction in basal diastolic blood pressure (down to 50-60 mm Hg). However, 5 h after administration purified LPF did not affect autonomic responsiveness and blood pressure.

Pure B.pertussis LPS elicited neither vascular beta 2-adrenergic nor cardiac cholinergic blockade 4 days following vaccination with different doses of LPS. However, 5 h after pretreatment with LPS a distinct beta 2-adrenoceptor blockade was measured. In contrast to the LPF effect this beta 2-lytic effect was not accompanied by a cholinergic blockade. Furthermore, at that time diastolic blood pressure of the LPS treated animals was significantly higher compared to the control group (107 $\pm$ 8 mm Hg versus 80 $\pm$ 8 mm Hg).

Recently a new and possibly safer pertussis vaccine has been developed in Japan according to a method of Sato. In contrast to a classical whole-cell vaccine we did not find any autonomic impairment 4 days using this vaccine. Since the purified vaccine contains neglectable amounts of LPS and consists mainly of detoxified LPF, these results substantiate the conclusion that LPF represents the pharmacological active component with respect to autonomic blockade within the circulatory system of rats.

In conclusion, LPF by interfering with the functioning of the autonomic nervous system may contribute to systemic adverse reactions like shock and convulsions which may occur after immunization of infants with B.pertussis vaccine. However, a short-term effect of LPS e.g. beta 2-adrenergic hyporesponsiveness cannot be excluded as an additional factor in the clinical manifestation of these effects.

De Wildt, D.J., Kreeftenberg, J.G. and Nijkamp, F.P. (1983a). Eur. J. Pharmacol. 86: 315

De Wildt, D.J., Kreeftenberg, J.G. and Nijkamp, F.P. (1983b). Inf. and Imm. 41:

CHLOROQUINE-DIGOXIN INTERACTION

P.F. D'Arcy, J.C. McElnay* and A.M. Sidahmed, Department of Pharmacy, The Queen's University of Belfast, Belfast BT9 7BL, Northern Ireland.

The interaction between digoxin and quinidine is now well recognised (Doering, 1979). Since the interaction was discovered many other drugs have also been shown to interact with digoxin e.g. amiodarone (Moysey et al., 1981), nifedipine (Belz et al., 1981), quinine (Aronson et al., 1981), verapamil (Pedersen et al., 1981) and more recently hydroxychloroquine (Leden, 1982). The chemical similarity between quinidine, hydroxychloroquine and chloroquine prompted the presently reported study which examined the effect of chloroquine on steady state digoxin serum concentrations in the dog. The clinical relevance of such an interaction is of particular importance in malaria endemic countries of the 'Third World' where digoxin is frequently used and where the malaria attack is often treated with chloroquine.

The present study was carried out in six dogs since this species appears to be a good predictive animal model of digoxin-drug interaction, for example, Wilkerson et al. (1980) showed that ibuprofen interacted with digoxin in the dog, and this prediction of interaction was subsequently confirmed in man (Quattrocchi et al., 1983).

The dogs (weight approximately 20kg) were given an intravenous loading dose of digoxin (25 $\mu g\ kg^{-1}$) on day 1 and then dosed orally each morning thereafter (14 $\mu g\ kg^{-1}$). This dosage regimen gave rise to serum levels equivalent to those used therapeutically in man. After eight days digoxin treatment (ensuring steady state had been reached) the dogs were given oral chloroquine at a dosage of one-third the quantity of the normal adult treatment regimen. Each dog received chloroquine phosphate 333 mg stat; 167 mg after 6 hours and then 167 mg each morning for two days. A series of venous blood samples were taken from each dog while receiving digoxin alone and while receiving the digoxin/chloroquine combination. Serum digoxin concentrations were estimated using radioimmunoassay and chloroquine was shown not to interfere with the assay technique.

Our initial results show that on the third day of combined therapy, chloroquine significantly ($P < 0.05$) increased mean digoxin peak serum concentrations by over 75% while the AUC values (calculated by the trapezoidal rule over a 24 hour dosage interval) were also increased to a significant extent ($P < 0.05$) by approximately 20%. Although this interaction has yet to be confirmed in patients it would seem prudent to monitor digoxin therapy carefully during concomitant therapeutic treatment of a malaria attack with chloroquine. High peak serum digoxin concentrations would certainly present an additional toxic hazard to an already debilitated patient suffering from malaria.

