EFFECT OF REPEATED INTRATHECAL THYROTROPHIN-RELEASING HORMONE (TRH) OR TRH ANALOGUE ADMINISTRATION ON SPINAL MOTONEURONES

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

Electronmicroscopic evidence shows that thyrotrophin-releasing hormone-like immunoreactivity (TRH-LI) is located in boutons making synaptic contact with spinal motoneurones (Ulfhake et al., 1987) but the role of TRH in regulating normal spinal motor output is unknown. TRH may exert a trophic-like action on motoneurones as suggested by the elevation of choline acetyltransferase (ChAT) activity in cultured motoneurones exposed to TRH (Schmidt-Achert et al., 1984). The present study compared the effect of repeated intrathecal administration of TRH (pGln-His-ProNH2), the C-terminal modified TRH analogue RX 77368 (pGln-His-3,3'-dimethyl-ProNH2) and N-terminal modified CG 3509 (orotyl-His-ProNH2) on ChAT activity and calcitonin-gene related peptide (CGRP)-LI in the rat ventral horn, which are both principally located in motoneurones in this region, as biochemical markers for motoneurones. In addition plasma thyroid stimulating hormone (TSH) and free thyroxine (T4) levels were measured to assess the neuroendocrine effects of the TRH peptides.

Male Wistar rats (270-320g) were anaesthetised with sodium methohexitone (60mg/kg i.p.) to permit implantation of an intrathecal cannula (Fone et al., 1987). After a seven day recovery period rats (n=8 in each group) received nine successive intrathecal injections of either saline (10µl + 15µl wash-in), TRH (20µg), CG 3509 (2µg) or RX 77368 (2µg), doses which were equipotent at producing wet-dog shakes when given intrathecally, twice daily (between 08.30-09.30 and 17.30-18.30h) for five days. Three to four hours after the last injection the brainstem, the thoraco-lumbar spinal cord divided into dorsal and ventral portions and a blood sample were taken from each rat and stored at $^{-80}$°C prior to assay. ChAT activity was measured using a radioenzymatic method (Fone et al., 1987), CGRP-LI levels by radioimmunoassay and plasma free T4 and TSH levels were determined using radioimmunoassay kits (Amersham). ANOVAR was used for statistical analysis and results are presented as mean ± s.e.mean.

Repeated intrathecal CG 3509 administration produced a small but significant ($P<0.05$) increase in ventral horn ChAT activity (1.716 ± 0.098 µmol h-1 g wet weight) compared with saline (1.401 ± 0.092) or RX 77368 (1.178 ± 0.129) treated rats. TRH peptide administration did not significantly alter CGRP-LI levels in any region from that in saline treated controls although CGRP-LI was significantly greater ($P<0.05$) in RX 77368 than CG 3509 treated rats in the brainstem and conversely, significantly lower ($P<0.05$) in the dorsal spinal cord. In contrast, ventral horn CGRP-LI levels and dorsal horn ChAT activity were comparable in all groups. Plasma free T4 levels were elevated by both TRH (40.27 ± 2.55 pmol l^{-1}, $P<0.05$) and RX 77368 (32.92 ± 1.95, not significant) compared with levels in either saline or CG 3509 treated rats (26.79 ± 3.42 and 24.05 ± 3.19, respectively). Similarly, only TRH (1.80 ± 0.09 µIU ml^{-1}) and RX 77368 (1.47 ± 0.13) significantly reduced plasma TSH levels from those in saline treated controls (2.54 ± 0.21, $P<0.05$).

These results confirm the previous observation that repeated intrathecal CG 3509 injection elevates ChAT activity in rat ventral horn (Fone et al., 1987) but suggests that this effect may be related to its N-terminal modification. In contrast, the C-terminal modified TRH analogue (RX 77368) appears to possess actions more similar to TRH than CG 3509 on both the pituitary-thyroid axis and CGRP-LI levels. Any relevance of the CG 3509-induced elevation in ventral horn ChAT activity to the suggested therapeutic use of TRH peptides in the treatment of motor neurone disease remains to be established.

We wish to acknowledge Reckitt and Colman, the Wellcome Trust and Action Research for the Crippled Child for financial support.

Fone K.C.F. et al., (1987) Brit J. Pharmacol. 91, 434P.
Schmidt-Achert K. M., Engel & Askanas (1984) J. Neurochem. 43, 586-589.
Ulfhake B. et al., (1987) Neurosci. 23, 917-929.

EFFECT OF IDAZOXAN AND A TRH ANALOGUE (RX 77368) ON ENDOGENOUS NORADRENALINE RELEASE FROM RAT SPINAL CORD IN VITRO

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

Intrathecal injection of thyrotrophin-releasing hormone (TRH) or TRH analogues produces a dose-related wet-dog shaking which is antagonised by prazosin (Fone et al., 1987) suggesting that spinal noradrenergic neurones may be involved in this motor behaviour. TRH has been shown to cause dopamine release in the nucleus accumbens (Sharp et al., 1982) and noradrenaline release in the hypothalamus (Heal et al., 1987). Therefore, the present study investigated whether the TRH analogue RX 77368 (pGln-His-3,3'-dimethyl-ProNH2) released endogenous noradrenaline from ventral horn spinal cord slices, using a fixed volume incubation technique.

The ventral thoracic spinal cord of rats was divided into bilateral halves, sliced and washed in calcium free Krebs before being resuspended in Krebs (1ml) containing pargyline (1μM) as described previously (Sharp et al., 1982). The tissue was gased with 95%O2/5%CO2 and pre-incubated with idazoxan or maprotiline at 37°C for five min in a shaking water bath before adding RX 77368 and/or potassium (20μl in Krebs) and shaking for a further 20 min. Slices were then rapidly centrifuged (2000g) and the supernatant removed, frozen and stored at -80°C. Noradrenaline content of the supernatant was determined following extraction with alumina using 3,4-dihydroxybenzylamine as internal standard and then detected using high performance liquid chromatography with electrochemical detection. Student's t-test was used for statistical analysis and results (n=6 in each group) are presented as mean ± s.e.mean.

Potassium (15 and 50mM) produced a dose-related increase (54.8%, $P<0.05$ and 218.4%, $P<0.001$ above the basal level of 2.80 ± 0.41 pmol mg protein^{-1}, respectively) in noradrenaline release from ventral cord slices which was significantly attenuated ($P<0.01$) by incubation in a calcium free medium containing EGTA (1mM). Pre-incubation with the selective α2-adrenoceptor antagonist idazoxan (10^{-6}, 10^{-5} and 10^{-4}M) potentiated the potassium (15mM) evoked release of noradrenaline in a dose-related manner (by 27.2% and 32.0%; not significant and 94.0%; $P<0.01$ above control levels, respectively). This effect of idazoxan was further enhanced by combined pre-incubation with the noradrenaline uptake inhibitor maprotiline (1μM), being 39.3% (not significant) and 42% ($P<0.05$) elevated by 10-6M and 10-5M idazoxan. The TRH analogue RX 77368 (10^{-5} and 10^{-4}M) increased potassium-induced noradrenaline release in a dose-dependent manner (by 8.3%, not significant and 27.5%, $P<0.05$, respectively), but this effect was significantly reduced ($P<0.05$) by adding maprotiline (1μM) to the incubation medium.

By using a fixed volume incubation technique a calcium dependent, potassium induced release of noradrenaline was demonstrated from rat ventral spinal cord slices. Noradrenaline release was potentiated by idazoxan suggesting that the slices possess viable autoreceptors of the α2-adrenoceptor subtype. Furthermore, the TRH analogue RX 77368 potentiated potassium-evoked noradrenaline release, although this effect required the presence of a noradrenaline uptake mechanism. These results are consistent with the proposal that noradrenaline release may contribute to the spinal motor effects produced by the local application of TRH analogues to the spinal cord.

We wish to acknowledge Reckitt and Colman, the Wellcome Trust and Action Research for the Crippled Child for financial support.

Fone K.C.F. Bennett G.W. & Marsden C.A. (1987) Neuropharmacol. 26,1147-1155.
Heal D.J. et al., (1987) Neuropharmacol. 26, 313-322.
Sharp T. Bennett G.W. & Marsden C.A. (1982) J. Neurochem. 39, 1763-1766.

DOPAMINE D2 RECEPTORS AND DOPAMINE UPTAKE SITES VISUALIZED AUTORADIOGRAPHICALLY IN HEMI-PARKINSONIAN MONKEYS

W.C.Graham*, C.E.Clarke, A.R.Crossman and G.N.Woodruff[1], Experimental Neurology Group, Department of Cell and Structural Biology, University of Manchester Medical School, Manchester M13 9PT and [1]Merck, Sharp and Dohme Research Laboratories, Terlings Park, Harlow, Essex, CM20 2QR.

Dopamine D2 receptor binding sites and dopamine uptake sites can be studied autoradiographically by using the radiolabelled ligands ^{3}H-sulpiride and ^{3}H-mazindol respectively. These two markers of the dopamine system were studied in the striata of monkeys rendered hemi-parkinsonian by the method first described by Bankiewicz et al. (1986).

Three monkeys (Macaca fascicularis) received infusions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (1.1 - 2.7 mg/kg), into the right common carotid artery. The animals developed varying degrees of tremor, hypokinesia and rigidity, on the contralateral side. When challenged with apomorphine the animals turned contralaterally reflecting an imbalance in the dopamine system. Animals were killed 11 - 14 weeks after the MPTP injections by barbiturate overdose, and the brains were rapidly frozen in isopentane at -50°C. For D2 receptor binding studies cryostat-cut sections (20µm) were preincubated in 50 mM Tris HCl (pH 7.4) for 15 minutes and then transferred to the same medium, with 120 mM NaCl and 10 nM ^{3}H-sulpiride, for 15 minutes at room temperature. Specific binding was defined as that displaced by 1 µM (-)sulpiride. Sections used for the visualization of dopamine uptake sites were preincubated in 50 mM Tris HCl (pH 7.9) at 4°C for 5 minutes followed by the incubation in the same buffer containing 300 mM NaCl, 5 mM KCl, 10 nM ^{3}H-mazindol and 50 nM desmethylimipramine, at 4°C for 40 minutes. Specific binding was defined as that displaced by 1 or 10 uM nomifensine. All sections were washed in ice-cold buffer, rinsed in water and rapidly dried in a stream of cool air. Sections were exposed to tritium sensitive film (Hyperfilm, Amersham) at -20°C. Sections from 4 control monkey brains were treated in the same manner.

Densitometric analysis of ^{3}H-mazindol autoradiographs revealed a widespread depletion of dopamine uptake sites in the ipsilateral caudate nucleus and putamen, indicating an extensive lesion of the nigrostriatal pathway (mean % depletion compared to control tissue > 90%). Small significant increases in the levels of D2 binding were seen in the most lateral areas of the ipsilateral putamen. This latter result contrasts with that of Joyce et al. (1985), who reported a 100% increase in ^{3}H-spiperone binding in the lateral putamen of a hemi-parkinsonian monkey. Two of the monkeys also showed a decrease (approx. 50%) in the number of ^{3}H-mazindol binding sites in the contralateral caudate nucleus and putamen, indicating a bilateral effect of the MPTP injection, even though behavioural signs were confined to the one side. This model should be used with caution and necessitates comparison with untreated controls, but again reveals an increase in D2 receptor binding in the same areas as those seen in monkeys treated systemically with MPTP (Graham et al., 1987).

Banciewicz, K.S. et al. (1986) Life Sci. 39, 7-16
Joyce, J.N. et al. (1985) Brain Research 382, 360-364
Graham, W.C. et al. (1987) Br. J. Pharmacol. Proc. Suppl. 91, 306

IN VIVO VOLTAMMETRIC EVIDENCE THAT SCHEDULE CONTROLLED BEHAVIOUR INCREASES DOPAMINE METABOLISM IN RAT CAUDATE

M.H.Joseph*, Helen Hodges & J.A. Gray. MRC Brain, Behaviour and Psychiatry Research Group, Department of Psychology, Institute of Psychiatry, Denmark Hill, London SE5 8AF

The neurotransmitter dopamine (DA) is widely held to be involved in the maintenance of positively reinforced behaviour, on the basis of self-stimulation experiments and on the effects of drugs which alter dopamine transmission on those behaviours (Phillips, 1984). Biochemical evidence for increased dopamine release has been reported in experiments in which rats were killed immediately after completing a 30-minute session of lever pressing (Heffner et al, 1981). We have now used in vivo voltammetry (IVV) at carbon paste electrodes to continually monitor the dopamine metabolite HVA, and other substances, in freely moving animals during and after lever pressing for food reward.

Male Sprague-Dawley rats (290-320g, OLAC) were stereotactically implanted with previously calibrated sets of two working electrodes in the caudate (AP +0.2, L ± 3.2, V -5.5), Ag wire auxilliary and Ag/AgCl reference electrodes. The methods of O'Neill & Fillenz (1985) were closely followed for electrode construction, chronic preparation and IVV recording (linear sweep 0-700 mV, 5mV/s, 12 min interval). The three electrochemical peaks attributed to ascorbate (pk 1; c.220mV), urate (pk 2; c. 380mV) and HVA (pk 3; c.550mV) were observed; local infusion of HVA confirmed the latter attribution.

Squads of four rats were shaped to lever-press on a variable interval-10 sec schedule, until each rat reached a stable baseline (3-600 presses/30 min). IVV recording preceded by 10 background sweeps included at least 2 hrs recording before and after a 30 min behavioural session (B). Results were expressed as % of mean of1h control period before B. Towards the end of B, peak 3 rose somewhat, and rapidly increased thereafter reaching a maximum of 132.8% (mean of 7; sem 7.8; $p<0.01$) of control at c.30 min after the end of B during the dark period. In contrast peak 1 was unaffected in both periods. Peak 2 increased during B and was maximal (156.4% of control; sem 9.0(8); $p<0.001$) c.10 min after B. Peak 2 increased at the beginning of the dark phase (+25%), and increased from this higher baseline to the same maximum (156.4% of control; sem 7.9(8)) 10 min after B in the dark phase.

These results indicate that dopamine metabolism is increased by schedule-controlled behaviour, the maximum change in HVA being seen after the offset of behaviour. The larger changes in peak 2 have an earlier onset, but drug and lesion based experiments will be needed to identify the substances responsible (uric acid/5HIAA). Further investigation of the relation of the HVA changes to the different components of the behaviour (motor activity, food consumption, arousal, positive reinforcement) is required.

We thank the Medical Research Council for financial support, and Robert O'Neill for many helpful personal communications.

Heffner, T.G., Luttinger, D., Hartman, J.A. & Seiden, L.S. (1981) Brain Research, 214, 215-218
O'Neill, R.D. & Fillenz, M. (1985) Neuroscience, 14, 753-763
Phillips, A.G. (1984) Brain Research Bulletin, 12, 195-201

DIFFERENTIAL EFFECTS OF ACUTE ADMINISTRATION OF SELECTIVE OPIOID AGONISTS UPON DOPAMINE BINDING SITE DENSITY IN THE RAT

K.F. Rooney*[1], R.D.E. Sewell & P.S.J. Spencer, Division of Pharmacology, Welsh School of Pharmacy, UWIST, Cardiff & [1]Department of Physiology & Pharmacology, University of Nottingham Medical School, Nottingham.

Both acute and chronic administration of morphine has been shown to elevate D-2 binding site population and potentiate dopamine mediated behaviours in mice (Martin & Takemori, 1985, 1987). Similar observations have been reported following acute and chronic administration of the more selective μ-agonist, levorphanol. The purpose of this study is to investigate the effect upon both D-1 and D-2 binding site populations induced by acute administration of selective mu-, kappa- and delta -opioid agonists.

