THE EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON THE BEHAVIOURAL RESPONSE TO A TRH ANALOGUE (CG 3509)

G.W. Bennett*, Celia Lighton & C.A. Marsden, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Thyrotrophin-releasing hormone (TRH) has been shown to co-exist with 5-hydroxytryptamine (5HT) in ventral spinal cord neurones and to interact with the amine in the nucleus accumbens (Bennett et al 1984). Following repeated electroconvulsive shock (ECS) (Lighton et al 1984a) or chronic antidepressant treatment (Lighton et al 1984b), levels and ex vivo release of TRH in the spinal cord and nucleus accumbens were altered. As both of these treatments affect the $5HT_2$ receptor, the results may provide further indication of a TRH-indoleamine interaction in these two regions.

In the present study, the effect of chronic amitriptyline treatment on behaviour induced by the TRH analogue CG 3509 (orotyl-L-histidyl-L-prolineamide) has been measured. Response to CG 3509 was assessed by the analogue's ability to induce hyperactivity (Sharp et al 1984a) and to reduce the duration of pentobarbitone sleeping time (Sharp et al 1984b).

Male Wistar rats were bilaterally implanted with guide cannulae into the nucleus accumbens under pentobarbitone anaesthesia (60 mg/kg i.p.).

CG 3509-induced hyperactivity: Following recovery, animals received intra-accumbens injections of CG 3509 (2x2.5 µg in 0.5 µl saline). Hyperactivity was then monitored in 15 min periods for 2 h using an Actimat doppler shift radar activity meter. 24 h later the procedure was repeated following control injections of saline.

CG 3509-reduction of pentobarbitone sleeping time: Sleeping time was measured as the duration of the loss of righting reflex (LRR) following sodium pentobarbitone (35 mg/kg i.p.). 20 mins after LRR, animals received intra-accumbens CG 3509 (2x2.5 µg in 0.5 µl saline). Response to central saline was assessed 24 h later. Rat rectal temperature and respiration rate were measured following LRR and every 10 min thereafter.

All animals then received amitriptyline (15 mg/kg, i.p., twice daily, n=9) or an equivalent volume of 0.9% saline (n=6) for 14 days. On days 13 and 14 animals were again tested for their response to central CG 3509 and saline respectively, in both behavioural models.

Chronic amitriptyline significantly reduced the hyperactivity produced by intra-accumbens CG 3509 when compared to both the pretreatment response (-21.0%; $p<0.001$) and that of the chronic saline treated group (-24.6%; $p<0.01$). Prior to antidepressant treatment, CG 3509 reduced the pentobarbitone sleep time by 60%. This was less effective (40% reduction) after amitriptyline although this change did not reach significance and the pentobarbitone sleep time was more than doubled, in treated animals tested with either CG 3509 or saline.

These results indicate that in the nucleus accumbens, the increased levels of TRH previously measured following amitriptyline are associated with a decreased responsiveness to a TRH analogue administered into the same region.

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Bennett, G.W. et al (1984) Br.J.Pharmac. 82, 267P.
Lighton, C. et al (1984a) Neuropharmacology 23, 963-966.
Lighton, C. et al (1984b) 14th C.I.N.P. Abstracts, P.134.
Sharp, T. et al (1984a) Reg.Peptides 9, 305-315.
Sharp, T. et al (1984b) Neuropharmacology 23, 339-348.

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LONG-TERM BLOCKADE OF NICOTINE CUE BY CHLORISONDAMINE IN RATS

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

Intraventricular injection of chlorisondamine, a bisquaternary ganglion-blocking drug, produces long-term blockade of nicotine-induced locomotor activity in rats (Clarke & Kumar, 1983). We have now studied whether chlorisondamine blocks nicotine in a standard, two-bar, drug discrimination procedure. Specificity of the block has been examined by tests on the discrimination of a short-acting benzodiazepine, midazolam.

Rats were trained to discriminate nicotine (0.1 mg/kg s.c.) from saline (Stolerman et al, 1984), or midazolam (0.1 mg/kg s.c.) from saline. Half of the rats in each group received an intraventricular injection of chlorisondamine chloride (5 μg/1 μl) and the remainder received isotonic saline (1 μl), under halothane anaesthesia. Extinction tests were conducted with training drug (0.2 mg/kg s.c.) or saline on two consecutive days each week, both before the operation and weekly for four weeks after operation. All doses are those of the bases.

In the tests 7-8 days after operation, chlorisondamine blocked the effects of nicotine. The mean percentage of drug-appropriate responding ($\pm$ s.e.m.) after 0.2 mg/kg nicotine was reduced to 26.1 $\pm$ 8.2% (chlorisondamine group), as compared with 90.9 $\pm$ 2.6% (saline group; $P < 0.001$). There was no significant difference in drug-appropriate responding after saline injection between the chlorisondamine group (21.8 $\pm$ 7.6%) and the saline group (11.7 $\pm$ 4.2%). In the second post-operative week, block of the nicotine discriminative stimulus was present but less pronounced. Drug-appropriate responding was 48.8 $\pm$ 10.2% after nicotine (chlorisondamine group) as compared with 89.2 $\pm$ 4.0% (saline group; $P < 0.01$). During the third and fourth post-operative weeks the block was maintained at 49.6 $\pm$ 10.4% ($P < 0.01$) and 50.2 $\pm$ 10.6% ($P < 0.01$) respectively, whereas chlorisondamine slightly increased drug-appropriate responding after saline (21.9 $\pm$ 6.7%, $P < 0.05$ and 35.2 $\pm$ 8.6%, $P < 0.01$ during the third and fourth weeks respectively). Thus, there was partial block of the response to nicotine even four weeks after injection of chlorisondamine.

Chlorisondamine had no effect on the midazolam discriminative stimulus. In the first post-operative test, drug-appropriate responding was 88.7 $\pm$ 2.9% after midazolam (chlorisondamine group) compared to 87.2 $\pm$ 4.0% after midazolam (saline group). There was no difference in drug-appropriate responding after saline between the chlorisondamine group (10.3 $\pm$ 2.5%) and saline group (8.7 $\pm$ 4.0%). Similar results were obtained in all subsequent tests.

These results show that chlorisondamine causes a long-term blockade of the nicotine cue. This is not a non-specific effect on any discriminative response as chlorisondamine did not block the cue produced by a non-nicotinic drug, midazolam. Whether the long-term effect is due to persistence of chlorisondamine in the brain or to an irreversible interaction of chlorisondamine with the site of nicotine action is not yet known.

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Clarke, P.B.S. & Kumar, R. (1983) Br.J.Pharmac. 80, 587-594
Stolerman, I.P., Garcha, H.S., Pratt, J.A. & Kumar, R. (1984) Psychopharmacology, 84, 413-419

IS THE ANALGESIC RESPONSE TO FOOTSHOCK A FORM OF PASSIVE AVOIDANCE LEARNING?

A.J. Gower* and M.D. Tricklebank, Merrell-Dow Research Center, 16, rue d'Ankara, 67084 Strasbourg Cedex, France.

Exposure of rats to brief, unavoidable footshock induces analgesia provided the animals have been subjected to the test of analgesia (e.g. determination of tail-flick latency) immediately prior to shock onset. No analgesic effects are obtained if either the pre-shock measurement or the shock are omitted (Tricklebank et al., 1984). This suggests that the phenomenon may be a form of passive avoidance learning. We examined this possibility in the rat using the tail-flick response to a focussed heat stimulus provided by an Appelex DS 20 tail-flick apparatus. The heat intensity was adjusted to produce control latencies of approximately 2-4 sec. A preliminary time-course study showed that the analgesia was maximal immediately after shock and decayed with time, returning to control values within 2 h. The ability to reinstate the analgesia was determined for the putative memory-enhancing drugs physostigmine (Baratti et al., 1979) and pramiracetam (Poschel et al., 1983) and also for morphine and naloxone.

Groups of naive male Sprague-Dawley rats (260-320 g) were tested for tail-flick latencies (pre-shock). Immediately after testing, each rat was placed in a chamber (22 x 20 x 9 cm high) and subjected to 6 x 5 s periods of scrambled footshock (3 mA) at 1s intervals. On removal from the chamber, the rats were injected subcutaneously with drug or saline. Two hours later the tail-flick latency was again measured. Additional groups were included: a) rats placed in the shock chamber for 35 sec without shock and b) rats subjected to shock without the pre-shock tail-flick test. Physostigmine (0.2 and 0.6 mg/kg) and pramiracetam (10 and 30 mg/kg) both increased tail-flick latency measured 2 h after unavoidable footshock but only in rats in which pre-shock tail-flick latency had been measured. In contrast, morphine (3 and 10 mg/kg) increased latencies in all rats regardless of shock treatment or pre-shock latency test. Naloxone alone (10 and 30 mg/kg) was without effect.

The ability of the same drugs to facilitate retention of a classical passive avoidance response in male albino mice (22-28 g) was determined using a 2-compartment, light-dark apparatus. Each compartment measured 15 x21 x22 cm high and access between them was by means of a guillotine door, 6 x 6 cm. The latency to enter the dark compartment from one corner of the illuminated section was noted. Immediately on entering the dark compartment, the door was lowered and the mouse subjected to 1.5 s, 27 V footshock. The mouse was removed, injected intraperitoneally and returned to the home cage. Twenty-four hours later, the time taken to again enter the dark compartment (retention latency) was measured. Physostigmine (0.1-0.4 mg/kg) and pramiracetam (10-40 mg/kg) both significantly increased retention latencies. Neither morphine (1-10 mg/kg) nor naloxone (5-20 mg/kg) had enhancing effects.

Thus, there was a correspondance between the drugs which were effective in reinstating tail-flick analgesia and those enhancing retention of a conventional passive avoidance response. Since physostigmine and pramiracetam were devoid of analgesic effects per se and reinstated analgesia only under conditions permitting an association between analgesia testing and footshock, the results provide support for the hypothesis that analgesia induced by brief footshock is, under these circumstances, a form of passive avoidance learning.

Baratti, C.M. et al. (1979) Psychopharmacol. 64, 85-88.
Poschel, B.P.H. et al. (1983) Drugs Exptl. Clin. Res. IX (12) 853-871.
Tricklebank, M.D. et al. (1984) Neuropharmacol. 23, 417-421.

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DIFFERENTIAL INDUCTION OF CLIMBING BEHAVIOUR IN RATS BY DOPAMINE AGONIST DRUGS

A.Davis*, P.Jenner & C.D.Marsden, MRC Movement Disorder Research Group,University Department of Neurology & Parkinson's Disease Society Research Centre, Institute of Psychiatry & King's College Hospital Medical School,Denmark Hill,London,UK.

Few attempts have been made to investigate the ability of dopamine agonist drugs to induce climbing behaviour in rodents. We compare the ability of a range of dopamine agonists to induce climbing in apomorphine prescreened 'climbing' and 'non-climbing' rats.

Groups of female Wistar rats (180-220 g) were designated as 'climbers' or 'non-climbers' on the basis of their response to the administration of apomorphine hydrochloride (0.5 mg/kg sc) as previously described (Davis et al.,1984). Dopamine agonists or vehicle were administered to animals previously acclimatised to the climbing cages for 1 h. Climbing behaviour was assessed continuously for up to 6 h.

In 'climbing' rats apomorphine induced a dose-related increase in climbing behaviour. The intensity of climbing behaviour induced by L-DOPA, (+)-amphetamine and pergolide mesylate was similar to that produced by apomorphine. Lergotrile, nomifensine and bromocriptine mesylate induced a less marked response. Lisuride induced only a weak climbing response. However, administration of lergotrile induced muscle hypotonia while lisuride induced ataxia. LY 141865, a selective D-2 agonist induced a dose-related climbing response, but the selective D-1 agonist, SKF 38393, induced only weak, non-dose-related climbing. In 'non-climbers', L-DOPA, amphetamine, nomifensine, bromocriptine and LY 14865 induced a climbing response equivalent to that observed in 'climbers'. Other drugs were ineffective. Pretreatment of the animals with the D-2 selective antagonist, sulpiride, antagonised the climbing behaviour induced by dopamine agonist drugs.

Table 1. The ability of dopamine agonists to induce climbing

Agonists	Dose (mg/kg)	'CLIMBERS'		'NON-CLIMBERS'	
		Maximum climbing score (Dose)	ED_{50} (mg/kg)	Maximum climbing score (Dose)	ED_{50} (mg/kg)
Apomorphine	0.063-2.0	10.9* (0.25)	0.15	2.0 -	-
L-DOPA	50-400	11.4* (400)	120	11.4* (400)	175
Bromocriptine	0.063-10.0	5.6* (5.0)	-	5.5* (5.0)	-
Pergolide	0.063-4.0	9.6* (1.0)	0.15	3.0 -	-
Lisuride	0.125-4.0	2.5 (1.0)	-	1.0 -	-
Lergotrile	1.25-20.0	6.7* (10)	10	1.0 -	-
LY 141865	0.125-2.0	7.5* (0.5)	0.35	7.3* (0.5)	0.44
SKF 38393	0.5-16.0	3.7* (2.0)	-	2.3 -	-
Amphetamine	0.63-10.0	10.5* (5.0)	1.25	4.8* (2.5)	-
Nomifensine	0.63-20.0	7.9* (10)	3.0	6.0* (5.0)	2.5

* $p < 0.05$ compared to vehicle; Mann Whitney U-test

Climbing can be initiated by a range of dopamine agonist drugs and may be mediated via D-2 receptors. However, the drugs differ in the intensity of the climbing response produced,particularly in 'non-climbing' rats.

Davis,A.S. et al. (1983) Br.J.Pharmac.80,549P.

FUNCTIONAL ANTAGONISM BETWEEN SUBTYPES OF 5-HYDROXYTRYPTAMINE RECEPTORS ON MORPHINE ANTINOCICEPTION

J.P.Gonzalez & J.F.Stolz*, Division of Pharmacology, The Welsh School of Pharmacy, UWIST, PO Box 13, Cardiff CF1 3XF

Distinct 5-hydroxytryptamine (5-HT) containing pathways exist in the fore brain and spinal cord which are believed to play a role in the modulation of pain perception and opioid antinociception (Messing and Lyttle, 1977). It is generally accepted that activation, either electrical or pharmacological, of 5-HT systems facilitates pain suppression and augments morphine analgesia (Yaksh and Wilson 1979, Malec and Langwinski 1980). There is now considerable biochemical evidence for the existence of multiple sub-types of 5-HT receptors (Peroutka & Snyder 1981), however, little information on the relative involvement of these receptor sub-types in the modulation of nociception is available. The present study examines the effect of the 5-HT agents, 5-methoxy-N',N'-dimethyltryptamine (5MeODMT), quipazine, 6-chloro-2-(1-piperazinyl)pyrazine (MK212) 5-Methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H-indole (RU 24969) and 8-hydroxy-dipropylaminotetralin (8-OHDPAT) on morphine antinociception using the mouse tail immersion test (48^{0}C). In all experiments morphine (2.5mgkg^{-1} s.c.) was administered simultaneously with the 5HT agonist. Control response latencies (saline treated) were 2.0±0.15 sec and 2.84±0.22 sec at 20 min and 80 min respectively, whilst mice treated with morphine alone showed response latencies of 6.31±0.54 sec and 4.59±0.31 sec at the same times (all values are mean SE n=40). The table summarizes the percentage change in morphine antinociception produced by co-administration of the 5HT agonists.

% Change in Morphine Antinociception produced by co-administration of various 5HT agonists

Interactant	Dose(mgkg^{-1} i.p.)	20 min†	80 min†
5MeODMT	5.0	-31 ± 11%*	+40 ± 14%*
	10.0	-47 ± 12%*	+72 ± 26%*
Quipazine	1.0	+ 3 ± 12%	+40 ± 9%*
	10.0	+75 ± 11%*	+159 ± 25%*
MK212	1.0	-24 ± 7%	-25 ± 18%
	10.0	+209 ± 25%*	+121 ± 48%*
RU24969	1.0	-38 ± 16%*	-31 ± 10%*
	10.0	-44 ± 11%*	-28 ± 10%*
8OHDPAT	0.1	-59 ± 5%*	-38 ± 16%*
	0.3	+25 ± 14%	+11 ± 19%

†Tested 20 and 80 mins after injection; - = reduction, + = potentiation.
*$P<0.05$ Students t-test

These data are consistent with a functional antagonism between subtypes of 5-HT receptors on morphine antinociception and may reflect the pre- and post-synaptic location of 5-HT receptors.

Malec,D. & Langwinski,R. (1980) Psychopharmacol. 69 79-83
Messing,R.B. & Lyttle,L.D. (1977) Pain 4 1-21
Peroutka,S.J. & Snyder,S.H. (1981) Brain Res. 208 339-347
Yaksh,T.L. & Wilson,P.R. (1979) J.Pharmacol.exp.Ther. 208 446-453

TOLERANCE TO THE ANTIPUNISHMENT EFFECTS OF DIAZEPAM IN MICE

D.N. Stephens, Research Laboratories of Schering AG,
Postfach 650311, Berlin 65 (West).