Aronson, J.K. and Carver, J.G. (1981) Lancet 1, 1418.
Belz, G.G. et al (1981) Lancet 1, 844-845.
Doering, W. (1979) N Engl J Med 301, 400-404.
Leden, I. (1982) Acta Med Scand 211, 411-412.
Moysey, J.O. et al (1981) Br Med J 282, 272.
Pedersen, K.E. et al (1981) Clin Pharmacol Ther 30, 311-316.
Quattrocchi, F.P. et al (1983) Drug Intell Clin Pharm 17, 286-288.
Wilkerson, R.D. et al (1980) Am J Cardiol 45, 1201-1210.

CHROMATOGRAPHIC RESOLUTION AND STEREO-SELECTIVE DISPOSITION OF ACEBUTOLOL AND DIACETOLOL IN DOG AND MAN

A.R. Boobis & A.A. Gulaid*[1], Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W.12 OHS and [1]Biopharmaceutical Research, May & Baker Ltd., Dagenham, Essex, RM10 7XS.

Acebutolol [1-(2-Acetyl-4-butyramidophenoxy)-2-hydroxy-3-isopropylaminopropane hydrochloride] is an important beta-adrenoceptor antagonist. Currently the commercially available preparation is a racemic mixture. The S-(-)-enantiomers of this class of drug generally exhibit greater cardiac beta-adrenoceptor blocking activity, while R-(+)-enantiomers have an important membrane stabilizing effect. A chiral reagent, R-(+)-or S-(-)-1-phenylethylisocyanate (Thompson et al., 1982) was found chemically quite stable. This reagent has been used for converting racemic acebutolol and diacetolol to diastereomeric derivatives which can be resolved chromatographically. This paper describes an h.p.l.c. method which can be used to quantitate separately S-(-)-and R-(+) acebutolol, and S-(-)- and R-(+)-diacetolol in dog and human plasma. The acetubolol and diacetolol enantiomers were separated from each other by formation of their diastereometic derivatives with R-(+)- or S-(-)-1-phenylethylisocyanate Figure 1. The assay procedure was Linear for each diastereoismer up to 1.5 µg/ml plasma with correlation coefficient circa 0.999. The limit of detection was about 0.05 µg/ml.The method was applied to the determination of acebutolol and diacetolol isomers in plasma obtained after oral administration to dogs and man.

1. J.A. Thompson et al. (1982), J. Chromatogr., 238 470.

Peak Identification

1. Solvent front
2. S-(-)-diacetolol derivatized with R-(+)-1-phenylethylisocyanate.
3. R-(+)-diacetolol derivatized with R-(+)-1-phenylethylisocyanate.
4. S-(-)-acebutolol derivatized with R-(+)-1-phenylethylisocyanate.
5. R-(+)-acebutolol derivatized with R-(+)-1-phenylethylisocyanate

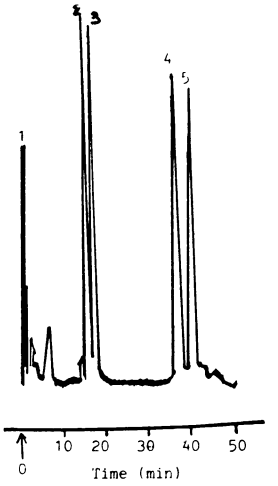

Figure 1

HPLC chromatogram of S-(-) and and R-(+)-diacetolol and S-(-) and R-(+)-acebutolol derivatized with R-(+)-1-phenylethylisocyanate. The column is spherisorb5 µm C_{18} (250 x mm x 4.6 mm id). The solvent is methanol-Water-Triethylamine (50:50 : 0.05v/v). The detector is Perkin-Elmer 3000 (excitation 238 nm, emission 470 nm).