For radioligand binding analyses, rats were sacrificed 3 hours after injection of either saline (i.p. or i.c.v.), sufentanil (10μg/kg, i.p.), U50,488H (10mg/kg i.p.) or D-Ala^2-D-Leu^5-enkephalin (DADL)(1μg/animal, i.c.v.) and striata removed. Pooled striata were homogenised in Tris-HCl containing 5mM KCl, 100mM $MgCl_2$, 200mM $CaCl_2$ and 120mM NaCl. Characterisation of D-1 binding sites was elicited using 0.03-0.3nM ^{3}H-SCH23390 and D-2 binding sites using ^{3}H-spiperone 0.1-8nM, with non-specific binding defined as that remaining following displacement with unlabelled SCH23390 and (+)-butaclamol respectively.

All three opioid agonists used in the study induced differential effects upon D-1 and D-2 binding site populations without affecting the affinity of the ligand for the binding site - see Fig. 1.

Fig. 1 Changes in Bmax (fmol/mg protein) and Kd(nM) induced by acute administration of selective opioid agonists

	^{3}H-SPIPERONE BINDING		^{3}H-SCH23390 BINDING	
	Bmax	Kd	Bmax	Kd
SALINE i.p.	378.5±29.3	0.21±0.04	1280.2±35.4	0.15±0.03
SALINE i.c.v.	418.5±27.2	0.20±0.05	1330.7±42.5	0.20±0.05
SUFENTANIL 20μg/kg i.p.	528.0±15.9**	0.22±0.03	1307.4±29.0	0.16±0.05
U50,488H 10mg/kg i.p.	513.2±20.8**	0.23±0.05	1300.3±41.6	0.15±0.05
DADL 1μg/animal	484.5±18.5*	0.19±0.04	1292.6±40.7	0.22±0.03

(**=$p<0.01$ *=$p<0.05$ Significantly different from appropriate vehicle control)

It is therefore concluded that all three types of opioid agonist studied induce a significant elevation in dopamine D-2, to the exclusion of D-1, binding site density. Although explanations of this change have been postulated (Martin & Takemori, 1987), relating in particular to a rapid decrease in dopamine levels after an initial increase following morphine administration (Broderick, 1985), the exact mechanism by which this selective increase in dopamine binding site density is induced remains to be elucidated.

We acknowledge the Newby Trust for financial support.

Broderick, P.A. (1985) Life Sci., 36, 2269-2275
Martin, J.R. and Takemori, A.E. (1985) Eur. J. Pharmac., 119, 75-84
Martin, J.R. and Takemori, A.E. (1987) J. Pharmac. Exp. Ther., 241, 48-55

CY 208-243: KINETICS OF BINDING TO D-1 AND D-2 DOPAMINE RECEPTORS

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

Animal studies and functional in vitro tests suggest that CY 208-243 ((-)-4,6,6a,7,8,12b-hexahydro-7-methyl-indolo(4,3-ab)-phenanthridine) is a centrally acting D-1 dopamine receptor agonist (Markstein et al, 1987). However, radioligand binding experiments indicate that CY 208-243 is not selective for D-1 receptors. We have investigated the action of CY 208-243 at D-1 and D-2 dopamine receptors in rat and post-mortem human tissues.

The binding of ^{3}H-SCH 23390 (^{3}H-SCH) and ^{3}H-spiperone (^{3}H-spip) to rat striatal and human putamen D-1 and D-2 receptors was carried out as previously described (O'Boyle & Waddington, 1984; 1987). The affinity of CY 208-243 for human putamen D-1 and D-2 receptors was determined in competition experiments using 0.6 nM ^{3}H-SCH and 0.45 nM ^{3}H-spip. Data were analysed by Hill analysis. The kinetics of the interactions between CY 208-243 and D-1 and D-2 receptors were determined in a series of saturation experiments in which rat striatal membranes were incubated with ligand and increasing concentrations of CY 208-243. Scatchard analysis was used to determine changes in Kd and Bmax.

CY 208-243 displaced specific ^{3}H-SCH or ^{3}H-spip binding from human putamen D-1 and D-2 receptors with Ki values of 180 and 750 nM respectively (n=3). The effects of 50, 500 and 2000 nM CY 208-243 on rat striatal D-1 and D-2 receptor characteristics are summarised in Table 1.

Table 1 Effect of CY 208-243 on D-1 and D-2 receptor characteristics

	D-1		D-2	
	Kd nM	Bmax pm/g	Kd nM	Bmax pm/g
Control	0.44±0.1	64.4±3.8	0.08±0.02	37.6±2.7
CY 50 nM	0.65±0.2	59.5±7.4	0.10±0.01	36.0±3.7
500 nM	1.22±0.3*	51.9±7.4	0.35±0.09*	39.6±4.7
2000 nM	3.69±0.68**	46.7±4.2**	1.42	34.5

mean±s.e.mean, n=2-6. *p<0.05, **p<0.01 Mann-Whitney U test

The presence of 50-2000 nM CY 208-243 caused a progressive increase in the Kd for ^{3}H-SCH binding and a decrease in the number of binding sites. In the presence of 2000 nM CY 208-243 D-1 receptor Bmax was reduced by 27% (p<0.01, Mann-Whitney U test). At D-2 receptors CY 208-243 caused a progressive reduction in the affinity of ^{3}H-spip binding without influencing Bmax.

These data indicate that CY 208-243 has only 4-fold higher affinity for D-1 vs D-2 receptors in human putamen and are in agreement with the lack of selectivity reported for rats (Markstein et al, 1987). CY 208-243 has the characteristics of a competitive antagonist of ^{3}H-spip binding to D-2 receptors. However, the interaction between CY 208-243 and ^{3}H-SCH binding appears to be more complex. At D-1 receptors CY 208-243 inhibits ^{3}H-SCH binding in the manner of a mixed antagonist.

This work was supported by the Health Research Board and RCSI.

Markstein, R. et al (1987) 6th International Catecholamine Meeting (in press)
O'Boyle, K.M. & Waddington, J.L. (1984) Eur.J.Pharmac. 98, 433-436
O'Boyle, K.M. & Waddington, J.L. (1987) J.Neurochem. 48, 1039-1042

IN VIVO VOLTAMMETRIC EVIDENCE THAT BALB/CJ MICE RELEASE MORE STRIATAL DOPAMINE THAN CBA/J MICE

F. Crespi*, C.A. Marsden, and M.K. Sanghera[1]. Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [1]Department of Psychiatry and Physiology, University of Texas, Health Science Center, Dallas, USA.

BALB/cJ mice have 20-50% more mid-brain dopamine (DA) neurons and exhibit greater spontaneous as well as DA-drug-induced behaviours than the CBA/J strain (Fink and Reis 1981). Furthermore, while the striatal levels of DA and its metabolites appear to be similar in both strains, the rate of DA synthesis measured in vitro appears to be 30-50% greater in the striatum of the BALB/cJ strain (Sved et al, 1984). The purpose of the present study was to determine whether the biochemical and behavioural differences between the two strains of mice were associated with differences in the release of DA.

Six mice of each strain were anaesthetised with chloral hydrate (400 mg/kg ip) held in a stereotaxic frame and implanted with carbon fibre electrodes (CFEs) in the striatum (1.5 mm A to bregma, 2.5 mm L and 3.6 mm below dura, tooth bar at 0). DA release was determined using differential pulse voltammetry (DPV) with 12 µm diameter CFEs electrically pretreated to detect catechol and indole peaks (Crespi et al, 1984). 3 h before DPV measurement of K^+-stimulated DA release the mice were injected with pargyline HCl (150 mg/kg ip) to remove DOPAC to avoid its detection (Gonon 1988). Prior to infusion of K^+ only three voltammetric peaks were recorded in vivo: Peak 1 (ascorbic acid at -50mV), Peak 3 (uric acid at +260 mV, Crespi et al 1983) and Peak 4 (at about 400 mV) while Peak 2 (DOPAC at +80 mV) was undetectable. Peak 4 may correspond to the oxidation of 3 methoxytyramine (3MT) as this DA metabolite, produced by the action of COMT, oxidised in vitro at the same potential as HVA, which was absent in vivo following MAO inhibition. When stable recordings had been obtained (15-25 min) a Hamilton needle containing KCl (0.1 M) was inserted close to the CFE and ten min later KCl (2 µl) was infused. The infusion was followed by the immediate appearance of a peak at 80 mV (Peak 2), in both strains of mice, which represented release and oxidation of DA (Gonon 1988). However, in the BALB/cJ mice this release was considerably larger than in the CBA/J (3.5 times) and the height of Peak 2 detected in vivo corresponded to in vitro DA levels of 24.5 ± 7.6 µM for BALB/cJ and of 7 ± 2 µM for CBA/J. In addition, in the BALB/cJ a similar magnitude of K^+-stimulated DA release could be evoked every 10-15 min, whereas in the CBA/J very little K^+-stimulated DA release was obtained when K^+ stimulation was repeated up to 45 mins after the first stimulation, and this was only observed in three of the CBA/J mice. In the BALB/cJ mice Peak 4 was also consistently larger than in the CBA/J mice during the control period. Furthermore, K^+ infusion was followed (15-20 min post infusion) by a greater increase of this peak in the BALB/cJ mice (255% of control) versus the CBA/J mice (160% of control). 5 min after death the extracellular DA level increased by a similar amount in both strains reaching 10-15 µm. The in vivo observations on K^+-evoked DA release and extracellular 3MT levels indicate that the major difference between the two strains of mice is the greater rate of DA turnover in the BALB/cJ.

Crespi et al (1983) Neurosci.Lett. 43, 203-207.
Crespi et al (1984) Neurosci. Lett. 52, 159-164.
Fink, J.S. & Reis, D.J. (1981) Brain Res. 222, 335-349.
Gonon et al (1980) Nature (Lond) 286, 902-904.
Sved, A.F. et al (1984) Brain Res. 303, 261-266.

GROOMING BEHAVIOUR INDUCED BY THE NEW PUTATIVE D-1 DOPAMINE RECEPTOR AGONIST CY 208-243

Angela M. Murray* & J. L. Waddington, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2.

CY 208-243 ((1)-4,6,6a,7,8-12b-hexahydro-7-methyl-indolo (4,3-ab) phenanthridine) is a D-1 dopamine receptor partial agonist for which there are marked differences between in vitro and functional indices of its selectivity. While it stimulates adenylate cyclase, its affinity for D-2 receptors in ligand binding assays approaches that for D-1 receptors; however it is inactive in functional D-2 models such as prolactin secretion or emesis (Markstein et al, 1988). As selective D-1 agonists such as SK&F 38393 and SK&F 77434 induce intense grooming as a characteristic response (Molloy & Waddington, 1987; Murray & Waddington, 1988), we have studied CY 208-243 in this behavioural model.

Male Sprague-Dawley rats were injected s.c. with CY 208-243, SK&F 77434 or vehicle; SCH 23390 or the selective D-2 antagonist R-piquindone were given s.c. 30 min before agonist challenge. They were observed using a rapid time-sampling behavioural checklist procedure which generates 'counts' for each individual behaviour evident (Molloy & Waddington, 1987).

Over a 1 h period, CY 208-243 variably induced intense grooming showing a bell-shaped dose-response relationship; it was active at 1.0 mg/kg but not at 0.1 or 10.0 mg/kg. Responses to CY 208-243 were blocked by 0.05-0.25 mg/kg SCH 23390, and were also blocked by 0.1-0.75 mg/kg R-piquindone.

Drug	mg/kg	counts for intense grooming
Vehicle	---	$0.1 \pm 0.1_a$
SK&F 77434	0.75	$3.2 \pm 0.8_a$
CY 208-243	1.0	2.1 ± 0.6
+ SCH 23390	0.05	0.9 ± 0.3
	0.25	0.1 ± 0.1*
+ R-piquindone	0.1	1.6 ± 0.6
	0.75	0.2 ± 0.2*

Means ± s.e. mean, n=8-16. [a], $p<0.05$ vs vehicle; *, $p<0.05$ vs CY 208-243

CY 208-243 failed to induce stereotyped behaviour, but reproduced the following effects of selective D-1 but not D-2 agonists: induction of intense grooming; sensitivity to selective D-1 antagonist blockade; sensitivity to D-2 antagonist blockade, consistent with D-2 tone enabling the expression of typical D-1-stimulated behaviours (Waddington & O'Boyle, 1987); failure to release myoclonic jerking, as seen with D-2 agonists after D-1 antagonist pre-treatment (Waddington et al, 1988). However, no vacuous chewing was seen under any conditions. These data suggest that CY 208-243 shows some of the properties of a D-1 agonist in the intact adult animal, though its in vitro binding characteristics remain enigmatic.

We thank HRB and RCSI for support, and Sandoz and SK&F for drugs.

Markstein, R. et al (1988) In: Proceedings of the 6th International Catecholamine Symposium (Eds. M. Sandler et al) Alan R. Liss (in press).
Molloy, A.G. & Waddington, J.L. (1987) Psychopharmacology 92, 164-168.
Murray, A.M. & Waddington, J.L. (1988) Irish J Med Sci 157, 17.
Waddington, J.L. & O'Boyle, K.M. (1987) Rev Neurosci 1, 157-184.
Waddington, J.L. et al (1988) In Pharmacology & Functional Regulation of Dopaminergic Neurones (Eds. P. Beart et al), MacMillan, London (in press).

THE EFFECT OF NIFEDIPINE ON NERVE-MEDIATED RESPONSES OF BLOOD VESSELS IN VIVO AND IN VITRO

J.M. Bulloch*, C.J. Daly, A MacDonald[1], J.C. McGrath and V.G.Wilson. Autonomic Physiology Unit, University of Glasgow, Glasgow G12 8QQ. Department of Biological Sciences, Glasgow College, Glasgow G4 OBA[1].

Non-α-adrenergic nerve-mediated contractile responses have been uncovered *in vivo* and *in vitro* and are susceptible to blockade by the P_{2x}-densensitizing agent α,β-methylene ATP (mATP) (Sneddon and Burnstock, 1984; Bulloch and McGrath, 1988). Similar responses in the rat isolated vas deferens are inhibited by both mATP (Sneddon and Burnstock, 1984) and the calcium channel blocker nifedipine (Blakeley *et al.*, 1981).We have, therefore, examined the effect of nifedipine on α-adrenergic and non-α-adrenergic nerve-mediated responses in the pithed rat and the rabbit isolated ileocolic and saphenous arteries (see: von Kügelgen and Starke 1985; Burnstock and Warland, 1987).

Rats were pithed under halothane anaesthesia (Gillespie *et al.*, 1970) and diastolic vasopressor responses to sympathetic nerve stimulation were monitored via the right carotid artery. Vasopressor responses to electrical field stimulation were elicited in the rabbit perfused isolated ileocolic artery as described by von Kügelgen and Starke (1985). 4mm ring segments of the rabbit isolated saphenous arteries were placed in physiological salt solution maintained at 37°C and gassed with 95%O_2:5%CO_2. Isometric contractile responses to electrical field stimulation were recorded under a resting tension of 0.5g wt.

Nifedipine (0.3mg/kg i.a.) attenuated pressor responses to sympathetic nerve stimulation (T_6-T_8, 1cm electrode, 1-20 Hz, 1sec) both in the absence (approx' 40% reduction at 20 Hz) and in the presence (approx' 70% reduction at 20 Hz) of the α-adrenoceptor antagonists prazosin and rauwolscine. However, following repeated intravenous injection of mATP, nifedipine produced only small attenuation of the residual (α-adrenergic) vasopressor response. Nifedipine attenuated the vasopressor response produced by intravenous injection of mATP. Electrically evoked vasopressor responses of the isolated perfused ileocolic artery (5 Hz, 50volts 10 & 100 pulses), previously shown to be prazosin-resistant (von Kügelgen and Starke, 1985), were not affected by 10μM nifedipine. Contractile responses of the rabbit isolated saphenous artery to electrical stimulation (16 Hz, 25-30volts, 0.1msec pulse width for 1 sec) were reduced but not abolished by 1μM prazosin. The remaining prazosin-resistant component was abolished by desensitization with 3μM ATP. Although 1μM nifedipine produced a variable reduction in the prazosin-resistant response (4/10 preparations unaffected, but an 18-67% reduction in 6/10 preparations), it had little effect on the response to nerve stimulation following mATP (α-adrenergic response).