Although the development of tolerance to the anticonvulsant and sedative effects of the benzodiazepines (BZ) is well documented, it is widely held that their anxiolytic activity is persistent. We investigated the antipunishment properties of diazepam (DZP) in mice which had been given 9 daily doses of the BZ (5mg/kg, p.o.).

Male or female mice (NMRI strain, 20-25 g) were given DZP suspended in Cremophor EL (CEL) or equivalent volumes (about 0.2 ml) of the vehicle. 48 h following the last chronic dose, the mice, together with previously untreated animals, were given a test dose of DZP (0.6 - 10 mg/kg, p.o.) or CEL, and tested 1 h later in the 4-plate test of anxiety (Boissier et al, 1968). Each mouse was allowed to explore a perspex box (23 x 18 x 30 cm) whose floor consisted of 4 metal plates. Electric shock (1mA, 60ms) could be delivered during crossings between the plates. The number of such crossings in a 1 min period, following 20 s habituation, was measured in independent shocked and unshocked groups (n = 8).

Table 1 shows that DZP given acutely increased punished crossings. Chronic DZP, but not chronic CEL, decreased the antipunishment effects of the test dose of DZP (ANOVA: $F(3,91) = 6.5$; $P<0.001$). No test dose of DZP was effective in the chronically treated mice (Scheffe, $P>0.05$). The ability of DZP to increase unpunished activity was unaffected by chronic treatment.

Chronic Treatment	Test Dose (mg/kg, p.o.) 0	0.6	2.5	10
None	3.9 ± 0.4	9.6 ± 0.9	13.6 ± 1.3	19.1 ± 2.8
Vehicle	6.1 ± 0.6	10.6 ± 0.8	15.9 ± 1.1	16.4 ± 1.7
Diazepam	7.4 ± 0.5	8.8 ± 1.1	10.0 ± 0.9	8.9 ± 1.2

Table 1 Effect of chronic treatment on DZP's ability to increase activity suppressed by punishment (number of crossings ± s.e.mean).

In a second experiment, the development of tolerance 48 h following 1,3,6 or 9 daily treatments was investigated. A single, or 3 daily treatments had no effect on the ability of DZP (0.6 - 10 mg/kg) to increase punished activity, but these test doses of DZP were significantly less effective following either 6 or 9 days pretreatment.

Boissier, J.R., Simon, P., and Aron, C. (1968) Eur. J. Pharmacol. 4, 145-150.

THE AMYGDALA, LATERALITY, AND THE CONTROL OF RAT LOCOMOTOR ACTIVITY

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

Experiments in the rat on laterality have been restricted mainly to studies on rotation or side/paw preference and the importance of asymmetry within the nigrostriatal system (Glick, 1983). In the present study we investigate the possibility of laterality in the control of locomotor activity from a limbic structure, the amygdala.

Male Sprague-Dawley (Bradford Strain) rats were initially assessed for laterality as a postural motor asymmetry of a turn preference observed in an open field. If the turn preference was to the left the right hemisphere was defined as dominant and vice versa. Only animals showing a consistent preference to turn to the right or left were used in further studies. Animals were then subjected to standard stereotaxic techniques for the implantation of chronically indwelling guide cannulae for subsequent infusion into the area of the central nucleus of the amygdala (Ant.5.8, Vert.-1.8, Lat.±4.5). Infusion was effected for 13 days from Alzet osmotic minipumps implanted subcutaneously in the back neck region (for experimental details see Costall et al, 1982). In one series of experiments the injection unit attached to the minipump was designed for removal during infusion to allow an acute injection of neuroleptic drug. At the completion of the study the animals were killed for histological assessment of the site of infusion, shown to be in the areas of the central or lateral nuclei of the amygdala or close to the nucleus amygdaloideus intercalatus.

The unilateral infusion of dopamine (0.48µl/hr, 25µg/24h) into the left amygdala of a rat with a right dominant hemisphere caused a marked increase in locomotor activity of between 200-400% of control values (vehicle infused rats) on all 9 days of testing (control counts on days 1 to 9 were 56.5±5.3-87.6±9.1 counts/60 min whilst peak responses from dopamine infused rats ranged from 158.5±15.2 to 256.4±27.3 counts/60 min, $n = 5$, $P<.001$). The infusion of dopamine into the right amygdala of a right dominant hemisphere caused only inconsistent increases in locomotor activity of low magnitude on only 3 days of testing and the infusion of dopamine into either the right or left amygdala of rats with a dominant left hemisphere failed to modify locomotor activity ($n = 5$, $P>.05$).

Groups of animals exhibiting hyperactivity to dopamine infusion into the left amygdala (right hemisphere dominant) were given acute unilateral injections of (-)sulpiride both into the infused and non-infused amygdala (tested on days 3 and 8 of infusion). (-)sulpiride was shown to antagonise the dopamine response from both hemispheres, but most potently from the side of infusion [reductions of 66, 54 and 83% ($P<0.001$) caused by 5, 10 and 50 pg (-)sulpiride respectively from the infused amygdala, 6 ($P>0.05$), 56 and 72% ($P<0.001$) by 50, 100 and 250 pg (-)sulpiride respectively from the non-infused amygdala, $n = 5$-8].

It is concluded that (a) dopamine can cause a locomotor hyperactivity when infused into the left amygdala but only when animals show a right hemispheric dominance in a turn preference test, (b) that (-)sulpiride can antagonise this hyperactivity from the left or right amygdala to show that (c) the activities of the two amygdalae are closely co-ordinated.

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Costall, B. et al (1982) Neuropharmacology 21, 327-335
Glick, S.D. (1983) In: Hemisyndromes, Psychology, Neurology, Psychiatry, Ed. Myslobodsky, M.S., pp. 7-26 London: Academic Press

SUBTYPES OF THE 5-HT RECEPTOR INVOLVED IN HYPOTHERMIA AND FOREPAW TREADING INDUCED BY 8-OH-DPAT

D.N. Middlemiss, J. Neill & M.D. Tricklebank*, Merrell-Dow Research Center, 16, rue d'Ankara, 67084 Strasbourg Cedex, France.

Pharmacological analysis of reciprocal forepaw treading induced in the rat by the 5-HT receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), suggests that the behaviour reflects activation of the putative 5-HT_{1A} receptor (Tricklebank et al., 1984). We have found that reductions in body temperature accompany this behaviour and, on the basis of blockade of these responses by β-adrenoceptor antagonists having appreciable affinity for 5-HT_{1A} recognition sites, provide evidence for an involvement of the 5-HT_{1A} receptor in their genesis.

Core temperature of male Sprague-Dawley rats (200-250 g) was recorded at an ambient temperature of 22°C by a rectal probe 30 min after injection (s.c.) of 8-OH-DPAT. Forepaw treading was quantified (Tricklebank et al., 1984) 3-18 min after injection of 8-OH-DPAT. Antagonists were given (s.c.) 30 min before 8-OH-DPAT. Receptor binding studies used membranes from rat frontal cortex (Middlemiss & Fozard, 1983) with [^{3}H] 8-OH-DPAT , [^{3}H] 5-HT, and [^{3}H] ketanserin as ligands for 5-HT_{1A} (Hamon et al., 1984), 5-HT_{1B} (Middlemiss & Fozard, 1983) and 5-HT_2 (Leysen et al., 1982) recognition sites respectively.

Following doses of 0.03-3 mg/kg 8-OH-DPAT, core temperature fell dose-dependently by a maximum of 2.6°C. Similar doses induced a dose-dependent increase in the intensity of forepaw treading. Pindolol (0.125-4 mg/kg) and alprenolol (1-4 mg/kg) dose-dependently and stereoselectively antagonised both hypothermia and forepaw treading induced by a submaximal dose of 8-OH-DPAT (0.125 mg/kg, Table 1). In contrast, the β_1 and β_2 selective adrenoceptor antagonists, betaxolol and ICI 118.551 (0.25-2.5 mg/kg), given either singly or combined, were ineffective in antagonising both responses. Core temperature was not altered by administration of antagonists alone. In receptor binding studies, pindolol and alprenolol had appreciable affinity and stereoselectivity for 5-HT_{1A} and 5-HT_{1B} recognition sites, but were only weakly and non-stereoselectively active at the 5-HT_2 recognition site (Table 1). Betaxolol and ICI 118.551 were inactive at all 5-HT recognition sites ($pIC_{50} < 5.5$).

Table 1. β-Adrenoceptor antagonists, responses to 8-OH-DPAT and 5-HT receptor binding.

	ID_{50} (mg/kg)		pIC_{50}		
	Hypothermia	Forepaw Treading	5-HT_{1A}	5-HT_{1B}	5-HT_2
(-)-Pindolol	0.18	0.88	7.35	6.32	4.22
(+)-Pindolol	NA	NA	5.45	5.08	4.25
(-)-Alprenolol	1.5	1.7	6.93	7.05	5.61
(+)-Alprenolol	NA	NA	5.51	5.57	4.92

NA = Inhibition < 30 % at highest dose used (4 mg/kg).

The results suggest that blockade by pindolol and alprenolol of both hypothermia and forepaw treading induced by 8-OH-DPAT does not occur by antagonism of β-adrenoceptors or 5-HT_2 receptors, but, in the absence of significant affinity of 8-OH-DPAT for 5-HT_{1B} recognition sites (Middlemiss & Fozard, 1983), are consistent with these responses arising by activation of the putative 5-HT_{1A} receptor.

Hamon, M. et al., (1984) Europ. J. Pharmacol. 100, 263.
Leyson, J. et al., (1982) Molec. Pharmacol. 21, 301.
Middlemiss, D.N. and Fozard, J.R., (1983) Europ. J. Pharmacol. 90, 151.
Tricklebank, M.D. et al., (1984) Europ. J. Pharmacol. 106, 271.

ANXIOLYTIC ACTION OF $GABA_A$ AND $GABA_B$ AGONISTS

Rammy Gill, Susan D. Iversen and S. R. Thomas,* Department of Behavioural Pharmacology, Merck Sharp and Dohme Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR.

It is widely accepted that the anxiolytic effects of benzodiazepines are mediated by facilitation of ongoing GABA-ergic transmission at GABA-A receptors (e.g. Biggio and Costa, 1982). GABA-A agonists should therefore produce anxiolytic effects. This study reports findings on the anxiolytic potency and properties of GABA-A agonists in comparison with the reference sedative compound diazepam, and the GABA-B agonist baclofen tested on the CER model of anxiety (Iversen, Thomas and Trimnell, 1983). All drugs were given i.p. 20 mins before testing. The results are shown in Table 1.

Table 1

Compounds	ED_{50A}	ED_{50S}	A/S Ratio	Relative Efficacy
Diazepam	0.7	4.2	7.0	1.00
THIP	6.2	8.7	1.4	0.80
Baclofen	0.7	1.5	2.1	0.48
Muscimol	Inactive	20.0	N/A	N/A
Thiomuscimol	Inactive	30.0	N/A	N/A

Table 1. Anticonflict effects of GABA agonists in comparison with diazepam. Activity is given as ED_{50} estimates in $mg.Kg^{-1}$ i.p. for anxiolysis (ED_{50A}) and sedation (ED_{50S}); and the ratio of these estimates (A/S ratio) gives a guide to the separation of anxiolytic and sedative effects. The ratio of the peak anticonflict effect of each compound to that of diazepam yields a measure of anxiolytic efficacy (Relative Efficacy).

It is concluded that GABA-A agonists exemplified by THIP can have an anxiolytic action but show poor separation of ED_{50} estimates for the anxiolytic and sedative properties (the A/S ratio). The apparent inactivity of muscimol and thiomuscimol is assumed to reflect their inability to penetrate the blood-brain barrier or rapid metabolism. The GABA-B agonist baclofen was much less potent than THIP and also had a low A/S ratio.

Biggio, G. and Costa E. (eds) (1980) Adv. Biochem. Psychopharm., Vol 38.

Iversen, S. D.; Thomas, S. R. and Trimnell, L. E. (1983) Brit Journal Pharmacology., 80 637P.

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THE ACTIONS OF VIP AND ACH ON THE VISUAL RESPONSES OF NEURONES IN THE STRIATE CORTEX

K.L. Grieve*, P.C. Murphy & A.M. Sillito, Department of Physiology, University College, Cardiff, CF1 1XL.

The peptides somatostatin (SSt), cholecystokinin, neuropeptide Y and vasoactive intestinal polypeptide (VIP) are found in multipolar, fusiform and bipolar cells in the visual cortex and there is considerable interest in their likely functional role. We have previously reported on the effects of SSt in the visual cortex (Salt & Sillito, 1984) and the present account extends these observations to VIP. Taking note of the possibility that VIP and ACh may be coexistent in some bipolar cells (Eckenstein & Baughman, 1984) we have compared the effects of VIP with those of ACh and looked for an interaction between the two drugs.

Experiments were carried out on anaesthetised (70% N_2O, 30% O_2 and 0.1-0.4% halothane), paralysed (10mg/kg/hr gallamine triethiodide) cats prepared as described elsewhere (Sillito & Kemp, 1983). Five barrel micropipettes were used for recording single unit activity and iontophoretic application of the drugs; VIP (C.R.B. 0.5mM in 165 mM NaCl) and ACh (Sigma 0.2M, pH 4.5). Drug effects were assessed on the computer averaged responses of cells to a visual stimulus.

Iontophoretic application of VIP with currents in the range 40-100 nA produced a facilitation of the visually driven responses of cells without reducing response selectivity to for example stimulus direction. These effects were qualitatively similar to those obtained by iontophoretic application of ACh to the same cells with currents in the range 0-80nA. We also observed iontophoretic application of VIP to depress the visually driven response at similar currents and when this occurred ACh also produced a depression of the visually driven response. Simultaneous application of VIP and ACh to cells facilitated or depressed by drug application did not reduce the effect of either alone (c.f. Lamour et al., 1983) and in several cases there was a clear summation of effect. As in the case of SSt (Salt & Sillito, 1984) there was no correlation of effect with cells falling into the the simple or complex groups, but in contrast to the variablity of SSt effects, the effects of VIP on any given cell were consistently reproducible. Our data raise the possibility that the effects VIP and ACh may interact in a synergistic fashion in the visual cortex.

The support of the Medical Research Council is gratefully acknowledged.

Eckenstein, F. & Baughman, R.W. (1984) Nature, 309, 153-155.
Lamour, Y., Dutar, P. & Jobert, A. (1983) Neuroscience 10, 107-117.
Salt, T.E. & Sillito, A.M. (1984) J. Physiol. 350, 28P.
Sillito, A.M. & Kemp, J.A. (1983) Brain Res. 289, 143-155.

THE EFFECTS OF NASAL AIRFLOW AND MENTHOL ON THE NASAL ELECTROMYOGRAPHIC ACTIVITY OF THE ANAESTHETISED CAT

By Alison M. Davies* and R. Eccles. Department of Physiology, University College, Cardiff, CF1 1XL.

Menthol inhalation is traditionally believed to be of use in the symptomatic treatment of nasal congestion. Despite the popularity of menthol preparations there has been little research on the effects of this substance on the nose. Studies in man have indicated that menthol may enhance the sensation of nasal airflow by acting on sensory receptors in the nose (Fox, 1927; Burrow, Eccles & Jones,1983). We present results which indicate that menthol may stimulate sensory receptors in the nasal vestibule and cause a reflex augmentation of activity in nasal muscles which dilate the nostril.

Electromyographic (E.M.G.) activity was recorded from the quadratus labii superioris muscle in 18 cats anaesthetised with chloralose (60-70 mg./kg.) using fine wire bipolar electrodes (Basmajian & Stecko, 1962). The cats breathed spontaneously through a tracheal cannula with a plastic end tube which was clamped down in order to increase respiratory resistance. Nasal E.M.G. exhibited phasic respiratory activity associated with a dilation of the nostril.

Application of a jet of air at room-temperature (2 L/min. at 20-25°C) to the nasal vestibule augmented the ipsilateral integrated E.M.G. activity by 233 $\pm$ 37% (mean $\pm$ S.E.M. n=31) during the first minute of airflow. With a continuous airflow (2 L/min.) applied to the nasal vestibule, application of a solution of menthol in liquid paraffin (24-28 % W/V) to the surface of the nasal vestibule caused an augmentation of the nasal E.M.G. activity of 48 $\pm$ 4% (mean $\pm$ S.E.M. n=29). Liquid paraffin applied to the nasal vestibule prior to the menthol application had no effect on nasal E.M.G. activity.

In addition, we have found that the same concentration of menthol in liquid paraffin applied to the nasal vestibule in the absence of airflow augmented the integrated nasal E.M.G. by 56 $\pm$ 11 % (mean $\pm$ S.E.M. n=5).