HISTAMINE SECRETION FROM MAST CELLS STIMULATED WITH NERVE GROWTH FACTOR

K.E. Barrett, F.L. Pearce & H. Thompson*, Department of Chemistry, University College London, 20 Gordon Street, London, WC1H OAJ

Nerve growth factor (NGF) is the generic name given to a group of proteins which stimulate the growth and development of sympathetic and embryonic sensory nerve cells. The factor is normally characterized by its ability to promote the proliferation of nerve fibres from explanted peripheral ganglia in tissue culture or to evoke hypertrophy of these structures *in vivo*. However, recent studies have indicated that the molecule has additional activities and may also induce the release of histamine from rat peritoneal mast cells (Bruni et al, 1982). The present investigation is designed to confirm and extend the latter observation.

NGF was purified from the submaxillary salivary gland of the adult male mouse (Chapman et al, 1979) and from the venoms of the poisonous snakes *Vipera russelli* and *Ancistrodon rhodostoma* (Pearce et al, 1972; Bailey et al, 1975). Histamine release was determined as previously reported (Atkinson et al, 1979).

In the presence of phosphatidylserine or its lyso-derivative, NGF (1-1000 ng/ml) produced a dose-dependent release of histamine ($\leqslant$ 80%) from isolated rat peritoneal mast cells. Similar results were obtained with the factors from all three sources. The process was almost totally dependent on the presence of the added lipid and showed a strict requirement for extracellular calcium ions. Lyso-phosphatidyl-serine was a more effective adjuvant than the parent phospholipid. Strontium could substitute for calcium but a ten-fold higher concentration of the ion was required for maximal release. The response was in this case also less dependent on phosphatidylserine and a significant effect was observed in the absence of the lipid. The secretion was non-cytotoxic, being inhibited by extremes of temperature (25 ^{o}C and 45 ^{o}C), metabolic poisons or the omission of glucose from the incubation medium. The release process was relatively slow, requiring 5-10 min for completion and having a half-life of *ca* 2 min. Secretion was maximal at physiological pH. The response was unaffected by high concentrations of glucose and low molecular weight dextran polymers, which act as specific antagonists of histamine release induced by clinical dextran, and by benzyldimethyltetra-decylammonium chloride, which prevents secretion induced by compound 48/80 and other polybasic histamine liberators. The receptor for NGF is thus presumably distinct from those of these agents. The release was also unaffected by soluble, myeloma rat IgE. The process is then apparently not mediated by cell-fixed reaginic antibody. Peritoneal mast cells of the hamster were much less responsive than those of the rat whereas mouse peritoneal cells and enzymically dispersed tissue mast cells of the guinea pig were essentially refractory to the protein. These results further emphasize the functional heterogeneity of mast cells from different sources.

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Atkinson, G. et al (1979) Br.J.Pharmac. 65, 395-402
Bailey, G.S. et al (1975) Comp.Biochem.Physiol. 51B, 429-438
Bruni, A. et al (1982) FEBS Letts 138, 190-192
Chapman, C.A. et al (1979) FEBS Letts 105, 341-344
Pearce, F.L. et al (1972) Eur.J.Biochem. 29, 417-425

EFFECT OF HISTAMINE ON BRADYCARDIA INDUCED BY ACETYLCHOLINE AND VAGAL STIMULATION IN THE GUINEA-PIG

I. Marshall & P.M. Roberts*, Department of Pharmacology and Therapeutics, The Middlesex Hospital Medical School, London, W1P 7PN

Histamine attenuates bradycardia caused by electrical stimulation of the right vagus in anaesthetized dogs, while having no effect on bradycardia induced by exogenous acetylcholine (Nandiwada et al, 1980). These results imply that histamine may act presynaptically to modify bradycardia caused by vagal stimulation. In the present study we have examined the effects of histamine on the bradycardia induced by acetylcholine and vagus nerve stimulation using the guinea-pig isolated right atrium.

Right atria with intact vagus innervation were removed from male guinea-pigs. Acetylcholine caused a dose related bradycardia (IC_{50} 1.0 ± 0.4 μM, n=6) which was inhibited by the muscarinic receptor antagonist atropine, 10 nM (log K_B 9.1 ± 0.3 mean ± s.e.mean, n=4). Stimulation of the vagi (square wave pulses, 0.5 ms duration, 1-50 Hz, supramaximal voltage) caused a frequency related bradycardia. Stimulation of the right vagus (50% reduction of resting rate, 6.0 ± 0.7 Hz, n=25) was more effective than was stimulation of the left (50% reduction, 16.6 ± 0.5 Hz, n=22). Responses were reproducible, and abolished by atropine, 0.3 μM.