In conclusion, non-α-adrenergic nerve-mediated responses in the rabbit isolated saphenous and ileocolic arteries were abolished by the mATP, but displayed varying degrees of susceptibility to nifedipine. However, similar responses in both the pithed rat (present study) and the rat isolated vas deferens (Blakeley *et al.*, 1981) were highly susceptible to nifedipine. This may reflect differences in the excitation-contraction coupling of non-α-adrenergic neurotransmission between large arteries and resistance vessels. In contrast, α-adrenergic nerve-mediated responses in both the pithed rat and rabbit isolated saphenous artery were relatively resistant to nifedipine.

Blakeley, A. *et al.*, (1981). Nature. 294, 759-761.
Bulloch, J.M. and McGrath, J.C. (1988). Br. J. Pharmacol., 94, 103-108.
Burnstock, G. and Warland, J.J. (1987). Br. J. Pharmacol., 90, 111-121.
Gillespie, J.S. *et al.*, (1970). Br. J. Pharmacol., 40, 257-267.
von Kügelgen, I. and Starke, K. (1985). J.Physiol., 367, 435-455.
Sneddon, P. and Burnstock, G. (1984). Eur. J. Pharmacol., 100, 85-90.

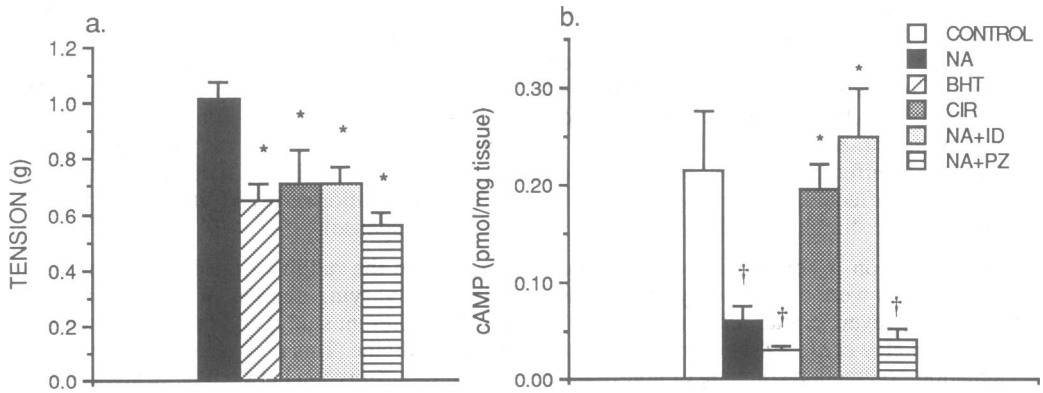
cAMP LEVELS IN THE FEMORAL VEIN OF THE RAT FOLLOWING α_1- AND α_2-ADRENOCEPTOR STIMULATION

D. Stubbs*, J.W. Smith, K.A. Wilson & O.A.Downing, Pharmaceutical Sciences Institute, Aston University, Birmingham B4 7ET.

We have previously reported, in the femoral vein of the rat, that stimulation of α_1- but not α_2-adrenoceptors gives rise to hydrolysis of phosphoinositides (Stubbs *et al*, 1988). In non-vascular tissues stimulation of α_2-adrenoceptors is associated with a decrease in intracellular cAMP (see Jacobs, 1985). In the present study we have investigated whether there are changes in tissue cAMP levels associated with stimulation of α_1- and α_2 -adrenoceptors in vascular muscle.

Femoral veins were removed from male Wistar rats (200-300g) and the wet weight determined. To assay cAMP, two vessels were incubated in 2ml of physiological salt solution (PSS) gassed with 5% CO_2 in O_2 at 37°C for 60 min. Noradrenaline (NA), B-HT920 (BHT) or cirazoline (CIR) was then added and after 3 min the tissues were removed and immersed in liquid N_2. Frozen tissues were homogenised in 6% trichloroacetic acid and cAMP was measured by radioimmunoassay. In some experiments, prazosin (PZ, 10^{-8}M) or idazoxan (ID, $5x10^{-6}$M) was added 15 min before NA. To study contraction, 5mm ring segments of femoral veins were mounted under 0.5g tension between 2 fine steel wires in PSS at 37°C gassed as above. After 60 min equilibration, four responses were determined to an agonist. In some experiments antagonist was added 30 min before the determination of the fourth response to NA. The results are summarised in figure 1.

Figure 1. The effects of agonists (10^{-5}M) and antagonists on a) contractile responses and (b) cAMP levels in the rat femoral vein.



Values are mean ± se mean (n≥6) and are significantly different from; †control, *NA, ($p<0.05$).

These observations indicate that while the contractile response of the femoral vein of the rat is mediated via α_1- and α_2-adrenoceptors it is only the α_2-mediated response that is associated with a reduction in cAMP levels.

Jakobs, K.H. (1985). J. Cardiovasc. Pharmac. 7 (Suppl. 6), S100-S112.
Stubbs, D. *et al* (1988). Br. J. Pharmac., 94, 364P.

STIMULATION OF cAMP-DEPENDENT PROTEIN KINASE THROUGH BOTH β_1- AND β_2-ADRENOCEPTORS IN HUMAN ATRIAL MYOCARDIUM

A.J. Kaumann*[1], K.J. Murray[1], J.A. Hall, F.C. Wells[2], M.J. Brown, Clinical Pharmacology Unit, Addenbrooke's Hospital, Cambridge, CB2 2QQ. [1]Smith Kline and French Research Limited, The Frythe, Welwyn, Herts, AL6 9AR. [2]Surgical Unit, Papworth Hospital, Papworth Everard, Cambridge CB3 8RE.

The adenylate cyclase of human myocardium is stimulated more through β_2- than through β_1-adrenoceptors by both adrenaline and noradrenaline (Gille et al. 1985, Kaumann & Lemoine 1987). Both β_1- and β_2-adrenoceptors contribute to inotropic responses to adrenaline in human atrium (Gille et al. 1985, Kaumann & Lobnig 1986) and ventricle (Kaumann & Lemoine 1987) from patients not pretreated with ß-blockers. Atrial inotropic responses to adrenaline, but not to noradrenaline, are increased in patients treated with the β_1-selective antagonist atenolol due to a predominant involvement of β_2-adrenoceptors (Hall et al. 1988). Do both β_1- and β_2-adrenoceptors stimulate cAMP-dependent protein kinase (cA-PrK) in human myocardium when inotropic responses to catecholamines match? To answer this question we carried out experiments on atria from 4 patients with coronary heart disease receiving atenolol (50-100 mg daily, >6 weeks pre-op). The right atrial appendage was dissected into between 3 and 8 strips incubated with phenoxybenzamine 5 µM for 2 h and paced at 0.5 Hz at 37°C. An inotropic response mediated through β_1-adrenoceptors was obtained with 1 µM noradrenaline in the presence of 50 nM ICI 118,551 to block β_2. An inotropic response mediated through β_2-adrenoceptors was obtained with 0.2 µM adrenaline in the presence of 300 nM CGP 20,712 A to block β_1. After 4 min exposure to a catecholamine, which caused a stable inotropic response, the atria were freeze clamped for biochemical assays. For the cA-PrK assay, soluble enzyme was incubated for 2 min at 30°C with 300 µM [^{32}P]-Y-ATP, 50 mM Na_2HPO_4, pH 6.8, 10 mM $MgCl_2$, 1 mM EGTA and 20 µM malantide in the absence and presence of 2 µM cAMP (Murray et al. 1988). We determined soluble cAMP by radioimmunoassay in this sample. Data are expressed as x ± sem.

Table 1

	CGP	Adren + CGP	ICI	Norad + ICI
cA-PrK activity ratio -cAMP/+cAMP	0.144±0.005	0.353±0.043	0.198±0.017	0.333±0.008
cAMP (pmoles mg^{-1} protein)	12±39+1.48	24.04±3.66	11.65±4.00	34.81±2.20
Δ Contractile force (µN mg^{-1} tissue)		173±56		264±73

The results are consistent with (1) Noradrenaline → β_1 → ΔcAMP → ΔcA-PrK → Δforce
(2) Adrenaline → β_2 → ΔcAMP → ΔcA-PrK → Δforce.

Gille E et al. 1985. Naunyn-Schmiedeberg's Arch Pharmacol 331: 60-70.
Hall JA et al. 1988. Br J Pharmac 93: 116P.
Kaumann AJ & Lemoine H 1987. Naunyn-Schmiedeberg's Arch Pharmacol 335: 404-411.
Kaumann AJ & Lobnig BM 1986. Br J Pharmac 89: 207-218.
Murray KJ et al. 1988. Biochem Soc Trans 16: 355.

CHARACTERISTICS OF α_1-ADRENOCEPTORS IN VASCULAR AND NON-VASCULAR SMOOTH MUSCLE

A.K. Mir*[1] and J.R. Fozard [1], Merrell Dow Research Institute, 16 Rue D'Ankara, 67084 Strasbourg, France. [1] Present address: Preclinical Research, Sandoz Ltd., 4002 Basel, Switzerland

Based on an analysis of literature data, Flavahan and Vanhoutte (1986) proposed that α_1-adrenoceptors of vascular smooth muscle are heterogeneous; one group shows high affinity for prazosin and yohimbine and a second shows relatively low affinity for these antagonists. This proposal has been criticized on a number of grounds including the fact that different experimental conditions could be important for the observations made (Docherty, 1987). In the present study we have further investigated the possibility of α_1-adrenoceptor heterogeneity using both vascular and non-vascular tissues, phenylephrine as a selective α_1-adrenoceptor agonist and identical experimental conditions. WB 4101, which has recently been claimed to discriminate between the putative α_{1A} and α_{1B} adrenoceptor subtypes (Han et al., 1987), was used as antagonist.

Spiral strips from guinea-pig, rabbit and rat aorta without endothelium, segments of portal vein and the longitudinal muscle of rabbit stomach fundus and whole rat anococcygeus muscles were set up for isometric tension recording in organ baths at 37°C in Tyrode solution (Fozard and Mobarok Ali, 1978) gassed with 95% O_2 : 5% CO_2. Cumulative concentration-response curves were obtained to phenylephrine and repeated following exposure for 30 min to each concentration of WB 4101. Time-dependent sensitivity changes were determined in parallel experiments in which incubation was with antagonist vehicle; the reproducibility of succesive concentration-response curves to phenylephrine in all tissues was excellent. pA_2 values were calculated by the method of Arunlakshana and Schild (1959) and mean data (± SEM) of the number of determinations indicated in parentheses are presented.

Table 1 Antagonism of phenylephrine by WB 4101

	Guinea-pig Aorta	Rabbit Aorta	Rabbit Fundus	Rat Aorta	Rat Portal vein	Rat Anococcygeus
pA_2	8.58 ± 0.07 (4)	8.29 ± 0.04 (5)	8.45 ± 0.20 (6)	9.48 ± 0.06 (4)	9.20 ± 0.26 (4)	9.34 ± 0.14 (6)
slope	-1.09 ± 0.07	-1.02 ± 0.06	-0.98 ± 0.08	-1.17 ± 0.06	-1.16 ± 0.14	-0.94 ± 0.08

Consistent with the proposal of Flavahan and Vanhoutte (1986), the data with WB 4101 allow the α_1-adrenoceptors in the different tissues to be divided into two groups based on their showing high (pA_2 values > 9.2) or relatively low (pA_2 values < 8.6) affinities for the antagonist. The data would also be consistent with the high affinity site being classified as α_{1A} and the low affinity site being α_{1B} (Han et al., 1987). Significantly, the discriminatory properties of α_1-adrenoceptor antagonists is not a general phenomenon since phentolamine has the same pA_2 value on a typical "low affinity" tissue (rabbit fundus; pA_2 7.94 ± 0.02 (5); slope - 1.09 ± 0.09) as on a "high" affinity tissue (rat aorta; pA_2 7.97 ± 0.02 (5); slope - 1.02 ± 0.05).

Arunlakshana, O. and Schild, H.O. (1959) Br. J. Pharmacol. 14, 48 - 58.
Docherty, J. (1987) Trends Pharmacol. Sci. 8, 123 - 124.
Flavahan, N.A. and Vanhoutte, P.M. (1986) Trends Pharmacol. Sci. 7, 347 - 349.
Fozard, J.R. and Mobarok Ali, A.T.M. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 301, 223 - 235.
Han, C. et al. (1987) Nature 329, 333 - 335.

COMPARISON OF THE EFFECTS OF FLESINOXAN AND URAPIDIL ON DIFFERENT REGIONAL SYMPATHETIC OUTFLOWS IN ANAESTHETISED CATS

A.G.Ramage* & S.J. Wilkinson, Academic Department of Pharmacology, Royal Free Hospital School of Medicine, Hampstead, London, NW3 2PF.

Recently, it has been shown that 8-OH-DPAT causes differential sympathoinhibition and hypotension in anaesthetised cats (Ramage & Wilkinson, 1988), presumably due to activation of central 5-HT_{1A} receptors. The present experiments were carried out to determine whether two other hypotensive agents, urapidil and flesinoxan whose hypotensive action has been suggested to be due to activation of central 5-HT_{1A} receptors (Ramage, 1986; Fozard & Mir 1987; Ramage et al,1988) can also cause a similar pattern of differential sympathoinhibition as 8-OH-DPAT in anaesthetised cats.

Cats were anaesthetised with α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (12 mg) and paralysed with vecuronium bromide (200 μg kg^{-1}). Simultaneous recordings of whole nerve were made from the inferior cardiac, splanchnic and renal nerves as described previously (Ramage, 1988), along with brachial arterial pressure (BP) and heart rate (HR). Nerve activity was tested to ensure that it was under baroreceptor modulation by its response to injections (i.v.) of sodium nitroprusside (2 μg kg^{-1}) and noradrenaline (0.25 μg). Following these tests the above variables were recorded for 20 min before a cumulative (cum.) dose response curve was produced for either urapidil (0.01-3.0 mg kg^{-1}) or flesinoxan (3-300 μg kg^{-1}).

Both urapidil (n=5) and flesinoxan (n=5) caused dose related falls in blood pressure and heart rate reaching maxima of -47 ± 4 (s.e. mean) and -63 ± 6 mmHg and -60 ± 8 and -130 ± 8 beats min^{-1} respectively at the highest dose. These decreases in both blood pressure and heart rate were associated with sympatho-inhibition in all three nerves. However, only with flesinoxan did the degree of sympathoinhibition differ between the nerves at certain doses (see Table 1), although by the highest dose both drugs caused nearly complete inhibition in all three nerves.

Table 1

Drug	Cum.Dose	ΔBP mmHg	ΔHR beats min^{-1}	ΔCNA %	ΔSNA %	ΔRNA %
Urapidil	1.0 mg kg^{-1}	-36 ± 3	-33 ± 5	-55 ± 15	-64 ± 11	-53 ± 16
Flesinoxan	30 μg kg^{-1}	-39 ± 5	-19 ± 12	-7 ± 32*	-42 ± 20■	-71 ± 12*■

CNA SNA and RNS - cardiac, splanchnic and renal nerve activity.
* ■ $p < 0.05$: (Least significant difference two way analysis of variance).

The results with flesinoxan are similar to those previously observed for 8-OH-DPAT and therefore support the view that different regional sympathetic outflows vary in their sensitivity to sympathoinhibition caused by activation of central 5-HT_{1A} receptors. The failure to observe this effect with urapadil is suggested to be due to its α_1-adrenoceptor antagonist action masking this effect, since in prazosin pretreated cats the differential sympathoinhibitory action of 8-OH-DPAT was not observed (Ramage, 1988).