These results indicate that both airflow into the nasal vestibule and the application of menthol onto the vestibule surface can cause an increase in the E.M.G. activity of nasal muscles which dilate the nostril.

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Burrow,A., Eccles,R.& Jones,A.S. (1983) Acta Otolaryngol. (Stockh.) 96, 157-161
Basmajian,J.V. & Stecko,G. (1962) J. Appl. Physiol. 17, 849.
Fox, N. (1927) Arch. Otolaryngol. 6, 112-122.

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EFFECTS OF INTRAVENOUS KETANSERIN, METHYSERGIDE AND MDL 72222 ON RESPONSES TO IONTOPHORETICALLY APPLIED 5-HT IN THE RAT BRAINSTEM

M. Davies, M.H.T. Roberts and L.S. Wilkinson*. Department of Physiology, University College, Cardiff, CF1 1XL.

Radioligand binding studies have revealed at least two main types of 5-HT recognition sites in the rat brain. Sites with high affinity for tritiated 5-HT have been termed 5-HT(1) and those with low affinity for tritiated 5-HT but high affinity for tritiated spiperone have been termed 5-HT(2) (Peroutka and Snyder, 1979). The 5-HT antagonist ketanserin displays high affinity for the 5-HT(2) site whilst being inactive at the 5-HT(1) site. Methysergide discriminates poorly between the two sites having only a slightly greater affinity for the 5-HT(2) site (Leysen et al. 1981). MDL 72222, a selective antagonist at certain peripheral neuronal 5-HT receptors, shows no ability to displace radioligand binding to either the 5-HT(1) or 5-HT(2) sites (Fozard 1984). Microiontophoretic application of 5-HT to single neurones in the rat brainstem has both excitatory and depressant effects on neuronal firing rate. In this study we have examined the effects of ketanserin, methysergide and MDL 72222 on both the excitatory and depressant effects of 5-HT to investigate the relationship between the responses and the 5-HT(1) and 5-HT(2) sites.

Adult male albino Wistar rats were anaesthetised with halothane. Tracheal, carotid and external jugular cannulae were inserted and the animal placed into a stereotaxic apparatus. Blood pressure, ECG and temperature were monitored and maintained within physiological limits. The activity of single brainstem neurones was recorded with 5 barrelled microelectrodes driven through the cerebellum. Permanent records were made of the rate and amplitude of the action potentials. 5-HT and glutamate were applied by microiontophoresis. Ketanserin, methysergide and MDL 72222 were applied intravenously in divided doses. When antagonism was evident no further antagonist was injected.

Selective and reversible blockade of 5-HT induced excitation of neuronal firing was observed with ketanserin (mean dose 300 ug/kg i.v.) and methysergide (mean dose 1 mg/kg i.v.). Ketanserin had no effects on 5-HT induced depression of neuronal firing rate with doses up to 2.1 mg/kg i.v. Methysergide at doses of 25 mg/kg i.v. also had no effect on depressions but on two occasions doses of 40 mg/kg i.v. reduced the depressant response. Recovery was not seen but spike amplitudes did not change. MDL 72222 at doses of 1 mg/kg occasionally reduced excitatory and depressant responses without recovery being seen but in the majority of studies had no effect. In view of the great sensitivity of the Bezold-Jarisch effect to MDL 72222 (39 ug/kg i.v.), 1 mg/kg must be considered a very high dose indicating that the peripheral and central neuronal receptors are different.

It may be concluded that the selective 5-HT(2) antagonist ketanserin potently blocks 5-HT induced excitation of brainstem neurones but that depressant responses are resistant. Methysergide, which requires an 8 fold higher concentration (Leysen et al. 1981) to displace 5-HT(1) binding than to displace 5-HT(2) binding, antagonised the excitatory effects of 5-HT at 1 mg/kg and failed to block the depressant effects at 25 mg/kg. However, Peroutka and Snyder (1979) report that approximately a 40 fold higher concentration is necessary to displace 5-HT(1) binding and higher doses of methysergide may be necessary.

Fozard,J.R. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 326, 36-44.
Leysen,J.E. et al. (1981) Life Sci. 28, 1015-1022.
Peroutka,S.J. and Snyder,S.H. (1979) Molec. Pharmacol. 16, 687-699.

TISSUE SPECIFIC MODULATION OF β-RECEPTOR NUMBER IN CHRONIC HYPOXIA WITH AN ATTENUATED RESPONSE TO DOWN REGULATION BY SALBUTAMOL

[2]K.E.J. Dickinson, [1]R.M. Rudd, [2]P.S. Sever, *[1]R.J.D. Winter. [1]Dept. of Thoracic Medicine, London Chest Hospital, Bonner Road, London, E2 9JX and [2]Dept. of Clinical Pharmacology, St. Mary's Hospital Medical School, Norfolk Place, London, W.2.

Reduction in β-receptor density has been reported in cardiac membranes prepared from rats exposed to chronic hypoxia (Voelkel et al., 1980). However, little information exists about changes in β-adrenoceptors in other tissues. We have used radioligand binding techniques to measure β-adrenoceptors in an animal model of human hypoxic disease (Hunter et al., 1974) and have investigated the possibility that there are changes in β-receptor number in lung and spleen with chronic hypoxia. We have also assessed β-adrenoceptor response to down regulation by salbutamol.

Male Wistar rats weighing 100-150g were exposed to continuous hypoxia (FiO_2 = 10%) for 28 days. Control hypoxic animals developed pulmonary hypertension (mean right ventricular systolic pressure 41.2, SEM 2.4 mmHg compared with 24.0, SEM 1.4 mmHg for normoxic controls), an increase in right ventricular weight corrected for body weight (RV/T = 9.5 X 10^{-4}, SEM 3.3 X 10^{-5}, compared with 5.06 X 10^{-4}, SEM 1.9 X 10^{-5}) $p < .001$ in every case. Treatment with salbutamol (2.5 mg/kg sc bd) did not influence the development of pulmonary hypertension. Immediately after the animals were sacrificed tissues were frozen in liquid N_2 and stored at -70° until use. Crude membrane preparations were made and assayed on the same day. In brief, tissues were homogenised in 10 vols of 50mM Tris HCl pH 7.4, 5mM EDTA, passed through two layers of muslin, centrifuged and washed once in Tris buffer. Pellets were taken up in 50mM Tris HCl pH 7.4, 0.5mM EDTA, centrifuged and resuspended in 50mM Tris HCl pH 7.4. β-Adrenoceptor number was measured by conventional radioligand binding techniques using the radioligand ^{125}I-iodocyanopindolol. Specific binding to β-adrenoceptors was defined as that displaceable by 200μM (-)Isoprenaline.

The table below shows the effects of hypoxia and salbutamol on β-receptor number in lung and spleen. There was no significant change in the dissociation constant (K_D) of the radioligand for β-adrenoceptors of either tissue following hypoxia or salbutamol treatment.

TABLE 1 β-Receptor number (fmoles/mg protein) in membrane preparations of tissues from normoxic & hypoxic animals following salbutamol treatment (mean ± SEM)

	Normoxic				Hypoxic			
	Lung		Spleen		Lung		Spleen	
Control	357 ± 28	(n=8)	76 ± 19	(n=5)	514 ± 33†	(n=8)	80 ± 15	(n=5)
Treated	470 ± 50	(n=6)	14 ± 7*	(n=6)	536 ± 88	(n=6)	77 ± 13	(n=6)

†$p < .004$ relative to normoxic control

*$p < .01$ relative to normoxic control, $p < .001$ relative to hypoxic treated

Following chronic exposure to hypoxia, β-adrenoceptor density in lung membranes was increased by 44% whereas β-adrenoceptor number in the spleen was unchanged. Salbutamol treatment during normoxia produced no down regulation in the lung but effected an 80% reduction in splenic β-adrenoceptors. This finding agrees with other reports of a difference between lung and spleen in their susceptibility to down regulation with β_2-agonists (Hasegawa et al., 1983). In contrast, salbutamol treatment during hypoxia failed to produce a down regulation of β-adrenoceptors in either lung or spleen. These data suggest that chronic hypoxia causes tissue specific attenuation of β-adrenoceptor number and reduces their susceptibility to down regulation.

Hasegawa, M. et al. (1983). J. Allergy Clin. Immunol., 71, 230.
Hunter, C. et al. (1974). Clin. Sci., 46, 375.
Voelkel, N.F. et al. (1981). J. Appl. Physiol., 50, 363.

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INTRINSIC ACTIVITY AND POTENCY OF GUINEA-PIG TRACHEAL RELAXANTS AFTER DIFFERENT SPASMOGENS IN VITRO

J.R.Carpenter, Dept. Pharmacology, Therapeutics & Materia Medica, University of Manchester, Manchester M13 9PT.

The isolated trachealis muscle of the guinea pig is often used to evaluate drugs that cause bronchodilatation via beta-adrenoceptors. However several workers have shown that the spasmogen carbachol (CCh) can change both the location of the dose-response curve to the relaxant ie potency and, for partial agonists, the maximal relaxation achieved ie intrinsic activity (O'Donnell & Wanstall, 1978; Jenkinson, 1979; Kenakin & Beek, 1980). Experiments were therefore carried out to compare the effects of the three spasmogens CCh, histamine and PGE_2, on concentration-effect curves to the full agonist isoprenaline (ISO) and the partial agonists salbutamol (SAL), prenalterol (PREN) and dichloroisoprenaline (DCI).

Tracheal segments comprising about 3 cartilaginous rings were opened by longitudinal cuts through the cartilage and set up for isotonic recording with tissue loadings of 200mg in a Krebs-Henseleit solution gassed with O_2 + 5% CO_2 at 37°C. Cumulative concentration-effect curves to the relaxant drugs were obtained firstly with spontaneously developed tone and then again in the presence of the EC90 of the spasmogen after spontaneous tone had been abolished by 60 min incubation in Krebs-Henseleit solution containing 5.6uM indomethacin. Potencies were estimated by interpolating the log EC50 on each concentration-effect curve. Maxima are expressed as percentages of the maximal relaxation to aminophylline (1mM) added at the end of each cumulative treatment. Statistical comparisons were made on pD_2 values (-log EC50) and maxima using Student's t test for paired data. In all cases n=4.

CCh caused significant changes in the potencies of all the relaxants used; log shifts were:-ISO 0.25; SAL 1.43; PREN 2.68; DCI 2.83. Histamine, however, only produced a significant change in the potency of DCI (log shift = 2.96) whereas PGE_2 changed the potency of only PREN (log shift = 0.83). None of the spasmogens altered the maximal response to ISO. Although CCh reduced the maximal responses to SAL and to PREN in all tissues, these reductions failed to reach significance ($p = 0.065$ & 0.053 respectively). Neither PGE_2 nor histamine produced appreciable changes in maximal responses to SAL or PREN; PGE_2 similarly had no significant effect on the maximal response to DCI. The right-ward shift in the DCI concentration-effect curve caused by CCh and histamine was sufficient to reveal a secondary relaxant property of DCI at concentrations above about 5×10^{-5}M which was slow to develop, near maximal and resistant to propranolol.

It is concluded that while CCh causes unacceptable changes in the concentration-effect curves, particularly of partial agonists, histamine and PGE_2 may prove to be suitable spasmogens if experimental conditions are such that spontaneous tone cannot develop.

Salbutamol was a generous gift from Glaxo & prenalterol from AB Hässle.

Jenkinson,D.H. (1979)Proc.VIthInt.Symp.Med.Chem.; ed: M.A.Simpkins, Cotswold Press, Oxford; pp 373-383
Kenakin,T.P. & Beek, Deborah (1980) J.Pharmac.Exp.Ther. 213,406-413
O'Donnell,Stella R. & Wanstall,Janet C.(1978) Eur.J.Pharmac. 47,333-340

CONCENTRATION-RATIO ANALYSIS OF THE INOTROPIC EFFECTS OF PHENYLEPHRINE IN GUINEA-PIG LEFT ATRIAL MUSCLE

J.W. Black, V.P. Gerskowitch, R.A.D. Hull* & P.Leff[1], Analytical Pharmacology, King's College School of Dentistry and Medicine, London SE5 9NU and [1]Department of Pharmacology, Wellcome Research Laboratories, Beckenham, BR3 3BS.

Sympathomimetic amines, in the presence of β-adrenoceptor blockade, can increase myocardial contractility by activation of α-adrenoceptors (Wenzel & Su, 1966). In guinea pig atrial muscle, low levels of α adrenoceptor stimulation produce a biphasic response in the force of contractions: a peak is reached after less than 3 min and then progressively fades. Higher levels of stimulation produce a triphasic response: during the period of fade, 5-10 min after exposure to the agonist, a secondary increase in the force of contraction occurs which now does not fade. Previous analyses of these responses (Hattori & Kanno, 1982) have suggested that the fading response is due to activation of negative inotropic receptors, possibly α_2-receptors, and that the primary and secondary positive inotropic responses are due to different mechanisms. These tentative conclusions were based on attempts to produce disproportionate reductions in the three phases using selective α-adrenoceptor antagonists. However, the usual problems associated with response-ratio analysis are compounded in this case by the dose-dependence and possible serial correlation of the different phases of the complex response. Therefore we have now carried out a more conventional concentration-ratio analysis on the assumption that the presence of prazosin-insensitive receptors should be exposed by non linearities in the Schild regression.

The antagonism by prazosin of the inotropic effects of phenylephrine was studied on left atrial muscle obtained from guinea pigs pretreated with 6-hydroxydopamine. The muscles were suspended in Krebs-Henseleit solution containing (l)-propranolol (5×10^{-7}M) and stimulated at 1Hz. Using a randomised block design, each preparation was exposed to only a single concentration of agonist and antagonist.

At concentrations between 10^{-9}M and 3×10^{-7}M, phenylephrine produced biphasic responses: secondary positive inotropic responses developed at concentrations higher than this. When the antagonism by prazosin was surmounted by appropriate increases in phenylephrine concentration, the time course of the responses had apparently not been changed. Log concentration-response curves based on the primary inotropic phase of discrete responses were displaced dextrad in parallel by prazosin: the slope of the Schild plot was not significantly different from unity and the calculated pK_B was 9.31 ± 0.06, a value not inconsistent with an α_1-receptor classification.

Concentration-response curves based on the secondary inotropic response were also constructed. At high agonist concentrations, the secondary response was measured when it reached a plateau. At low agonist concentrations when the secondary response was not clearly visible, the measurement was taken at 10 min after agonist addition. The effects of prazosin on the secondary response measurement were virtually indistinguishable from the primary response measurements and produced a pK_B of 9.25 ± 0.17

We conclude that because the whole response profile to phenylephrine is equally susceptible to prazosin the primary and secondary responses may be sequentially linked events. Furthermore, this study provides no evidence for the involvement of prazosin insensitive receptors.

Hattori, Y. & Kanno, M. (1982) Japan J. Pharmacol. 32, 359-368.
Wenzel, D.G. & Su, J.L. (1966) Arch. Int. Pharmacodyn. 160, 379-389.

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ALTERED PERIPHERAL α_2 ADRENOCEPTOR RESPONSES IN RABBITS WITH PERINEPHRITIS HYPERTENSION

C.A. Hamilton, C.R. Jones, J.L. Reid, University Department of Materia Medica, Stobhill General Hospital, Glasgow, G21 3UW.

There are numerous reports of increased reactivity to catecholamines in hypertensive man and in animal models of hypertension. We have observed increased responses to α_1 adrenoceptor agonists in rabbits with perinephritis hypertension (PHT) which may be related to post receptor events linking receptor activation to smooth muscle contraction (Hamilton and Reid, 1983). These studies have been extended to investigate the role of peripheral α_2 adrenoceptors in the same model. Hypertension was induced in male New Zealand white rabbits, wrapping one kidney in cellophane and removing the other. The pressor responses to a range of agonists with α_2 adrenoceptor activity were examined in groups of conscious hypertensive (PHT) and sham operated animals (NT). In vitro the pro-aggregatory response of rabbit platelets to adrenaline (A) and the specific binding of [^{3}H] yohimbine to the platelets were examined. Pressor responses to noradrenaline (N) and α-methyl-noradrenaline (αMN) were increased over the dose range examined (Table 1). The sensitivity but not the maximum response of platelets to A was increased in PHT animals (EC_{50} µM .017 ± .003 PHT, .086 ± .015 NT, maximum ΔOD 54 ± 2PHT, 48 ± 1 NT). In contrast pressor responses to the partial agonists guanabenz (G) and BHT 920 (B) were not significantly changed (Table 1). The increased responses to N and αMN were not due to α_1 adrenoceptor activity of the agonists as they could not be blocked by prazosin up to 1.0 mg/kg. There was no indication that either the vascular or platelet responses reflected changes in β receptor mechanisms.