Histamine, 0.1-10 μM, and the selective histamine H_2-receptor agonist dimaprit, 0.3-30 μM, caused a dose related tachycardia. The effects of histamine were inhibited by the histamine H_2-receptor antagonist cimetidine, 10 μM, (log K_B 6.2 ± 0.1, n=6). The effects of histamine, 0.1-10 μM, and dimaprit, 0.3-30 μM, were studied on the response to acetylcholine, 1 μM, and to right vagal nerve stimulation, 5 Hz.

Histamine, 3 and 10 μM, significantly reduced responses to acetylcholine, 1 μM ($P<0.05$, n=5). This inhibition was not affected by the selective histamine H_1-receptor antagonist mepyramine, 0.3 μM. Dimaprit, 3-30 μM also significantly reduced responses to acetylcholine, 1 μM ($P<0.05$, n=6). Histamine, 10 μM, and dimaprit, 30 μM, reduced responses to acetylcholine 1 μM by 82.9 ± 6.6% and 90.1 ± 1.2% respectively. Histamine 0.1-3 μM had no effect on the response to vagal stimulation, 5 Hz, although histamine, 10 μM, reduced the responses by 24.5 ± 11.1% ($P<0.05$, n=22). To determine whether the overall lack of effect might be the result of mixed excitatory and inhibitory actions, the effects of histamine were studied on the response to vagal stimulation in the presence of mepyramine, 0.3 μM, or cimetidine, 100 μM. Neither antagonist had any effect on the response to vagal stimulation, 5 Hz. In the presence of mepyramine, 0.3 μM (n=8) or cimetidine, 100 μM (n=6) histamine 0.1-10 μM and 0.1-1000 μM respectively had no effect on the bradycardia caused by vagal stimulation, 5 Hz. Dimaprit, 0.3-30 μM also had no effect on the response to vagal stimulation, 5 Hz.

In conclusion, histamine had a positive chronotropic effect on guinea-pig right atrium which was mediated by histamine H_2-receptors. Histamine and dimaprit inhibited (>80%) the bradycardia caused by acetylcholine. The effect does not involve histamine H_1-receptors, and is probably mediated by histamine H_2-receptors. In contrast to the inhibitory action against acetylcholine, histamine and dimaprit had no effect on bradycardia caused by vagal stimulation. These effects of histamine are different from those seen on the anaesthetized dog heart.

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Nandiwada, P.A. et al (1980) Eur.J.Pharmacol. 63, 281-286.

LYMPHOCYTES, INFLAMMATION AND FIBRIN IN A RAT CARDIAC ALLOGRAFT REJECTION MODEL

Gillian R. Bullock, S.E. Christmas[+*], M.K. Jasani, M.I.V. Jayson[+], B.H. Kemmenoe, P.D. Sibbons. Research Centre, CIBA-GEIGY Pharmaceuticals Division, Horsham, West Sussex, RH12 4AB, and [+]Rheumatic Diseases Centre, University of Manchester, Salford, M6 8HD.

Inflammation characterised by the appearance of extravascular (e-v) fibrin deposits and oedema has been shown to develop in association with tissue localisation of lymphocytes in rabbit skin homografts and in the foot-pads of immunised rats inoculated with M. tuberculosis (Bullock, et al., 1983). The present studies were carried out to determine the role of re-circulating T lymphocytes as compared with bone marrow derived mononuclear cells in the development of e-v fibrin deposits and oedema during rejection of rat cardiac allografts.

Allografts: hearts from AO rats were transplanted heterotopically into age-matched male DA strain recipients as previously described (Christmas and MacPherson, 1982). Syngrafts: DA hearts were similarly transplanted into age-matched DA recipients and were studied for comparison. Graft survival was monitored daily by palpation of heart beat through the flank. Formation of e-v fibrin deposits was detected microscopically and quantified using ^{125}I-human fibrinogen, B.P., 5 µCi (Amersham International, PLC) administered i.v. 24 h before removal of the graft post-mortem. ^{125}I-fibrin accumulation in the graft ventricles was measured as per cent of values found in the corresponding recipient's ventricles. Oedema was measured by calculating the percentage change in the weight of donor ventricles following transplantation.