This work was supported by Byk Gulden Pharmazeutika and Duphar B.V.

Fozard, J.R. & Mir, A.K. (1987) Br. J. Pharmac. 90, 24P.
Ramage, A.G. (1986) Eur. J. Pharmac, 129, 129, 307-314.
Ramage, A.G. (1988) J. Cardiovasc. Pharmac.11, (Suppl.1) S30-S34.
Ramage, A.G.& Wilkinson, S.J. (1988) Br. J. Pharmac. 93, 121P.
Ramage, A.G., Wouters, W. & Bevan, P. (1988) Eur. J. Pharmac. In press.

5-METHYL-URAPIDIL - AN ANTAGONIST WHICH DISCRIMINATES BETWEEN α_1-ADRENOCEPTOR SUBTYPES

G. Gross*, G. Hanft (*introduced by O.-E. Brodde), Department of Pharmacology, University of Essen, D-4300 Essen, Fed. Republic of Germany

There is increasing evidence from radioligand as well as from functional studies that α_1-adrenoceptors can be devided into pharmacologically distinct subtypes designated α_{1A} and α_{1B} (Morrow and Creese, 1986, Han et al., 1987). However, the lack of antagonists with sufficient selectivity makes the investigation of these subtypes difficult. Recently, we reported that derivatives of the antihypertensive agent urapidil have high affinities for 5-HT_{1A} and for α_1-adrenergic receptors (Gross et al., 1987). In the present study we investigated whether these compounds discriminate between α_1-adrenoceptor subtypes.

The affinity of 5-methyl-urapidil for α_1-adrenoceptors labelled by ^{3}H-prazosin (0.2 nmol/l) was determined in membranes of 6 different tissues of the rat. Computerized curve fitting of the data revealed two populations of binding sites with significantly different affinities in vas deferens, hippocampus, brain cortex and heart. Mean pK_I values amounted to 9.1 to 9.4 (-log mol/l) at the high affinity site (α_{1A}) and to 7.2 to 7.8 at the low affinity site (α_{1B}). In liver and spleen membranes, however, only a single population of α_1-adrenoceptors with low affinity for 5-methyl-urapidil (pK_I 7.6) was found. Similar results were obtained with WB 4101 (2-(2,6- dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane). 5-Formyl- and 5-acetyl-urapidil also had higher affinities for α_{1A}-adrenergic binding sites. However, 5-methyl-urapidil was clearly the most subtype-selective of these compounds. Unlabelled prazosin, on the other hand, inhibited ^{3}H-prazosin binding in a monophasic manner in all tissues (pK_I 9.8 - 10.5). In contrast to the agonist adrenaline, no GTP shift was found for 5-methyl-urapidil. In saturation experiments with ^{3}H-prazosin on liver membranes addition of 5-methyl-urapidil apparently increased the K_D but did not alter B_{max} values indicating a competitive type of interaction.

5-Methyl-urapidil antagonized the α_1-adrenoceptor-mediated contraction of rat vas deferens induced by adrenaline with a significantly higher pA_2 value as compared to the α_1-adrenoceptor-mediated positive inotropic effect in right ventricles of the rat heart suggesting a functional relevance of both α_1-adrenoceptor subtypes. No intrinsic activity could be detected on these in vitro preparations.
These results demonstrate that 5-methyl-urapidil has a high affinity for α_{1A}-adrenoceptors and is the most subtype-selective antagonist known at present.

Supported by a grant of the Deutsche Forschungsgemeinschaft

Gross, G., Hanft, G. Kolassa, N. (1987) Arch. Pharmacol. 336, 597.
Han, C., Abel, P.W. Minneman, K.P. (1987) Nature, 329, 333.
Morrow, A.L. Creese, I. (1986) Mol. Pharmacol. 29, 321.

POSTJUNCTIONAL α_2-ADRENOCEPTORS IN BOVINE LYMPHATIC SMOOTH MUSCLE

J.M. Allen*, M. Arnott[1], C. Campbell, J.G. McCarron & K.D. Thornbury[1] Biomedical Sciences Research Centre, University of Ulster, Newtownabbey BT37 0QB, N.Ireland & Department of Physiology, [1]The Queen's University of Belfast, Medical Biology Centre, Lisburn Road, Belfast BT9 7BL.

Bovine mesenteric lymphatic vessels are spontaneously contractile and respond to field stimulation of their adrenergic nerves or exogenous noradrenaline by an increase in contraction frequency. This excitatory effect is mediated via postjunctional α-adrenoceptors (McHale et al, 1980; Allen & McHale, 1986). It was the purpose of the present study to examine the effect of the α_1-adrenoceptor antagonist prazosin and the α_2-antagonist yohimbine on the response to both endogenous and exogenous noradrenaline. Isolated segments of lymphatic vessels were mounted in an organ bath at 37°C for the recording of isometric tension and perfused with Krebs solution containing cocaine (10^{-6}M), normetanephrine (10^{-6}M) and propranolol (10^{-6}M). Vessel response to field stimulation in the absence and presence of prazosin (10^{-6} & 10^{-5} M) is summarized in fig.1. Vessels were stimulated at 10min intervals (0.3ms pulses, 40V nominal, 1min train) on six consecutive occasions (S1-S6). Prazosin at a concentration of 10^{-6}M was added between S2 & S3 trains and its concentration increased to 10^{-5}M between the S4 & S5 stimulations. Mean contraction frequency (N=6) for the 1min period before (open bar) and during (closed bar) stimulation is plotted. Vertical bars show +1s.e.m. Field stimulation remained effective in ($P<0.005$) increasing spontaneous contraction rate at both concentrations of the drug (S3-S6). In contrast, in a similar series of experiments the response to S5 and S6 trains was lost in the presence of yohimbine (10^{-5}M).

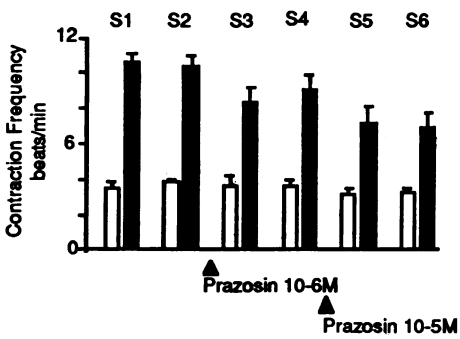


Fig .1. Mean contraction frequency (N=6) for the 1min period before (open bars) and during six consecutive periods (S1-S6) of 4Hz stimulation (closed bars). Pulse trains were separated by 10min periods. Prazosin (10^{-6}M & 10^{-5}M) was added as indicated by the arrows. Vertical bars show +1s.e.m.

Exogenous noradrenaline (10^{-6}M, 5min addition) increased contraction frequency by approximately 160% from 4.3±0.7 to 11.3±1.8 beats/min (N=5, $p<0.03$). This effect persisted in the presence of prazosin (10^{-6}M) where noradrenaline (10^{-6}M) increased contraction frequency by 140% from 3.0±0.8 (mean ± s.e.m.) to 7.2±1.2 beats/min (N=5, $p<0.05$). In contrast, when yohimbine (10^{-6}M) was present noradrenaline (10^{-6}M) had no significant effect, with frequency rising only from 3.5±0.5 to 3.7±0.2 beats/min (N=5). Similarly, the higher dose of 10^{-5}M noradrenaline produced an approximate 250% increase in frequency whether or not prazosin (10^{-6}M) was present, while in the presence of yohimbine (10^{-6}M) this was reduced to <90%.

These findings suggest that the postjunctional α-adrenoceptors on lymphatic smooth muscle are mainly of the α_2 sub-type.

Allen, J.M. & McHale, N.G. (1986). Microvasc. Res. 31, 77-83.
McHale, N.G. et al. (1980). J. Physiol. 309, 461-472.

COMPARATIVE EFFECTS OF CROMAKALIM ON CONTRACTIONS TO NORADRENALINE AND CAFFEINE IN RABBIT ISOLATED RENAL ARTERY

C. WILSON, Beecham Pharmaceuticals, Coldharbour Road, Harlow, Essex.

Relaxation of isolated blood vessels by cromakalim (CRK) (BRL 34915) results from hyperpolarisation of the smooth muscle cell membrane, a consequence of increased outward K^+ conductance (Hamilton et al., 1986). Thus, CRK inhibits contractions to low [KCl] and to noradrenaline (NA) (Clapham & Wilson, 1987) which involve Ca^{2+} influx through voltage- and receptor-operated channels respectively. Contractions to NA may also involve the release of intracellular Ca^{2+}, a property shared by caffeine (CAF). The present study compares the effect of CRK on contractions to NA and CAF in rabbit isolated renal artery, a vessel reported to have a high dependence on intracellular Ca^{2+} release for contraction to NA (Bevan et al. 1986).

Rings of renal artery (n=5) were prepared for isometric tension recording (Clapham & Wilson, 1987) in Krebs-Henseleit solution (KHS) containing 2.5 mM Ca^{2+}. Three concentrations of NA, 0.27, 2.3 and 21 µM, corresponding to 30, 60 and 90% respectively of maximum response, were used to construct two sequential concentration response curves per tissue. The first curve was made in KHS and the second in either a. KHS, b. KHS + CRK 1µM, c. Ca^{2+} free KHS containing EGTA 0.1 mM (CFKHS) or d. CFKHS + CRK 1 µM. Inhibition was quantified by calculating the % reduction in area under the NA curve. Contractions to NA were maintained on repeating the curve in KHS, but significant (p 0.05, Dunnett's test) inhibition (39.1±5.2%) was observed with the inclusion of CRK 1 µM. Incubation (for 15 min) in CFKHS gave a large and significant inhibition (80.0±2.4%). However there was no further reduction with CFKHS + CRK 1 µM (78.4±2.6%).

In separate experiments, contractions to CAF 10 mM either a. alone, b. with CRK 0.1, 1, or 10 µM or c. with CRK + glibenclamide (GLIB) 3 µM were obtained in KHS. Whereas contractions to CAF alone (time-matched controls) were maintained, CRK inhibited CAF contractions in a concentration-related manner (Table 1). Furthermore, this inhibition was antagonised by GLIB. GLIB itself had no significant effect on caffeine contractions.

Table 1. Mean (± s.e.mean, n=5) contractions (mg) to CAF 10 mM .

Time-matched controls	[CRK]	CRK	CRK + GLIB 3 µM
967 ± 140	Pre-dose	847 ± 122	887 ± 164
870 ± 127	0.1 µM	613 ± 61**	900 ± 127
880 ± 132	1.0 µM	410 ± 68**	887 ± 141
847 ± 144	10 µM	273 ± 33**	533 ± 83*

Sig. diff. from own pre-dose value ** p 0.01, * p 0.05, Dunnett's test.

Similar results were obtained when this experiment was repeated in CFKHS. The data show that in CFKHS the contraction to NA, which presumably results from the intracellular release of Ca^{2+}, appeared to be unaffected by CRK. In contrast, CRK inhibits contractions to caffeine which are also attributed to the intracellular release of Ca^{2+}. The antagonism of this effect by the K^+ channel blocker, GLIB (Wilson et al., 1988) indicates a K^+ channel involvement. This may indicate a difference in the mechanism by which NA and CAF release intracellular Ca^{2+}. In the physiological situation, however, the effect of CRK on Ca^{2+} movements in this tissue would be limited to the indirect inhibition of Ca^{2+} influx.

The author acknowledges the technical assistance of Mrs F. Hicks.

Bevan, J.A. et al., 1986, J. Cardiovasc. Pharmacol., 8(suppl.8), S71-S75.
Clapham, J.C. & Wilson, C. 1987, J. Autonom. Pharmacol., 7, 233-242.
Hamilton, T.C. et al., 1986, Br. J. Pharmacol., 88, 103-111.
Wilson, C. et al., 1988, Br. J. Pharmacol., 93, 126P.

GLIBENCLAMIDE BLOCKS THE TRANSMEMBRANE ACTION POTENTIAL SHORTENING EVOKED BY CROMAKALIM IN GUINEA-PIG PAPILLARY MUSCLE

M. Mestre, D. Escande & I. Cavero, Rhône-Poulenc Santé, 13, quai J. Guesde, BP 14, 94403 Vitry-sur-Seine Cedex, France.

Cromakalim, at high concentrations, shortens the transmembrane action potential in guinea pig cardiac preparations (Cain and Metzler, 1985 ; Grosset and Hicks, 1986 ; Scholtysik, 1987). This finding is consistent with an increase in an outward K^+ current although theoretically it could also result from the inhibition of the inward Ca^{++} current. The aim of this study was to exclude the latter hypothesis and to assess whether glibenclamide, an antagonist of ATP-dependent K^+ channel, modifies the effects of cromakalim on the action potential recorded from guinea pig papillary muscle.

Papillary muscles ($\simeq$ 1 mm diameter) obtained from right cardiac ventricles of guinea pigs (400-500 g) were mounted horizontally in 4 ml tissue chambers and superfused with oxygenated (95% O_2 + 5% CO_2), prewarmed (37°C) Tyrode solution (NaCl 136 mM ; KCl 5 mM ; $CaCl_2$ 1.8 mM ; $MgCl_2$ 0.5 mM ; $NaHCO_3$ 12 mM ; NaH_2PO_4 0.35 mM ; Glucose 11.1 mM). The preparations were preloaded with 0.2 *g* and paced (1 Hz). Conventional microelectrodes were used to record transmembrane action potentials (AP). Resting membrane potential, duration of action potential at 90% repolarization (ADP_{90}) and maximal upstroke velocity (V_{max}) were measured. The effects of 30 min exposure to cromakalim (30 µM), nitrendipine (3 µM), Bay k 8644 (3 µM) and glibenclamide (30 µM) were studied. Furthermore, cromakalim and nitrendipine were also investigated in preparations pretreated 30 min earlier with the latter two compounds.

During the 30 min following the addition of cromakalim to the medium perfusing the papillary muscle, the duration of the transmembrane action potential underwent progressive shortenings up to a maximum of 53% (± 3.5, n=4) of the control (ADP_{90}). V_{max} and resting membrane potential were not modified by cromakalim. Nitrendipine also shortened AP (34%) whereas prolongation occurred with Bay k 8644 (22%). Glibenclamide (30 µM) did not change AP parameters but prevented entirely the effects of cromakalim without affecting those of nitrendipine. In contrast, Bay k 8644 antagonized the shortening of the action potential produced by nitrendipine but not that due to cromakalim.

These results show that the effects of cromakalim on papillary muscle preparations can be antagonized by a blocker of ATP-dependent K^+ channels (Fossed *et al.*, 1988) but not by an activator of the slow inward Ca^{2+} current. The lack of effects of glibenclamide on the AP profile indicate that the latter sulfonylurea does not inhibit outward K^+ currents involved in cardiac repolarization otherwise it would have increased the duration of the action potential. Furthermore, this finding is in agreement with the physiology of cardiac ATP-dependent K^+ channels which are believed to be silent under normoxic conditions (Weiss and Scott, 1987). Finally, the antagonism by glibenclamide of the electrophysiological effects of cromakalim lead us to suggest that the latter compound activates cardiac ATP-modulated K^+ channels.

Cain, C.R., Metzler, V. (1985) Naunyn-Schmiedeberg's Arch. Pharmac. 329, R53.
Fosset, M., De Weille, J.R., Green, R.D., Schmid-Antomarchi, H. & Lazdunski, M. (1988) J. Biol. Chem. (in press).
Grosset, A., Hicks, P.E. (1986) Br. J. Pharmac. 89, 500P.
Scholtysik, G. (1987) Naunyn-Schmiedeberg's Arch. Pharmac., 335, 692-696.
Sturgess, N.C., Ashford, M.L.J., Cook, D.L. & Hales, C.N. (1985) Lancet **8453**, 474-475.
Weiss, J.N. & Lamp, S.T. (1987) Sciences **238**, 67-69.