Table 1 Pressor responses to α adrenoceptor agonists

Agonist	Dose (µg/kg)	Pressor response (mmHg) NT	PHT
Noradrenaline	0.5	19 ± 4	39 ± 15*
Noradrenaline	1.0	32 ± 5	56 ± 30*
α Methyl-noradrenaline	2.0	23 ± 18	40 ± 13*
α Methyl-noradrenaline	4.0	31 ± 18	50 ± 8*
Guanabenz	50	16 ± 4	14 ± 5
BHT 920	10	16 ± 6	17 ± 5
BHT 920	35	26 ± 5	29 ± 8
Noradrenaline + Prazosin	20	28 ± 4	55 ± 13*
α Methyl-noradrenaline + Prazosin	10	29 ± 6	47 ± 3*

n = 6-10 animals per group * PHT significantly different to NT

Previously we have shown that clearance of N and specific [^{3}H] clonidine binding to brain and spleen membranes was unchanged in PHT (Hamilton and Reid 1983). In this study [^{3}H] yohimbine binding to platelet membranes from PHT and NT rabbits was similar (Bmax 24±5 and 29±9 fmoles/mg protein K_D 6±2 and 7±3 nM for PHT and NT). Thus the increased responses are unlikely to be due to altered clearance of the agonists or an increase in α_2 adrenoceptor binding sites. The ability of the antagonist idazoxan to attenuate responses to N, αMN, G and B was also unchanged in PHT. As the coupling of full agonists to the guanine nucleotide binding protein and adenylate cyclase differ from that of partial agonists and antagonists the increased responses of N αMN and A in PHT may be related to altered α_2 adrenoceptor activation.

Hamilton, C.A., Reid, J.L. 1983. Hypertension. 5, 958.

AN INVESTIGATION OF AGE-RELATED CHANGES IN VASCULAR α-ADRENOCEPTORS IN THE ANAESTHETISED RAT

J.R.Docherty*and L. Hyland, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2, Ireland.

We have recently reported that there is a reduced potency of the alpha-2 adrenoceptor antagonist yohimbine with increasing age, without change in the potency of the alpha-1 adrenoceptor antagonist prazosin, at inhibiting stimulation-evoked contractions in the human saphenous vein (Docherty and Hyland, 1985). The object of this study was to extend this work to the vascular system of the rat.

Old male Sprague-Dawley rats (22-24 months, 500-700 g) and weight-matched young animals (6-7 months) were anaesthetised with pentobarbitone sodium and ventilated with 100% O_2. The carotid artery was cannulated for blood pressure recording, and the jugular vein was used for the administration of drugs.

Resting diastolic blood pressure (DBP) was 114.0±10.8 mmHg (n=6) in young and 69.3±13.7 mmHg (n=6) in old rats ($P<0.05$). Noradrenaline (NA) (1 ug/kg) produced a rise in DBP of 27.8±5.5 mmHg in young and 36.7±5.3 mmHg in old rats (no significant difference). Prazosin (1 mg/kg) lowered DBP to 47.6±5.4 mmHg in young and 22.0±2.2 mmHg in old rats ($P<0.01$). In the presence of prazosin, NA was significantly more potent in young than in old with ED50 values (dose producing 50% of the maximum rise in DBP to NA) of 0.61 ug/kg (95% confidence limits 0.19-0.87 ug/kg) and 10.0 ug/kg (95% confidence limits 5.0-20.0 ug/kg) in young and old, respectively (n=4 each, $P<0.001$). The maximum pressor response to NA in the presence of prazosin did not differ between young and old rats (71.2±7.5 mmHg and 70.5±5.7 mmHg in young and old, respectively, n=4 each).

Hence, we can demonstrate that there is a reduced responsiveness of postsynaptic alpha$_2$-adrenoceptors without loss of overall alpha-adrenoceptor responsiveness in the vasculature of the anaesthetised rat. This is in agreement with data obtained in the rat vas deferens (no age-related change in the responsiveness of postsynaptic alpha-1 adrenoceptors: Docherty and O'Malley, 1983) and in the human saphenous vein (see above). There appears to be a selective loss of alpha-2 adrenoceptor responsiveness postsynaptically with increasing age.

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Docherty,J.R. & Hyland,L. (1985). Proceedings of the B.P.S., London, December 1984.
Docherty,J.R. & O'Malley,K. (1983). Eur. J. Pharmacol.,95, 171-176.

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RELATIVE α- AND β-ADRENOCEPTOR DENSITY DETERMINES WHICH RECEPTOR MEDIATES THE CARDIAC RESPONSE TO PHENYLEPHRINE

K.J.Broadley, R.G.Chess-Williams*, D.J.Sheridan+ & K.L.Williamson, Departments of Pharmacology, UWIST, and Cardiology+, UWCM, Cardiff.

Phenylephrine is a classical α_1-receptor agonist which also has partial agonist activity at cardiac β_1-adrenoceptors. This study examines cardiac responses to phenylephrine and the relative density of α- and β-adrenoceptor binding sites in the ventricular tissue of the rat and guinea-pig.

Papillary muscles from rat and guinea-pig hearts were set up in Krebs-bicarbonate solution containing metanephrine (10μM) and gassed with 5% CO_2 in oxygen at 32°C. Tissues were paced at 1Hz (threshold voltage + 50%, 5msec pulse-width) and cumulative concentration-response curves determined to isoprenaline and after washout to phenylephrine. In the rat papillary muscle, phenylephrine had a greater ($P<0.01$) maximum response (67.7±6.4%) relative to isoprenaline than in the guinea-pig (28.5±4.1%). Furthermore, the EC_{50} value for phenylephrine was lower ($P<0.001$) in the rat, 3.6 (1.2-10.9)μM compared with 51.6 (26.3-100)μM for the guinea-pig papillary muscle. In the rat, phenylephrine concentration-response curves were shifted to the right by prazosin (3nM), the EC_{50} being increased ($P<0.05$) to 8.1 (4.9-13.5)μM by this antagonist. The presence of propranolol (1μM) however had no effect on the responses of this tissue to phenylephrine, the EC_{50} value being 2.6 (1.8-3.9) M. In contrast, the phenylephrine responses of guinea-pig papillary muscles were abolished by propranolol (1μM) but were unaffected by prazosin (10nM), in the presence of which the EC_{50} value was 38.7 (23.0-65.9)μM.

$[^3H]$Prazosin and $[^3H]$dihydroalprenolol binding was examined in membranes prepared from the remaining ventricular muscle from these animals. Scatchard analysis of $[^3H]$prazosin saturation curves yielded similar dissociation constants (0.31±0.08nM and 0.49±0.10nM) but different ($P<0.001$) number of binding sites (104.6±9.5 and 17.9±1.5 fmol mg^{-1} protein in the rat and guinea-pig respectively). $[^3H]$dihydroalprenolol binding experiments also yielded similar dissociation constants (1.62±0.08nM and 1.63±0.11nM) but different ($P<0.001$) number of binding sites in the two species (25.9±3.1 and 114.0±5.1 fmol mg^{-1} protein in the rat and guinea-pig respectively).

We conclude that in the rat where the α: β-adrenoceptor ratio is 4.0 the response to phenylephrine is mediated through α-adrenoceptors whereas in the guinea-pig where the α:β ratio is 0.16 the phenylephrine response is mediated via β-adrenoceptors.

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α_1-ADRENOCEPTOR RESERVE AND NIFEDIPINE SENSITIVITY OF NORADRENALINE PRESSOR RESPONSES IN RAT SUPERIOR MESENTERIC ARTERIAL BED

C.R. Hiley and A.J. Nichols*, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD

It has been suggested from experiments in the pithed rat that the sensitivity of α_1-adrenoceptor mediated responses towards calcium antagonists is increased by elimination of spare receptors (Ruffolo et al, 1984). Since the response observed was that of the central arterial pressure, it was not possible to determine which vascular beds were involved. Thus we have studied the relationship between α_1-adrenoceptor reserve and sensitivity to calcium channel blockade in the superior mesenteric arterial bed of the rat which we have shown to possess no α_2-adrenoceptors (Nichols & Hiley, 1985).

Male Wistar rats (250-280g; Bantin & Kingman) were anaesthetised with thiobutabarbitone (120 mg/kg i.p.; Inactin-BYK) and prepared for in situ blood perfusion of the superior mesenteric arterial bed as described by Nichols & Hiley (1985). Recordings were made of central arterial pressure (from the right carotid artery) and mesenteric arterial perfusion pressure using Bell & Howell transducers (4-422-0001) connected to a Grass 7D polygraph.

Noradrenaline (0.63-63 nmol) gave dose-related increases in mesenteric perfusion pressure with mean log (mole ED_{50})=-8.07±0.04 and maximum recorded response = 151±9 mmHg (n=8). Phenoxybenzamine (3 µg/kg i.v.) significantly reduced ($P<0.01$, analysis of variance) the maximum response to 105±9 mmHg (n=6) without causing a significant change in the ED_{50}. Phenoxybenzamine at 10 µg/kg i.v. further reduced the maximum response ($P<0.01$) to 45±4 mmHg (n=7), again without changing the ED_{50}.

Nifedipine (0.1 µg/kg, i.v.) had no effect on the noradrenaline log dose/response curve although it reduced diastolic pressure by 45%. At 1 mg/kg i.v. it significantly reduced both the responses to 3 out of 7 doses of noradrenaline and diastolic pressure by 60%. After pretreatment with 3 µg/kg phenoxybenzamine there were again no significant differences between the noradrenaline log dose/response curves obtained before and after the administration of 0.1 mg/kg nifedipine but diastolic pressure was 48% lower. Increasing the dose of nifedipine to 1 mg/kg significantly reduced both diastolic pressure by 64% and the maximum response to noradrenaline by 36% when compared to the values obtained before administration of nifedipine but after phenoxybenzamine. Responses to 3 of the 6 other doses of noradrenaline were also reduced. Rats exposed to 10 µg/kg phenoxybenzamine gave noradrenaline responses that were no more sensitive to nifedipine. Nifedipine had no significant effects on the ED_{50} values for noradrenaline both in those experiments carried out with, and in those performed without, α_1-blockade with phenoxybenzamine.

We conclude that there are no spare α_1-adrenoceptors in the superior mesenteric arterial bed of the rat when noradrenaline is used as the agonist. Also, the responses to this agonist had little sensitivity to nifedipine even at doses which gave large depressions in central diastolic arterial pressure and pretreatment with phenoxybenzamine increased only slightly the effectiveness of nifedipine on the pressor responses. The results are consistent with the hypothesis of de Jonge et al (1983) that there are two subtypes of α_1-receptor response one of which is more sensitive to phenoxybenzamine and the other to calcium channel blockade; the present preparation containing the former subtype.

AJN is an MRC research student.

de Jonge, A. et al (1984) Br.J.Pharmac. 81, Proc.Suppl., 80P
Nichols, A.J. & Hiley, C.R. (1985) J.Pharm.Pharmacol. In press.
Ruffolo, R.R. et al (1984) J.Pharmacol.exptl.Ther. 230, 587-594

α,β meATP DOES NOT INHIBIT [^{3}H]-NORADRENALINE RELEASE IN THE RABBIT EAR ARTERY

R.J. Allcorn, T.C. Cunnane, T.C. Muir & Kay A. Wardle, Dept. of Pharmacology, Glasgow University, Glasgow G12 8QQ.

On the basis of electrophysiological experiments in guinea-pig arterioles a unique class of adrenoceptor, the γ-receptor, has been proposed to explain the failure of α1-adrenoceptor antagonists to block excitatory junction potentials (e.j.p.s) (Hirst, 1984). The alternative explanation is that two transmitters, ATP and NA, are released from sympathetic nerves, and that ATP mediates the e.j.p.. Support for the latter hypothesis has come from experiments using two drugs which apparently block e.j.p.s by a postjunctional action; namely arylazido aminopropionyl-ATP ($ANAPP_3$) (Sneddon & Westfall, 1984) and α,β methylene-ATP (α,β meATP) (Burnstock & Sneddon, 1984). Hirst (1984) has recently reported that α,β meATP indeed blocks γ-receptor mediated e.j.p.s in guinea-pig arteriolar muscle but it does so by inhibiting NA release. The purpose of the present investigation was to determine whether this agent had any effect on ^{3}H-NA release in a tissue where it abolishes the e.j.p..

The responses of the rabbit ear artery to field stimulation were assessed by measuring changes in (a) membrane potential with intracellular micro-electrodes (b) electrically-evoked release of ^{3}H-NA from spiral strips pre-incubated with ^{3}H-NA and superfused with Krebs' solution containing 114 μM ascorbic acid, 2.6μ m atropine, 0.6 μm desmethylimipramine and 10 μm normetanephrine (Alberts et al., 1981) (c) pressure of isolated perfused tissues and contraction of superfused spiral strips. Field stimulation (0.05-0.5 ms, 5-60 V), at various frequencies was carried out via Ag/AgCl ring electrodes. α,β meATP and NA were applied either to the solution bathing the artery or into the lumen by bolus injection.

Contractile responses to field stimulation of perivascular arterial nerves were accompanied by e.j.p.s and 'spikes' and followed by a small slow depolarization lasting some 5-15 s. α,β meATP (1-10 μm) abolished e.j.p.s and spikes but surprisingly, neither the pressure response nor the contraction of arterial strips was reduced. Responses to exogenous NA were not reduced by α,β meATP (Allcorn et al., 1985). Although α,β meATP abolished e.j.p.s it had no detectable effect on electrically-evoked ^{3}H-NA release (1-20 Hz, 300 pulses).

The results of the present study clearly indicate that α,β meATP has no inhibitory effect on the release of ^{3}H-NA from sympathetic nerves.

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Alberts, P. et al. (1981) J. Physiol. 312, 297-334
Allcorn, R.J. et al. (1985) Physiological Society Proceedings, Guy's Hospital Meeting, Jan. 1985, C23
Burnstock, G. & Sneddon, P. (1984) J. Physiol. 351, 28P
Hirst, G.D.S. (1984) 9th IUPHAR International Congress (London) L20
Sneddon, P. & Westfall, D.P. (1984) J. Physiol. 347, 561-580

EFFECTS OF α-ADRENOCEPTOR ANTAGONISTS ON BLOOD PRESSURE IN THE SPONTANEOUSLY HYPERTENSIVE RAT

J.R. Docherty, and P. Warnock* Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin.

We have recently reported that the alpha$_2$-adrenoceptor antagonist rauwolscine, but not Wy 26392 (Lattimer et al., 1982), lowers diastolic blood pressure (DBP) subsequent to prazosin in the anaesthetised Spontaneously Hypertensive Rat (SHR), and that this blood pressure lowering action of rauwolscine was prevented by removal of the adrenal medulla (Docherty and Sawyer, 1985). We now report further data in support of our contention that circulating catecholamines are responsible for the alpha$_2$-adrenoceptor mediated control of blood pressure in the anaesthetised SHR.

Male SHR (250-350g) were anaesthetised with pentobarbitone. The left carotid artery was cannulated for recording of blood pressure and the left jugular vein was used for drug administration. Some animals were pretreated with 6-hydroxydopamine to produce a chemical sympathectomy or with vehicle (see Gibson and Pollock, 1973). Other animals underwent surgical removal of the adrenal medulla or sham operation.

Resting DBP was significantly lower in sympathectomised (126.8+9.7 mmHg, n=5) and adrenal demedullated animals (122.2+5.4 mmHg, n=17) than in unoperated (148.2+4.6 mmHg, n=12) or sham treated animals. There was no significant difference between unoperated, sham operated or demedullated animals in the fall in DBP produced by prazosin (1 mg kg^{-1}) (approximately 60 mmHg fall) but prazosin was significantly less effective in sympathectomised animals producing a fall of 38.0+10.2 mmHg ($P<0.05$ from other groups).

Subsequent to prazosin, rauwolscine (0.1 & 1 mg kg^{-1}) produced significant further reductions in DBP in all groups of animals except the demedullated. This failure of rauwolscine in demedullated animals was not due to the low resting DBP in these animals following prazosin (65.2+7.1 mmHg, n=6) since rauwolscine was effective in sham or unoperated animals when DBP, subsequent to prazosin was lowered to 67.8+2.5 mmHg (n=5) by hydralazine. Even in demedullated animals, isoprenaline (1 µg kg^{-1}), subsequent to prazosin was able to lower DBP to a level of 30.5+2.0 mmHg (n=4) (not significantly different from resting DBP in pithed SHR). Rauwolscine, subsequent to prazosin, was as effective at lowering DBP in sympathectomised as in unoperated or vehicle treated animals.

Hence, alpha$_2$-adrenoceptor mediated pressor responses in the anaesthetised SHR involve predominantly circulating catecholamines, whereas alpha$_1$-adrenoceptor mediated pressor responses involve both nerve released noradrenaline and circulating catecholamines. It seems that alpha$_2$-adrenoceptors are predominantly extrasynaptic on the arterial side of the circulation, at least in the rat.