Electron microscopically e-v fibrin deposits were found in both types of graft. Quantitatively, though, ^{125}I-fibrin accumulation in the syngrafts averaged only 159% ($\pm$ 60, s.e., n = 3) by day 7; whereas in the allografts it was 1460 $\pm$ 420% by day 5, 9710 $\pm$ 2100% by day 6 and 12920 $\pm$ 4700% by day 7 ($p < 0.001$, n = 6). Weight (mg) of syngrafts was found to decline post-transplantation by 44.2 $\pm$ 5.8%, whereas that of the comparable day 7 allografts was found to be increased by 42.3 $\pm$ 22.7%. Syngrafts were beating strongly on day 7 but most allografts had stopped beating by this time.

Effects of myelotoxic immunosuppressive drugs and depletion and adoptive transfer of mononuclear cells. A single dose of cyclophosphamide (100 mg/kg) administered i.v. on day 2 post-transplantation and once daily doses of methotrexate (2.5 mg/kg, i.v.) from day 0 were both found to preserve heart beat and to reduce both ^{125}I-fibrin accumulation and weight change in the allografts to levels found in the comparable day 7 syngrafts. Depletion of re-circulating T lymphocytes either by drainage of thoracic duct lymph or by adult thymectomy followed by whole-body irradiation (600 rads x 2) was also found to exert a similar effect on the heart beat and ^{125}I-fibrin accumulation. Oedema of ventricles was reduced in severity, but not to levels as low as in the syngrafts. Finally, repletion of the irradiated recipients with syngeneic thoracic duct lymphocytes (5×10^8 cells) administered i.v. on the day of transplantation was found to increase the ^{125}I-fibrin content of day 7 allografts to 1800 $\pm$ 270% and oedema to levels found in the comparable allografts of unmodified recipients.

The data reinforce the involvement of re-circulating T lymphocytes in the development of e-v fibrin deposits and oedema in immunological inflammation, and suggest that in their absence the radioresistant bone marrow derived monunuclear cells contribute to the formation of oedema but not fibrin deposits, nor the stoppage of heart beat.

Bullock, G.R. et al. (1983). Br. J. Pharmacol. 79, 240P.
Christmas, S.E. and MacPherson, G. (1982). Cell Immunol. 69, 248-290.

A COMPARISON OF CIMETIDINE AND RANITIDINE: EFFECT ON LIVER BLOOD FLOW

H.E. Barber, J.C. Petrie and K.J. Smith*, Department of Pharmacology and Department of Therapeutics and Clinical Pharmacology, University Medical Buildings, Foresterhill, Aberdeen. AB9 2ZD

Cimetidine (5mg kg^{-1}), given as a single intraportal dose in the pithed rat produced an increase in the area under the blood concentration versus time curve (AUC) of propranolol (200 µg kg^{-1}) given intravenously;no effect was observed on the AUC of propranolol given intraportally (Barber et al. 1983a). Ranitidine (2.5 mg kg^{-1}) given intraportally produced no significant difference in AUC of propranolol given by either route (Barber et al. 1983b). These results suggested that the cimetidine-induced increase in AUC of intravenously administered propranolol may be explained by a reduction in liver blood flow (Wilkinson and Shand, 1975).

The aim of the present study was to compare the relative effects of cimetidine and ranitidine on liver blood flow using radiolabelled microspheres (McDevitt and Nies, 1976).

Male Sprague-Dawley rats were pithed under halothane anaesthesia and ventilated with 100% oxygen (Gillespie et al., 1970). Cimetidine (5mg kg^{-1}) or ranitidine (2.5mg kg^{-1}) was infused over 30 minutes into a side branch of the hepatic portal vein to simulate oral administration. ^{57}Co microspheres were injected over 15 seconds into the left ventricle. During and 75 seconds after injection, blood was withdrawn from the right femoral artery at 1.8 ml min^{-1}. Blood flow to specific organs was calculated by comparison of the radioactivity trapped within each organ with that in the reference sample.

TABLE 1: EFFECTS OF CIMETIDINE AND RANITIDINE ON HEPATIC PORTAL AND HEPATIC ARTERIAL BLOOD FLOW.