CONTRACTIONS TO THE α_2-ADRENOCEPTOR AGONIST RILMENIDINE IN RAT AND RABBIT AORTAS AND IN PERFUSED RAT KIDNEY

T.J. Verbeuren*, E. Koenig-Bérard & A.G. Herman, Institut de Recherches Servier (Fondax), 7 rue Ampère, F92200 Puteax, France & Division of Pharmacology, Univ. of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk, Belgium.

The oxazoline derivative rilmenidine ([(dicyclopropylmethyl)amino]2Δ2 oxazoline; S3341) is a new α_2-adrenoceptor agonist with potent antihypertensive properties (Koenig-Bérard et al. 1988). In the dog saphenous vein rilmenidine evoked contractions by stimulating the postjunctional α_2-adrenoceptors; in the rabbit pulmonary artery the compound inhibited the release of noradrenaline by activiting the prejunctional α_2-adrenoceptors (Verbeuren et al. 1986). At the α_2-adrenoceptors, rilmenidine was about 25 times less potent than clonidine.

The aim of the present investigation was to further study the interaction of rilmenidine with the vascular alpha-adrenoceptors; the contractile effect of the drug was studied in rat and rabbit aortas and in isolated perfused rat kidneys. Segments of the aortas, with or without endothelium, were mounted for isometric tension recording in organ chambers filled with Krebs-Ringer solution. Kidneys of Wistar rats were perfused with tyrode solution; changes in vascular resistance were recorded as changes in perfusion pressure (Collis and Vanhoutte, 1977).

Rilmenidine and clonidine caused concentration-dependent contractions in rat and rabbit aortas; in the rat, but not in the rabbit tissues, these contractions were enhanced after endothelium-removal. In the aortas of both species, the responses to the agonists were inhibited by prazosin and not by rauwolscine indicating that they were due to stimulation of α_1-adrenoceptors. From the pD_2 values (Table 1) it can be derived that rilmenidine was about 125 times less potent than clonidine at the α_1-adrenoceptors. In the perfused rat kidney rilmenidine and clonidine caused constrictions which maximally averaged 5% of the maximal responses to noradrenaline (263 ± 18 mmHg). Both compounds inhibited the responses to noradrenaline but not those to serotonin. In the presence of prostaglandin $F_{2\alpha}$(constriction : 63 ± 5 mmHg), a constrictor response to increasing doses of rilmenidine was unmasked (Max. response : 58 ± 7 mmHg).

Table 1 : pD_2 values for rilmenidine and clonidine.

Aorta		pD_2 (Mean ± SEM) Rilmenidine	Clonidine	Ratio Clonidine/Rilmenidine
Rat	+Endothelium	4.55 ± 0.06	6.77 ± 0.11	166
	-Endothelium	4.82 ± 0.15	7.00 ± 0.18	151
Rabbit	+Endothelium	4.57 ± 0.15	6.58 ± 0.09	102
	-Endothelium	4.56 ± 0.05	6.55 ± 0.13	98

Our studies show that rilmenidine is about 125 times less potent than clonidine at the vascular α_1-adrenoceptors. Since rilmenidine was only 25 times less potent than clonidine for the α_2-adrenoceptors (Verbeuren et al., 1986) rilmenidine appears to be 5 times more selective for the α_2-adrenoceptors than clonidine. In the rat kidney both rilmenidine and clonidine possess partial agonistic properties; elevation of tone with prostaglandin $F_{2\alpha}$ unmasks a constrictor response to the α_2-adrenoceptor agonists.

Collis, M.G. & Vanhoutte P.M. (1977) Circ. Res. 41, 759-767.
Koenig-Bérard, E. et al. (1988) Am.J.Cardiol.: 61, 22D-31D.
Verbeuren, T.J. et al. (1986) Arch. int. Pharmacodyn. 284, 38-52.

CYCLOSPORIN AND THE VASCULAR REACTIVITY OF ISOLATED PERFUSED RABBIT KIDNEYS

HS Cairns*, L Fairbanks, GH Neild and J Westwick, Institute of Urology, St Philip's Hospital, Sheffield St., London WC2A 2EX and Dept. of Pharmacology, Royal College of Surgeons, London WC2A 3PN

Nephrotoxicity is the major problem associated with the use of cyclosporin(CS).The mechanism of this toxicity is unclear although intrarenal haemodynamic changes, and in particular afferent and efferent glomerular arteriolar constriction, appear to play a major role (Thiel,1986).

We have examined the effects of vasodilators in isolated perfused kidneys (IPK) from rabbits treated with CS or CS vehicle (VEH). The left kidney from male NZW rabbits, weight 2.5-3 kg, was first perfused in-situ at 4°C with 100ml Krebs buffer containing 20 amino acids (KHB/AA)(Epstein et al.,1982), indomethacin 5.6 µM and prostacyclin 10^{-9}M and then in an organ chamber at 37°C with KHB/AA and indomethacin gassed with 95%O_2/5%CO_2. Vascular tone was induced with noradrenaline(150nM) and the perfusion rate adjusted to a perfusion pressure of 90mmHg. In kidneys from untreated rabbits endothelium derived relaxant factor (EDRF) appeared to play a role in regulating vessel tone in the IPK as infusions of methylene blue (10 µM) and oxyhaemoglobin (10 µM), both EDRF inhibitors (Griffith et al.,1984;Moore et al,1987), resulted in increased perfusion pressure while infusion of a cGMP phosphodiesterase inhibitor (M&B 22948A) produced a fall in perfusion pressure. Changes in response to bolus injections of vasodilators were measured as maximum fall in perfusion pressure(δP). Dose response curves were established for acetylcholine(ACh) and substance P, endothelium-dependent vasodilators, and prostacyclin (PGI_2) and nitroprusside (NP), endothelium-independent factors. Quinacrine (4 & 8 µM infusion), an EDRF inhibitor, resulted in a dose dependent inhibition of vasodilatation by ACh but not by NP.

Treated animals were given CS 15 mg/kg/day sc or (VEH) for 20 days (6 each grp). CS animals had a significant fall in creatinine clearance (12.0±1.5(s.e.mean) to 7.8±1.2ml/min, $p<0.05$) compared with controls (12.4±0.6 to 10.85±0.8),NS). δP after ACh, PGI_2 and NP was reduced in CS compared with VEH kidneys.

Drug		CS	VEH	CS	VEH	CS	VEH
ACh:	Dose (nmols)	0.27		1.375		13.75	
	δP (mmHg)	6.56	10.60*	11.20	16.60*	16.64	20.16*
	s.e.mean	(±0.7)	(±0.7)	(±1.0)	(±0.7)	(±1.3)	(±1.3)
PGI_2:	Dose (nmols)	0.028		0.28		2.8	
	δP	-	4.58	8.24	10.30*	11.31	17.41*
	s.e.mean		(±0.6)	(±0.5)	(±1.7)	(±1.3)	(±3.1)
NP:	Dose (nmols)	9.5		95		380	
	δP	5.04	8.24	7.64	15.27*	8.85	14.67*
	s.e.mean	(±1.4)	(±0.6)	(±1.2)	(±1.5)	(±2.1)	(±1.2)

* = $p<0.05$

These data show that response to all vasodilators tested was reduced by CS treatment, at least in vessels which control overall renal vascular resistance, and that this effect was not mediated specifically via an inhibition of EDRF.

Epstein, F.H. et al.(1982) Am.J.Physiol. 243,F284-F292
Griffith, T.M. et al. (1984) Nature 308,645-647
Moore, P. (1987) International Seminar on Relaxant Factors, IBC Conference, London
Thiel G (1986) Clin.Nephrol. 25(Suppl.1),205-210

ERGOMETRINE: A POTENT AGONIST AT 5-HT_1- BUT NOT 5-HT_2-RECEPTORS MEDIATING VASCULAR CONTRACTION

S.J. MacLennan*, G.R. Martin & P. Leff[1], Department of Biochemical Sciences, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS and [1]Fisons plc, Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire LE11 0RN.

Ergometrine contracts the coronary arteries of various species by activating 5-hydroxytryptamine (5-HT) receptors (e.g. Muller-Schweinitzer, 1980). In patients with vasospastic angina, ergometrine-induced coronary artery spasm is not prevented by ketanserin (Freedman et al., 1984), implying that in man 5-HT_2 receptors are not involved. However, ketanserin-resistant 5-HT_1 receptors also mediate vascular contraction, therefore, in this study, we examined the 5-HT_1 and 5-HT_2 receptor activities of ergometrine. Comparison was made with the chemical congener methysergide, its primary metabolite methylergometrine and the selective 5-HT_1 receptor agonist 3-[2-(dimethylamino)-ethyl]-N-methyl-1-H-indole-5-methanesulphonamide (prepared at W.R.L.), first described as GR43175 (Humphrey et al., 1987).

5-HT_1 and 5-HT_2 receptor mediated changes in tissue isometric force were recorded from ring preparations of rabbit saphenous vein (RbSV: Martin et al., this meeting) and rabbit aorta (RbA: Leff and Martin, 1986) respectively. Antagonist effects were studied as described previously (Leff and Martin, 1986). Agonist affinity (pK_A) and efficacy (τ) estimates were obtained by operational model-fitting of concentration-effect curve data before and after partial, irreversible receptor occlusion (Black et al., 1985).

At 5-HT_2 receptors in RbA, ergometrine, methysergide and methylergometrine did not express agonism but antagonised 5-HT effects with high (sub-micromolar) affinity. Only methysergide behaved competitively (pK_B = 8.25). GR43175 (30μM) displayed neither agonism nor antagonism. By contrast, each compound induced contraction of RbSV. From the concentration-effect curves the following parameters of 5-HT_1 receptor agonism were estimated (Table 1).

TABLE 1. pK_A and efficacy (τ) estimates for 5-HT, ergot alkaloids and GR43175 in RbSV.

AGONIST	pK_A	τ
5-HT	7.33	2.64
Ergometrine	7.83	1.80
Methylergometrine	7.68	2.11
Methysergide	6.84	0.99
GR43175	6.07	3.11

The results show that like 5-HT and GR43175, ergometrine is a potent 5-HT_1 receptor agonist, comparable in efficacy to both agents, but higher in affinity. They also demonstrate that at the 5-HT_1 receptor the metabolite methylergometrine is both higher in affinity and greater in efficacy than its parent, methysergide. It is concluded that 5-HT receptor-mediated vasoconstriction by these ergot alkaloids involves receptors of the 5-HT_1-, rather than the 5-HT_2-type.

Black, J.W. et al. (1985) Br. J. Pharmac., 84, 561-571.
Freedman, S.B. et al. (1984) Circulation, 70(2), 178-183.
Humphrey, P.P.A. et al. (1987) Br. J. Pharmac., 92, 616P.
Leff, P. and Martin, G.R. (1986) Br. J. Pharmac., 88, 585-593.
Muller-Schweinitzer, E. (1980) J. Cardiovasc. Pharmac., 2, 645-655.

ANTAGONISM OF 5-HT BY METHYSERGIDE ON THE RAT MESENTERIC VASCULAR BED IN VITRO

Mina Patel* and D.F. Weetman, Dept. Pharmacology Sunderland Polytechnic SR1 3SD.

Methysergide antagonises 5HT non-competitively on some tissues containing $5HT_2$-receptors, either by an allosteric (Kaumann & Frenken, 1985) or hemi-equilibrium (Martin & Leff, 1988) mechanism. The interaction of these two drugs has now been examined on the perfused mesenteric vascular bed (MVB).

The MVB was prepared from male Wistar rats (250-300g) and perfused with McEwen's solution (2ml/min from a peristaltic pump) at 37°C, which had been gassed with 95% O_2 & 5% CO_2. The endothelium was removed by a 30s perfusion with sodium deoxycholate (1mg/ml in 0.9% w/v NaCl) (Byfield, et al, 1986). Bolus doses (0.1ml) of 5-HT were administered at 10min intervals and the rise in peak perfusion pressure monitored. Subsequently, methysergide (0.1-1nM) was added to the perfusate and the reactivity to 5HT re-determined 30min later. The effects of ICI 169 369, a competitive $5HT_2$-receptor antagonist (Blackburn, et al, 1987), on the 5HT-methysergide interaction were determined.

5HT caused a dose-related rise in perfusion pressure (max = 105 ± 12mmHg, dose for 65mmHg rise: ED_{65} = 2 ± 0.2nmol, increment in dose to elevate pressure by 10mmHg measured by extrapolation at the midpoint of the curve, i.e. slope of the dose-response curve = 2.0 ± 0.4, n=4). These values did not change significantly when 5HT was re-tested 30 min later ($p>0.05$, n=4). Methysergide (0.1-1nM) antagonised 5HT, prolonging the time to maximal rise in perfusion pressure (i.e. the response was "slowed" when responses of equal magnitude were compared, e.g. 5HT control response = 7.5 ± 2.5s, after 0.3nM methysergide = 27 ± 6s, n=4, $p<0.05$). Furthermore, the dose-response curve to 5HT was rotated to the right; for example, after 1nM methysergide (max = 16 ± 3mmHg, ED_{65}>5.7 µmol, slope = 340 ± 30 compared with the slope of 5HT control curves = 2.1 ± 0.5, n=4, all $p<0.05$). In separate experiments, pretreatment with ICI 169 369 (10nM) 30 min before methysergide prevented a methysergide-induced rotation of the dose-response curve to 5HT (max = 64 ± 15mmHg, slope = 3.3 ± 0.6, n=4, $p<0.05$). Responses to 5HT in the presence of methysergide and ICI 169 369 were not "slowed" significantly (9 ± 1.7s for 5HT control and 12 ± 2.5s in the presence of the two antagonists, n=4, $p>0.05$), being similar to those caused by 5HT alone.

Thus the MVB is an ideal tissue to analyse the mechanism of non-competitive antagonism of 5HT by methysergide.

Blackburn, T.P., Cox, B., Growcott, J.W. & Hutton, R., (1987) Br. J. Pharmacol. 90 279P.
Byfield, R.A., Swayne, G.T.G. & Warner, T.J., (1986) Br. J. Pharmacol. 88 151P.
Kaumann, A.J. & Frenken, M., (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 328 295-300.
Martin, G.R. & Leff, P. (1988) Proc. Br. Pharmacol. Soc. Spring Meeting.

AMINE METABOLISM IN ISOLATED PERFUSED MESENTERIC ARTERIES OF THE RAT

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

Semicarbazide sensitive amine oxidase activity (SSAO) was first shown to occur in rat arteries by Coquil et al (1973), where it resides mainly in the plasma membranes of the smooth muscle cells (Wibo et al, 1980; Lyles & Singh, 1985). Although this activity has been demonstrated in homogenates of many rat blood vessels including the mesenteric arteries and veins, its ability to metabolise amines perfusing through intact vessels has not been shown.

Male Wistar rats (200 to 350g) were given 500 units of heparin, anaesthetised, the cranial mesenteric artery cannulated, and the mesenteric arterial bed perfused according to the method of McGregor (1965). Some rats were treated with 1 or 2mg/kg of (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145), a selective SSAO inhibitor (Lyles & Fitzpatrick, 1985), 1h before dissection. Clorgyline (Clorg; 10μM) was added to the perfusing fluid of those preparations where the effects of MAO inhibition were to be studied. Subsequently, 10ml of 25μM ^{14}C-benzylamine (BZ; 5μCi/umole) or 20ml of 100μM ^{3}H-tyramine (TYR; 6μCi/umole) in Krebs-Henseleit solution were perfused. A total of 30 and 100ml of perfusate were collected in 0.5 and 1ml fractions in the BZ and TYR experiments respectively. Each fraction was sampled and its total radioactivity counted. Deaminated products were extracted into organic solvent following acidification. Pressor responses to the amines and to noradrenaline were also measured.