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Docherty, J.R. & Sawyer, R. (1985). Br. J. Pharmacol, 84, 145P.
Gibson, A. & Pollock, D. (1973). Br. J. Pharmacol., 49, 726-7.
Lattimer, N. et al. (1982). Br. J. Pharmacol., 75, 154P.

α_2-ADRENOCEPTOR COUPLING TO ADENYLATE CYCLASE AND ADRENALINE-INDUCED AGGREGATION OF HUMAN FOETAL PLATELETS

C.R. Jones., J.L. Reid., I.W. Rodger[1] & M.A. Giembycz.[1] Department of Materia Medica, University of Glasgow, and Department of Physiology and Pharmacology,[1] University of Strathclyde, Glasgow.

Adrenaline (ADR)-stimulated human platelet aggregation is mediated through α_2-adrenoceptors. These receptors are negatively coupled to adenylate cyclase in human platelets such that their activation attenuates the cyclase activity, and, thus cyclic AMP levels within the platelets fall (Jakobs et al, 1976). This reduction in cyclic AMP, with subsequent lowering of the active/inactive cyclic AMP-dependent protein kinase ratio, is believed to trigger aggregation presumably through phosphatase-induced dephosphorylation of a specific platelet protein(s). Human fetal platelets do not aggregate in response to ADR (Mull & Hathaway, 1970), yet possess α_2-adrenoceptor binding sites. It has been recently suggested that this may represent an uncoupling of α_2-adrenoceptors from adenylate cyclase (Jones et al, 1985). This abstract describes the results from a study in which we examined this possibility.

Maternal venous and fetal cord blood was obtained at delivery by elective caesarean section under epidural anaesthesia. Cyclic AMP levels of whole platelet suspensions, following ethanol extraction, were assayed by competitive protein binding, and aggregation was assessed by the turbidometric method of Born (1962). Values in the table and text refer to the mean ± s.e.m. of six paired observations. Differences between means were determined non-parametrically using one-tailed Wilcoxon matched pairs signed-ranks test, and considered significant when $P < 0.05$. The results are summarised in table 1.

The inability of fetal platelets to aggregate in response to ADR (1-100 μM) was confirmed. The potentiation of adenosine diphosphate-induced aggregation by ADR (10 μM) was inhibited by prostaglandin E_1 (PGE_1; 4 μM). There was no significant difference between the cyclic AMP rises induced by PGE_1 in fetal (22.7 ± 6 fold) and maternal (15.1 ± 2 fold) platelets, although the maximum ADR-induced inhibition obtained in fetal (52 ± 3%) and maternal (67 ± 5%) platelets was significantly different ($P < 0.05$). ADR was more potent at reducing basal cyclic AMP levels than the elevated cyclic AMP levels induced by PGE_1 ($P < 0.05$). Phentolamine (10 μM) abolished ADR-induced cyclic AMP reduction in both fetal and maternal platelets.

Table 1. Effect of Adrenaline on Basal and PGE_1 Stimulated Cyclic AMP Formation in Human Fetal and Maternal Platelets.

SUBJECT	BASAL (pmol/mg protein)	PGE_1 (4 uM) (pmol/mg protein)	ADR-INDUCED INHIBITION IC_{50} (nM)	
			Basal	PGE_1
MATERNAL	9.41 ± 1.94	142.0 ± 33.9	130 ± 80	730 ± 260
FETAL	6.46 ± 2.14	106.1 ± 18.9	90 ± 80	580 ± 380

These results demonstrate that both PGE_1-receptors and α_2-adrenoceptors are functionally coupled to adenylate cyclase in human fetal platelets. Swart et al (1985) proposed that the inability of ADR to stimulate aggregation of platelets derived from a sub-group of patients suffering from proliferative myelomas was unrelated to a defect in the coupling of α_2-adrenoceptors to adenylate cyclase. Their results and ours may reflect an inability of the catalytic subunit of cyclic AMP-dependent protein kinase to reassociate with the regulatory subunit of the enzyme when the levels of cyclic AMP within the platelet fall.

Born, G.V.R (1962). J.Physiol.(Lond)., 194, 927-929.
Jakobs, K., et al. (1976). J.Cyclic Nucl.Res., 2, 381-392.
Jones, C.R., et al. (1985). Thromb.Haem., (in press)
Mull, M.M. & Hathaway, W.E. (1970). Paed.Res., 4, 229-237.
Swart, S.S., et al. (1985). Br.J.Pharmac., 84, 63P.

INFLUENCE OF CHOLESTEROL FEEDING ON ENDOTHELIUM-DEPENDENT VASOMOTOR RESPONSE IN RABBIT AORTIC STRIPS

Chappell, S.P., Griffith, T.M., Henderson, A.H., Lewis, M.J. Departments of Cardiology, Radiology and Pharmacology and Therapeutics, UWCM, Heath Park, CARDIFF.

Previous studies in rabbits and monkeys (Henry and Yokoyama, 1980, Yokoyama et al 1983; Heinstad et al 1984) have shown that both hereditary and induced hypercholesterolaemia result in increased constrictor responses to noradrenaline, 5-hydroxytryptamine (5HT) and ergometrine. One possible explanation might be reduced endothelium-derived relaxant factor (EDRF) activity. We have investigated this possibility.

New Zealand white rabbits (2-2.5 kg) were given a diet containing 2% cholesterol for 9-10 weeks. Control age and sex matched animals were fed a standard diet of Pilsbury modified SG1 ACS. After stunning the descending thoracic aorta was cut into rings 2-3 mm wide and endothelium removed by gentle abrasion from half the rings. Rings were mounted isometrically in Holman's solution at 37°C gassed with 95% O_2 , 5% CO_2 and equilibrated for 90 min at resting tension 1.2 gm. Drugs were added cumulatively. EC_{50} and maximum constrictor responses (MCR) were estimated by computer best fit. For acetylcholine (ACh)-induced relaxation, endothelialised strips were preconstricted with 5 HT (10^{-5}M). Results (±SEM) were compared using unpaired student's t test.

In control animals the cumulative dose responses were significantly different with and without endothelium, MCR for 5HT being 1526±109 (n=23) and 1251±82 mg(n=25) respectively ($p < 0.05$), and EC_{50} values 7.0 x 10^{-7}M and 1.1 x 10^{-7}M ($p < 0.01$). Responses to ergometrine were not significantly different with and without endothelium, MCR being 1894±185 (n=20) and 1689±155 mg (n=20) and EC_{50} values 7.6 x 10^{-7}M and 7.2 x 10^{-7} M respectively.

In cholesterol-fed rabbits the responses to 5HT and to ergometrine were similar with and without endothelium, MCR being 1463±115 and 1300±90 mg (n=34) with and without endothelium and 1818±182 (n=20) and 1798±168 (n=20) respectively, EC_{50} 10^{-6}M and 2.2 x 10^{-6}M respectively.

ACh-induced endothelium-dependent relaxation in preparations preconstricted with 5HT (10^{-5}M) was significantly greater in strips from control than from cholesterol animals at all concentrations used (e.g. at 10^{-6}M 70±5% cf. 10±5% n=23, $p < 0.001$).

Maximum responses to 5HT or ergometrine, with or without endothelium, did not differ between preparations from control and cholesterol fed animals, implying similar smooth muscle constrictor responses.

Thus cholesterol feeding reduces both basal and stimulated EDRF-mediated activity, although the effect was demonstrable only to 5HT and not to ergometrine. These findings are consistent with a recent brief report of Habib et al (1984). EDRF is shortlived and increased diffusional distance between endothelium and smooth muscle could account for the observation. Unlike previous studies we were unable to demonstrate greater constrictor responses to ergometrine in deendothelialised preparations even at low concentrations (Henry and Yokoyama 1980; Yokoyama et al, 1983).

Henry, P.D., Yokoyama, M. (1980) J. Clin. Invest. 66, 306-313.
Yokoyama , M., Akita, H., Mizutoni, T., Fukuzaki, H., Watanabi, Y. (1983) Circ. Res. 53 , 63-71.
Heinstad, D.D., Armstrong, M.L., Marcus, M.L., Piegors, D.J., Mark, A.L. (1984) Circ. Res. 54, 711-718.
Habib, J.B., Wells, S.T., Williams, C.L., Henry, P.D. (1984) Circ. 70, Abstract No.490

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ENDOTHELIUM-DEPENDENT RELAXATIONS IN ISOLATED ARTERIES OF CONTROL AND HYPERCHOLESTEROLEMIC RABBITS

M.-C. Coene, A.G. Herman, F. Jordaens, C. Van Hove, T.J. Verbeuren*, L. Zonnekeyn, Pharmacol Div. , Departments of Medicine and Pharmacy, Universitaire Instelling Antwerpen, University of Antwerp, B-2610 Wilrijk, Belgium

A variety of vasodilator substances evoke relaxations of isolated blood vessels by releasing the endothelium-derived relaxant factor (EDRF) from the endothelial cells (Furchgott & Zawadski 1980; De Mey & Vanhoutte 1981; Furchgott 1983). It has been speculated that dysfunction or loss of endothelium in atherosclerosis may impair the release of EDRF thereby augmenting vasoconstrictor responses (Vanhoutte 1983; Heistad et al. 1984).

The aim of the present study was to compare endothelium-dependent relaxations in four arteries (aortic arch, brachiocephalic artery, pulmonary artery and abdominal aorta) obtained from control and hypercholesterolemic rabbits. Hypercholesterolemia was induced by feeding the rabbits a cholesterol-rich (0.3%) diet during 8 or 16 weeks. Segments of the isolated arteries were mounted in organ chambers for isometric tension recording. In some abdominal aortas the release of EDRF was measured using a bio-assay technique (Griffith et al. 1984).

In the hypercholesterolemic arteries, the contractile responses to acetylcholine (ACh) and prostaglandin $F_{2\alpha}$ were not significantly altered; those to serotonin were augmented in the most severely affected tissue, the aortic arch. In the aortic arch, the brachiocephalic artery and the pulmonary artery, the contractions caused by noradrenaline and clonidine were significantly decreased; they were not altered in the artery the least affected by the hypercholesterolemia: the abdominal aorta.

The relaxations to ACh and ATP were markedly reduced in arteries denuded of the endothelium;those to nitroglycerin were endothelium-independent. Hypercholesterolemia reduced the relaxations to ACh and ATP; the reduction was more pronounced after 16 than after 8 weeks high cholesterol diet and was dependent on the degree of fatty streak formation in the tissues (e.g. the relaxation to 10^{-6} 92 $\pm$ 3 % in control arteries and 14 $\pm$ 8 % after 16 weeks hypercholesterolemia in aortic arches; in abdominal aortas, these values averaged 97 $\pm$ 1% and 57 $\pm$ 7% respectively). The relaxations to nitroglycerin were either not or only moderately affected by the hypercholesterolemia.

ACh evoked release of EDRF in control and 16 weeks hypercholesterolemic abdominal aortas; no significant difference between both groups was noted (e.g. 22 $\pm$ 4% relaxation of bio-assay tissue evoked by perfusate from control and 21 $\pm$ 4% evoked by perfusate from hypercholesterolemic aortas).

We conclude that in arteries obtained from hypercholesterolemic rabbits :
(1) the contractile responses to alpha-adrenergic agonists are reduced while those to serotonin tend to be augmented.
(2) the endothelium-dependent relaxations to ACh and ATP are markedly reduced; in abdominal aortas, this is not due to a decreased EDRF-release.

Our results indicate that reduced endothelium-dependent relaxations in hypercholesterolemic arteries probably result from the thickening of the intimal layer which then prevents the EDRF from reaching the vascular smooth muscle cells.

De Mey, J.G. & Vanhoutte, P.M. (1982) Circulation Res. 51, 439-447.
Furchgott, R.F. (1983) Circulation Res. 53, 557-573.
Furchgott, R.F. & Zawadski, J.V. (1980) Nature 288, 373-376.
Griffith, T.M., et al. (1984) Nature 308, 645-647.
Heistad, D.D., et al. (1984) Circulation Res. 54, 711-718.
Vanhoutte, P.M. (1983) Fed. Proc. 42, 233-237.

INHIBITION OF HUMAN GASTRIC MUCOSAL THROMBOXANE SYNTHESIS BY WHR2348A

Baskar N K, Filipowicz B, Hawkey C J*, Department of Therapeutics, University Hospital, Nottingham. NG7 2UH

WHR2348A (1-cyano-3-(3-(5 isoquinolyloxy)propyl)-2-methyl-pseudothiourea methane sulphonate) has previously been shown to protect the gastric mucosa against the macroscopic signs of damage caused by hydrochloric acid, alcohol or sodium hydroxide (W H Rorer Inc. Personal communication). Since the mode of action of WHR2348A has hitherto been unknown its affects on synthesis of immunoreactive prostaglandin E_2 ($iPGE_2$) and thromboxane B_2 ($iTXB_2$) have been investigated using human gastric mucosa in vitro.

Intact human gastric mucosa was obtained at partial gastrectomy from 5 patients. Small fragments of mucosa were dissected from underlying sub-mucosa and frozen at -50 °C until used (within one week). After thawing, the mucosa was homogenised on ice for 15 seconds in Tris HCl 0.05M, pH 7.4 using an Ultra Turax homogeniser, and divided into aliquots (final tissue content 20 mg wet weight, volume 1 ml). The aliquots were pre-incubated on ice with or without WHR2348A for 15 minutes. Aliquots were then incubated in duplicate at 37 °C for 30 minutes with arachidonic acid, 2 ug (6.6×10^{-6}M, final concentration). The reaction was stopped by placing the tubes on ice and adding BW755C 100 ug/ml, (3.8×10^{-4}M, final concentration) before centrifuging for two minutes at 10,000 G to remove tissue debris. Specific radioimmunoassays were used to measure $iPGE_2$ and $iTXB_2$ in unextracted supernatant.

Neither arachidonic acid nor WHR2348A affected the radioimmunoassay blank values. Increments of $iPGE_2$ or $iTXB_2$ caused by adding authentic prostanoids to samples were quantitated accurately. The effects of WHR2348A on $iPGE_2$ and $iTXB_2$ synthesis were expressed as per cent control after allowance for synthesis during homogenisation. WHR2348A caused dose dependent inhibition of $iTXB_2$ synthesis (by 56 $\pm$ 7%, mean and SEM, at 10^{-6}M, by 63 $\pm$ 8% at 4.8×10^{-6}M and by 75 $\pm$ 3% at 3.2×10^{-5}M, n = 5, $p < 0.01$ for all doses compared with control, $p < 0.05$ for 10^{-6}M compared with 3.2×10^{-5}M, analysis of variance/t test). Synthesis of $iPGE_2$ was 117 $\pm$ 13% control at 10^{-6}M, 139 $\pm$ 24% control at 4.8×10^{-6}M and 135 $\pm$ 20% control at 3.2×10^{-5}M. These values for $iPGE_2$ were not significantly different from control.

These data suggest that WHR2348A acts as an inhibitor of gastric mucosal thromboxane synthesis. WHR2348A should be investigated for possible gastric mucosal protection in man. The possibility that it may enhance PGE_2 synthesis pari passu with its inhibition of thromboxane synthesis, although not clearly established in this work, is also worth further investigation.

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NO EVIDENCE FOR AN ENDOTHELIAL COMPONENT IN THE RELAXANT EFFECT OF HYDRALAZINE ON RAT AORTA

G.R. Bullock[1], S.G. Taylor[2*] and A.H. Weston[2]. [1]Ciba Geigy Research Centre, Wimblehurst Road, Horsham, Sussex RH12 4AB and [2]Department of Pharmacology, Medical School, University of Manchester, Manchester M13 9PT.

Hydralazine (Hyd) has been used in the treatment of hypertension for over 30 years and yet its precise mechanism of action is still unknown. In vivo Hyd is a potent antihypertensive drug, the final plasma Hyd concentration correlating well with its antihypertensive effect (Zacest and Koch-Weser, 1972). In vitro, however, some groups have found it necessary to use high (mM) concentrations of Hyd to achieve vascular relaxation (e.g. Mclean et al, 1978).

Furchgott and Zawadski (1980) showed that acetylcholine(ACh)-induced relaxation in blood vessels was dependent on the presence of the vascular endothelium. Subsequently Spokas et al (1983) reported that in rabbit aorta the relaxant effect of Hyd (<1μM) was endothelium-dependent whilst at higher concentrations (>1μM) an endothelium-independent mechanism existed. In the present study the importance of the vascular endothelium in the relaxant effect of Hyd on rat aorta has been investigated.