FLOW (ml min^{-1})	Control	Cimetidine	Ranitidine
Hepatic Portal Vein (HPV)	5.86 ± 1.08	2.57 ± 0.68	3.73 ± 0.95
		p = 0.023	p = 0.122
HPV g^{-1} liver	0.46 ± 0.07	0.23 ± 0.07	0.32 ± 0.09
		p = 0.046	p = 0.196
Hepatic Artery (HA)	1.45 ± 0.39	0.42 ± 0.14	0.87 ± 0.30
		p = 0.027	p = 0.189
HA g^{-1} liver	0.11 ± 0.03	0.04 ± 0.01	0.07 ± 0.03
(n=6 for all groups)		p = 0.040	p = 0.259

Bonferroni tests of simultaneous comparisons of all pairs of means. To be significant at the 0.05 level the p value must be less than 0.017 (BMDP-81, 1981).

Simultaneous comparisons of all pairs of means showed that not even the absolute flow rates reached significance at the 0.05 level. Nevertheless examination of the means may indicate that cimetidine reduces both hepatic portal and hepatic arterial flow to a greater extent than ranitidine. Certainly the magnitude of reduction in blood flow correlates closely with the previously reported increase in AUC of intravenously administered propranolol in the presence of cimetidine.

Barber, H.E. et al., (1983a) Br. J. Pharmac. 78 (Supp) 59P
Barber, H.E. et al., (1983b) Proc. 2nd World Conf. Clin. Pharmac. Ther. (in press)
Gillespie, J.S. et al., (1970) Br. J. Pharmac., 40, 257-267.
McDevitt, D.G. and Nies, A.S. (1976) Cardiovasc. Res., 10, 494-498.
Wilkinson, G.R. and Shand, D.G., (1975) Clin. Pharmac. Ther., 18, 377-390.
BMDP-81 (1981) Biomedical Computer Programs. University of California, Berkely USA

ENHANCEMENT OF VOLTAGE-SENSITIVE CALCIUM CHANNELS BY A NOVEL DIHYDROPYRIDINE

S.B. Freedman* and R.J. Miller, Department of Pharmacological and Physiological Sciences, Univ. of Chicago 947 E. 58th Street, Chicago, IL 60637.

We have previously shown (Freedman et al, 1982) that several neuronal cell lines possess voltage sensitive calcium channels (VSCC). There are particularly evident when cells are grown in the presence of agents that increase intracellular levels of cyclic AMP. A striking feature of these VSCC is that they are blocked low concentrations of organic calcium channel blockers. In particular, dihydropyridines such as nifedipine and nitrendipine are extremely potent with IC_{50}'s in the nM range. In the present experiments, we demonstrate that a novel dihydropyridine, CGP 28392, [4,2-(Difluoromethoxy)phenyl-1,4,5,7-tetrahydro-2-methyl-5-oxo-furo-3,4-pyridine-3-carboxylic acid ethyl ester] enhances rather than inhibits the movement of calcium through VSCC.

NG108-15 cells were grown on 60 mm Falcon plastic dishes in EMEM supplemented with 10% FBS with 10 µM PGE_1 and 50 µM IBMX for periods between 6-8 days. Cells were depolarized by the addition of medium containing 25 or 50 mM KCl or 50 µM veratridine. $^{45}Ca^{2+}$ (1 µCi/ml) was added at the start of the depolarizing stimulus. Assays were allowed to continue at 37°C for various times and were terminated by aspiration of the medium. Cells were washed four times in 175 mM choline chloride, 2 mM EGTA. Cells were then extracted in 0.2% SDS and radioactivity and protein determined.

At a concentration of 1 µM CGP 28392 sightly enhanced the uptake of $^{45}Ca^{2+}$ in low (5 mM) K^+ medium. When added to high (50 mM) K^+ medium, the drug enhanced Ca^{2+} uptake 2-3 fold resulting in a total 4-6 fold increase in the uptake over low K^+ conditions. The effect of CGP 28392 (EC_{50} 150 nM) was apparent at concentrations as low as 30 nM and maximal effects were observed at a concentration of 1 µM. 1 µM CGP 28392 also enhanced the effects of a submaximally depolarizing concentration of K^+ (25 mM). The stimulatory effects of other depolarizing agents (50 µM veratridine) were also enhanced by CGP 28392. The effects of CGP 28392 were inhibited by the organic calcium blockers nitrendipine (IC_{50} 16 nM) and D-600 (IC_{50} 310 nM) The divalent cations Cd^{2+} and Co^{2+} also blocked with approximate IC_{50}'s of 14 µM and 789 µM respectively.