The total radioactivity recovered in these experiments was 70% for BZ and 80% for TYR and there was no difference between treated and control groups. The total amounts of metabolites extracted from the perfusate are given below:

	Control(BZ)	MDL(BZ)	Control(TYR)	MDL(TYR)	Clorg(TYR)	MDL+Clorg(TYR)
Metabolites	30.8	5.4*	69.2	33.9*	70.6	29.2*
mean ± sem	±0.9	±0.4	±3.5	±4.1	±4.1	±2.9
(n)	(4)	(4)	(8)	(6)	(6)	(4)

(Metabolites are given in total nmoles extracted; *; $P \leqslant 0.001$ vs control)

No significant increase in the pressor response to tyramine could be detected in either the MDL or Clorg treated groups but a significantly larger area under the response was found in the MDL + Clorg group.

Thus SSAO in situ in the blood vessel wall is able to metabolise benzylamine and tyramine from the perfusing fluid. MAO-A does not appear to contribute to the metabolites collected with tyramine, while more than 50% were formed by SSAO. How the rest of the metabolites are produced remains to be established since there is no MAO-B present. Although tyramine is metabolised by SSAO, it is unlikely that this enzyme is simply a scavenger for dietary or circulating amines, since MAO in the gut wall and liver and uptake processes would be more suited for this purpose. Generation of reactive products, such as hydrogen peroxide, in the vicinity of the plasma membrane by SSAO could well have more relevance to its function within smooth muscle cells.

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

Coquil,J.F., Gordis,C., Mack,G. & Neff,N.H. (1973) Br. J. Pharmac. 48, 590-599
Lyles, G.A. & Fitzpatrick, M.S. (1985) J. Pharm. Pharmac. 37, 329-335
Lyles, G.A. & Singh, I. (1985) J. Pharm. Pharmac. 37, 643-647
McGregor, D.D. (1965) J. Physiol. 177, 21-30
Wibo,M., Duong,A.T. & Godfraind,T. (1980) Eur. J. Biochem. 112, 87-94

EFFECTS OF NEUROMEDIN-N AND NEUROTENSIN ON NET FLUID FLUX ACROSS THE SMALL BOWEL OF THE ANAESTHETISED RAT

R.A. Spokes*, Y.C. Lee & S.R. Bloom, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN.

Neuromedin-N, a six amino acid peptide isolated and purified from porcine spinal cord (Minamino *et al*, 1984) has close sequence homology with neurotensin, a thirteen amino acid brain/gut peptide. Both peptides have a remarkably similar peripheral distribution (Carraway & Mitra, 1987, Lee *et al*, 1987). Neuromedin-N enhances the contractility of guinea-pig ileum with about 15% the activity of neurotensin (Minamino *et al*, 1984) and increases pancreatic exocrine secretion and pancreatic, mesenteric and portal blood flow (Sumi *et al*, 1987). Neurotensin is a potent stimulant of net fluid secretion into the small intestine of the anaesthetised rat (Mitchenere *et al*, 1981). We have now tested the activity of neuromedin-N in this system and compared it with that of neurotensin.

Female Wistar rats (200g) were starved of solid food for 18-24 hr then anaesthetised with fentanyl, fluanisone and midazolam (i.p.). Segments of the small bowel, each 5cm long, were tied off, one in the duodenum, three in the jejunum and one in the ileum. Each segment was injected with 0.5ml of Krebs solution and the rat infused, via the femoral vein, with neurotensin (20, 50 or 100 pmol/kg/min) or neuromedin-N (400, 1000 or 2000 pmol/kg/min). Peptides were made up in saline containing 1% BSA, control animals received vehicle alone. After 40 min the rat was killed, the gut segments removed and weighed, before and after being opened, drained and gently blotted. Fluid accumulation (secretion) or loss (absorption) was measured as ml/g of blotted tissue weight.

Fluid absorption in the control rats decreased along the gut. In the jejunum and ileum low doses of neuromedin-N or neurotensin caused a reduction in net absorption while larger doses caused net secretion of fluid into the gut. The results for the four post-duodenal segments of each rat were meaned to give a single value. Dose-response curves were then constructed for the two peptides. One way analysis of variance indicated that neurotensin was 22 times more potent than neuromedin-N with 95% confidence limits of 16 and 32, (n = 6-10). Neither peptide had any effect in the duodenum. It is interesting to note that concentrations of both peptides in the duodenum were found to be low compared with the rest of the small intestine (Carraway & Mitra, 1987, Lee *et al*, 1987).

The results presented here suggest that neuromedin-N may have a role in the control of fluid movement across the gastrointestinal tract.

Carraway, R.E. & Mitra, S.P. (1987) Endocrinology 120, 2092-2100
Lee, Y.C., Ball, J.A., Reece, D. & Bloom, S.R. (1987) Febs Lett. 220, 243-246.
Minamino, N., Kangawa, K. & Matsuo, H. (1984) Biochem. Biophys. Res. Commun. 122, 542-549.
Mitchenere, P., Adrian, T.E., Kirk, R.M. & Bloom S.R. (1981) Life Sci. 29, 1563-1570.
Sumi, S., Inoue, K., Kogire, M., Doi, R., Takaori, K., Yajima, H., Suzuki, T. & Tobe, T. (1987) Neuropeptides 9, 247-255.

INVESTIGATION OF GENTAMICIN, VERAPAMIL AND MAGNESIUM INTERACTIONS ON CALCIUM TRANSPORT IN RAT RENAL BRUSH BORDER MEMBRANE VESICLES

C. Godson * & M.P. Ryan, Department of Pharmacology, University College Dublin, Belfield, Dublin 4, Ireland.

Use of the aminoglycoside (AG) antibiotics is limited by dose-dependent nephrotoxicity. We have previously reported on gentamicin-induced nephrotoxicity in cystic fibrosis patients (Godson et al., 1986). The renal pathogenesis of the AG can be attributed to their selective accumulation within renal proximal tubular cells. Reabsorption via the brush border membrane is thought to constitute the dominant route of tubular accumulation (Williams et al., 1986). Because the initial event in the renal tubular reabsorption of AG involves binding to the brush border membrane, the use of brush border membrane vesicles (BBMV) may provide useful models for studying in vitro the mechanisms of gentamicin-induced nephrotoxicity. We have previously reported (Godson & Ryan, 1987) that gentamicin dose-dependently reduced ^{45}Ca uptake into BBMV from rat renal cortex. The present investigations were aimed at further elucidation of this action and in particular the effects of verapamil and Mg on ^{45}Ca uptake in the absence and presence of gentamicin. The effects of gentamicin on ^{28}Mg uptake were also studied.

BBMV were prepared by a divalent cation precipitation technique from renal cortex obtained from male Wistar rats (Biber et al., 1981). Purity of the membrane preparations was assessed by marker enzyme assays. Vesicle morphology was examined by electron microscopy. Transport studies were carried out using ^{45}Ca or ^{28}Mg tracer and a rapid filtration technique.

Gentamicin (100 µg/ml) reduced ^{45}Ca uptake (n mole/mg protein; mean ± s.e. mean) in 0.1 mM $CaCl_2$ over a 60 min time period. For example, at 60 min; ^{45}Ca uptake was reduced from 4.37 ± 0.08 to 3.02 ± 0.20 ($p<0.05$) by gentamicin (100 µg/ml). Verapamil (0.1 mM) alone did not significantly affect ^{45}Ca uptake. Gentamicin (100 µg/ml) reduced ^{45}Ca uptake by similar amounts in the absence and presence of 0.1 mM verapamil. ^{45}Ca uptake into control BBMV was not affected by incubation in equimolar $MgCl_2$ (0.1 mM). Likewise, gentamicin (100 µg/ml) reduced ^{45}Ca uptake by similar amounts in the absence or presence of 0.1 mM $MgCl_2$. Gentamicin (0 - 200 µg/ml) significantly ($p<0.01$) reduced ^{28}Mg uptake over time periods between 0 and 60 min. For example, at 60 min gentamicin (200 µg/min) reduced the Mg uptake (nmol/mg protein) from 2.73 ± 0.30 to 1.07 ± 0.06 ($p<0.01$). The effects of gentamicin on the initial rate of ^{28}Mg uptake was investigated in media containing final concentrations 0.25 - 4.0 mM $MgCl_2$. Uptake of ^{28}Mg did not reach saturation at these concentrations during the 20 second initial uptake studies. Gentamicin (25 - 200 g/ml) significantly ($p<0.05$) reduced the initial rates of ^{28}Mg uptake.

These results provide further evidence for the effects of gentamicin on ^{45}Ca uptake in BBMV from renal cortex. The effect does not seem to be mediated by a verapamil-sensitive Ca ion channel. The inhibition of ^{28}Mg uptake into BBMV may provide insights into the mechanisms of gentamicin-induced renal Mg wasting. The failure of equimolar $MgCl_2$ concentrations to alter the gentamicin inhibition of ^{45}Ca uptake suggests relatively specific effects of gentamicin on Ca and Mg transport in these BBMV preparations.

This work was supported by the Cystic Fibrosis Association of Ireland.

Biber, J. et al (1981) Biochem. Biophys. Acta 647, 169-176.
Godson, C. et al (1986) Ir. J. Med. Sci. 155, 130.
Godson, C. and Ryan, M.P. (1877) Br. J. Pharmacol., 490P.
Williams, P.D. et al (1986) J. Pharmacol. Exp. Ther. 237, 919-925.

THE DISPOSITION OF DIGOXIN-SPECIFIC ANTIBODIES/ANTIBODY FRAGMENTS IN THE RAT

P. C. Johnston*, I. H. Stevenson, D. S. Hewick, Department of Pharmacology and Clinical Pharmacology, Ninewells Hospital and Medical School, DUNDEE. DD1 9SY.

Fragments (Fab) of sheep immunoglobulin G (IgG), specific for digoxin, are used to reverse severe cardiac glycoside toxicity (Wenger et al, 1985). The use of heterologous drug-specific antibody fragments could also have a role in the treatment of poisoning by other drugs and toxins. However, despite their current and potential use, knowledge regarding the disposition of Fab is limited. This situation would be improved with the availability of a more convenient assay, which unlike previous procedures, did not involve the use of radiochemicals. Accordingly, we have developed an enzyme-linked immunosorbent assay (ELISA) which has been used to compare in rats, the disposition of Fab, IgG and Digibind (the commercial Fab preparation produced by Wellcome Ltd).

Female Sprague-Dawley rats (210-280g) were anaesthetised with pentobarbitone (55 mg kg^{-1} i.p.), cannulated (carotid artery, jugular vein and bile duct) and given Digibind, Fab or IgG (1 mg kg^{-1} i.v.) followed by NaSCN (0.25 ml, 100 mg ml^{-1}). Blood samples and bile were collected over a 2h period, after which time urine produced was also collected. A 40 min plasma sample was used to measure extracellular fluid volume (ECFV) (Bianchi et al, 1981). Digibind, Fab or IgG concentrations were determined by an ELISA according to the following general procedure. Wells of a microtitre plate were coated with a dilution of donkey anti-sheep serum, solutions containing Digibind/Fab/IgG added, and the plate incubated allowing binding to the first antibody to occur. Next, the plate was incubated with anti-sheep antibody alkaline phosphatase conjugate (binds to the Digibind/Fab/IgG) and then p-nitrophenyl phosphate. The resulting absorbances (proportional to Digibind/Fab/IgG concentrations) were read at 405 nm. For each preparation, log plasma antibody concentration versus time plots allowed the calculation of distribution and elimination half-lives ($t\alpha_{\frac{1}{2}}$ and $t\beta_{\frac{1}{2}}$), and the apparent volume of distribution (Vd).

The sensitivity of the ELISA increased towards the antibody preparations in the order Digibind, Fab, IgG. For instance, to measure a concentration of about 20 µg ml^{-1}, 20 µl of plasma had to be diluted by 20 and 100 for Digibind and IgG respectively to obtain absorbance values suitable for reading on the calibration curve. The plasma disposition of Digibind, Fab and IgG appeared to fit a 2-compartment model giving the following respective parameters: $t\alpha_{\frac{1}{2}}$, 1.4, 1.7, 1.3 min; $t\beta_{\frac{1}{2}}$, 86, 113, 358 min; Vd, 45, 45, 34 ml kg^{-1}. The respective numbers of rats used were 6, 4 and 4 with an overall mean (± s.e. mean) ECFV of 305 ± 8 ml kg^{-1}. The percentage of the dose excreted in the urine was 0.92 ± 0.19, 0.48 ± 0.17, 0.07 ± 0.02 respectively. No antibody excretion occurred in the bile.

The increased assay sensitivity associated with IgG is presumably associated with enhanced antigenicity of the whole antibody. The two antibody fragment preparations had similar dispositional profiles. The longer $t\beta_{\frac{1}{2}}$ for IgG reflected the fact (as shown by the urinary data) that the whole antibody is only relatively poorly renally excreted. The Vd of IgG approximated the plasma volume (35 ml kg^{-1}, Waynforth, 1980). Surprisingly, and in contrast to data reported for the baboon (Smith et al, 1979), the corresponding values for Digibind and Fab are only slightly larger, and are much smaller than the ECFV.

Bianchi, M. et al (1981). Clin. Sci. 61, 685.
Smith, T.W. et al (1979). Clin. & Exp. Immunol. 36, 384.
Waynforth, H.B. (1980). In "Experimental and Surgical Technique in the Rat". pp 239-257. London, Academic Press.
Wenger, T.L. et al (1985). J. Am. Coll. Cardiol. 5, 118A.

THE PERCUTANEOUS ABSORPTION OF BENZYL ACETATE THROUGH RAT SKIN IN VITRO

S.A. Hotchkiss*, S. Fraser, J.M. Miller & J. Caldwell, Department of Pharmacology & Toxicology, St. Mary's Hospital Medical School, London W2 1PG.

Benzyl acetate is a naturally occurring ester, widely used as a fragrance agent and food additive and currently undergoing extensive safety evaluation. As a major route of human exposure is via the skin, a knowledge of its percutaneous absorption, and factors affecting this absorption, is important.

Circles (0.32cm^2) of full thickness shaved dorsal skin from male Fischer 344 rats were used in the Bronaugh flow-through diffusion apparatus (1). (Methylene-^{14}C) benzyl acetate (5*u*l) was applied to the epidermal side either neat or in an ethanol vehicle, so that mixtures containing 10-100% benzyl acetate were applied. The skin was occluded and acceptor phase perfusate (0.9% saline) was collected hourly for up to 48h and assayed for radioactivity. The amount of radioactivity remaining on/in the skin at the end of the experiment was also determined.

The absorption of benzyl acetate appeared to be a first order process, reaching 47-52% of the applied dose after 48h. The amount absorbed through skin in 24h increased proportionally from 0.66 $\pm$ 0.042 to 10.27 $\pm$ 0.51 mg/cm^2 as the amount of benzyl acetate applied to the skin was increased from 1.66 to 33.13 mg/cm^2 ($r = 0.996$). The use of ethanol as a vehicle had no effect on benzyl acetate absorption, and did not affect the recovery in the skin at the end of the experiment, which was consistent at ca. 10%.

Data generated from *in vivo* experiments (2) correlate very well with these percutaneous absorption data, supporting the use of this *in vitro* system as a model for the evaluation of the penetration of benzyl acetate *in vivo*.

Bronaugh, R.L. and Stewart, R.F. (1984) Methods for *in vitro* percutaneous absorption studies IV: the flow-through diffusion cell. Journal of Pharmaceutical Science 74 (1), 64-67.

Chidgey, M.J., Kennedy, J.F. and Caldwell J. (1987) Studies on benzyl acetate III. The percutaneous absorption and disposition of (methylene-^{14}C) benzyl acetate in the rat. Food and Chemical Toxicology 25 (7), 521-525.

THE EFFECTS OF FRUSEMIDE ON THE DISPOSITION OF SALICYLIC ACID IN THE HORSE

Gwyn A. D'Souza,* J. Caldwell, R.L. Smith, M. Horner[1] & M.S. Moss[1], Department of Pharmacology and Toxicology, St. Mary's Hospital Medical School, London W2 1PG and [1]Horseracing Forensic Laboratory, Newmarket, Suffolk CB8 7DT.