Pairs of aortic rings from male Wistar rats (300-450g) were opened to form transverse strips one of which was gently rubbed to remove the endothelium. Each strip was set up in a 20 ml tissue bath containing Krebs bicarbonate solution at 37°C gassed with 95% O_2/5% CO_2 and attached to an isometric transducer under 1g tension. After inducing contraction with noradrenaline (NA:20nM), cumulative challenge with ACh (10nM-10μM) induced dose-dependent relaxations only in those preparations with an intact endothelium. One hour later the tissues were again exposed to NA (20nM) and challenged cumulatively with Hyd (15μM-1mM at 10-15 min intervals). Hyd produced relaxations only at concentrations >100μM. These responses were not significantly different in tissues with or without endothelium. After a further hour, the tissues were rechallenged with NA (20nM) and the ACh dose-response experiment repeated. ACh once again produced significant dose-dependent relaxations only in preparations with an intact endothelium.

In a second series of experiments, tissues were pre-incubated with Hyd (10μM-1mM) for 40-50 min before challenging first with NA and then with ACh. Again only high concentrations of Hyd (>100μM) produced a significant reduction of the NA (20nM)-induced contraction with ACh inducing relaxations in tissues with an intact endothelium.

In a third series of experiments, tissues were superfused with Krebs solution and contracted by continuous injection of NA into the flowing solution to give a final NA concentration of 20nM. When the contraction was established, Hyd was also injected into the superfusing solution to give a final concentration of 10-100μM. At these concentrations, Hyd failed to produce significant relaxations of the tissues whereas ACh (10nM-10μM) produced relaxations in tissues with an intact endothelium.

It is concluded that the presence of an intact endothelium plays no role in the relaxant effect of Hyd on rat aorta.

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Furchgott, R.F. & Zawadski, J.V. (1980) Nature 288, 373-376.
Mclean, A.J. et al (1978) J.Pharmacol.Exp.Ther. 207, 40-48.
Spokas, E.G. et al (1983) Hypertension 5(2), I-107-111.
Zacest, R. & Koch-Weser, J. (1972) Clin.Pharmacol.Ther. 13, 420-425

LEUKOTRIENES C_4 AND D_4 DECREASE INTRACRANIAL BLOOD FLOW IN THE ANAESTHETISED PIG

Priscilla J. Piper & A.W.B. Stanton*, Department of Pharmacology, Royal College of Surgeons, Lincoln's Inn Fields, London, WC2A 3PN.

Leukotrienes (LTs) C_4 and D_4 are active vasoconstrictors in several vascular beds of the anaesthetised pig (see Piper et al., 1984). Furthermore cysteinyl LTs and LTB_4 are generated from porcine blood vessels in vitro on incubation with calcium ionophore A23187 (Piper & Galton, 1984).

We have examined the effects of infusion of LTC_4, D_4 and another vasoconstrictor, arginine vasopressin (Pitressin; AVP) on intracranial blood flow in the pig and measured the generation of LT-like material from cerebral arteries in vitro.

Ten female pigs (29-39 kg) were sedated with azaperone (Suicalm, Janssen) and anaesthetised with metomidate (Hypnodil, Crown Chemicals) given i.v. The animals were placed on a heating pad, intubated through a tracheotomy incision and ventilated with room air. Systemic arterial BP, ECG, heart rate and rectal temperature were recorded. Blood gases were closely monitored and PCO_2 was maintained at 5.61 $\pm$ 0.60 kPa (SD). The left common carotid artery (CCA) and its branches were isolated. The thyroid artery was cannulated for administration of drugs and measurement of perfusion pressure (PP). All remaining branches except the internal carotid artery (ICA) were ligated. Extracranial facial and scalp soft tissue were resected ipsilaterally. ICA blood flow was recorded from the CCA using an electromagnetic flow cuff. LTC_4, LTD_4 and AVP were each infused for two-minute periods. The brains were removed post mortem and the larger cerebral arteries dissected free. The vessels were chopped and incubated with A23187 and the LT-like material generated assayed on guinea-pig ileum smooth muscle.

LTC_4, LTD_4 (1, 3 and 9 x 10^{-8} mol min^{-1}) and AVP (2 x 10^{-4} - 2 x 10^{-1} units min^{-1}) produced dose-dependent reductions in ICA flow. LTC_4 and D_4 caused long-lasting effects (up to 25 min) and had steep dose-response relationships over the range tested. LTC_4 was significantly more potent than LTD_4 at the highest dose (means $\pm$ SEM were 77.9 $\pm$ 5.9% [n=4] vs. 47.2 $\pm$ 4.4% [n=3] respectively, $p < 0.02$). BP and PP were raised during LT infusion. AVP (2 x 10^{-1} units min^{-1}, approximately 4.5 x 10^{-10} mol min^{-1}) produced a 52.0 $\pm$ 5.7% reduction in ICA flow [n=3]. The cerebral arteries generated LT-like material in quantities equivalent to 174.0 $\pm$ 17.5 ng g^{-1} LTD_4 [n=5]. This is more than is generated by other blood vessels so far studied.

These results show that exogenous LTC_4 and LTD_4 can decrease intracranial blood flow, which probably reflects active cerebral vasoconstriction. If generation of LTD_4-like substance(s) from the cerebral arteries occurs in vivo, LTs must be considered as possible mediators of pathological cerebral vasospasm in man.

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Piper, P.J. and Galton, S.A. (1984) Generation of leukotriene B_4 and leukotriene E_4 from porcine pulmonary artery. Prostaglandins 28, 905-914.

Piper, P.J., Stanton, A.W.B., McLeod, L.J., Galton, S.A. & Letts, L.G. (1984) Actions of leukotrienes in the circulation. IUPHAR 9th Int.Cong.Pharmac., Vol.3, 63-67P, eds. W. Paton, J. Mitchell and P. Turner, Macmillan Press Ltd., London.

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TEMPORAL STUDIES OF THE FORMATION OF CYCLO-OXYGENASE AND LIPOXYGENASE METABOLITES IN YEAST-INDUCED INFLAMMATION

F. Carey* and D. Haworth (Introduced by M.J. Rance), ICI Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.

The yeast-inflamed rat paw model has been used extensively to evaluate the analgesic potential of NSAIDs. While prostaglandins, in particular PGE_2, appear to mediate yeast-induced hyperalgesia, the role (if any) of lipoxygenase metabolites in this model remains less clear. We previously identified and characterised by RPHPLC the immunoreactive LTB_4 present in yeast-inflamed paw exudates (Haworth and Carey, 1984) and have now undertaken a temporal study to include the radioimmunoassay and characterisation of immunoreactive PGE_2, TXB_2 and LTB_4 in the yeast-induced lesion up to 24hr post injection. Immunoreactive PGE_2 and TXB_2 were characterised by extraction of paw exudates using the procedure of Powell (1981) and separated by RPHPLC using a Spherisorb 5ODS column eluted with 32.5% (v/v) acetonitrile in aqueous phosphoric acid (pH2.0). Approx. 90% of immunoreactive PGE_2 and TXB_2 co-eluted with their respective [^{3}H] standards. Female rats (approx 60g) were given subplantar injections (0.1ml) of 20% (w/v) brewer's yeast (right paw) or saline (left paw). Before and up to 24hr after injection, the extent of hyperalgesia and paw oedema was determined. At 0.5,1,2,4,6,8 and 24hr paw exudate was obtained by lavage with 0.2ml saline and LTB_4, PGE_2 and TXB_2 measured by direct radioimmunoassay. In separate experiments, paws were fixed in 10% (v/v) formal saline for histology.

Paw exudate LTB_4 reached a maximum of 20.5 ± 3.9ng ml^{-1} (mean ± sem, n=12) 4hr after yeast injection but reduced to 4.2 ± 0.7ng ml^{-1} (n=6) by 6hr and was little changed thereafter. In contrast, PGE_2 and TXB_2 were elevated to 5.4 ± 0.9ng ml^{-1} and 21.6 ± 7.3ng ml^{-1} respectively (n=12) at 4hr and further increased to 47.3 ± 9.0ng ml^{-1} and 69.3 ± 19.3ng ml^{-1} (n=6) at 24hr. In saline injected paws, levels of immunoreactive eicosanoids were close to the detection limit of the radioimmunoassays (0.5 to 1.0ng ml^{-1}). Elevation of LTB_4, PGE_2 and TXB_2 correlated with the development of hyperalgesia and invasion of yeast bodies by PMN (2-4hr) but not with the rapid onset of oedema (maximal at 30 min). Although by 24hr hyperalgesia, oedema and LTB_4 levels were reduced, both PGE_2 and TXB_2 levels remained elevated. Accordingly, measurement of inflammation, hyperalgesia and paw exudate eicosanoids, at 4hr post injection in the yeast inflamed rat paw model allows the bioavailability of novel agents that inhibit eicosanoid biosynthesis to be measured.

Haworth, D. and Carey, F. (1984) In Inflammatory Mediators edited by G.A. Higgs and T. Williams, in press.
Powell, W.S. (1980) Prostaglandins 20, 947-957.

INDOMETHACIN-INDUCED GASTRO-INTESTINAL LESIONS: PROTECTION BY SALICYLATE AND RELATIONSHIP TO PLASMA ACUTE PHASE PROTEINS

M.E.J. Billingham, F. Carey, J.W. Growcott* and C.L. Hughes, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG

Plasma acute phase proteins (APP) have been demonstrated to be elevated following tissue damage due to a variety of injurious substances (Darcy, 1968). Moreover, a correlation between indomethacin (IND)-induced gastrointestinal tract (GIT) toxicity and elevation of APP has been previously reported in the rat (Billingham and Tucker, 1979). We now describe changes in plasma levels of an APP-haptoglobin in the mouse following treatment with IND and the correlation between the inhibitory effects of sodium salicylate (SAL) on IND-induced GIT toxicity (Ezer et al., 1976) with reduction in elevated APP levels.

Preliminary experiments using female Alderley Park/CBU mice fasted for 24 hrs demonstrated that IND given as a single oral dose at 2, 4, 8 or 16mg kg^{-1} caused a dose-related increase in plasma APP levels, significant rises being detected as early as 12 hrs post administration, these levels returning to baseline by 240 hrs. In subsequent experiments 8mg kg^{-1} IND was used as this produced a reproducible sub-maximal rise in APP levels. Thus IND was given as a single oral dose 30 min after carboxymethylcellulose (CMC) 1% w/v or SAL 200mg kg^{-1} p.o. Two further groups of mice received SAL 200mg kg^{-1} or CMC vehicle as single oral doses. Following treatment at 12, 24, 48 and 72 hrs animals were tail bled into heparinized tubes and the plasma assayed for haptogolbin by radial immunodiffusion assay. Cyclo-oxygenase activity was then determined following incubation of blood with 10µg ml^{-1} A23187 and subsequent radioimmunoassay of TXB_2. At post morten, the whole GIT was examined macroscopically for evidence of damage (e.g. Arrigoni et al., 1984).

Increases in haptoglobin correlated well with the degree of macroscopic damage which were both reduced in IND-dosed mice pretreated with SAL. Prior administration of SAL reduced APP levels by 54%, 71%, 74% and 35% (all $p<0.001$ Student's t-test) and lesion scores by 71%, 32%, 100% and 100% at 12, 24, 48 and 72 hrs respectively. Moreover, in mice pretreated with SAL, IND was able to produce maximal inhibition of serum TXB_2 throughout the duration of the experiment. These data support the known cytoprotective action of SAL on IND-induced GIT toxicity (Ezer et al., 1976) and indicate that measurement of plasma APP may have utility in the non-invasive assessment of GIT toxicity.

Arrigoni, C. et al (1984) Prostaglandins Leukotrienes and Medicine 15, 79
Billingham, M.E.J. and Tucker, M.E. (1979) Br. J. Pharmac. 67, 450P
Darcy, D.A. (1968) Br. J. Exp. Pathol. 49, 614
Ezer, E. et al. (1976) J. Pharm. Pharmacol. 28, 655

AH6809, A PROSTANOID EP_1 RECEPTOR BLOCKING DRUG

R.A. Coleman, I. Kennedy & R.L.G. Sheldrick*, Department of Respiratory Pharmacology & Biochemistry, Glaxo Group Research, Ware, Herts, SG12 0DJ.

Few truly specific, competitive prostanoid receptor blocking drugs have been reported, but one example is SC-19220 (Sanner, 1969) which blocks some but not all receptors sensitive to PGE_2, these SC-19220-sensitive receptors being termed EP_1 receptors (Kennedy et al., 1982). The usefulness of SC-19220 is however limited by low potency and poor solubility. We now report on another prostanoid antagonist, AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid), which may also be an EP_1-receptor blocking drug, but is more potent than SC-19220 and is water soluble.

AH6809 has been compared with SC-19220 for antagonist activity against the agonist actions of prostanoids and, where appropriate, acetylcholine (ACh) in some smooth muscle preparations containing receptors sensitive to PGE_2 (EP_1- and EP_2-receptors), $PGF_{2\alpha}$ (FP-receptors) and TxA_2 (TP-receptors) (Kennedy et al., 1982). The methods are as described in Kennedy et. al., (1982).

Table 1 pA_2 values for AH6809 and SC-19220 against prostanoids and ACh on some isolated tissues

Preparation	Agonist	Receptor	AH6809		SC-19220	
			pA_2 (95% C.L.)	slope	pA_2 (95% C.L.)	slope
guinea-pig	PGE_2		6.8 (6.5-7.3)	1.02	5.4 (5.3-5.5)	1.24
ileum	$PGF_{2\alpha}$	EP_1	6.9 (6.5-7.5)	0.66+	5.3 (5.2-5.5)	0.80+
	ACh		<5.0	-	<4.0	-
guinea-pig	PGE_2		6.6 (6.4-6.8)	0.86	5.6 (5.4-5.9)	1.12
fundus	$PGF_{2\alpha}$	EP_1	6.6 (6.4-7.1)	0.78	5.2 (5.1-5.4)	0.93
	ACh		<5.0	-	<4.0	-
dog	PGE_2		6.6 (6.5-6.9)	1.00	5.6 (5.4-6.1)	0.94
fundus	$PGF_{2\alpha}$	EP_1	6.5 (6.1-6.9)	0.99	5.4 (5.3-5.5)	1.35
	ACh		<5.0		<4.0	-
cat trachea	PGE_2	EP_2	<5.0	-	<4.0	-
chick ileum	PGE_2	EP_2	<5.0	-	<4.0	-
dog iris	$PGF_{2\alpha}$	FP	<5.0	-	<4.0	-
guinea-pig lung	U-46619	TP	<5.0	-	<4.0	-

numbers of determinations >3. + slope differs significantly from unity

The results summarised in Table 1 show that on the preparations studied, AH6809 has a profile of action identical to that of SC-19220. Thus they both appear to act as specific, competitive prostanoid antagonists on preparations containing EP_1-receptors, but are both weak or inactive on those containing EP_2-, FP- or TP-receptors. AH6809 is however consistently more potent (10-40 fold) than SC-19220. Hopefully, AH6809 will prove a useful tool in future studies on prostanoid receptor classification.

Kennedy, I. et. al. (1982). Prostaglandins, 24, 667.
Sanner, J.H. (1969). Arch. int. Pharmacodyn., 180, 46.

M. Rampart* and T.J. Williams, Vascular Biology Section, MRC Clinical Research Centre, Harrow, Middlesex.

Intradermal injection of chemotactic factors such as C5a, f-met-leu-phe and leukotriene B4 induces increased microvascular permeability in the rabbit by triggering a rapid interaction between PMN-leukocytes and venular endothelial cells (Wedmore & Williams, 1981). Oedema formation induced by chemotactic agents is dramatically enhanced by locally-injected vasodilator prostaglandins. The mechanisms involved in the phenomenon of PMN-dependent increased permeability are unknown, but one possibility is that PMN's are triggered to release oxygen radicals. H_2O_2 has been shown to trigger PGI_2 production by vascular endothelial cells in vitro (Ager & Gordon, 1984). Further, chemotactic factors have been shown to stimulate PGI_2 production from endothelial cells when in contact with PMN's; a response which is blocked by catalase (Rampart, Jose & Williams, 1985). PGI_2 can inhibit PMN function (including H_2O_2 production) and may thus provide negative feedback control.

Our current experiments are aimed at evaluating the following hypothesis:

a) that local extravascular chemotactic agents can trigger PMN's in contact with endothelial cells to release H_2O_2;
b) that H_2O_2 stimulates endothelial cells to increase permeability and release PGI_2, and
c) that released PGI_2 down regulates PMN's and consequently permeability.

The role of H_2O_2 was investigated by testing the effect of catalase on PMN-dependent plasma leakage. Attenuation of PMN function in vivo by prostaglandins was investigated by treating the animals systemically with a stable protaglandin analog, 15-methyl-PGE_1. Zymosan (100µg), rabbit C5a des Arg (5.10^{-11}moles), bradykinin (10^{-10}moles), each with or without PGE_2 (3.10^{-10}moles) were injected intradermally in the dorsal skin of rabbits. Plasma leakage was measured over a 30 min period as the local accumulation of i.v. injected ^{125}I labelled serum albumin. Ten minutes before intradermal injections, animals were systemically treated with saline (1 ml/kg, n=6), catalase (220,000 U/kg, iv, n=6) or 15-methyl-PGE_1, (300 ng/kg, s.c., n=6).