These results indicate that CGP 28392 as well as the recently reported Bay K8644 (Schramm et al, 1983) represents a new class of dihydropyridine able to enhance Ca2+ flow through VSCC acting at the same site at which other dihydropyridines block these channels.

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Freedman, S.B. et al (1983) Br.J.Pharmac. 78, 80P
Schramm, M. et al (1983) Nature 303, 535-537

BINDING OF (^{3}H)-YOHIMBINE IN RAT CEREBRAL CORTEX

A.D. Michel and R.L. Whiting, Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

The selective α_2-adrenoceptor antagonist, yohimbine (Starke et al, 1975) has recently been introduced as a ligand for direct binding studies (Motulsky et al, 1980; Cheung et al, 1982). The present study describes the binding of [^{3}H]-yohimbine in rat cerebral cortex.

Saturation binding assays were performed at 25^{0}C using a Tris-EDTA-ascorbate buffer (Tris 50 mM, EDTA 0.5 mM, ascorbate 0.5 mM; pH 7.8 at 25^{0}C). [^{3}H]-yohimbine (0.5 - 50 nM) and an EDTA washed cerebrocortical membrane preparation (250 - 400 µg membrane protein) were incubated to equilibrium (30 min) and bound ligand separated from free by vacuum filtration (2.04 x 10^4 Pa) over Whatman GF/B filters with 3 x 5 ml washes of ice cold buffer. Total binding was determined in triplicate and non specific binding (nsb) in duplicate. Data were analysed using the iterative non-linear curve fitting programme LIGAND (Munson & Rodbard, 1980).

In preliminary competition experiments using 3 nM [^{3}H]-yohimbine, noradrenaline at concentrations between 30 µM and 300 µM produced maximal inhibition of total binding and so a concentration of 300 µM was chosen for definition of specific binding in saturation experiments. Scatchard analysis of specific binding isotherms assuming a homogenous population of receptors produced a dissociation constant (Kd) of 17.2 nM and binding capacity (B_{max}) of 305 fmol.mg.protein^{-1} (n = 10).

Graphical analysis of the data revealed concave curvature of Scatchard plots and a significant improvement on the one site model was produced when specific binding isotherms were resolved into two components. The two site analysis produced a well-defined high affinity site (Kd 6.3 nM, B_{max} 90 fmol.mg.protein^{-1}) and a more variable low affinity component (Kd 90 nM, B_{max} 457 fmol.mg.protein^{-1}). The high capacity and large variability of the low affinity site indicated the possibility that it represented displacement of nsb of [^{3}H]-yohimbine by the concentration of noradrenaline used to define specific binding. This was tested by analysis of specific binding isotherms assuming the presence of a nsb component which was estimated along with the Kd and B_{max}.

With this approach the best fit to the experimental data was produced with a one site model (Kd 9.1, B_{max} 151 fmol.mg.protein^{-1}) and represented a significant improvement on the one site model assuming the absence of nsb. However, it was statistically indistinguishable from the two site model and consequently it was not possible to determine if the deviations from the single site model for [^{3}H]-yohimbine binding represented displacement of nsb or the presence of a second low affinity site. Quantitatively similar results were obtained using a lower concentration of noradrenaline (100 µM) or phentolamine (5 and 10 µM) to define specific binding of [^{3}H]-yohimbine.

The results of the present study thus confirm the presence of a high affinity binding site for [^{3}H]-yohimbine in rat cerebral cortex, but, in addition, also indicate either the presence of a second lower affinity binding component or the displacement of nsb of [^{3}H]-yohimbine by the ligand chosen to define specific binding.

Cheung, Y-D. et al (1982) Eur.J.Pharmac. 84, 79-85.
Motulsky, H.J. et al (1980) Biochem.Biophys.Res.Commun. 97, 1562-1570.
Munson, P.J. & Rodbard, D. (1980) Anal.Biochem. 107, 220-239.
Starke, K. et al (1975) Eur.J.Pharmac. 34, 385-388.