The high-ceiling diuretic frusemide is used in race horses for the treatment of oedema and for the prophylaxis of epistaxis. Due to the increased use of frusemide in recent years, this study was designed to determine (a) the potential pharmacokinetic interaction between frusemide and the non-steroidal anti-inflammatory drug salicylic acid, and (b) whether the forensic detection of salicylic acid is hindered by frusemide administration.

Three horses received on separate occasions in a randomized cross-over design frusemide (1mg/kg; i.v.) alone, [^{14}C]-salicylic acid (40mg/kg; 20μCi/horse p.o.) alone and [^{14}C]-salicylic acid (40mg/kg; 30μCi/horse p.o.) followed immediately by frusemide (1mg/kg; i.v.). Serial venous blood samples were collected from an indwelling jugular cannula for 24h and urine was collected as voided for 48h. Salicylate concentrations were determined by liquid scintillation spectrometry since, in the horse, salicylate has been shown to be excreted almost totally unchanged in the urine (Marsh *et al.*, 1981).

Plasma pharmacokinetic parameters (Table 1) show that the peak salicylate concentration (C_{max}) was significantly higher after administration with frusemide and was attained at later times. After absorption was complete, the elimination rate constant (K) and the half-life ($T_{0.5}$) were unaltered by frusemide, resulting in persisting salicylate concentration differences in plasma for up to 12h. There were significant reductions in volume of distribution and clearance, and an increase in AUC when salicylate was administered with frusemide. Frusemide had an immediate but short-lasting diuretic effect with 7-9 litres of urine produced in the first hour. It significantly (29.3% *vs* 37.6%, $p<0.05$) reduced the excretion of salicylate in the first 4h after administration. Due to a five-fold increase in urine volume during this time there was an approximately 10-fold dilution in urinary salicylate concentrations. However total salicylate recovery was 95.6 - 100.9% of the dose, and was unaffected by frusemide co-administration.

Table 1. Pharmacokinetic parameters for salicylate with and without frusemide (mean ± SD; n = 3)

	C_{max} (μg/ml)	t_{max} (h)	$T_{0.5}$ (h)	K (h^{-1})	App.V_d (l/kg)	Cl (ml/h/kg)	AUC (μg/ml.h)
Salicylate	123 ± 5	1.67 ± 0.3	3.40 ± 0.6	0.208 ± 0.04	0.22 ± 0.02	53 ± 12	756 ± 137
Salicylate+ Frusemide	160 ± 9	2.37 ± 0.7	3.61 ± 0.7	0.197 ± 0.04	0.15 ± 0.006	37 ± 10	1122 ± 260
Paired t-test	<0.05	NS	NS	NS	<0.05	<0.05	<0.05

The higher plasma levels of salicylate after frusemide may result from haemoconcentration as a consequence of diuresis. The delay in salicylate excretion is likely to be because both salicylic acid and frusemide are actively secreted into equine urine by the organic acid transport system. Competition between these compounds is likely to reduce the concentration of salicylate in urine. This effect however will be short lasting due to the rapid elimination of frusemide in the horse (Roberts *et al.*, 1978). These data show that frusemide administration results in reduced urinary salicylate concentrations, which may result in inconsistent drug detection in routine screening tests. However, the increased salicylate concentrations in plasma should enhance the detection of the drug in blood samples.

Marsh M.V. *et al.* (1981) Xenobiotica 11, 655-663.
Roberts B.L. *et al.* (1978) J. Eq. Med. Surg. 2, 185-194.

PLACENTAL TRANSFER OF MEPTAZINOL IN THE RABBIT

R J Carson* and F Reynolds, Anaesthetic Unit, St Thomas' Hospital, London SE1 7EH.

Meptazinol has been advocated for use in labour, some reports (DeBoer et al, 1987) suggesting it produces less neonatal depression than pethidine. The placental transfer of these two drugs and antipyrine, as an index of placental exchange, have therefore been compared. Near term, pregnant, anaesthetised New Zealand White rabbits were infused intravenously with meptazinol (1.25mg/ml), pethidine (1.25mg/ml) and antipyrine (4mg/ml) simultaneously at a rate of 12ml/h initially, declining to 3ml/h. Umbilical vessels of a single placenta were perfused with Krebs buffer (pH 7.4) containing 3% Dextran as described by Hamshaw-Thomas et al (1984). Drug concentrations were measured in the umbilical effluent (UV) and maternal arterial plasma (M) by HPLC (meptazinol) and GLC. Protein binding by ultrafiltration of fetal and maternal plasma and partition coefficients for oleyl alcohol/buffer pH 7.4, were determined. Placental clearance of unbound drug (UV concentration x flow/M free concentration) was used as a measure of transfer rate.

Table 1 Physicochemical data. Mean ± SD

Drug	pKa	Protein binding (%) Maternal	Fetal	Partition coefficient
Meptazinol	8.7	41.4±12.1 (n=13)	31.4±19.4 (n=7)	8.18±2.20 (n=5)
Pethidine	8.5	39.5±19.7 (n=13)	23.0±14.2 (n=4)	19.5 ±4.05 (n=5)
Antipyrine	1.4	0	0	3.15±1.39 (n=5)

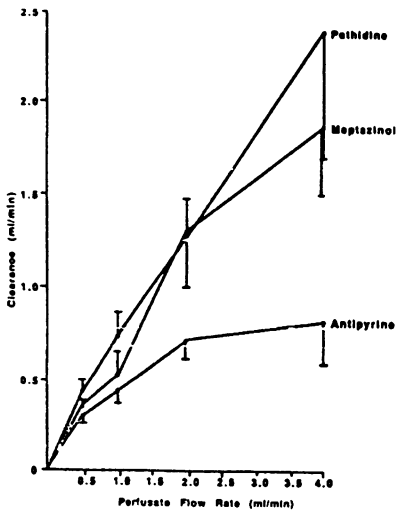


Fig 1 Clearance of unbound drugs (means and standard errors, in 13 experiments)

Placental clearance of pethidine and meptazinol increased with flow rate up to 4ml/min while that of antipyrine increased only up to 2ml/min. The findings for pethidine and antipyrine are similar to those reported previously (Hamshaw-Thomas et al, 1984). There were no significant differences in clearance between the drugs, all showing flow dependent transfer, though there was a tendency for the transfer of the less lipid soluble meptazinol, and antipyrine, to show permeability dependence at high flows. Antipyrine is essentially non ionised and unbound with an equilibrium UV/M ratio approaching 1 (Hamshaw-Thomas and Reynolds, 1985). Both opioids are basic and weakly protein bound with a tendency to reduced fetal binding, suggesting that, in vivo, protein binding disparities would counteract ion trapping, and at equilibrium UV/M ratios would also be near unity.

DeBoer, F.C. et al (1987) Br.J.Obstet.Gynaec. 94, 256.
Hamshaw-Thomas, A. et al (1984) Placenta 5, 61.
Hamshaw-Thomas, A. & Reynolds, F. (1985) Br.J.Obstet.Gynaec. 92, 706.

UPTAKE OF [^{195}Pt] CISPLATIN INTO LLCPK$_1$ AND MDCK RENAL EPITHELIAL CELL LINES

B.A. Casey[1]*, C.A. McAuliffe[2], I. Pratt[1], M.P. Ryan[1], H.L. Sharma[2] & N.D. Tinker[2]
[1]Department of Pharmacology, University College Dublin, Dublin 4, Ireland and [2]Department of Chemistry, UMIST, Manchester, England.

Cisplatin (CP), a platinum containing co-ordination complex, is an effective anti-neoplastic agent. The clinical use of CP is limited chiefly by dose-related nephrotoxicity. While CP is known to accumulate in the kidney the mechanism of renal cellular accumulation is unclear. Renal epithelial cell lines provide useful models for studying the cellular uptake of CP. We have investigated the uptake of [^{195}Pt] CP in two established renal cell lines, LLCPK$_1$ which exhibit properties of the proximal tubule and MDCK cells which exhibit properties of the distal tubule.

Cells were cultured in Dulbeccos modified Eagles medium (DMEM) supplemented with 10% foetal calf serum and glutamine (2 mM) and maintained in an atmosphere of 9% CO_2/91% air at 37°C. Cellular uptake of ^{195}Pt-CP (5 μg/ml and 40 nCi/ml) was examined at 2, 4, 8, 12, 24 and 48 hours in the absence and presence of four potential nephroprotectants: diethyldithiocarbamate (DDTC 1 mM), mercaptoethane-sulphonate (MESNA 5 mM), methionine (10 mM) and amiloride (0.1 mM).

LLCPK$_1$ cells were found to accumulate CP to a greater extent than MDCK cells at all time points measured ($p<0.005$). For example, at 48h the LLCPK$_1$ cells had accumulated 5209±457 cpm/10^6 cells compared to an accumulation of 1950±38 cpm/10^6 cells in the MDCK cell line. The presence of serum in the culture medium resulted in a decreased uptake of CP compared to that found in serum-free medium ($p<0.005$). The presence of certain cytoprotectants reduced significantly CP accumulation in both LLCPK$_1$ and MDCK cells. The mean CP accumulations at 48 h expressed as % of appropriate control values in LLCPK$_1$ and MDCK cells respectively were: in 1 mM MESNA, 22 ± 2% and 34 ± 5%; in 10 mM methionine, 41 ± 6% and 43 ± 6%; in 0.1 mM amiloride, 54 ± 3% and 82 ± 2%. At these concentrations, MESNA and methionine demonstrated cytoprotective actions against CP. Marked increases in the accumulation of CP (40-fold in LLCPK$_1$ and 20-fold in MDCK even after 2h) were observed in the presence of DDTC (1 mM) and no cytoprotective actions were observed.

The difference in uptake of CP *in vitro* by the two cell types may reflect the differing susceptibility of proximal and distal tubules to the nephrotoxic action of CP *in vivo*. DDTC did not protect against CP-induced nephrotoxicity in cultured cells although it has been reported to have a protective effect *in vivo* (Borch & Pleasants, 1979). The increased uptake of CP in the presence of DDTC may be due to the formation of a lipophilic chelate which crosses cell membranes more readily that the parent drug and increased cellular levels of CP may account for the absence of cytoprotective effects. MESNA and methionine may decrease CP uptake by extra-cellular chelation by virtue of the sulphydryl groups which would also explain the observed antagonism of the antiproliferative effects of CP in this model.

This work was supported by a grant from St. Lukes Cancer Research Fund.

Borch, R.F. & Pleasants, M.E. (1979) Proc. Natl. Acad. Sci. (USA) 76, 6611-6614.

REDUCED NEPHROTOXICITY IN VIVO AND IN VITRO OF CISPLATIN-METHIONINE COMPLEX

T. Ormond*, I. Pratt, and M.P. Ryan, Department of Pharmacology, University College Dublin, Belfield, Dublin 4, Ireland.

Cisplatin (CP) is an effective antineoplastic agent used in a variety of human malignancies including ovarian, testicular and small cell lung tumours. Its clinical use is complicated by nephrotoxicity. It would be useful to devise means of reducing the nephrotoxicity of CP while retaining the cellular anti-proliferative actions. It has been suggested that a methionine complex is one of the biotransformation products of CP (Daley-Yates & McBrien, 1982). Melvic & Pettersen (1987) demonstrated that simultaneous treatment with CP and high dose methionine was less lethal in terms of colony forming ability in NHIK 3025 cells than treatment with CP alone. We have compared the nephrotoxicity and cytotoxicity both in vivo and in vitro of CP and a CP-methionine complex.

The methionine complex of CP was prepared by incubating molar ratios of L-methionine with CP at 37°C overnight. Complex formation was confirmed by thin layer chromatography. The relative nephrotoxicity in vivo of CP and CP-methionine complex was assessed in male Wistar rats 5 days after i.p. administration of 6 mg CP/kg body weight and the equivalent amount of CP as the methionine-complex. The renal handling of CP-methionine was assessed by investigating effects on [^{3}H]-p aminohippuric acid (PAH) and [^{14}C]-tetraethylammonium bromide (TEA) accumulation in renal cortical slices. The cytotoxicity of CP-methionine was assessed by [^{3}H]-thymidine incorporation in a C6 glioma cell line.

CP (6 mg/kg) was nephrotoxic in Wistar rats as indexed by significant increases in plasma creatinine ($p<0.005$), blood urea nitrogen ($p<0.001$) and significant decreases in [^{3}H]-PAH uptake to 28 ± 4% of control ($n=6$, $p<0.001$) and [^{14}C]-TEA to 15 ± 6% of control ($n=6$, $p<0.02$) in renal cortical slices taken from treated animals. In marked contrast, CP-methionine was not nephrotoxic in Wistar rats as reflected by any of the nephrotoxic markers studied. In addition, in renal slices from untreated rats, in vitro, CP-methionine at concentrations up to 4 mM did not decrease [^{14}C]-TEA uptake, whereas CP dose-dependently (1-4 mM) reduced [^{14}C]-TEA uptake. In C6 glioma cells, the cytotoxicity of CP-methionine was less then that of CP alone and the relative cytotoxicity was dependent on the ratio of methionine to CP.

These results indicate that CP-methionine complex is much less nephrotoxic in vitro and in vivo than CP. CP-methionine retained some cytotoxic properties against C6 glioma cells. The in vitro renal slice transport studies suggest that CP-methionine is not transported by the proximal tubules. This finding is supportive of the report by Daley-Yates & McBrien (1983) that the mono-methionine substitution complex of cisplatin has a fractional clearance close to unity suggesting that filtration is the main mechanism for excretion. The observations that CP-methionine is less nephrotoxic than CP may be due to the complex not being transported by the renal proximal tubule.

This work was supported by the Health Research Board of Ireland.

Daley-Yates, P.T. & McBrien, D.C.H.(1982) Chem-Biol. Interactions., 40:25-334.
Daley-Yates, P.T. & McBrien, D.C.H. (1983) Biochem. Pharmacol., 32:181-184.
Melvik, J.E. & Pettersen, E.O. (1987) Inorg. Chem. Acta. 137, 115-118.

EFFECTS OF STREPTOZOTOCIN-INDUCED DIABETES ON GENTAMICIN NEPHROTOXICITY

H. McGlynn, M.P. Ryan & E. Stuart*, Department of Pharmacology, University College Dublin, Belfield, Dublin 4, Ireland.

Nephrotoxicity is a dose-limiting feature of aminoglycoside nephrotoxicity. The renal pathogenesis of the AG can be attributed to their selective accumulation within renal proximal tubular cells. Reabsorption via the brush border membrane is thought to constitute the dominant route of tubular accumulation (Williams et al., 1986). In rats, streptozotocin(STZ)-induced diabetes mellitus has been shown to provide protection against aminoglycoside nephrotoxicity (Cronin et al., 1983). Previous reports from our laboratory (Godson & Ryan, 1987, 1988) have indicated that gentamicin affects ^{45}Ca transport and phosphatidylinositol metabolism in brush border membrane vesicles (BBMV) from rat renal cortex. The present study concerned the effects of STZ-induced diabetes on gentamicin nephrotoxicity in vivo and in vitro in rat renal BBMV.

Diabetes mellitus was induced in male Sprague Dawley rats (200-250 grams) by administering STZ (60 mg/kg i.p. in a citrate buffer), non-diabetic animals received the vehicle only. Animals were housed in individual metabolism cages with free access to food and water. Induction of diabetes was monitored by polyuria and glycosuria. 14 days after induction of diabetes, animals were treated with gentamicin (60 mg/kg/12h i.p. for 5 days). Animals were killed and indices of in vivo nephrotoxicity were assessed. BBMVs were prepared using a magnesium precipitation technique (Biber et al., 1981) from diabetic and non-diabetic animals with and without gentamicin treatment. Purity of BBMV was assessed by marker enzymes and Ca transport was studied using ^{45}Ca and a rapid filtration technique.