Systemic treatment with 15-methyl-PGE_1 and catalase significantly ($P<0.05$, Wilcoxon test) reduced PMN-dependent plasma leakage (exudation, controls: Zymosan + PGE_2 = 59 ± 12µl, C5a des Arg + PGE_2 = 97 ± 15µl, 15-methyl-PGE_1: Zymosan + PGE_2 = 26 ± 9µl, C5a des Arg + PGE_2 = 36 ± 11µl, catalase; Zymosan + PGE_2 = 29 ± 6µl, C5a des Arg + PGE_2 = 23 ± 5µl). The response to bradykinin + PGE_2 (whose action does not involve PMN-leukocytes) was not affected by either 15-methyl-PGE_1 or catalase (exudation induced by bradykinin + PGE_2, controls = 94 ± 15µl, 15-methyl-PGE_1 = 88 ± 15µl, catalase = 103 ± 13µl). At higher doses of 15-methyl-PGE_1 all responses were suppressed as has been observed previously (Fantone et al, 1980). This may be caused by a fall in systemic blood pressure.

Our data suggest: I) that H_2O_2, or peroxide derived products may be important for PMN-mediated plasma exudation in vivo; II) that prostaglandins of the E-type may have either pro- or anti-inflammatory properties depending on whether they act systemically or locally.

Ager, A. & Gordon, J.L. (1984). J. Expt. Med. 159: 592-603.
Fantone, J.C., Kunkel, S.L., Ward, P.A. & Zurier, R.B. (1980). J. Immunol. 125: 2591-2596.
Rampart, M., Jose, P.J. & Williams, T.J. (1985). Agents & Actions (in press).
Wedmore, C.V. & Williams, T.J. (1981). Nature, 289: 646-650.
Williams, T.J. & Jose, P.J. (1981). J. Exp. Med. 153: 136-153.

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EFFECT OF INHIBITORS OF PROSTAGLANDIN (PG) INACTIVATION ON PG SYNTHESIS FROM EXOGENOUS AND ENDOGENOUS ARACHIDONATE IN LUNG

Y.S. Bakhle and J.J. Pankhania, Department of Pharmacology, Royal College of Surgeons, London, U.K.

The lung is capable of metabolizing arachidonic acid(AA) to a variety of biologically active cyclooxygenase products(COP) including prostaglandins(PG) and thromboxanes(Tx). The lung will also metabolize these PGs and Tx to inactive forms. It seemed reasonable therefore that inhibitors of PG inactivation should increase the net amount of biologically active COP formed by lung. We have tested this hypothesis in rat and guinea pig isolated lungs perfused with Krebs solution, using two inhibitors of PG inactivation, dipyridamole (Uotila and Mannisto, 1981) and nafazatrom (Wong et al., 1982).

Inactivation of PGE_2 was measured by bioassay on the rat stomach strip superfused with lung effluent. Synthesis of COP from exogenous AA was measured similarly, expressing COP in lung effluent as ng PGE_2 equivalents. Each function was assayed before (control) and after drug treatment. Drugs were infused for 10min before and during the second assay at a single concentration which gave clear inhibition of PG inactivation (dipyridamole, 10uM and nafazatrom, 37uM). The results of the bioassays are given in the Table (mean±s.e.mean; n=4-8 lungs).

	PGE_2 survival (%injected dose)		COP synthesis (ng PGE_2 eq/ug AA)	
	Rat	Guinea-pig	Rat	Guinea-pig
Control	4±1	2±1	1.3±0.1	14±3
Dipyridamole	22±7	42±10	1.0±0.1	15±4
Nafazatrom	47±5	22±7	1.0±0.1	6.8±0.2

Although the drugs markedly increased PGE_2 survival in either rat or guinea pig lung, neither increased the amounts of bioassayable COP formed from exogenous AA. We also estimated amounts of 6-oxo-$PGF_{1\alpha}$ in the lung effluent, by radioimmunoassay. In the presence of either drug, no change in 6-oxo-$PGF_{1\alpha}$ formation from exogenous AA was observed in rat lungs and in guinea pig lungs, only nafazatrom decreased formation to 31±7% of control values.

However, 6-oxo-$PGF_{1\alpha}$ synthesis from endogenous AA stimulated by the calcium ionophore, A23187, was increased during infusion of dipyridamole or nafazatrom. In rat lung, 6-oxo-$PGF_{1\alpha}$ levels increased to 522±188% and 770±190% with dipyridamole or nafazatrom respectively and, in guinea pig lung, increases to 413±115% and 303±77% respectively were observed (all control values set at 100%).

Clearly, the effects of these drugs on PGI_2 production depend on the mechanisms involved in stimulating that production. There are important differences in the enzymic environment for exogenous and endogenous AA, in terms of COP synthesis in lung (Bakhle et al., 1985). Our present results suggest an equivalent variation in the effects of drugs acting on the metabolic pathway of COP thus formed.

Bakhle, Y.S., Moncada, S., de Nucci,C. & Salmon,J.A. (1985) Br. J. Pharmac. 84, 34P..
Uotila, P. and Mannisto, J. (1981) Prostaglandins, 21, 413-416.
Wong, P.P.-Y., Chao, P.H.-W. and McGiff, J.C. (1982) J. Pharm. exp. Therap. 223, 757-760.

EICOSANOID PRODUCTION DURING THE DEVELOPMENT OF ANTIGEN-INDUCED CHRONIC ARTHRITIS

Prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) are found in the synovial fluid of patients with established rheumatoid arthritis and it is likely that these eicosanoids contribute to the pathology of this disease.

We have now investigated the synthesis of eicosanoids following the induction of immune arthritis in rabbits (Dumonde& Glynn, 1962). New Zealand White or Old English rabbits were sensitized to ovalbumin and challenged by injection of antigen into the knee joint of one hind limb. The contralateral joint received a similar injection of saline. Animals were killed 1-16 days after challenge and joint fluids were collected from both knees by washing the joint space with saline. Samples of synovial lining were removed from each joint and maintained for 24h in non-proliferative organ culture (Poulter et al., 1970). The concentrations of PGE_2, LTB_4, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 (TXB_2) in joint washes and organ culture fluids were determined by specific radioimmunoassays (Salmon et al., 1983).

Antigen-challenged joints developed an arthritic response which was sustained until the animals were killed and did not occur in the control joints. Joint fluids collected 24h after induction of arthritis contained 10.5 ± 2.5 ng/ml immunoreactive PGE_2 (mean ± s.e. mean) and 0.23 ± 0.16 ng/ml immunoreactive LTB_4. After 12-16 days PGE_2 concentrations in arthritic joint fluids had fallen to about 0.5 ng/ml and LTB_4 was undetectable (<0.05 ng/ml). At 24h control joint fluids contained 0.4 ± 0.2 ng/ml PGE_2 but no detectable LTB_4. From 5-16 days there was no detectable PGE_2 or LTB_4 in the control joint fluids. The concentrations of PGE_2 found in the joint washes from arthritic joints were similar to those reported by Blackham et al. (1974).

Prostaglandin production in cultures of synovial explants from arthritic joints increased progressively from 1-16 days reaching peaks of 14.2 ± 3.6 ng PGE_2 per mg wet weight of tissue and 1.8 ± 0.38 ng/mg immunoreactive 6-keto-$PGF_{1\alpha}$. There was a lower production of immunoreactive TXB_2 (0.35 ± 0.021 ng/mg) and LTB_4 (0.0091 ± 0.0023 ng/mg). Interestingly, in tissues taken 24h after antigen challenge, the production of PGE_2 and 6-keto-$PGF_{1\alpha}$ by control tissues was significantly higher than in arthritic tissues. In explants taken at 5-16 days, however, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesis in control tissues declined to approximately 20% of arthritic values. The synthesis of TXB_2 and LTB_4 by arthritic synovium was significantly greater than that of control tissues at all the sampling times.

These results show that in an experimental model of chronic arthritis the synovial lining has an elevated capacity for eicosanoid synthesis and that the development of the response is accompanied by the production of prostaglandins, thromboxanes and leukotrienes.

Blackham, A., Farmer, J.B., Radziwonik, H. & Westwick, J. (1974). Br. J. Pharmac., 51, 35-44.
Dumonde, D.C. & Glynn, L.E. (1962) Brit. J. Exp. Path., 43, 373-383.
Poulter, L.W., Bitensky, L., Cashman, B. & Chayen, J. (1970) Virchows Arch. 4, 303-309.
Salmon, J.A., Simmons, P.M. & Moncada, S. (1983) J. Pharm. Pharmacol., 35, 808-813.

HISTAMINE AND PEPTIDOLEUKOTRIENE RELEASE FROM IMMUNOLOGICALLY CHALLENGED GUINEA-PIG LUNG

C.T. Dollery, D.J. Heavey, R. Richmond*, G.W. Taylor & J. Vial, Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 OHS.

Leukotrienes C_4 and D_4 (LTC_4, LTD_4) are lipoxygenase metabolites of arachidonic acid with potent inflammatory effects. To help define their biological role, a specific and sensitive method for quantitation in biological fluids is required. We have developed an assay using high pressure liquid chromatography (HPLC) and radioimmunoassay (RIA) for measurement of LTC_4 and LTD_4 and have applied it to measure these leukotrienes in guinea-pig lung perfusates. Five guinea-pigs were sensitised with ovalbumin, their lungs removed and perfused at 10 ml/min with Krebs solution. Perfusate (90 ml) was collected before and after challenge with ovalbumin. Internal standards (^{3}H-LTC_4, ^{3}H-LTD_4) were added to aliquots (15 ml), the pH adjusted to between 7.0 and 7.5 and the sample loaded in stages onto a Brownlee C_{18} pre-column using a 5 ml injection loop. When the complete sample had been loaded, HPLC mobile phase was diverted through the pre-column, delivering the leukotrienes directly onto the C_{18} analytical column. A gradient of methanol:water:acetic acid was used to elute leukotrienes from this second column. LTC_4 and LTD_4 were widely separated on HPLC with retention times of 26 and 48 minutes respectively. Fractions were collected and a small aliquot counted to locate the internal standards. Those fractions containing LTC_4 and LTD_4 were vacuum dried and resuspended in RIA buffer. An aliquot was counted for estimation of final recovery and the remainder used for RIA using an antibody with a high affinity for LTC_4 and LTD_4 (Hayes et al, 1983). The RIA allows sample size of 150 µl, uses ^{3}H-LTC_4 as the radioactive ligand and dextran/charcoal for phase separation; 50% inhibition of binding occurs with 106 pg of LTC_4 and 258 pg LTD_4 (n = 3). Separate standard curves were constructed for both leukotrienes. Histamine was measured using a double isotope radioenzymatic method (Brown et al, 1982). Mean overall recoveries of LTC_4 and LTD_4 were 20.1% and 23.1% respectively. The results are summarised in Table 1.

Table 1 LTC_4, LTD_4 and histamine release from immunologically challenged, ovalbumin sensitised guinea-pig lung (ng/90 ml perfusate).

	LTC_4		LTD_4		Histamine	
Animal No.	pre-	post-	pre-	post-	pre-	post-
1	2.5	4.6	5.4	79.8	20	3807
2	1.8	3.5	7.0	12.1	22	680
3	2.9	3.0	12.8	18.7	38	2133
4	2.7	4.7	5.4	34.3	27	402
5	2.9	25.0	12.4	446.0	33	10800
6*	1.8	2.8	7.3	4.4	34	29

* Sham sensitised.

There was a rise in LTD_4 and histamine in all sensitised animals with a positive correlation between histamine and LTD_4 post-challenge ($r = 0.96$, $p < 0.01$). The levels of LTC_4 were just above the limit of sensitivity but showed a clear rise after challenge in the animal which showed the largest LTD_4 response. This assay for LTC_4 and LTD_4 combines the specificity of HPLC and sensitivity of RIA. The on-line concentration column minimises losses due to handling. The use of identical internal standards in this form of assay is mandatory since the overall recoveries are variable.

Brown, M.J. et al (1982) J.Allergy Clin.Immunol. 69, 20-24
Hayes, E. et al (1983) J.Immunol. 131, 429-433.

[^{3}H]-DILTIAZEM LABELS A SPECIFIC RECOGNITION SITE ASSOCIATED WITH THE CALCIUM CHANNEL IN THE RAT CEREBRAL CORTEX

S.Z. LANGER and H. SCHOEMAKER*, Department of Biology, L.E.R.S. Synthélabo 58, rue de la Glacière, 75013 Paris, France

The binding of [^{3}H]dihydropyridine (DHP) calcium channel antagonists in various tissues is competitively inhibited by chemically related calcium agonists and antagonists. Other calcium channel antagonists such as diltiazem (DTZ) affect the binding of [^{3}H]DHP through a mechanism of heterotropic cooperativity, implying the presence of distinct recognition sites for these drugs (Glossmann et al., 1982 ; Yamamura et al., 1982). This hypothesis was evaluated using [^{3}H]-labeled DTZ, a specific calcium channel antagonist of the benzothiazepine series.

The cerebral cortex of male Sprague-Dawley rats was homogenised in 50 mM ice cold Tris-HCl buffer (pH 7.4), and washed by three centrifugation cycles (48,000 x g, 10 min). After resuspension of the final pellet, an aliquot equivalent to 15 mg original tissue was incubated with [^{3}H]d-cis-DTZ (New England Nuclear, 72 Ci/mmol) in a final volume of 1 ml Tris buffer (pH 7.4). Following incubation (180 min at 0°C, or 60 min at 30 or 37°C), membranes were isolated by filtration over 0.05 % polyethylenimine pretreated Whatman GF/B filters, and washed with three 5 ml volumes of buffer. Dissociation experiments from equilibrium were performed at 30°C as in Yamamura et al. (1982). Specific [^{3}H]DTZ binding was defined using 10 µM unlabeled d-cis-DTZ. The binding of [^{3}H]nitrendipine (New England Nuclear, 70.0 Ci/mmol) was measured under identical experimental conditions using the equivalent of 10 mg original tissue.

At 0°C, [^{3}H]DTZ binding is inhibited with high affinity by unlabeled DTZ (IC_{50} = 40 nM). Whereas at 30°C identical results were obtained (IC_{50} = 38 nM), a significantly higher IC_{50} was found at 37°C (170 nM). L-cis-DTZ, the pharmacologically less active isomer, is less active than DTZ with IC_{50}'s ranging from 1.3 µM at 0°C to 5.1 µM at 37°C.

Saturation analysis of [^{3}H]DTZ binding at 30°C yielded a Kd of 50 $\pm$ 3 nM and a Bmax of 11.9 $\pm$ 0.5 pmol/mg tissue. The Bmax of [^{3}H]DTZ is similar to that observed using [^{3}H]nitrendipine (9.4 $\pm$ 0.2 pmol/mg tissue), a high affinity (Kd = 0.49 $\pm$ 0.03 nM) DHP calcium channel antagonist.

Consistent with the hypothesis that [^{3}H]DTZ binding is associated with the calcium channel, binding was potently inhibited by verapamil with IC_{50} values ranging from 19 nM (0°C) to 59 nM (37°C).

The DHP calcium channel antagonists affect [^{3}H]DTZ binding through a temperature dependent mechanism. Thus, nitrendipine inhibits [^{3}H]DTZ binding at 0°C with an IC_{50} of 1.8 nM (maximal inhibition 75 %). In contrast, at 30°C nitrendipine enhances [^{3}H]DTZ binding by 30 % with an IC_{50} of 9 nM. Enhancement of [^{3}H]DTZ binding is even more pronounced at 37°C (+ 108 %, EC_{50} : 2.7 nM). The DHP calcium agonist Bay K 8644 also inhibits [^{3}H]DTZ binding at 0°C with an IC_{50} of 3.8 nM. However, at 37°C, Bay K 8644 fails to affect [^{3}H]DTZ binding when present in concentrations up to 100 nM, even though its affinity for the DHP receptor ([^{3}H]nitrendipine binding) under these conditions is 14 nM.

Enhancement of [^{3}H]DTZ binding by nitrendipine at 30°C is at least partly mediated by an increase in the Kd of [^{3}H]DTZ. Thus, after equilibration for 60 min using 4 nM [^{3}H]DTZ, the addition of unlabeled DTZ (10 µM) results in the dissociation of the ligand-receptor complex with a half-life of 2.1 min. In contrast, the half-life observed after simultaneous addition of DTZ (10 µM) and nitrendipine (1 µM) is 34.0 min.

[^{3}H]Diltiazem binding to the rat cerebral cortex is stereospecific, of high affinity, and appears to label a calcium channel antagonist recognition site allosterically coupled to the previously identified [^{3}H]DHP recognition site.

Glossmann, H. et al. (1982) Trends Pharmacol. Sci. 3, 431.
Yamamura, H.I. et al. (1982) Biochem Biophys. Res. Comm. 108, 640.

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8-BROMO-cGMP AND CALCIUM FLUX IN ARTERIAL SMOOTH MUSCLE

Collins P., Griffith T.M., Henderson A.H., Lewis M.J. Departments of Cardiology, Radiology, Pharmacology and Therapeutics, UWCM, Heath Park, CARDIFF

The smooth muscle relaxant effect of nitrovasodilators and of endothelium-derived relaxant factor (EDRF) has been attributed to an increase in arterial cyclic guanosine monophosphate (cGMP) on the basis of measured tissue levels (Rapoport and Murad, 1983), the use of pharmacological probes in bioassay experiments (Griffith et al, 1984) and the demonstration that it stimulates soluble guanylate cyclase(Forstermann et al, 1985). cGMP is thought to mediate relaxation through an effect on contractile proteins associated with dephosphorylation of myosin light chains (Rapoport et al, 1983). An effect of cGMP on calcium (Ca) movements and free cytosolic Ca is not known. We have measured the effect of the lipid soluble analogue of cGMP, 8-bromo cGMP on calcium flux in rabbit aorta.

3-5 mm-wide ring segments from the descending thoracic aorta were prepared. Endothelium was removed from the rings by gentle abrasion. Rings were immersed in Hepes buffer at 37°C, pH 7.3, gassed with 100% O_2 and equilibrated for 90 min. For influx studies, rings were then placed in buffer containing ^{45}Ca (c. 1.5 μCi/ml) for 1.5 min, transferred into ice-cold buffer containing 2 mM EGTA for 45 min then gently blotted, weighed and placed in 5 mM hypotonic EDTA solution overnight after which the radioactivity in the supernatant was measured. For efflux studies, rings were equilibrated for 3 hrs in buffer containing ^{45}Ca, then placed in ice-cold buffer containing 5 mM EGTA and 6.5 mM $CaCl_2$ for 45 min. Rings were transferred through a series of 3 ml aliquots of buffer at 5 min intervals for measurement of efflux rates over 60 min, blotted, weighed and their residual ^{45}Ca measured. Efflux was expressed as fractional ^{45}Ca loss/min. Results are given as mean ± SEM and compared using students t test for unpaired data.

Noradrenaline (10^{-5}M) significantly increased ^{45}Ca influx from 30±2.3 μM/kg to 50±4.7 μM/kg (n=6, $p<0.001$). 8-bromo cGMP (10^{-3}M) did not affect basal ^{45}Ca influx but significantly reduced the noradrenaline-induced increase in influx to 32±2.7 μM/kg (n=6, $p<0.001$). ^{45}Ca efflux was significantly increased by noradrenaline (10^{-5}M) from 0.029±0.003/min to 0.063±0.004/min (n=6, $p<0.001$). This noradrenaline-induced efflux was significantly reduced by 8-bromo cGMP (10^{-3}M) to 0.044±0.003/min (n=6, $p<0.005$).

These data indicate that the smooth muscle relaxant effect of 8-bromo cGMP in noradrenaline stimulated preparations is associated with a reduction of net ^{45}Ca influx and efflux. This suggests that cGMP can reduce Ca influx and that this may contribute to its relaxant action. The reduction of Ca influx by EDRF and nitrovasodilators would appear to be due to cGMP rather than an independent effect of these agents.

Rapoport, R.M., and Murad, F. (1983) Circ. Res. 52, 352-357.
Griffith, T.M., Henderson, A.H., Hughes-Edwards, D., Lewis, M.J. (1984) J. Physiol. 350, 46P.
Forstermann, M., Mulsch, A., Bohme, E., Busse, R. (1985) J. Cardiovasc. Pharmac. (in press).
Rapoport, R.M., Draznin, M.B., Murad, F. (1983) Nature 306, 174-176.

DIPYRIDAMOLE, DILAZEP AND HEXOBENDINE INHIBIT BOTH ADENOSINE UPTAKE INTO AND RELEASE FROM NEONATAL RAT HEART CELLS IN CULTURE

P. Meghji and A.C. Newby, Department of Cardiology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, WALES (introduced by M.J. Lewis).

The coronary vasodilator action of dipyridamole, hexobendine and dilazep has been explained by their ability to inhibit nucleoside transport hence preventing uptake and inactivation of the endogenous vasodilator adenosine (Kolassa et al, 1971; Fujita et al, 1980). This inhibition raises the interstitial fluid concentration of adenosine promoting action at its cell-surface receptors (Schrader et al, 1977). Since, the nucleoside transporter is symmetrical, we investigated the effect of dipyridamole, hexobendine and dilazep on both uptake and release of adenosine using cultured heart cells from neonatal rats.

Cell culture, according to Harary and Farley (1963) and uptake measurements of [^{3}H] adenosine (10 μM) were conducted as previously described (Newby et al, 1983). Adenosine formation and release were measured in the presence of 10 μM - 2 - deoxycoformycin, an inhibitor of adenosine deaminase, and 1 μM 5-iodotubercidin, an inhibitor of adenosine kinase, as previously described (Newby et al, 1983).

Incorporation of [^{3}H] adenosine into cellular nucleotides was inhibited concentration dependently by dipyridamole (EC_{50} 0.75 μM), hexobendine (EC_{50} 0.26 μM) and dilazep (EC_{50} 0.22 μM) (n=7 in each case). Adenosine formation was stimulated 2.2 fold by 30 mM-2 deoxyglucose and 2 μg/ml of oligomycin to a rate of 0.23$\pm$0.03 nmol/min per 10^7 cells (n=6). After 10 min 9.5$\pm$2.0% of the newly formed adenosine was found inside the cells and the remainder in the medium. Dipyridamole, dilazep and hexobendine (10 μM) increased the amount of adenosine inside the cells and reduced that in the medium such that 45-50% (n=6 in each case) was present inside the cells.

Transport inhibitors prevented both uptake of extracellular adenosine and release of adenosine formed during ATP catabolism whereas their vasodilator action implies a selective inhibition of uptake. It may be concluded that dipyridamole, dilazep and hexobendine have another, common but as yet undiscovered mechanism of action. Alternatively it must be explained how a symmertric inhibition of adenosine transport can lead to an increase in extracellular adenosine concentration in the physiological situation.

Fujita et al (1980) Br. J. Pharmac. 68, 343-349.
Harary, I. & Farley, B. (1963) Exp. Cell Res. 29, 451-465.
Kolassa et al (1971) Eur. J. Pharmac. 13, 320-325.
Newby, A.C. et al (1983) Biochem. J. 214, 317-323.
Schrader, J. et al (1977) Pflugers Arch., 369, 251-257.

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AMLODIPINE, A NEW DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKER WITH SLOW ONSET AND LONG DURATION OF ACTION

R.A. Burges*, A.J. Carter, D.G. Gardiner and A.J. Higgins (Introduced by V.A. Alabaster), Pfizer Central Research, Sandwich, Kent CT13 9NJ.

Amlodipine (2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4 -dihydropyridine) is a new calcium channel blocker, structurally related to nifedipine, but which displays distinct pharmacological properties. Both drugs inhibited the contractile response of the rat aorta to 45 mM KCl but their equilibration rates with the tissue were markedly different. Thus nifedipine (5 nM) induced 43±1.3% (mean ± s.e. mean, n = 5) inhibition after 30 min tissue contact, with little change over the following 3 h; in contrast, inhibition by amlodipine (50 nM) was 31.2±0.9% (n = 5) after 30 min but had increased to 63.6±2.1% after 3.5 h. Accordingly Ca^{2+}-antagonist potency in the K^{+}-depolarised rat aorta was estimated for both drugs after 3 h contact with the tissue. Amlodipine and nifedipine induced parallel, rightward shifts of dose-response curves to $CaCl_2$ with estimated pA_2 values of 9.2 and 9.1 respectively.

To compare association rates at the receptor level, amlodipine (30 nM) and nifedipine (5 nM) were pre-incubated with a rat cardiac membrane preparation for various times before determination of (^{3}H) nitrendipine binding. The displacement of specific binding by nifedipine was complete within 30 min whereas the effect of amlodipine was still increasing after 2 h.

The drugs also differed in their rates of recovery of response after washout. KCl-induced contractions of rat portal veins were found to be highly reproducible over at least 10 h; after 1 h exposure to nifedipine (10 nM) followed by washout, the response recovered to 95% of its pre-drug value within 2 h. With amlodipine (2 μM), no recovery of the response was observed after 9 h.

The haemodynamic effects of amlodipine and nifedipine were compared in chloralose/urethane anaesthetised dogs. Coronary blood flow was measured by hydrogen-clearance (Auckland et al, 1964) and cardiac output by thermodilution. Intravenous amlodipine (25 to 800 μg/kg) and nifedipine (2 to 128 μg/kg), given cumulatively at 30 min intervals, caused dose-related coronary and peripheral vasodilatation, associated with reflex increases in heart rate, LV dp/dt max and cardiac output. Neither drug adversely influenced cardiac electrical conduction, as judged from the ECG and only nifedipine caused a transient negative inotropic effect at high doses. Whereas responses to nifedipine were rapid in onset (0.5 to 2 min) and of short duration (5-30 min), those to amlodipine occurred gradually (5 to 30 min) and showed no decline over each dosing period. Moreover, whilst nifedipine caused acute hypotensive responses at all doses, amlodipine did not affect blood pressure in these normotensive animals, a difference which may be ascribed to the slower onset of action of amlodipine, allowing adequate reflex compensation. However, amlodipine is a highly effective, long-acting antihypertensive agent in hypertensive dogs (Dodd and Machin, 1985).

We conclude that these pharmacological properties, combined with favourable pharmacokinetics (Beresford et al, 1985) distinguish amlodipine from other calcium channel blocking drugs.

Auckland, K. et al (1964) Circulation Res. 14, 164-187
Beresford, A.P. et al (1985) This Meeting
Dodd, M.G. and Machin, I. (1985) This Meeting

CALCIUM ANTAGONIST INHIBITION OF RESPONSES OF RAT UTERUS TO CALCIUM, OXYTOCIN AND POTASSIUM

Susan E. Granger, M. Hollingsworth* and A.H. Weston, Department of Pharmacology, Medical School, University of Manchester, Manchester M13 9PT

Hollingsworth et al (1983) and Granger et al (1985) have demonstrated that several calcium (Ca) antagonists are potent inhibitors of spontaneous and oxytocin-induced tension development in the uterus of the day 22 pregnant rat. The rank order of potency was nifedipine > gallopamil > diltiazem > cinnarizine. We have now examined the hypothesis that the inhibitory actions of these compounds on this tissue involve prevention of Ca influx from the extracellular medium into the cell.

Tonic tension development of longitudinal strips of whole uterus was produced by cumulative additions of Ca to a depolarising (40mM K^+) MOPS-buffered physiological salt solution (PSS). There were parallel rightward shifts in the Ca concentration-effect curves with lower Ca antagonist concentrations and in addition reductions in maximal responses to Ca by higher antagonist concentrations. The rank order of potency was nifedipine = gallopamil > diltiazem > cinnarizine. K-concentration-effect experiments were carried out on endometrium-free longitudinal uterine strips by cumulative addition of KCl (10-40 mM) to the PSS. The Ca antagonists produced a concentration-dependent reduction of both the slopes and maxima of the K-concentration-effect curves with a rank order of potency of nifedipine > gallopamil > diltiazem > cinnarizine.

The 'lanthanum technique', modified from Allen et al (1985), was used to assess the effects of the Ca antagonists on KCl-induced Ca-45 uptake into strips of endometrium-free uterus. The incubation sequence was:- 45 min equilibration; 25 min with or without Ca antagonist; 5 min with Ca-45 (250 nCi/ml) ± Ca antagonist; 10 min with Ca-45 ± Ca antagonist ± KCl (20 or 40mM). Tissues were then washed in a Ca-free MOPS-buffered medium containing 10mM lanthanum chloride at 0°C for 120 min. The Ca-45 content of tissues and treatment media was determined by scintillation counting. KCl induced a significant ($2P<0.05$) and concentration-dependent increase in Ca-45 uptake (20mM-27%; 40mM-63%). The increase in Ca-45 uptake by 20mM KCl was completely prevented by cinnarizine (5 μM) or diltiazem (500 nM) but not affected by nifedipine (2.5 nM). The increase in Ca-45 uptake by 40 mM KCl was prevented by gallopamil (100 nM) and significantly reduced by nifedipine (2.5nM), diltiazem (500nM) or cinnarizine (5μM). In contrast, oxytocin produced only a small (18%), but significant, increase in Ca-45 uptake at a low concentration (0.1 mU/ml) with no detectable change in Ca-45 uptake at higher concentrations (1 and 10 mU/ml).

These results support the idea that the Ca antagonists inhibit Ca- and KCl-induced tension development of rat uterus by reduction of Ca influx from the extracellular medium into the cell. The small increase in Ca-45 uptake with oxytocin, by comparison with KCl, suggests oxytocin-induced tension development involves both extracellular Ca and other mechanisms.

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Allen, S.L. et al (1985) Br.J.Pharmac. (In press).
Granger, S.E. et al (1985) Br.J.Pharmac. (In press).
Hollingsworth, M. et al (1983) Br.J.Pharmac. 79, Proc.Suppl., 208P.

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THE RAT ISOLATED INFERIOR TARSAL MUSCLE: A NEW ADRENERGICALLY INNERVATED PREPARATION

K. LAWSON* and D.F. WEETMAN, Department of Pharmacology, Sunderland Polytechnic, Sunderland SR1 3SD

Tension in the lower eyelid of anaesthetised or pithed rats is frequently measured to provide information about adrenergic nerve function (Gertner, 1956). Most of the response of the eyelid is brought about by contractions of the inferior tarsal muscle (ITM). The isolation of this muscle, its response to drugs and electrical field stimulation are now described.

Female Sprague Dawley (200-250g) rats were killed by cervical dislocation. The part of the jugal (cheek bone) forming the floor of the orbit was removed, together with the mastication muscles of the cheek. The Harderian gland was removed to reveal the ITM, enclosing the eyeball. The ITM was dissected from the eyeball and superior TM. Each ITM was suspended in an isolated organ bath containing McEwen's solution at 37° ± 1°C gassed with 95% O_2 and 5% CO_2. Tension was detected with a Grass FTO3 force-displacement transducer and recorded on a Grass 79D polygraph; the initial load on the preparations was 0.5g. An equilibration period of 30 min was allowed before drugs were administered cumulatively at 2 min intervals by the method of van Rossum (1963), until a maximum response was obtained.

The isolated ITM contracted to noradrenaline (NA EC_{50} = 20.4 ± 3.1 μM, maximal response = 670 ± 35 mg, n = 31), acetylcholine (EC_{50} = 25.2 ± 6.4 μM, max = 590 ± 80 mg, n = 4), methoxamine (EC_{50} = 0.71 ± 0.23 μM, max = 530 ± 50 mg, n = 6), 5HT (EC_{50} = 106 ± 17 μM, max = 550 ± 60 mg, n = 4) and indanidine (EC_{50} = 59 ± 13 μM, max = 590 ± 40 mg, n = 9). Isoprenaline (up to 300 μM) and histamine (up to 400 μM) did not contract the ITM, but tests for sm relaxant effect have not been made. The NA concentration-response curve was displaced to the left by 30 min pretreatment with cocaine (Reciprocal Dose Ratio = 9.3 at 3.3μM, 64 at 33μM n = 4 for both concentrations), but the max response was unchanged. Concentration-response curves to acetylcholine indanidine and methoxamine were not changed by cocaine (3.3 μM), but there was a small increase in RDR after 33 μM cocaine (methoxamine 4.3, indanidine 2.1, n = 4 for both agonists). Prazosin (in the presence of 3.3 μM cocaine) competitively antagonised contractions of the ITM to NA (pA_2 = 8.94 ± 0.07, slope of the Schild plot 0.95 ± 0.08, n = 12) and indanidine (pA_2 9.01 ± 0.18, slope 0.92 ± 0.12, n = 12): these pA_2 values were not significantly different (P>0.05). Electrical field stimulation of the ITM via Birmingham and Wilson (1963) type electrodes (0.5 ms duration pulses at 140v for 5 s every 30 s) produced monophasic, frequency-dependent contractures that were sensitive to tetrodotoxin (0.3 μM n = 4) and guanethidine (50 μM n = 4). Dexamphetamine (270 μM) restored the guanethidine-depressed contractions of the ITM to field stimulation without affecting the resting tone (n = 4).

Thus it is possible to isolate the muscle responsible for lower eyelid contractions in rat. The excitatory innervation of the ITM appears to be adrenergic.

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Birmingham, A.T. and Wilson, A.B. (1963) Br. J. Pharmac., 21, 569-580.
Gertner, S.B. (1956). Br. J. Pharmac., 11, 147-150.
Van Rossum, J.M. (1963) Archs int Pharmacodyn, 143, 299-330.