STZ-treated animals exhibited polyuria and glycosuria. Gentamicin nephrotoxicity as assessed by plasma creatinine and blood urea nitrogen was significantly reduced ($p<0.05$) in STZ-treated animals compared to appropriate controls. The brush border marker enzymes γ GT and alkaline phosphatase were enriched 14.7 ± 1.4 and 7.2 ± 1.8-fold respectively compared to homogenates. Gentamicin (12.5 to 200 µg/ml) inhibited the transport of ^{45}Ca in a dose-dependent manner in BBMV preparations form both diabetic and non-diabetic animals.

Animals treated with gentamicin in vivo showed a greatly lowered rate of ^{45}Ca-transport into BBMVs when compared with non-gentamicin treated animals. For example, Ca uptake (nmol/mg protein; mean ± s.e. mean) in diabetic animals at 40 min was reduced from 7.79 ± 0.20 to 2.66 ± 0.06 by in vivo gentamicin treatment. Significant differences were also detected in ^{45}Ca uptake at 40 min in diabetic (2.66 ± 0.06) versus non-diabetic (1.55 ± 0.05; $p<0.05$) rats treated with gentamicin in vivo.

These results confirm findings (Cronin et al., 1983; Pastoriza-Munoz et al., 1987) that STZ-induced diabetes has protective effects against gentamicin nephrotoxicity. The investigation of ^{45}Ca transport in BBMV may provide useful insights into the protective mechanisms involved.

Biber, J. et al (1981) Biochem. Biophys. Acta. 647, 169-176.
Cronin, R.E. et al (1983) Mineral Electrolyte Metab. 9, 38-44.
Godson, C. & Ryan, M.P. (1987) Br. J. Pharmacol., 490P.
Godson, C. & Ryan, M.P. (1988) Biochem. Soc. Trans. (in press).
Pastoriza-Munoz, E. et al (1987) J. Pharmacol. Exp. Ther. 241, 166-173.
Williams, P.D. et al (1986) J. Pharmacol. Exp. Ther. 237, 919-925.

HISTAMINE AS AN INHIBITOR OF CYTOCHROME P-450-CATALYSED DRUG METABOLISM - METABOLIC AND SPECTRAL STUDIES

C.Q. Morris, H.K. Crewe, J. Harlow, G.T. Tucker, H.F. Woods and M.S. Lennard*, University Department of Pharmacology and Therapeutics, Royal Hallamshire Hospital, Sheffield S10 2JF.

Cimetidine is one of a number of imidazole derivatives that are potent inhibitors of drug metabolism. Cimetidine is a close structural analogue of histamine and in a preliminary report the latter compound was also shown to inhibit drug oxidation (Lennard et al, 1986). Metabolic and spectral studies extending this work are now described.

Microsomes were prepared from male Wistar rat livers and from human liver obtained from three renal transplant donors. Rat livers were isolated and perfused in the recirculation mode with a semi-synthetic medium (volume = 150 ml) at a flow rate of 15 ml/min. Metoprolol, α-hydroxymetoprolol, O-demethylmetoprolol, lignocaine, 3-hydroxylignocaine and histamine (after derivatisation with O-phthaldehyde) were assayed by HPLC.

Histamine was a competitive inhibitor of both α-hydroxymetoprolol (Ki = 164 μM; IC_{50} at 20 μM substrate concentration = 308 μM) and O-demethylmetoprolol (Ki = 243 μM; IC_{50} at 20 μM = 400 μM) appearance in rat liver microsomes. The metabolites of histamine, N-acetylhistamine, imidazolyl acetic acid, 1-methylhistamine and methylimidazolyl acetic acid, were less potent inhibitors of metoprolol metabolism in this system (range of IC_{50} values at 20 μM = 4.7 to 9.5 mM for α-hydroxylation and 4.8 to >10 mM for O-demethylation). The oxidation of metoprolol by human liver microsomes was also inhibited by histamine (IC_{50} values for O-demethylmetoprolol appearance at 25 μM: Liver HL4 = 3.7, HL3 = 3.8 and HL1 > 10 mM). By comparison cimetidine gave an IC_{50} value of 1.5 mM for liver HL2. Histamine impaired the disappearance of lignocaine (4.17 μM) from rat liver microsomes, increasing the area under the incubation mixture drug concentration - time curves (AUC) by 47 $\pm$ 13% s.d. (n=4, p = 0.017) at 250 μM and by 109 $\pm$ 35% s.d. (n=6, p < 0.001) at 1000 μM histamine. This was accompanied by a corresponding inhibition of 3-hydroxylignocaine appearance (mean $\pm$ s.d. % decrease in AUC = 16 $\pm$ 8% at 250 μM (p = 0.046) and 49 $\pm$ 17% at 1000 μM histamine (p = 0.001)). Histamine interacted reversibly with rat liver microsomes in an apparently Type II fashion giving an absorption maximum of 432 nm, a minimum of 408 nm and an apparent spectral dissociation coefficient (K_s) of 0.11 mM. A K_s value of 0.04 mM was obtained for cimetidine. When histamine (1.7-8.3 μM) was incubated alone with rat liver microsomes, no loss of substrate was detected. Histamine impaired the elimination of metoprolol (1 μM) from the isolated perfused rat liver in a dose-dependent manner (p < 0.001) (mean $\pm$ s.d AUC at 0,10,50,100,500 and 1000 uM histamine = 2588 $\pm$ 155, 2721 $\pm$ 36, 2980 $\pm$ 192, 3601 $\pm$ 463, 4876 $\pm$ 278 and 5769 $\pm$ 420 ng.ml^{-1}.min, respectively).

The findings indicate that histamine can enter hepatocytes, interact with cytochrome P-450 and inhibit drug oxidation in the rat and in man. Histamine does not appear to be a substrate for cytochrome P-450. Localised hepatic histamine concentrations may be as high as 100 mM (W. Lorenz, personal communication) but further studies are required to determine whether histamine plays a role in the regulation of drug metabolising enzymes.

Lennard M.S. et al (1986) Biochem. Pharmac. 35, 2459.

THE INFLUENCE OF SINGLE DOSE RIFAMPICIN ON PYRAZINAMIDE KINETICS IN RABBITS

K. Chan*, R. Teoh[1], C.L. Wong & Jean Woo[1], Departments of Pharmacology and Medicine[1], The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

Pyrazinamide (PZA) and rifampicin (RIF) are often prescribed in combination as first line treatment in tuberculous meningitis. Very little information is available on the penetration of these anti-TB drugs into the CSF when they are given in combinations. In rabbits, there was no apparent barrier for the transfer of PZA into CSF and no difference in the area under plasma concentration-time curves (AUC) after either intravenous (i.v.) or oral (p.o.) doses (Chan & Wong, 1988). However, the penetration of RIF into the CSF appeared to be limited and saturable (Woo et al, 1988). We now report the effect of RIF after a single dose on the i.v. and oral disposition of PZA in rabbits.

Six New Zealand white rabbits (5F, 1M, mean weight 5.0 ± 0.42 kg) after an overnight fast, were given simultaneous PZA (30 mg/kg) and RIF (10 mg/kg) i.v. and orally on two separate occasions in a cross over design. Blood samples were collected at intervals in the conscious animal from a cannula in the central ear artery and CSF from the cisterna magna under light thiopentone anaesthesia. PZA and RIF concentrations in biological fluids were simultaneously measured by high performance liquid chromatography (Woo et al, 1987). Results were compared with those obtained from a previous study on 10 rabbits (9 F, 1 M, mean weight 5.02 ± 0.29 kg) following separate i.v. and p.o. dose of PZA (Chan & Wong, 1988). Using noncompartmental analysis, kinetic parameters of PZA under 4 different conditions were derived and summarised in Table 1. A 47.6% (after i.v.) and 51.2% (after p.o.) reduction in AUC of PZA was observed when RIF was co-administered but the mean residence times (MRT), mean absorption times (MAT) and elimination half-lives ($t_{\frac{1}{2}}$) were similar with or without RIF. The bioavailability of PZA (0.92) was slightly but not significantly reduced, suggesting a possible increase in first-pass metabolism of PZA when given orally in conjunction with RIF. In conclusion, the increase in plasma clearance (Cl), hence a reduction in AUC, of PZA in the presence of RIF, was due mainly to an increase in apparent volume of distribution (Vss) of PZA, and partially to the enzyme induction effect of RIF. RIF did not alter the CSF penetration of PZA as the CSF to plasma ratios were consistently maintained around unity throughout the time course, but the absolute concentrations were lower.

Table 1 Pharmacokinetic data

Parameters		After i.v. route			After p.o. route		
		PZA only	PZA + RIF	P	PZA only	PZA + RIF	P
AUC	(μg/ml.h)	71.1 (13.8)	37.2 (9.1)	<0.001	70.3 (17.4)	34.3 (9.9)	<0.0005
MRT	(h)	1.4 (0.3)	1.6 (0.2)	<0.1	2.7 (0.8)	2.7 (0.3)	<0.4
MAT	(h)	-	-	-	1.4 (0.7)	1.1 (0.2)	<0.2
$t_{\frac{1}{2}}$	(h)	1.0 (0.2)	1.1 (0.2)	<0.1	1.9 (0.6)	1.8 (0.2)	<0.4
Cl	($l\ h^{-1}$)	2.2 (0.4)	4.2 (0.8)	<0.0005	2.3 (0.7)	4.5 (1.3)	<0.001
Vss	(l)	2.9 (0.4)	6.7 (1.8)	<0.0005	6.0 (1.6)	11.7 (2.9)	<0.0005

References
Chan, K. & Wong, C.L. (1988), Eur. J. Drug Metab. Pharmacok., 13, in press.
Woo, J. et al (1987), J. Chromatogr. Biomed. Appl., 420, 73-80.
Woo, J. et al (1988), Neuroscience Lett., suppl. 31, S62.

THE USE OF MOLECULAR GRAPHICS IN THE DESIGN OF ANTI-INFLUENZA AGENTS

R.C. Wade (introduced by P.J. Goodford), Laboratory of Molecular Biophysics, University of Oxford, The Rex Richards Building, Oxford OX1 3QU.

There is a need for a specific drug which is effective against influenza despite the mutation of the virus which causes periodic epidemics of the disease. It is now possible to use the method of drug design by receptor fit to address this problem since the three-dimensional structures of the two antigenic surface coat proteins, haemagglutinin (HA) (Wilson et al,1981) and neuraminidase (Colman et al,1983), of the influenza virus have been solved. One of the functions of HA is to attach the virus to the host cell receptors by binding to sialic acid. In the present work, peptides were designed to block the host cell receptor binding site of HA by binding specifically to the highly conserved residues in this receptor site. The designed peptides should prevent attachment of the virus to the host cell and thus halt infection.

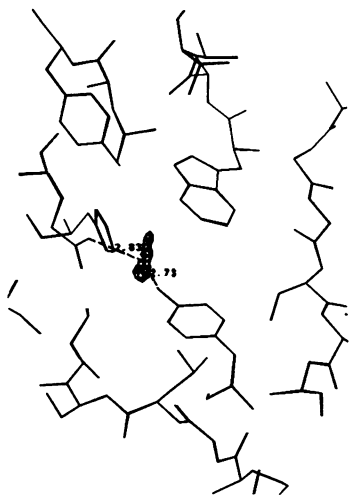


Figure 1

The HA receptor site was studied using the GRID method (Goodford,1985). This is a method of detecting energetically favourable ligand-binding sites on a chosen target molecule of known structure. The interaction energy between a small probe group, such as a carbonyl oxygen atom or an amine nitrogen atom, and the target molecule is calculated at evenly spaced points throughout the receptor site. The calculated energies can then be displayed as energy contours over the target region using computer graphics. Interaction energies with HA were calculated throughout the receptor site for a variety of probes and a number of favourable binding regions were identified. For instance, a binding site for an ammonium nitrogen NH_3^+ group is predicted at the base of the receptor site and is shown by the contours at about -10 kcal/mol in Figure 1. The probe can make two hydrogen-bonds of length 2.73Å and 2.83Å to the protein at this position.

Peptides were then modelled, using molecular graphics, to incorporate the appropriate probe groups in the energetically favourable positions predicted by GRID and to sterically fit the receptor site. The modelled peptides were energy minimised using program AMBER (Weiner et al,1984,1986). These studies demonstrate the viability of this approach for the design of drugs with suitable therapeutic properties.

Colman, P.M., Varghese, J.N. & Laver, W.G. (1983) Nature 303, 41-44.
Goodford, P.J. (1985) J. Med. Chem. 28, 849-857.
Weiner, S.J., Kollman, P.A., Case, D.A., Singh, U.C., Ghio, C., Alagona, C., Profeta, S. & Weiner, P. (1984) J.Am.Chem.Soc. 106, 765-784.
Weiner, S.J., Kollman, P.A., Nguygen, D.T. & Case, D.A. (1986) J.Comp.Chem. 7, 230-252.
Wilson, I.A., Skehel, J.J. & Wiley, D.C. (1981) Nature 289, 366-373.

ARE INDOMETHACIN INTESTINAL AND HEPATIC SIDE EFFECTS RELATED TO CLOSTRIDIUM PERFRIGENS TOXIN IN THE RAT?

Fracasso,ME, Leone, R, Del Soldato,P*, Cuzzolin, L,and Benoni,G,
Inst. Pharmacology, University of Verona, Verona and Italfarmaco S.p.A., Milano, Italy.

It has been observed that indomethacin causes toxic alterations in rat liver, kidneys and intestines (Fracasso et al, 1987).A relationship was found among these effects and intestinal microflora (particularly anaerobic bacteria such as Clostridii).
The aim of this paper was to detect Clostridium perfrigens enterotoxin in the faecal content of rats having intestinal and hepatic alterations induced by indomethacin. Fed male Sprague Dawley rats (150-200 g) were divided into groups of ten and treated for three days with an oral suspension (0.5% carboxymethylcellulose) of indomethacin (10 mg/kg) or placebo. Feces were collected for enterotoxin determination 24 hours after the second and third drug administration. The enterotoxin was determined by means of the late agglutination test of Sakaguchi et al (1973), by using a commercial kit (Oxoid). The results were expressed as 1-5 scores on the basis of concentration ranges 0-40 to 640-1280 ng/g feces.
Animals were killed by cervical dislocation 24 hours after the last administration of indomethacin. Organs were removed immediately: intestines were examined macroscopically for the presence of lesions and liver was prepared for microsomes, where cytochrome P450, cytochrome b5, NADPH-cytochrome C, N demethylase activity was assayed as described by Falzon et al (1984). Plasma was also collected for determination of transaminases which were assayed by conventional methods. Indomethacin caused intestinal lesions in all the animals. It also increased enterotoxin feces content and decreased hepatic enzyme activities, as shown in the following table (values as mean ±SE):

GROUP	ENTEROTOXIN SCORE		ENZYME ACTIVITIES (n moles/mg protein)			
	48 hr.	72 hr.	P450	b5	N Demethyl	NADPH/cytC
CONTROL	1.6±0.2	1.8±0.3	0.29±0.01	0.04±0.00	6.52±0.43	125.2±5.0
INDOMETH.	3.2±0.3*	2.7±0.4*	0.08±0.02*	0.01±0.00*	3.57±0.35*	63.2±7.6*

* $P < 0.05$ as compared to control group.

No significant change was observed in plasma enzyme activity in indomethacin treated rats as compared to controls.
These findings should indicate that, following indomethacin administration, the development of intestinal lesions is accompanied by release of Clostridium perfrigens enterotoxin and hepatic alterations in the rat.

Falzon,M, et al (1984) Biochem. Pharmacol.33,2285-1292
Fracasso,ME,et al (1987) Agent Actions 22,310-313
Sakaguchi,G, et al (1973) Appl. Microbiol. 26,762